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Miodrag Ristic
and Ian McIntyre
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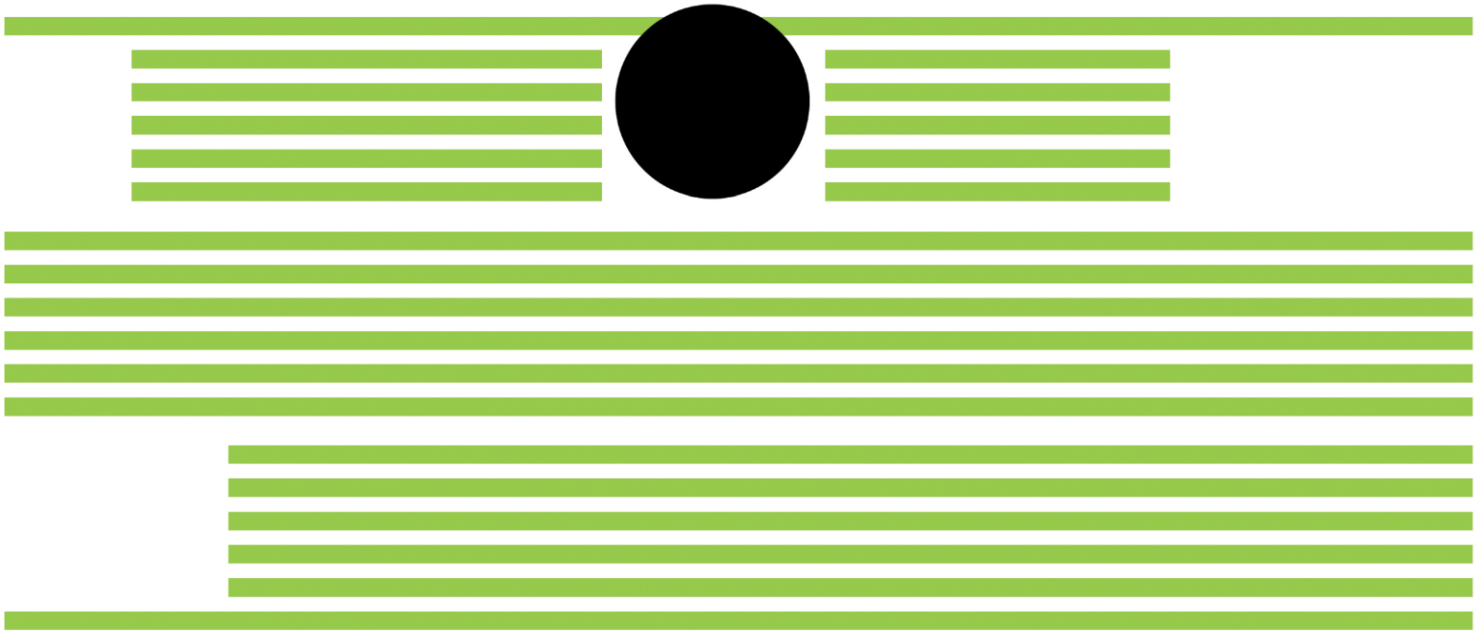

Diseases of Cattle in the Tropics

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DISEASES OF CATTLE IN THE TROPICS

Current Topics in Veterinary Medicine and Animal Science

Volume 6

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DISEASES OF CATTLE IN THE TROPICS

ECONOMIC AND ZOONOTIC RELEVANCE

Edited by

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PREFACE

Most of the future increase in livestock production is expected to occur in the tropical and subtropical regions of the world. Cattle are the most numerous of the ruminant species in the tropics and provide the largest quantity of animal food products. More than one-third of the world's cattle are found in the tropics. Disease is the major factor which prohibits full utilization of these regions for cattle production. Various infectious and transmissible viral, rickettsial, bacterial, and particularly protozoan and helminthic diseases, are widespread in the tropics and exert a heavy toll on the existing cattle industry there. This uncontrolled disease situation also discourages investment in cattle industries by private and government sectors. In Africa alone, it is estimated that 125 million head of cattle could be accommodated in the tropical rainbelt if the disease and other animal husbandry factors could be resolved. The potential of efficient cattle production under more favorable conditions prompted various international agencies to establish a multimillion dollar International Laboratory for Research in Animal Diseases (ILRAD) in Nairobi, Kenya, Africa.

In South America, principal sites for raising cattle are shifting to the savannah lands because the more fertile soils are being used for crop production, however, in the savannahs also, disease remains the most powerful deterrent in implementing the cattle industry. A small but well-organized research team in the Americas sponsored by The Rockefelier Foundation recently achieved an eminent breakthrough in a century-long struggle against bovine babesiosis. This team developed a method for a continuous *in vitro* propagation of *B. bovis* and used this cell culture-derived antigen to produce an effective vaccine against bovine babesiosis. Based upon past experience of the successful development of immunoprophylactic procedures for viral diseases, i.e., rinderpest and foot and mouth disease, this recent advancement with a protozoan disease fully attests that investment in research is the prime guarantee of a successful future for the cattle industry in the tropics.

In 1956 Henning published an excellent book, *Diseases of Animals in Africa,* which served as a source of reference for many years until it went out of print. Although several authors have made reference to Payne's books on *Animal Husbandry in the Tropics* and *Cattle Production in the Tropics,* the

editors have not included many of the topics so excellently covered by Payne. This book with its detailed treatment of disease in cattle should therefore be looked on as complementary to those by Payne.

This book is designed as an assemblage of modern basic and practical information on diseases of cattle in the tropics. It is not limited to diseases caused by infectious agents but also covers special topics associated with the epidemiologic, ecologic, and certain animal husbandry aspects of these diseases. Environmental effects such as those caused by tick bites and adverse climate are considered. Also included are some economic aspects of animal losses due to disease and how it relates to the world demand for animal protein. As a matter of reference, an appendix has been included which provides a full set of information on disease syndromes caused by toxic plants indigenous to the tropics as well as other geographical regions.

The book does not include every condition or disease of cattle known to occur in the tropics although the editors would claim that all of the conditions of economic importance have been included.

The book is not meant to serve as a massive source of information for research workers; rather it is a guide for graduate and undergraduate students of veterinary and agricultural sciences, field veterinarians and allied professions interacting with animal production and disease control. Consequently, the authors have made every effort to select key references which will lead the reader into the literature, sometimes vast in its entirety.

In each chapter, emphasis has been given to epidemiology, clinical signs, pathogenesis and control. Much of the laboratory details and scientific deliberation of certain specific aspects have been left for more appropriate texts.

Many diseases covered in this book are zoonotic. Some of these are old well-established zoonoses for which public health regulations are in existence. There are also new, more recently recognized zoonotic diseases, i.e., babesiosis, which need further studies before similar regulations can be recommended. Thus the book also serves as a source of information for medical and public health workers.

The authorship of the book is truly international and is represented by scientific eminence, intense practical experience, and comprehensive knowledge of the respective subject matter. To these authors, the editors express their esteem, thanks and appreciation. An acknowledgement of thanks is also extended to the publisher and staff of Martinus Nijhoff Publishers for their continued support.

Finally, the editors wish to acknowledge their appreciation to their able and patient secretarial staffs led by Ms. Lois Stauter which greatly contributed to the production of this book.

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PART I

SPECIAL TOPICS

1. CHARACTERISTICS OF TYPES AND BREEDS OF CATTLE IN THE TROPICS

Marvin Koger

Abstract. The origin and general characteristics of the principal types of cattle currently inhabiting the various tropical areas of the world are considered. The major groups included were the humped Zebu (*Bos indicus*), non-humped European (*Bos taurus*), intermediate crosses of these two types, and the close relatives of cattle, the Banteng and Water Buffalo.

The Zebu and European types of cattle both are thought to have been domesticated in western Asia. The Zebu spread across southern Asia, immigrating later to the African continent where they migrated slowly southward. Beginning about the middle of the 19th century, Zebu cattle principally from India were transported to the Americas, Australia and various tropical islands.

The European types spread over central Asia and Europe and also migrated to Africa where they mixed with the Zebu types. European cattle from the Iberian peninsula were introduced by the Spaniards into the Caribbean Islands, and onto the American continents in the early 1500s. They spread over all of South America, Central America and the western part of the United States. These cattle later became known as the Criollo cattle of South and Central America. They merged with European cattle introduced onto the North American Continent by the English colonists to give rise to the Texas Longhorn.

In the latter half of the 19th century, Criollo cattle were topcrossed with Zebu bulls. At present, the majority of the cattle of the tropics are of Zebu or Zebu-European breeding. For the most part, they are of poor to fair productivity for either milk or meat. These cattle are used widely also for work and as prestige or ceremonial animals. Water Buffalo are used for work, meat and milk in many areas. Banteng cattle are utilized for meat in southeastern Asia and Indonesia.

The art and science of animal husbandry is that of man striving to optimize to his advantage the interaction between his animals and the environment in which they are maintained. This relationship is governed by the genetic attributes of the animals involved and the quality of the environment including nutrition, disease, climatic influences and management. Since cattle were first domesticated 5000 to possibly 10 000 years ago, man has influenced both the genotypes of his cattle and the environments under which they have existed. These influences have been exerted both intentionally through

selection of breeding stock and management, and incidentally through usage of animals and the impact of man on the environment.

The importance of genetic adaptation of organisms to their environment was a basic concept embodied in the tenets of Charles Darwin relative to evolving populations. Survival of the fittest leads to genotypes that differ in their adaptation to varying ecological and climatic environments. Environmental differences with respect to diseases, parasites and insects are no exception. This should not be interpreted to imply that genetic resistance to disease is absolute and should be pursued as the principal means of disease control. The point is that different genetic stocks vary in their response to various diseases, pests and other components of the environment. These differences should be recognized and utilized to the extent that it is practical.

Information on the characteristics of animals is of importance in designing effective health and production programs for cattle in the tropical and subtropical areas of the world. The objective of this presentation is to give a brief overview of the distinctive features of some of the more important groups of cattle presently found in these areas. For additional information on specific breeds the reader is referred to more extensive works included among the references cited.

Origin of modern types and breeds

The origin, present distribution and examples of domesticated groups of cattle, including water buffalo, are shown in Table 1. Included are six groups representing four genera of the Bovidae family. Except for the Yak (*Poëphagus*), all of these groups are presently found in the tropics and sub-tropics. The most important groups in terms of numbers, utility and economic significance are included in the *Bos indicus* (humped cattle) and *Bos taurus* (humpless) groups. The former are frequently referred to as Zebu and the latter as European cattle.

The progenitors of modern-day *Bos indicus* and *Bos taurus* groups were domesticated principally in western Asia over a period of time extending possibly from 8000 to 3000 BC. The *Bos indicus* types spread eastward and southward into Asia and southwest into Africa. The *Bos taurus* types migrated south and west into Africa and spread over Europe and into the temperate regions of Asia. Later both types were transported to other parts of the world.

Table 1. Origin of modern domesticated cattle.

Group and area of origin	*Modern descendants*	*Present distribution*	*Examples of modern breeds or types*
Bos taurus – Western Asia, Central Europa	European types and breeds	Temperate, tropical and semitropical areas	Brown Swiss, Holstein, Angus, Hereford
Bos indicus – Southern Asia	Zebu types and breeds	Tropics and semitropics	Guzerat, Sahiwal, Gir, American Brahman
B. taurus × *B. indicus* – Asia, Africa, The Americas, Australia	Sanga types Yallow Chinese, Brahman × British derivatives	Africa, South China, The Americas, Australia	Africander Bonsmara Beefmaster Santa Gertrudis
B. sondaicus – Indonesia	Banteng Gaur	Indonesia Malaysia	Bali Gayal
Poëphogus – Central Asia	Yak	Central Asia	Yak
Bubalus bubalus – Asia	River Buffalo (milk type) Swamp Buffalo (work type)	Mostly in southern Asia and northeast Africa	Murrah, Nili

Current Zebu types

The Zebu Breeds of Asia

The humped cattle of southern Asia gradually became separated into more or less distinct types, generally acquiring names associated with the regions where the types predominated. Many of these types have come to be regarded as breeds. Phillips (1963), for example, listed 27 such breeds in India and Pakistan. These were classified into six groups based on phenotypic similarities. Payne (1970) included 32 breeds in the Indian subcontinent and two from south China and Taiwan. A list of the Asian Zebu breeds described by the above authors is shown in Table 2.

The Zebu Breeds of the Americas

The Brazilian Breeds. Cattle of three of the more distinct breeds of Indian origin were imported into Brazil and continued as closed breeds in that country. These included the Gir, Kankrej and Ongole. Their respective Bra-

Table 2. The Zebu groups of Asia.

Group	*Location*	*Description*
Alambadi	North Salem and Northern Coimbatore and Bangalore districts of Madras	Long backswept horns. Medium-sized and generally rangy in build. Coat color is gray or black in males, broken colors in females. A hardy draft animal.
Amrit Mahal	Vicinity of Mysore, India, latitude 11° to 15° N	Horns grow upward and back. Compact to medium type. Color varies from gray to almost black. Dewlap, navel flap and sheath tight. Used for work.
Bachaur	Bihar State, India	Shorthorned, compact, commonly gray, medium dewlap, tight sheath. Used for draft.
Bagur	Madras area of southeast India	Horns grow back and up. Medium size, color usually red and white but may be gray. Dewlap moderate, sheath is not excessive. Valued for their endurance and speed.
Bhagnari	Northeastern India, semiarid, subtropical	Shorthorned, deep-bodied; color white or gray to black in males. Used primarily for draft.
Dangi	Western coast of India, north of Bombay, latitude ±21° N	Horns usually short and lateral. Medium in size, compact in shape. Coat color broken red or white. Hardy, slow draft animal.
Deoni	Northwestern Hyderabad of Gir and Dangi background	Curled horns, medium size, compact. Black and white spotted. Long ears, loose skin or underline. Used primarily for work.
Dhanni	Punjab region of Pakistan at latitude 33° to 34° N	Horns are small, lateral and turn upward. Of medium size and compact bulld. Coat color is black and white with occasional red spots. Tight skinned, durable draft animal.
Gir	Kathiwar peninsula of western India and adjacent areas	Moderate-sized curled horns. Moderately large in size. Variable in color with brown spots. Pendulous sheath. Used for milk and work.
Gaolao	Central Provinces of India	Shorthorned, light-framed, long-bodied, loose-skinned, large dewlap, medium sheath. White to gray in color. Fast moving work animal.
Hallikar	Southern India	Horns sweep back then upward. Compact in build and of medium size. Color is gray to dark gray, frequently with white markings on underline. Ears are short, sheath is tight but dewlap is moderate. Valued as work animal.

Table 2. (continued)

Group	*Location*	*Description*
Hariana	Semi-arid, subtropical area of eastern Punjab and environs	Shorthorned, white to gray in color. Fine textured, relatively tight skin, medium to large hump. Used for work and milk.
Hissar	Eastern Punjab	Upcurved medium horns. Medium size. Color white to dark in bulls. Small dewlap, tight sheath. Used mostly for draft.
Kangayarm	Southern India, Latitude 10° to 11° N	Type not well fixed, influenced by Ongale and Mysore breeding. Of moderate size. Color varies from almost white to almost black. Valued draft animals.
Kankrej. The Guzerat of Brazil is a derivative of this breed	Northeastern section of Gujerat State, India	Lyre shaped horns. Large in size. Skin on underline is thin with pendulous dewlap navel and sheath. Variable shades of gray in color. Work breed.
Khillari	Western India, latitude 16° to 22° N	Unique shaped horns grow back then turn upward. Color varies with segment of breed, varying from white to gray, some with a red tint. The ears are small, skin on underline is tight. Valued for work.
Krishna Valley	Krishna Valley of south central India, latitude ±16°8′N	Shorthorned, variable in type. Generally large framed, deep bodied and loose-skinned. Color usually gray to white. Used mostly as a heavy draft animal.
Kumauni	Kumaun hill region of the United Provinces	Short-lateral horns, small compact cattle. Color may be black or red with white spots. Used for work and manure.
Lohani	Baluchistan and Northwest Frontier Province Pakistan	Short lateral horns, small type, tight dewlap and sheath. Color red or red and white. Used mainly for draft.
MALVI Group	North Central India, vicinity of latitude 24° N, longitude 76° E	Includes three groups varying in size, similar to Kankrej except for being shorter of leg and more compact in type.
Mewati	Mewat region of the Punjab – Uttar Pradesh border	Short horns, turning back at the tips. Color white to gray upstanding in stature. Skin folds on neck and underline loose but not pendulous. Used for work or milk.
Nagori	Northeast of Jodhpur in midwestern Rajasthan State, India	Moderate horns. Upstanding and sturdy of frame. White to gray in color. Ears are pendulous. Dewlap and sheath are fine textured and not pendulous. Used for rapid work.
Nimari	Eastern Madhya Pradesh, India of Gir and Khillari influence	Curled horns. Moderately long and compact in build. Color usually red with white splotches. Skin is loose, pendulous sheath is common.

Table 2. (continued)

Group	*Location*	*Description*
Ongole	Southeastern coastal area of India at latitude of ±15°30′ N	Shorthorned, large, long body and legs; short neck. Color normally is white with gray on neck, hump and points of males. Red color and splotching of skin may occur. Used for work and milk.
Ponwar	Uttar Pradesh and northern Bihar	Lyre shaped horns, medium in size. Color usually black and white or black and red with white markings. Dewlap and sheath are tight. Used for draft animals.
Punganoor	Chitaor district of Audhra Pradesh, S.E. India	Horns are small and often loose. Very small in size. Coat color varies. Most animals are white with brown spots. Almost extinct. Some say prospects for "small farmer".
Rath	Alwar region of Rajasthan State, India	Shorthorned, medium sized, short pendulous ears, white to gray color, small dewlap with tight sheath. Used for work and milk.
Red Sondhi	Region extending north from Karachi, Pakistan	Horns are lateral and upturned. Small in size and compact of build with pendulous dewlap, udder and sheath. Color varies from dark red to yellow. Used primarily for milk, secondarily for work.
Sahiwal	West Punjab, India	Horns are short, sometimes loose in females. Variable in size, compact in form. Pendulous dewlap. sheath and udder. Red tint in color is common. Used mostly for milk.
Shahabadi	Shahabad and Saron districts of Bihar; Ballia and Ghozipar districts of Uttar Pradesh (N.E. India)	Horns are thick and blanted. Dewlap is medium. Hump is prominent. Milk cows and work males.
Sinhala	Ceylon	Short stumpy horns, some polled animals. Small fine-boned. Coat color variable with reds and blacks being common. Dewlap and sheath are not pendulous. Useful as draft animal. Reproductive performance reported to be good.
South Chinese Zebu	Probably derived from cattle of southeastern Asia	The group includes three related types, Kwantung, Hainan and Kwangsi. All are short horned. Colors include yellow, brown, dun and roan. Used for draft.

Table 2. (continued).

Group	*Location*	*Description*
Taiwan Zebu	Present cattle mostly derived by topcrossing earlier stocks with Red Sindhi and Kankrej stock from the Mainland	See description for Red Sindhi and Krankrej.
Tharparker	District of Tharparker near Hyderabad, Pakistan	Horns of lyre type. Medium in size. Color usually white to gray with light gray backstripe. Pendulous dewlap, navel and sheath common. Use: Milk and work.

zilian descendants are the Gir, Guzerat and Nelore. A fourth Brazilian Zebu breed, the Indubrasil, was developed from crosses among the Gir, Guzerat and Nelore.

The American Brahman. This breed was developed in the Gulf Coast area of southern United States and Mexico. Although a few Indian cattle were imported into the United States prior to the introduction of the Zebu breeds into Brazil, the American Brahman was developed principally through topcrossing to bulls of the Brazilian Zebu breeds introduced into the United States through Mexico. Commercial cows of the area were used as foundation females. When the proportion of Zebu breeding reached 31/32, females which passed inspection were entered into the Brahman registry as foundation females. The relative proportions of the various Zebu breeds represented in foundation animals was not recorded but undoubtedly varied widely depending on the breeder. Over the entire breed, the influence of the Guzerat and Nelore appears to have been approximately equal, followed by that of the Gir, Indubrasil and others. Visual appraisal suggests that the importance of the various foundation breeds still varies considerably from one segment of the breed to another. This would appear to be reasonable in view of breed history. During recent years the use of Brahman bulls for crossbreeding beef cattle has steadily extended northward in the United States. The breed has enjoyed a continuing export trade to Central and South America and to Africa. Limited numbers of Brahman cattle have been introduced also into Northern Australia.

The Jamaican Brahman breed was developed from the descendants of Indian Zebu cattle introduced into Jamaica from 1850 to 1921 and the American Brahman which was introduced first in 1948. The herd book was

closed in 1955. The Jamaican Brahman and American Brahman are similar in appearance.

The Romana Red was developed in the Dominican Republic from a base of red Criollo type cows topcrossed to Mysore and Nelore bulls. While being somewhat intermediate in type, the breed phenotypically appears to fit better into the American Zebu breeds than into the American crossbred derivative group.

The Zebu Types of Africa

The humped cattle of western Asia early found their way into Africa. They drifted slowly southward, mixing with humpless cattle which also originated in western Asia, to produce a variety of crossbreds known as the Sanga types

Table 3. The Zebu types of Africa.

Group	*Location*	*Description*
Abyssinian	Central highlands of Ethiopa and Eritrea	Horns of medium length, variable in shape. Small of size with skin folds on underline. Color is variable. Used for work, milk and meat.
Adamawa	Adamawa provinces of Cameroun and Nigeria	Horns are small. Medium-sized, moderately loose skinned. Color is highly variable. Triple purpose in use.
Angoni	Zambia, Malawi and Mozambique	Shorrhorned type, some horns are rudimentary or absent. Medium to small in size. Color is variable. Used for draft, milk, meat and ceremony.
Azaouak	Azaouak Valley, latitude 15° to 20° N, longitude 3° to 7° E	Horns are short in female, medium in males, medium-sized, ear length and skin folds are medium. Color variable.
Boran	South Ethiopia, Somalia and north Kenya	Group includes related types. Colors are variable utilized by nomads and by ranchers.
Diali	Flood plains of the Niger River and surrounding valleys	Horns and general type are variable. Medium-sized. Coat color is usually white but black and white, red and white and roan animals also occur. Used mostly for meat.
Fulani, Senegal	Western Senegal and south Mauritania	Lyre-type horns. Tall of stature and looseskinned. Color is highly variable with white preferred. Triple-purpose breed.

Table 3. (continued).

Group	*Location*	*Description*
Falani, Sudanese	Flood plain of the Niger River from Ségou to Timbuktu	Horns usually long and lyre-shaped but are variable. Medium-sized, long of leg with moderate skin folds. Color usually gray with dark splotches. Flightly of temperament, used for meat.
Fulani, White	Northern Nigeria and Cameroon	Horns are medium to long, lyre-shaped. Large in size with heavy dewlap but moderate navel flap and sheath. Coat color usually is white with black points. Variants include flecked black and red and white. Triple-purpose, popular for milk.
Karamajong	Region of the junction of Sudan, Uganda and Kenya	Similar to Boran group. Origin uncertain. Not a distinct type. Generally rangy in frame, with pendulous dewlap and navel flap. Used mostly by Nomadic tribes for milk and blood.
Madagascar Zebu	Introduced from Africa or India	Horns usually are medium, curve out, up and forward but vary and may be absent. Small to intermediate in size. Used for beef and work.
Maure	Mauritania, Mali and Upper Volta	Horns are fine, short in males, medium in females. Medium to large in size. Color usually black or black and white, some brown. Triple purpose.
Red Bororo	Now found in Nigeria, Cameroon and Chad	Long, lyre-shaped horns. Large, upstanding with loose skin. Dark red to reddish brown in color. Hardy and nervous. Valued mostly for their skins.
Small East Africa Zebus	Presently found in most areas of East Africa	Group includes many varieties of the same general type. Subtypes include the Mongalla, Lugware, Bukedi, Nandi, Masai, Tanzania and Zanzibar, etc. Many of these cattle used by tribes for milk, blood, work and ceremonial purposes.
Small Somali Zebus (north Somali, Gasari and Garre)	North Somalia and Eritrea	Horns lyre-shaped or small. A few are polled. Small in size with a small hump. Color is variable. Used for milk, meat and work.
Shuwa	Chad region	Shorthorned, medium to small in size, color is usually red, brown or broken. Triple purpose.
Sakato	Northwest Nigeria	Short, lateral upturned horns. Medium sized, pronounced dewlap and navel fold. Used for milk and work.
Sudanese	Northeast Africa	Highly variable group of Zebu cattle which inhabit the region.

which will be discussed later. The unmixed Zebu cattle of Africa did not separate into groups as distinct as those of India; consequently, they have not become generally recognized as distinct breeds. Payne (1970) tentatively classified the African Zebus into 18 types as shown in Table 3.

European types found in the tropics

Asia

Few *Bos taurus* type cattle are found in the tropical and subtropical regions of Asia. Payne (1970) listed these to include the Oksh types of western Asia along with the Chinese Yellow and Batangas types of southeastern Asia.

Africa

Shorthorned *Bos taurus* (*B. brachyceros*) cattle from western Asia immigrated into north Africa prior to 2500 BC. Descendants of these cattle survived to become European types indigenous to Africa. Payne (1970) listed seven such groups including the Libyan, Brown Atlas, Kuri, N'Dama, Dwarf Shorthorn, N'Dama × Shorthorn crosses and the Muritius Criollo.

Criollo Cattle of the Americas

Bos taurus type cattle from the Iberian Peninsula of Europe were introduced into the Caribbean Islands, South America, Central America and the Gulf Coast region of North America by Spanish conquistadores beginning in 1493. Records are not adequate to document trends. It is evident, however, that in many areas these cattle increased rapidly in numbers for a period of approximately 300 years, after which time diseases and malnutrition (compounded with over-grazing, over-burning and mismanagement) began to take a severe toll. When Zebu cattle were introduced, the F_1 Zebu × Criollo crosses exhibited a favorable combination of heterosis, tolerance for tick-borne diseases and the ability to utilize fibrous roughage. As a consequence, upgrading to Zebu was accelerated as bulls became available. This practice has continued to the point of virtual extinction of Criollo stocks. There are a number of groups, however, that have been maintained as pure Criollos. Nine such groups are shown in Table 4. Numbers of cattle in these groups, however, are not large. The number that can continue as viable breeds is questionable.

Table 4. Improved Criollo breeds.

Name	*Location*	*Use*
Blanco Orejinegro	Colombia	Meat and milk
Chino Santandereano	Colombia	Meat
Costeño con Cuernos	Colombia	Meat and milk [a]
Romo – Sinuana	Colombia	Meat [a, b]
San Martinero	Colombia	Meat
Vallecaucano	Colombia	Primarily milk
Cuban Criollo [c]	Cuba	Meat and milk
Costa Rican Criollo	Costa Rica	Milk
Limonero	Venezuela	Milk

[a] Originally selected also for draft.
[b] Polled gene possibly introduced from Angus or Red Polled.
[c] Some animals of this group show indications of Zebu influence.

The Shorthorn of Northern Australia

Shorthorn cattle were introduced into Australia during the first half of the 19th century. The breed gradually underwent adaptive changes and eventually occupied the tropical region of Northeastern Australia. Recent experimental results and breeder experience, however, have shown Zebu crosses to be better adapted to the region. Consequently, the Shorthorn is being replaced by crossbred types. Temperament, however, is reported to be a problem in some crossbreds under extensive range conditions.

The Highly Improved Bos taurus *Breeds*

One of the great achievements of man has been the development of the highly productive improved breeds of Europe and the British Isles, especially the modern dairy breeds. It is natural that their proponents, including breeders and professional geneticists alike, have advocated replacement of local types with cattle of the highly improved breeds as the logical solution to cattle production in all areas. These expectations, however, have not been realized in the harsh tropics. The cost of high quality feed and effective health programs usually have been prohibitive. In general, the highly improved temperate-zone stocks are more sensitive to unfavorable conditions than less productive animals. Reproduction and survival are the traits most severely affected.

Under appropriate management the highly improved breeds may be used for production in limited areas of the more favorable tropics and semitropics. Their greatest potential utility in tropical areas, however, appears to be for crossbreeding and in generation of crossbred foundations for new strains.

Intermediate Zebu × European types

Bos indicus and *Bos taurus* cattle were domesticated, at least in part, contemporaneously in the same general region of the Eurasian continent. Since the two groups intermate freely, intermediate crossbred types arose in areas occupied jointly by the two groups. Some of the more distinctive of these intermediate groups as described by Phillips (1963), Payne (1970) and Rouse (1970a, b, 1973) are indicated below for various regions.

Asia

Payne (1970) included nine intermediate groups currently found in Asia. They include the Damascus, Lebanese and Persian of western Asia; the Siri from the mountainous regions of India; and the Burmese, Thai, Indo-Chinese, Thai-Kedah and Chinese yellow of China and southeast Asia.

Africa

Since both *Bos taurus* and *Bos indicus* cattle migrated into Africa approximately 4000 years ago it is not surprising that many intermediate crossbred types evolved on that continent. Cattle of these crossbred groups are known as Sanga cattle. Payne (1970) listed 36 named groups.

The two most notable groups are the Africander, predominantly of Zebu breeding, and the Bonsmara of South Africa. The Africander has enjoyed wide acceptance in Africa and has been exported to the United States and Australia. The Bonsmara is a recently-formed breed developed by selection from a crossbred foundation of 5/8 Africander, 3/16 Hereford and 3/16 Shorthorn breeding. The production performance reported for this breed is impressive.

Other intermediate African types are shown in Table 5.

The Zebu-European Types of the American Tropics

The grade Zebu and Zebu-European crossbred groups of the American tropics are of relatively recent origin. Zebu stocks were introduced into North America in small numbers beginning about 1850 and into South America beginning about 1870. European-Zebu crosses appeared soon after these introductions but did not become numerous until the early part of the 20th century when Zebu bulls from Brazil, Mexico and the United States became generally available to cattle producers. By this time the descendants of cattle introduced by the Spanish conquistadors had grown into vast Criollo populations extend-

Table 5. The major *Bos indicus – Bos taurus* crossbred (Sanga) types of African cattle.

Breed name	*General location*	*Group*	*General characteristics and use*
Egyptian	North Africa	Stabilized	Medium size, color variable. Mostly for draft.
Mekne's Black Pied	North Africa	Stabilized	Of multibreed origin including Holstein. Moroccan milk breed.
Bambari	West Africa	Stabilized	Small, yellow to red, used for beef.
Biu	West Africa	Stabilized	Shows influence of Shorthorn and white Fulani.
Borgu	West Africa	Stabilized	Of N'Dama, Shorthorn with Fulani background. Small, variable color.
Kuri Zebu	West Africa	Intermediate	Variable Zebu influence.
N'Dama × Zebu	West Africa	Intermediate	Variable Zebu influence.
N'Dama × Sh × Z	West Africa	Intermediate	Heterogeneous in ancestory. Mostly for meat.
Ankole	Northeast Africa	Stabilized	Large lyre-type horns. Used for milk and ceremony.
Danikil	Northeast Africa	Stabilized	Longhorned, lyre or crescent. Color variable.
Nilotic	Northeast Africa	Stabilized	Narrow, upstanding, mostly off-white. For milk and ceremony.
Alur	Northeast Africa	Intermediate	Medium sized. Red, red pied and black colors. For beef and milk.
Arado	Northeast Africa	Intermediate	Highly variable in size, type and color. Docile work animal.
Nganda	Northeast Africa	Intermediate	Light reds common but other colors occur. Variable size. Mostly for meat.
Nuba Mountain	Northeast Africa	Intermediate	Very small, variable in color, for social purposes.
Tuni	Northeast Africa	Intermediate	Large, thick, color is red or red and white. Multiple use.
Sukumu	Northeast Africa	Intermediate	Medium-sized with large upturned or crescent horns. Color variable.
Kasai	Northeast Africa	Recent	Hereford-Africander cross. Scarce in numbers.
Angolan	Central Africa	Stabilized	Medium large, upstanding. Large horns, color variable.
Barotse	Central Africa	Stabilized	Large, heavy-boned, loose skinned. Color variable. Multipurpose.
Tuli	Central Africa	Stabilized (registry)	Large-framed. Color red, intermediate to white. Beef type.
Mashona	Central Africa	Stabilized	Medium-horns, small, fine boned. Color red, black, brown. Beef and draft.
Tonga	Central Africa	Stabilized	Medium of size and horn. Color variable. Multiple purpose.
Baila	Central Africa	Intermediate	Intermediate between Barotse and Tonga breeds.
Matabele	Central Africa	Intermediate	Multiple breed ancestry. Medium-size, mostly for milk.
Kisantu	Central Africa	Recent	Type and color unfixed. Not important.
Mateba	Central Africa	Recent	Multiple breed origin. Not important.
Bechuana	South Africa	Stabilized	Long-horned, large, red or black, variable spotting. Multipurpose.
Ovambo	South Africa	Stabilized	Sanga type, variable size, color variable, flecked. Multipurpose.
Basuto	South Africa	Stabilized	Mixed ancestory, moderate size, roan color common. Multipurpose.
Nguni	South Africa	Stabilized	Medium small, lyre horns in cows. Color variable. Good milkers.
Africander	South Africa	Stabilized	Lateral-horned, large, hardy, red in color. Meat and work type.
Drakensberger	South Africa	Recent	Shorthorned, large, black, white or underline. Mostly for meat.
Bonsmara	South Africa	New breed	Medium horns, large, red productive.
Baria	Madagascar	Stabilized	Primitive type, no longer of importance.
Rana	Madagascar	Recent	Multibreed origin, small. Color variable. Mostly for milk.

ing from Argentina in South America to southern Canada in North America.

The first of these hardy cattle to be replaced were those of the temperate zones of both North and South America. Bulls of the improved British breeds were introduced into Argentina and the United States where the Criollos were upgraded into probably the most productive beef cattle ever tended by man. The contribution of Criollo genes to these cattle has yet to be generally recognized. The mounting problems of the current "improved" stocks, however, are beginning to arouse some appreciation.

As the density of Criollo cattle in the tropical and semitropical areas increased, so did the ravages of tropical diseases, starvation and mismanagement by man. As Zebu bulls became available, experimental Zebu × Criollo crosses were made. To the disbelief of promoters of the improved European breeds, such matings resulted in highly favorable responses in growth rate, thrift and general productivity. Unfortunately this response was interpreted as being due to superiority of the Zebu rather than to the phenomenon of hybrid vigor. Extensive topcrossing of local stocks with Zebu bulls led to grade Zebu types becoming the predominant cattle of the American tropics. As should have been anticipated, production performance declined with succeeding generations of top-crossing and the resulting loss of hybrid vigor. Now there is much confusion on what course to pursue.

Improved American Breeds Based on Crossbred Foundations

The favorable initial responses to crossing Zebu and European stocks led naturally to interest in establishing new breeds based on European × Zebu crossbred foundations. While growth in numbers within these groups has not been impressive in past years, interest in such groups has shown indications of some acceleration. Some of the more widely recognized of these breeds include the following:

Santa Gertrudis. The Santa Gertrudis was the first recognized breed of cattle to be developed in the United States. It was developed by the King Ranch of Texas, USA, in a semi-arid environment. In terms of breed composition, Santa Gertrudis cattle are approximately 3/8 Zebu and 5/8 Shorthorn. The breed is red in color with limited white spotting being permissible on the underline. The breed is of moderate to large size with mature bulls in pasture conditions weighing from 1700 to 2200 pounds. Cows under similar conditions weigh up to 1300 pounds.

Beefmaster. This breed was developed in Texas and Colorado, USA, by the Lassiter Ranch beginning in 1931. The exact percentage of blood of each

parent breed is not known. It is estimated, however, that a combination of about 25% Hereford, 25% Shorthorn and 50% Brahman breeding was incorporated into the breed. A unique feature of this breed is that selection has been entirely for economic traits with no attention being given to items such as color or horns.

Brangus. The Brangus breed was developed by combining Brahman and Angus breeding in a 3/8 – 5/8 combination and selecting for the desired traits of both breeds. Interest in developing the breed was stimulated by the early work in crossing of Brahman and Angus cattle at the United States Department of Agriculture Station at Jeanerette, Louisiana, USA. The introduction of newlyformed 3/8 – 5/8 foundation animals is still permitted.

Barzona. The foundation for the Barzona breed was formed during the early 1940s when the Bard Ranch of Arizona, USA instituted a crossbreeding program using Angus and Santa Gertrudis bulls on Afrikander – Hereford cows. From this crossbred foundation, Barzona cattle were selected for performance under the rigors of the sparse feed supply and high temperatures of the semi-desert rangelands. One of the characteristics for which selection was practiced was the ability to utilize coarse, fibrous browse plants. The number of cattle in this group is small.

Braford. Braford cattle in the United States were developed principally by the Adams Ranch of Ft. Pierce, Florida. In terms of foundation breeding they are approximately 2/3 to 3/4 Hereford and 1/4 to 1/3 Brahman. Superior Hereford-sired bull progeny from a Brahman-Hereford sire rotation were mated to females of the same breed composition to form first-generation Brafords. These cattle have been straightbred for 3 to 7 generations. The production traits which have been emphasized in selection include fertility, calving ease, calf survival, weaning weight, postweaning gain, moderate mature weight and adaptability to their environment.

Braford type cattle have been developed also in Australia.

Charbray. Brahman females were used extensively as foundation females in the process of upgrading to Charolais bulls in the USA. Crosses of these two breeds attracted wide attention because of their size and rapid rate of growth. The term Charbray was copyrighted at one time to include Charolais-Brahman crosses containing 1/16 to 1/2 Brahman. The requirement on breed proporations recently was changed to a 3/8 – 5/8 combination. Charbray bulls were used initially in the United States mainly as a substitute for Charolais bulls before the latter became plentiful. During recent years, they have been

exported principally for use in the more tropical areas where purebred European bulls are not well adapted.

Jamaica Black. This breed descended from Zebu × Angus crosses. It is similar in characteristics to the Brangus of the USA.

Jamaica Red. The foundation for this breed was formed by topcrossing Red Poll bulls on Creole × Zebu females, followed by selection for beef characteristics. This breed has been exported to a number of tropical countries.

Jamaica Hope. This dairy type animal is predominantly of Jersey background with an undercurrent of Sahiwal and other breeds. The breed was closed and a breed association formed in 1953. The breed is similar in appearance to the Jersey. While not as productive as the Jersey under ideal conditions, the breed has demonstrated the capability to perform satisfactorily as a dairy type under less than optimum environment.

Senepol. The Senepol breed originated on the island of St. Croix in the Virgin Islands. It was developed from a crossbred foundation formed by mating descendents of Senegal N'Dama cattle to Red Poll bulls. It is a well fixed beef type of medium size. They are red in color and show excellent adaptation to the island environment.

Canchim. The breeding of this group is 3/8 Zebu – 5/8 Charolais. The Zebu genes were derived from various Zebu breeds. The Canchim is a beef type animal developed by the Brazilian Ministry of Agriculture. The breed is not important in terms of numbers but is reported to be increasing in popularity.

Pitangueira. This Brazilian selection includes 3/8 Guzerat and 5/8 Red Poll breeding. It is being developed as a dual purpose type. Numbers in this breed are small.

Banteng cattle

Domesticated Banteng (*Bos sondaicus*) cattle are found in Indonesia with small numbers having been exported to neighboring areas. They exist in the wild state also in Malaysia, Thailand and Burma. The Banteng types are said to be fertile and tractable but have not been exploited as extensively as *Bos indicus* and *Bos taurus* cattle.

Water buffalo

The water buffalo, *Bubalis bubalis,* originated in southern Asia. Domesticated buffalo are of two types, the "river buffalo" and "swamp buffalo." The swamp type is used primarily for work. Wallows generally are utilized by this type. The River buffalo are used for milk and work. There are a number of breeds of river buffalo, the Murrah and Hili being seen most frequently. Water buffalo are of importance in southeastern Europe and Egypt, southern Asia, and in Taiwan, the Philippine Islands and Indonesia. Wider exploitation of the buffalo has been encouraged by various developmental agencies in recent years. Additional research on which to base recommendations is needed.

Some characteristics of cattle in various tropical regions

The American Tropics

The majority of the beef cattle of the American tropics now are grade Zebu or Zebu-Criollo crosses in the process of being further upgraded to Zebu. Sadly, the Criollo which undergirded the economy of South America and a good portion of North America for a period of 400 years has been nearly eliminated by topcrossing to Zebu bulls. The prospects for generating enough support to salvage effective remnants of the Criollo do not appear bright. Nine of the more distinctive groups remaining are shown in Table 4.

Survival characteristics of the current Zebu stocks are moderate to good but productivity, including both reproduction and rate of maturity, is low. The genetic attributes of the cattle and husbandry practices both are in need of improvement if significant progress is to be achieved. While most of these cattle are used primarily in beef production enterprises, they are used also in dual purpose enterprises where the cows are milked and also permitted to nurse their calves. While not regarded as progressive by some, economic studies tend to vindicate the practice. The more modern specialized dairies generally utilize cows representing one or more topcrosses of highly improved dairy sires. There is little unanimity of opinion, however, on the most favorable proportion of breeding from the highly improved dairy breeds. Production per lactation tends to improve with increasing levels of improved dairy breeding, providing nutrient requirements are met. Reproduction, however, tends to decline in the tropics with increasing levels of specialized dairy breeding. Where management and nutrition are poor, native types may perform better than high grade improved animals.

A number of breeds based on crossbred foundations of the improved European and Zebu breeds currently are being promoted. Numbers in these groups, however, are not large. The impact of these crossbred derivative groups on cattle production in the tropical areas remains to be determined.

There are a few water buffalo in South America and the Caribbean area but numbers are too limited to be of much economic significance. While favorable reports on the potential of water buffalo appear periodically, future exploitation of the animal in the area appears to be problematical because of extended dry seasons, manageability and rate of reproduction.

Asia

The outlook for the future of cattle in southern Asia is not a happy one. Having cradled the *Bos indicus* cattle which now dominate most of the tropical world, the future of cattle in southern Asia appears now to be one of decline. The improved Zebu breeds of the Americas have supplanted Indian cattle as the major source of export Zebu seedstock. Economic considerations, religious influences and the stresses of an excessive human population would seem to point to a probable decline in numbers and importance of cattle in most of tropical Asia.

Africa

The majority of the cattle of Africa show Zebu characteristics in varying degrees. Payne (1970) lists 19 types as being of Zebu parentage, 36 types as being of intermediate Zebu $\times$ European origin and eight groups considered to be of *Bos taurus* origin. Improved breeds of European origin are found only in the northernmost and southernmost portions of the continent, at higher elevations or in specialized dairies where adequate nutrition and good management are provided.

The majority of African cattle may be classified with respect to usage as those maintained principally as prestige stock. Attempts to improve the productivity of such animals have been minimal. These cattle have survived for centuries, however, where the environment is generally harsh and troublesome diseases and pests abound. Under such conditions it is natural that genetic tolerance for some of those conditions would be acquired over time. With increasing restrictions on use of chemicals in the control of diseases and pests, some of the African stocks may play a role in future genetic programs for cattle production in various parts of the world. Some of the more widely known of the African breeds currently include the N'Dama (*B. taurus*), Boran

(*B. indicus*), Nguni (*B. indicus* × *B. taurus*), Africander (*B. indicus* × *B. taurus*) and Bonsmara (5/8 Africander, 3/16 Hereford, 3/16 Shorthorn).

Water buffalo are important in Northeastern Africa where they are utilized for production of milk and for work. Males generally are slaughtered as calves for meat.

Indonesia

The cattle of Indonesia include principally the Bali, an indigenous breed of Banteng cattle; the Madura, a stabilized indigenous breed descending from Zebu × Bali crosses; the Java Ongole developed by upgrading Javanese cattle to Indian Ongole oxen imported for draft purposes and the Grati, a dairy type resulting principally by topcrossing Friesian bulls on Javanese cows. There are also a few pure Friesian cattle and recently Zebu × European cattle have been introduced on an experimental basis.

Indonesia is considered to be an area where cattle production potentially could be increased, economic and political conditions permitting. Much of the area has enough elevation to reduce some of the stresses of the lower tropics.

Australia

The cattle presently inhabiting the tropical area of northern Australia have descended through the female line from the Shorthorn introduced into Australia during the early 1800s. During recent years Zebu breeding has been introduced into the population through the use of Africander, American Brahman, Santa Gertrudis, Droughtmaster and Belmont Red bulls. The crossbred progeny from these matings have shown significant advantages over straightbred Shorthorns for heat tolerance, resistance to ticks and productivity. A systematic research program has been organized to evaluate physiological differences between the various types of cattle being produced.

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2. THE TROPICS AND THE WORLD DEMAND FOR ANIMAL PROTEIN

John A. Pino

Abstract. Much of the future growth in livestock production is likely to occur in the tropical and subtropical belt of the world. In the tropics the now unexploited areas with the greatest potential for expanding livestock production occur in South America and Africa. Intensive research currently under way is beginning to identify and resolve such problems as management practices, nutrition, genetic improvement, and disease control. Infectious blood diseases, such as anaplasmosis and babesiosis in South America and trypanosomiasis and East Coast fever in Africa, along with parasitic-helminth diseases, constitute the greatest single deterrent to expansion of livestock production in these tropical regions. There is considerable promise that the major disease deterrents are amenable to solution with the appropriate preventive technology and management. In Africa alone it is estimated that 125 million head of cattle could be accommodated in the tropical rain belt.

Although products of poultry, swine, and other non-ruminants can and do constitute a substantial part of the protein resources available to populations in the tropics and have a great potential for expansion (Loosli *et al.*, 1974) cattle are currently still the most important single animal resource of food. Cattle are the most numerous of the ruminant species in the tropics and provide the largest quantity of animal food products. More than one third of the world's cattle are found in the tropics (Payne, 1970).

According to recent information (Winrock, 1978), animal products from all sources provide roughly 32% of the world's protein consumption and approximately 17% of the energy component (Table 1). Behind such global figures are the obscured variations which occur from region to region, community to community, and even among members within a family. In the latter case, in some societies children are denied access to meat products, which are reserved for adult members of the family and, in some cases, only the male members. The Masai of Kenya subsist largely on diets consisting of milk, blood, and meat.

Argentinians annually consume approximately 100 kg of livestock product per person, mostly from beef animals. Similarly, consumption of animal

Table 1. Contribution of various food groups to world food supply, 1964-1966.

Category	*Calories* %	*Proteins* %
Cereals	52.4	47.4
Roots and tubers	7.8	4.8
Sugar and sugar products	8.8	0.2
Pulses, nuts and oilseeds	5.1	12.0
Vegetables and fruits	3.5	4.3
Vegetable fats and oils	5.3	5
All animal products	16.7	31.7
Meat	(7.1)	(14.0)
Milk	(4.9)	(10.8)
Eggs	(0.8)	(2.1)
Animal fats	(3.1)	(0.2)
Fish	(0.8)	(4.6)

Source: Winrock International (1978, p. 102).

products is high in North America and Oceania. Mutton and lamb form a large proportion of total meat consumption in the latter region. In the Indian subcontinent, meat products are not a significant part of the diet although the consumption of milk products is more common. Data regarding regional variations in per capita consumption of ruminant livestock products are reported by Winrock (1978) and presented in Table 2.

The world demand for livestock products is expected to continue to increase both as a result of population increases and in response to improved economic status of consumers. Whether or not livestock production will expand at a rate adequate to meet the world demand is conjectural. Projections based on current trends in population growth and consumption patterns would require approximately a 2% growth compounded annually. Developing market economies are expected to show higher relative growth levels in demand for animal products than developed market economies. Compared to current consumption levels in those nations, however, the real increases are relatively modest.

Historically, the strong economic demand of developed market economies for animal products has resulted in a flow of those products from economically poor countries to the more affluent regions, even though from a nutritional point of view those products could have filled an important need in the exporting countries. Gradually, some of those nations which have been traditional exporters have had to reduce their export levels as internal demand outpaced increases in production, and indeed some formerly exporting nations have become net importers (ILCA, 1979).

Table 2. Per capita consumption of ruminant livestock products 1970, and projected to 1985, by region.

Region	*All dairy products (milk equivalent, kg)*		*Beef and veal (carcass weight, kg)*		*Mutton and lamb (carcass weight, kg)*	
	1970	*1985*	*1970*	*1985*	*1970*	*1985*
1. North America	277	296	52	64	2	2
2. Middle America	79	99	9	11	1	1
3. South America	104	119	26	29	2	2
4. Western Europe	374	398	23	29	3	4
5. Eastern Europe	136	159	14	18	2	3
6. USSR	272	343	21	27	4	5
7. China	5	6	2	3	1	1
8. North Africa and Middle East	110	129	5	6	5	6
9. Central Africa	27	31	6	6	2	2
10. Southern Africa	139	158	23	24	9	10
11. India	69	82	–	–	1	1
12. S. and S.E. Asia	40	52	2	3	1	1
13. Japan	46	102	3	9	2	2
14. Oceania	489	480	43	41	39	37
15. Rest of world	40	52	3	3	1	2
World average	111	119	11	12	2	2

Source: Winrock International (1978, p. 107).

The long biological cycle of cattle, coupled with climatic changes and shifts in market, results in wide fluctuations in movement of livestock products, their prices, and ultimately in their consumption. Such dramatic variations occur in range-dependent livestock production systems as well as in the grain-dependent cattle industry of the United States.

Much of the future growth in demand for livestock products is likely to occur in the tropical and subtropical belt. This is where the fastest rate of growth in populations is occurring and where high elasticity in demand prevails. Changes in demand will not necessarily be met with increases in production or with increased availability of products on the market. Assuming the existence of favorable demand stimuli as seen in Colombia, Venezuela, Nigeria, the Philippines, and Indonesia, the capacity of the livestock sector to increase productivity will depend upon availability of land, feed, and animal resources necessary to sustain increases in livestock numbers; availability and adoption of technology which will economically increase productivity and efficiency; and distances of resources and access to markets.

Assuming the availability in a given region of land, forage, and other resources including improvements in technology, expansion in productivity

would be determined by structure of the production sector, price incentives, and other public policies which reflect the decision-making process of resource holders, biological time lag to expand herd numbers, and rapidity of adoption of improved technologies.

Public policies will determine whether the potentially larger supplies of animal products will be channeled to local consumption or diverted to exportation for foreign exchange. In some contiguous frontier areas, cattle may be moved across national boundaries to those markets offering the greatest access or return regardless of the existing public policies. To a large extent, the international flow of animal products will be conditioned by prevailing world market prices. Traditionally, the developed market economies of Europe, England, the United States, Japan, and more recently the Middle East, absorb the bulk of animal products which are exported. The principal sources of fresh, frozen, or processed animal meat products which move in international trade have been Australia, Argentina, Brazil, and to a lesser degree eastern and southern African countries, Mexico, and Central America. The large cattle populations of the Indian subcontinent, while contributing little to international trade in meat products, are of great importance domestically in providing milk, draft power, and dung for fuel, not to mention their vast genetic resource value to the rest of the world.

The tropics represent a diverse range of ecological conditions upon which varying cultural systems are superimposed. Payne (1970) summarized in the first chapter of his book the characteristics of the tropical world as these relate to cattle production. In those regions of the tropics where climate is moderated by virtue of altitude, it is possible to find established systems of milk or meat production similar to those of temperate climates. On the other hand, there are vast areas of desert, semi-desert, savannah lands, tropical forests, and scrub in which topography, climate, vegetation, and prevalence of disease have strongly infuenced the nature of the livestock enterprise.

Generally speaking, tropical livestock productivity is quite low. Not only is the extraction rate (in numbers) very low but the carcass yields are only a fraction of what is expected under improved management conditions. Thus, to meet the projected requirements for meat products by the year 2000, it will be necessary not only to increase herd numbers but also to increase the carcass yield per livestock unit. Levels of productivity of existing cattle-producing regions are influenced by climate, disease, quality and quantity of available forage, management systems, distance to markets, and genetic characteristics of the stock. To bring about changes in even a few of these factors is extremely difficult. In the first place, certain improvements may not be economically feasible even in commercial ranching schemes. Secondly, the technology on which change can be based may not exist. And finally, the

capacity to generate, deliver, and gain acceptance of new production methods is generally very weak or non-existent in many tropical regions.

For the most part, cattle in the tropics are maintained under extensive management systems. These systems vary considerably but are characterized by a minimum of livestock-environment manipulation. Under commercial ranching schemes in Brazil and Australia, for example, cattle may seldom be rounded up except for cutting, branding, and vaccinating. On the other hand, cattle may be constantly under surveillance while moving great distances as in the nomadic herding systems of Africa. For the most part, there is little stratification or specialization in tropical cattle production systems. Animals are born and reared to market size in mixed age groups mostly under pasture conditions. Separation and/or concentration of cattle in "feeder" systems in preparation for market is not practiced except in rare instances. The degree of herd management is generally unrelated to the value placed on each animal. There is now greater awareness, however, of the need for sound range and livestock management interventions if these systems are to be optimally productive.

Milk may be an important product of cattle operations in some locations, although not uniformly so. Nevertheless, the production of milk in tropical environments is an important consideration and is usually achieved by the limited milking of the indigenous breeds, the use of crossbreeding to increase milk potential, and careful management of the usual milking breeds. In the latter case, a high degree of management skill is required.

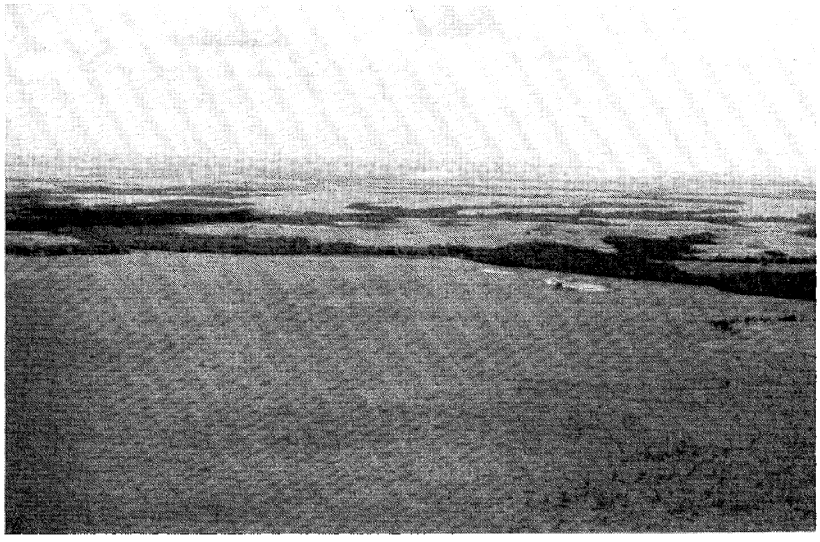

Fig. 1. Aerial view of the Colombian Llanos.

In the tropics the now unexploited areas offering the greatest potential for expanding livestock production occur in South America and Africa. There are approximately 828 million hectares of tropical soils (classified as oxisol and Utisol) in South America characterized by high annual rainfall, a dry season, and low soil fertility. Savannah lands cover some 300 million hectares of the region, most of which is poorly utilized by extensive cattle production (Fig. 1).

In the opinion of scientists at the Centro Internacional de Agricultura Tropical (CIAT), the savannah lands of South America will come under increasing pressure for cattle production as the more productive soils are shifted to crop production. The soils of the savannah lands as well as those of the semi- and dense forest regions are characterized by low pH and limited soil nutrient availability.

Intensive research now under way in Brazil, Venezuela, and Colombia is beginning to identify the problems which limit livestock productivity of the savannahs and forest regions. With guarded optimism, scientists are pointing to encouraging results achieved with new forage species, better management practices, mineral supplementation, and good herd health (Fig. 2). The effects on liveweight gains, fertility calving rate, low mortality, and days-to-market weight are very impressive. An impression of the potential livestock productivity of the vast savannah region alone can be gained from the data given in Table 3 (Paladines and Leal, 1979). These authors clearly recognize the crux of the problem by stating:

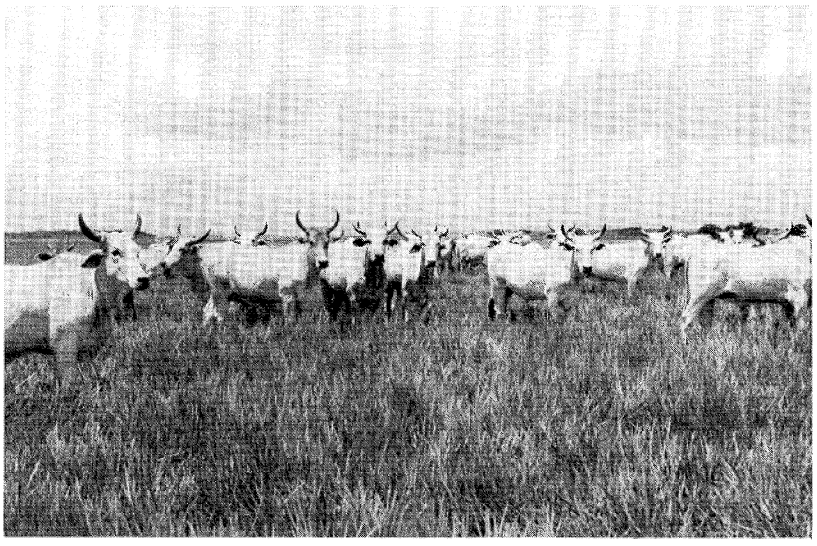

Fig. 2. An experimental lot of native Zebu-type cattle on improved pastures in the Colombian Llanos.

Table 3. Liveweight gain of steers under increasing technologies in the Llanos Orientales of Colombia.

	Maximum/animal [a]		*Maximum/ha* [b]	
	Per animal	*Per ha*	*Per animal*	*Per ha*
	(kg/gr)		*(kg/gr)*	
Unburnt savanna,				
0.35/an/ha	38	13	38	13
Savanna burnt in the dry season,				
0.20 an/ha	75	15	–	–
0.35 an/ha	–	–	67	23
Savanna burnt sequentially during the year,				
0.20 an/ha	95	19	–	–
0.35 an/ha	–	–	62	22
M. minutiflora, grazed all year,				
0.44 an/ha	98	43	–	–
0.44/0.88 an/ha	–	–	58	69
M. minutiflora with urea+molasses during the dry season,				
0.44 an/ha	130	58	–	–
0.44/0.88 an/ha	–	–	102	81
H. rufa, grazed during rainy season,				
0.70 an/ha	34	24	–	–
1.40 an/ha	–	–	24	34
P. plicatulum, grazed during rainy season,				
0.70 an/ha	66	40	–	–
1.40 an/ha	–	–	32	45
B decumbens, grazed all year,				
0.90 an/ha	118	106	–	–
1.70 an/ha	–	–	86	147

[a] Weight gain corresponding to the stocking rate in which per animal weight gain was maximum.

[b] Weight gain corresponding to the stocking rate in which per ha weight gain was maximum.

Source: Paladines and head (1979, p. 324).

The native savannah is a natural resource which is only now beginning to be known. It would seem unlikely, at this point, that a grass species could be found that is adapted to the conditions of the area, particularly the very low soil fertility.... The challenge continues to be finding a tropical legume capable of producing in this environment, competing with the grasses and providing a higher nutritive value to the pasture.

Although CIAT continues to emphasize adequate nutrition of animals, which is the main constraint for increased cattle productivity in the savannah regions, a great deal of research will also be required in the aspects of health, marketing, management systems, and genetic improvement of livestock.

Another major land mass where technology has inadequately been advanced to fully exploit the livestock production potential is Central Africa. Current livestock populations are found in the arid and semi-arid regions of the continent, principally in the periphery of the tsetse fly belt, so as to avoid parasitic infections associated with the more humid, high rainfall areas lying between 10° N and 10° S latitude. The major deterrent to livestock production in this region, variously estimated at between four and five million square miles, is the presence of the tsetse fly, the vector of human and animal trypanosomiasis. This area is virtually excluded from livestock production. There are other serious disease impediments such as concurrent hemoparasitic infections including anaplasmosis, babesiosis, and East Coast fever, as well as helminth infections. Undoubtedly these infectious diseases constitute the greatest single deterrent for expansion of livestock production in the humid tropics of Africa.

Diseases, however, are not the only factor. To date there has been little research on the management of the tropical pastures in the semi-arid lands of Africa. There is considerable promise and expectation that the major disease deterrents are amenable to some practical and effective solution. There is, however, little agronomic and husbandry research being conducted in anticipation of adequate disease-control measures. The great hope is that when the high forage potential areas become accessible to livestock, some form of stratified production system could be instituted to relieve the present grazing pressures in the semi-arid zones. While it is too soon to predict the impact on total cattle productivity which such changes could bring about, the theoretical potential is estimated at an additional 125 million head. At the same time, it could be anticipated that with the appropriate technology and management, expansion could also occur in sheep and goat production as well.

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3. EFFECT OF CLIMATE AND MANAGEMENT SYSTEMS ON PRODUCTION OF CATTLE

James E. Johnston

Abstract. Environment in the tropics varies from arid deserts to island paradises to steamy jungles. Climate directly affects cattle through heat, radiation and humidity in the ability to maintain a normal range of body temperature; indirectly, climate affects plant growth and nutrients available to livestock, and exposure to diseases and parasites. Cattle have a poorly-developed sweating mechanism. High temperatures, solar radiation, and high humidity deters maintenance of internal heat thus interfering with efficient cattle production.

Animal husbandry systems vary markedly, depending upon the type of animals involved and the nature of the major product to be produced. Tropical dairy management is the most difficult, partly because the tropical breeds do not have the capability of high levels of milk production. In less humid regions, it is possible to utilize pure breeds when they are provided adequate nutrition and are protected from environmental stress factors. Good management includes the year-round provision of high-quality forage supplemented by concentrate feeding to meet nutritional requirements. Implementation of the highest levels of sanitation and disease prevention methods are mandatory if animals are to maintain reasonable production. While disease may contribute to low productivity and high calf mortality rates, the principal cause of these losses is poor nutrition. The most costly nutritional improvements usually result in increased calving rates, lower mortality and more rapid growth rates. Management practices should also prevent or limit exposure to insect vectors and other direct and indirect means of infection. Improved marketing should assure reasonable prices for animals and animal products and result in better disease and parasite control practices.

The term "tropics" geographically refers to the portion of the earth located between the Tropics of Cancer and Capricorn in the area lying $23\frac{1}{2}^{\circ}$ N and S of the equator. The term also brings visions of steaming jungles and/or palm trees waving over sandy beaches. Actually, conditions in the tropics vary greatly, depending on altitude, rainfall patterns, and moderating influence of adjacent water; other factors are environments ranging from nearwaterless deserts through island paradises to steamy jungles. Except for the high altitude regions such as the Andean Cordillera of western South America, climates tend to be warm to hot with relatively narrow ranges of seasonal

temperature variation. Seasonal changes are most frequently identified with dry and wet periods associated with changes in wind speed and direction, the oceanic monsoons, which have a major influence on the climates of many tropical zones.

Payne (1970), in a more detailed description of tropical climates, classifies them into four types: (1) equatorial (hot, humid with little seasonal variation; (2) tropical, having three seasons (cool dry, hot dry, hot wet); (3) desert (hot with little and irregularly distributed rainfall); (4) montane (relatively low but uniform temperatures and high solar radiation). Climate affects cattle directly through the influence of temperature, radiation, humidity and air movement, and in the ability to maintain body temperature within the normal range; indirectly, the climate affects plant growth and quantity and quality of nutrients available to the animal as well as exposure to diseases and parasites. The level of productivity of domestic cattle is normally maximized when animals are maintained free of diseases and parasites, provided they have adequate nutrients and protection from environmental stresses. The climate found in the tropics influences an efficient implementation of these modern husbandry requirements. In the following pages, the role of management in minimizing the deleterious tropical climate influences will be discussed.

Cattle are kept by man for many purposes. In the temperate zones we have developed specialized breeds which are very efficient in producing milk and/or meat. In doing so we have selected animals which are large at maturity and require large quantities of nutrients to maintain the rapid growth rates and high levels of milk production. Such animals adapt well to cool or cold climates where elimination of heat from the body is seldom a problem. Unfortunately, cattle as a species have a poorly developed sweating mechanism. The heat produced in the body as a result of digestion in the rumen and basal productive metabolic functions is eliminated primarily by convection through the skin to cooler air, conduction from the skin when lying on cool ground or other surfaces, and through warming of cool air and vaporization in the respiratory tract and on the skin surface. When such animals are exposed to hot climates where air temperatures may approach or exceed body temperature, high humidity limits evaporation from both the respiratory tract and skin. When solar radiation is also high, maintenance of thermal balance becomes difficult, if not impossible. Since level of production, in terms of rate of growth and milk yield, is directly related to internal heat, the most productive animals experience the greatest difficulty when exposed to hot conditions.

Until recent times, cattle found in the tropics have not been so carefully selected for high levels of milk production and rates of growth. In most areas they have been kept primarily for draft use, production of manure and/or

accumulation of capital. Where milk is used by the owner, it is usually a by-product and meat and hides are residual benefits. Contrary to popular opinion, physiological studies have shown that the major reason for the greater adaptability of tropical cattle, such as the Zebu, to hot conditions is their lower internal heat production rather than ability to lose heat. All the evidence indicates that if the growth and/or milk production capabilities of these animals were equal to that of their temperate-zone counterparts, they would lose most of their heat tolerance. McDowell (1972) provides an excellent discussion of responses of animals to warm environments.

The problems of maintaining high levels of cattle productivity are most severe in areas when the humidity is high. This is due to the fact that when air temperatures are near animal body temperature, most or all of the excess body heat must be lost by evaporation. This is not very effective when the water vapor in ambient air is already high. Animals producing more than a few liters of milk per day are more severely affected than non-lactating animals because of high levels of internal heat production associated with digestion of feed, metabolism of nutrients and milk secretion. Internal heat production is augmented by radiation from solar, ground, and other surrounding sources. When heat gain exceeds heat loss, body temperature begins to rise. The first indication of this thermal imbalance is usually a rise in respiration rate and an increase in secretion by the sweat glands. Both of these reactions are designed to increase heat loss by evaporation. If the air is dry enough and excess body heat is not too great. the rise in body temperature may be prevented from reaching physiologically debilitating levels. Excess heat accumulated during the day can usually be eliminated during cooler night hours. It may be noted that relative humidity of ambient air may not be a good index of evaporation efficiency. Saturated air at 40 °C holds nearly three times as much water vapor as it does at 20 °C. Evaporation from the respiratory tract takes place at body temperature and from the skin at a temperature close to it.

When the body temperature of the animal rises above the normal level, one of the early effects is a reduction in appetite. If animals have a choice of feeds ranging from course roughages to grains, they will reduce their intake of roughage and increase intake of grain. This is advantageous since the metabolizable energy content of roughage is considerably lower than that of grains, and the heat associated with ruminal digestion is considerably higher. It is thus possible for the animal to maintain its metabolizable energy intake while reducing its heat loss requirements when provided this choice of feeds. To achieve this result without change in feedstuffs, one option is to provide the animal with access to the better quality feeds during the warmer parts of the day and to the coarser portions during the cooler night hours. This becomes

desirable when the roughage portion of the diet is obtained from grazing. The animal may then be protected from the added heat of the sun by shading during the day and the loss of radiant heat is emitted in the cool of the night.

Ideally, management practices in the tropics should be designed to (1) prevent or minimize rise in body temperature; (2) provide and encourage consumption of a diet containing adequate quantities of all nutrients required for body maintenance and current productive functions; (3) protect from exposure to infection by diseases and parasites and (4) avoid injury due to rough handling, predators, etc. Unfortunately, the ideal is seldom achieved. It may not be economically feasible or it may be impossible to effectively control all these elements due to problems such as theft of grazing animals at night or lack of adequate feedstuffs during certain seasons of the year. There are usually some management options, however, which can be implemented to improve efficiency of cattle production.

Management systems vary markedly, depending upon the type of animal employed and the product to be produced. Tropical dairy management is unquestionably the most difficult. This is due partly because none of the tropical breeds of cattle have the capability of high levels of milk production, therefore, most intensive dairy production units utilize either crossbred or pure temperate-origin dairy breeds. In the less humid regions it is possible to utilize pure breeds such as the Holstein-Friesian when they can be provided adequate nutrition and protection from environmental stresses. In humid zones it is almost always necessary to use crosses between tropically-adapted and European dairy breeds. This reduces maximum yield potential but simultaneously increases resistance to heat; also reduced is the cost of protection from other environmental stresses including some disease problems. Good management include the year-round provision of high-quality forage supplemented by concentrate feeding to meet individual nutritional requirements based on production level. The same basic feeding standards apply in tropical as in temperate-zone dairy management. Cut-and-carry forage systems protect animals from heat stress by the use of well-ventilated shaded feeding areas; also, confinement feeding frequently simplifies protection from tick and insect-born diseases and parasites. The highest levels of sanitation and disease prevention are mandatory in tropical dairy management if animals are to maintain a reasonable level of production. Reproduction is critical to dairy operations and is frequently a problem in the tropics. Nutritional, heat and disease (non-reproductive as well as reproductive) stresses all tend to reduce fertility and, even under the best management conditions, high-producing dairy cattle are usually subjected to one or more of these stresses. It is not unusual for conception to be delayed until near the end of the lactation

period. The problem is usually most severe in high-production animals. There is no absolute cure, but conditioning of animals prior to calving is important to provide adequate body reserves that are drawn upon during the peak of lactation. Use of artificial insemination is desirable, particularly during the period when herds are being graded up to temperate breeds. Many good managers also keep a bull and use it when a third or later service is required. Pregnancy diagnosis after non-return to service is good practice since many animals will exhibit "silent" heats during lactation peaks. Dairying is difficult, but not impossible in the tropics. The manager must have a good understanding of the stresses which affect the animal and how they can be reduced to a minimum. Veterinarians must be familiar with the symptoms of stress since they may frequently be similar to symptoms of disease and, in any case, reduce the animals' resistance to disease.

Beef, draft and other types of cattle operations have many more options than dairy production. Animals kept for these purposes have much lower nutritional requirements and, unlike the milking animal, can withstand short periods of deficiency without serious impact on future performance. Lower rates of metabolism associated with maintenance and normal growth result in comparatively lower levels of internal heat production so they are able to withstand much higher external heat loads before suffering physiological distress. Tropical breeds such as the Zebu cattle of Asia and Africa and the Criollo of Latin America are considerably more resistant than the European beef breeds. The American Brahman is an excellent compromise and has been used extensively in many parts of the world.

Raising cattle in the tropics has varied tremendously around the world. Methods range from the nomadic systems of the Sahelian zone of Africa to the extensive ranches of South America, and the village systems of Asia. A few management principles are outlined here. Most traditional systems suffer low reproductive rates in breeding-age females and high calf mortality. While disease is a factor in some cases, the principal cause of these problems is poor nutrition. These problems can be alleviated by any management system based on balanced nutritional feed. The animals should gain weight during the breeding period and they should be maintained in good condition during the first four or five months of the suckling period. In areas where rainfall is seasonal this can sometimes be achieved by scheduling breeding toward the end of the rainy season; calving will then take place during the early part of the subsequent rainy season to take advantage of good nutritional conditions during those times. Pasture improvement may also help through the incorporation of legumes for improved nutritional balance, increase total pasture production and extend the effective grazing period further into the dry season. When conditions permit, roughages should be stored to feed during critical

periods. Mineral deficiencies which are also frequently encountered can be overcome by use of salt and trace minerals, either in block or loose form. There are many approaches to the improvement of nutrition but the practical decision must be based on whether the resultant increase in income justifies the investment required.

Shade may improve reproduction and growth, particularly during periods of high solar radiation; this may be provided by leaving groups of trees when clearing pastures or by erection of artificial shade. Shade provisions, however, must be used with caution when animals are forced to cover large areas daily to obtain sufficient nutrients. In such cases they may remain in or near the shade, leaving other areas ungrazed, thus suffering from inadequate feed intake.

While some practices may cost little or nothing, such as adjusting the time of the year when animals are bred, most improvements entail some expense. The most costly are usually nutritional improvements resulting in increased calving rates, lower mortality of young stock, and more rapid growth rates. For the greatest economic benefits, management practices should also prevent or limit exposure to insect vectors and other routes of infection. Improved marketing arrangements should assure reasonable prices for animals and animal products and result in better disease and parasite control practices.

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4. HUMAN-BOVINE ECOSYSTEMS: REFLECTIONS ON ZOONOSES IN THE TROPICS

I. Kakoma and M. Ristic

Abstract. Various categories of zoonoses are maintained between cattle and man. These may be direct zoonoses, transmitted directly from cattle to man or vice versa; cyclozoonoses requiring more than one vertebrate host to complete the developmental cycle needed for transmission; metazoonoses requiring an invertebrate vector for transmission, or saprozoonoses which involve vertebrate and invertebrate hosts in the process of transmission. Social, cultural and religious attitudes to cattle are important factors in the development and maintenance of diagnostic, epidemiologic and control measures of bovine-human zoonoses. It is suggested that priority of disease control measures should start with intensive mass education in standard public health concepts; provision of good communication facilities, implementation of vector control and specific immunoprophylaxis.

Introduction

The economic, aesthetic and religious associations between cattle and man have had tremendous impact on the evolution of diseases common to man and his domestic animals. According to Schwabe (1979) the history of human-bovine interaction dates back to between 4000 and 300 BC, during which time it is referred to as the "bull-cow" culture of Egypt preceding the horse-culture. The "cattle-complex" culture is still represented in India for religious reasons and in East Africa for basic economic needs. The "cow-boy" culture of North and Central America is a very recent off-shoot of cattle culture in a highly commercial setting. Whatever the level of interaction between cattle and man, ecologic nooks exist which facilitate transmission of diseases between man and his bovine companions, thereby contributing to the zoonosis situation.

The term zoonosis has been variously defined but perhaps the most authoritative definition is that proposed by the Expert Committee on Zoonoses of the United Nations (WHO, 1967). The basic criterion emphasizes epidemiologic features and categorizes zoonoses into (1) direct zoonoses which are

transmitted from one vertebrate host to another by direct contact, fomite or mechanical vector (rabies, trichinosis, brucellosis); (2) cyclozoonoses whereby more than one vertebrate host species is required to complete the developmental cycle of the agent (Echinococcosis, Taeniasis); (3) metzoonoses transmitted by invertebrate hosts in which some developmental stages of the parasite occur (Rocky Mountain spotted fever, malaria, babesiosis); (4) saprozoonoses which involve both vertebrate and invertebrate hosts (larva migrans).

In this review an attempt will be made to examine the role of bovine-human interactions in the maintenance of zoonoses. Particular attention will be paid to those husbandry systems in the tropics where traditional cattle husbandry methods and other practices appear to favor transmission of various diseases common to man and cattle.

Conditions favoring spread of zoonoses

Elton (1958) observed that human and "animal" ecology have developed and evolved in curious contrast to each other. He further noted that human ecology has been concerned almost entirely with the effects of man upon man, disregarding the other animals among which we live. Schwabe (1969), on the other hand, has cautioned that however secure and well-regulated civilized life may become, bacteria, protozoa, viruses, infected fleas, lice, ticks, mosquitoes and bedbugs will always lurk in the shadows when neglect, ignorance, poverty and famine prevail. It is therefore clear that the success of preventive medicine will depend on a clear understanding of the interaction of man and his environmental animal partners. Chief among the time-honored socio-economic and religious companions of man is the cow. The frequency and intimacy of bovine-human interactions are almost inversely proportional to urbanization. The problem of zoonosis is principally, but not exclusively, a rural one.

Consider a typical human-bovine ecosystem among the Dodos of East Africa as described by Deshler (1965). There are 20 000 people on 3000 square miles where 75 000 heads of cattle are kept. The people are outnumbered by cattle by a factor of 4 to 1. These people consume bovine milk, meat and blood. Animals that die from miscellaneous diseases are also used as a source of meat.

The majority of human population who interact freely with cattle live in the highly disease-ridden rural tropical and subtropical parts of the world. In some of these countries (e.g., East Africa) there is intimate continuous interaction between cattle and human beings. In small households very young or

very sick calves are often removed from overcrowded calf-houses and transferred to human premises. Thus in a 50 square foot house, one may find three or four calves tethered in different corners of the living room and children and guests may virtually be surrounded by cattle during meal times, bedtime and on other occasions. In this environment there is aerosol, fecal, urinal and direct contact between healthy and diseased animals and human beings. In these communities where people depend so much on milk, no modern milk sterilization facilities are available. Milk is frequently consumed immediately after milking while it is still "warm" before any milk-inherent bacteriostatic or bacteriocidal effects on pathogens have taken place. In some of these communities bovine urine is also used to wash milk containers thus contributing to the spread of leptospirosis (see chapter 17 on Leptospirosis). Populations of some communities, e.g., the Masai of Kenya, also consume enormous amounts of raw meat and milk-blood cocktails providing a direct means for spread of zoonoses.

There is no data to substantiate grave public health dangers in these practices, but it would seem important to examine their relevance to disease transmission. From an immunological point of view, the level of antigenic bombardment of human beings by tissues and fluids derived from bovine species is phenomenal in these communities. Consider the following systems: (1) gastrointestinal – from milk, meat and blood; (2) cutaneous – directly from rubbing against animals during milking, etc.; (3) aerosol exposure – (a) droplets from animals and human beings breathing in a closed environment, (b) smoke from burning cow manure, especially in India where cow dung is a major source of energy. Although no concern has arisen regarding possible consequences of these multi-route exposures, one may classify these as potential environmental hazards with unpredictable immunological implications.

Specific human-bovine zoonoses

Cattle and man are susceptible to a number of diseases transmissible from cattle to man and sometimes vice versa (see Table 1). These may be viral, bacterial, protozoal, rickettsial, mycotic or helminth infections. The range of these infections include direct zoonoses, cyclozoonoses, metazoonoses and saprozoonoses.

Table 1. Major zoonotic diseases of man and cattle [a].

Infection	*Category*	*Incidence*	*Clinical picture*
Cowpox	Direct	Common	Mild
Foot-mouth disease	Direct	Sporadic	Mild
Vesicular stomatitis	Direct or indirect (mechanical)	Sporadic	Mild
Pseudorabies	Direct	Rare	Mild
Reovirus infection	Direct	Sporadic	Mild
Anthrax	Direct	Sporadic	Severe to mild
Brucellosis	Direct	Sporadic	Variable
Streptococcosis	Vehicle or direct	Common	Severe
Colibacillosis	Vehicle	Common	Severe
Leptospirosis	Vehicle or contact	Sporadic	Severe
Listeriosis	Contact	Sporadic	Severe
Klebsiella infection	Direct	Common	Severe
Pasteurellosis	Direct	Sporadic	Severe
Pseudotuberculosis	Direct	Sporadic	Severe
Tuberculosis	Direct	Common	Severe
Vibriosis	Direct	Sporadic	Severe
Ringworm	Direct	Common	Severe
Sacrosporidiosis	Direct	Sporadic	No disease
Taeniasis	Cyclo-	Common locally	Mild
Pentastom	Cyclo	Sporadic	Mild
Echinoccosis	Cyclo-	Sporadic or common locally	Mild
Pixuma	Meta-(mosquito)	Sporadic	Mild
Japanese B encephalitis	Cyclo-(mosquito)	Sporadic	Serious
Tick fever	Cyclo-(tick or milk)	Sporadic	Serious
Rift Valley fever	Cyclo-(mosquito)	Sporadic	Serious
Vesicular stomatitis	Cyclo-(phlebotomus)	Sporadic	Mild
Q-fever	Cyclo-(tick or direct milk)	Sporadic	Severe
Trypanosomiases	Cyclo-(tsetse fly)	Sporadic	Severe
Babesiosis	Cyclo-(tick)	Sporadic	Severe
Dicrocoeliasis	Cyclo-(snail)	Sporadic	Variable
Schistosomiasis	Cyclo-(snail)	Sporadic	Severe
Botulism	Sapro-(vehicle)	Sporadic	Severe
Rhinosporidiosis	Sapro-(vehicle)	Sporadic	Severe
Mucormycosis	Sapro-(vehicle)	Sporadic	Severe
Streptricosis	Sapro-(vehicle)	Rare	Mild
Syrigamus infection	Sapro-(vehicle)	Sporadic	Mild

[a] Modified from Schwabe (1969).

Direct Zoonoses

Brucellosis. Bovine brucellosis caused by *Brucella abortus* is transmissible to men by consumption of raw milk and milk products. Direct contact with infected tissues, fetuses and fetal membrane discharges are the most common

modes of transmission among farmers, herdsmen, veterinarians, butchers and many others.

Bovine brucellosis is a world-wide disease and its incidence corresponds with the density of cattle populations. Considering that no tropical or subtropical country has achieved an eradication program for brucellosis, it can be safely assumed that in these regions most herds of cattle are infected to varying degrees. In Colombia, for example, the cattle population of the "entire country" may be infected with *Brucella* (Manrique, 1976). In some areas of Subsahara Africa the incidence of brucellosis may be as high as 42 to 100% (Thimon and Wundt, 1976).

Aborted fetuses, membranes and discharges are generally not properly disposed of. In most cases, roaming herds of cattle stampede on the aborted tissues which usually are not found and disposed of by the cattlemen. Vaccination has not been universally adopted and slaughter-compensate policies are impractical because of economic reasons. The exact economic impact of brucellosis in nomadic communities has not been assessed since mobilization of cattle and owners is virtually impossible.

Staphylococcosis. This disease syndrome is a typical example where men may act as a reservoir for infection of his cattle and vice versa (Courter and Galton, 1962).

Tuberculosis. Tuberculosis caused by *Mycobacterium bovis* is one of the oldest and perhaps commonest diseases of cattle and man. Man may contract infection from drinking infected bovine milk and by direct contact (aerosol) with infected cattle or other human beings. Constraints due to difficulties of enforcing stringent public health programs in the tropics have perpetuated this disease in man and his domestic animals. Conditions of crowding of cattle and people in humid, poorly ventilated places are strong predisposing factors for overt or subclinical tuberculosis.

Approximately two-thirds of the world's one billion cattle live in regions perpetually threatened by tuberculosis (Myers and Steele, 1969), particularly affecting the third-world countries. As indicated above, living conditions in some developing countries favor intimate contact between cattle and human beings through exhaled air, feces, vaginal discharges, urine and milk. According to Myers and Steele (1969), *M. bovis* is excreted for long periods of time and persists in humid and dark conditions. It is estimated that feces may remain infective for up to 2 months and the organism may survive in slurry for up to 5 months. The majority of individuals perhaps become infected by the aerosol route (via droplet). The risk in households where pneumonic calves are tethered is obvious; Henning (1956) has cautioned that working in

an infected cattle herd is more risky than working in a human tuberculosis hospital. Developing countries will probably continue to be infested with tuberculosis since rigorous control measures, e.g., eradication, cannot be implemented due to economic constraints as in the case of brucellosis control measures.

Tuberculosis occurs in two predominant forms. Inhalation infection is commonly produced by the human mycobacterium type but may also be caused by the bovine type, especially in rural areas with a high incidence of bovine tuberculosis. An alimentary infection is caused almost exclusively by the bovine type. The alimentary infection is generally less severe than the inhalation type.

Among human populations in rural areas, the inhalation form of infection due to bovine type may be as high as 50% (Jensen, 1953). This infection arises almost invariably in stables. The alimentary type, on the other hand, arises most frequently from consumption of infected raw milk. Among the associated complications of the alimentary type are tuberculous meningitis and cervical adenitis in infants (Jensen, 1953). In a comprehensive study in Denmark, Jensen (1953) found that in areas with a high incidence of tuberculosis of children (7 to 8 years), 47% residing in urban areas and 23.3% of those from rural areas were tuberculin positive. A parallel study in areas free of bovine tuberculosis showed that there were significantly low tuberculin reactors. These figures obtained from Denmark about 30 years ago may be closely applicable to a number of developing countries today.

Leptospirosis. According to Hanson (see chapter 17 on Leptospirosis), tropical cattle appear to harbor a great number of leptospiral serovars and human exposure to bovine leptospires in these areas is a common occurrence. Chronic carriers continuously shed the organism in urine. Given the commonly humid conditions in the tropical rain forest, one would expect optimal conditions for the survival of leptospires in the environment. The chances, therefore, of human infection through either urine or milk are maximal under tropical living conditions in cattle-keeping societies.

Anthrax. Bacillus anthracis is pathogenic for almost all mammals. Glassman (1958) presented an extensive review of the world incidence of anthrax. In general, human anthrax is contracted through direct contact and fomites. Two categories of human anthrax are well-known: (1) non-industrial type affecting farmers, butchers, veterinarians and laboratory workers and (2) the industrial type affecting humans handling hides and hair products as exemplified by woolsorter's disease.

The non-industrial type is characterized by malignant pustules while the

industrial type may show both malignant pustules and/or pulmonary disease. Fatal septicemic anthrax frequently follows consumption of meat from infected carcases. The victims develop severe gastrointestinal disturbance with profuse diarrhea. Fatal cases have been reported among the Masai people of Kenya who, according to Cachia (1960) knowingly eat animals which have died of anthrax because they reason that not everyone who eats the infected meat becomes infected with anthrax and of those who contract the disease, few will die, so why waste the meat? Consumption of meat of moribund or dead cattle is a very common practice in rural Africa and incineration or burial of such carcases has to be strictly supervised by health and law enforcement officials.

Metazoonoses

Rift Valley Fever (RVF). This is an acute arthropod-borne virus disease with a strikingly wide host range. Humans associated with diseased animals present an influenza-like syndrome with varying degrees of fever. In sub-Sahara Africa, *Aedes lineapennis* and *A. coustani* are the major mosquito vectors of RVF. The geographic distribution of RVF coincides with the presence of heavy populations of cattle within the so-called "cattle-complex" societies of East Africa and in areas where various mosquito species exist.

The perpetuation of RVF depends on cattle among other mammals. This zoonosis can be transmitted through direct or indirect contact with diseased or dead domestic stock (see chapter 12 on RVF). The species of mosquitoes which transmit RVF feed on both cattle and human beings and, obviously, in a tight human-bovine ecologic niche (e.g., East Africa) transmission from one vertebrate host to another is a continuous cycle. Aerosol and wound contamination are the most common modes of infection for human beings in Kenya, Uganda, Tanzania, Sudan, Malawi, Zambia, Zimbabwe, South Africa and Namibia. Among animal and human populations, epizootics occur at intervals of 4 to 15 years, perhaps reflecting herd immunity and density of the invertebrate host. An element of zooprophylaxis may also be a significant factor.

Babesiosis. One of the most widespread hemotropic diseases is protozoan babesiosis caused by various *Babesia* spp. In the last two decades, babesiosis has been recognized as a new zoonotic disease (Ristic and Smith, 1974). For nearly a century babesiosis has caused enormous losses in cattle (Smith and Kilborne, 1893). Splenectomized human beings were the first reported to develop babesiosis (Skrabalo and Deanovic, 1957; Scholtens *et al.*, 1968; Fitzpatrick *et al.*, 1968). Human beings with spleen *in situ* can also succumb

to clinical babesiosis (Western *et al.*, 1970; Ristic *et al.*, 1971; Anderson *et al.*, 1973), thereby indicating that the incidence of babesiosis may be higher than was originally expected. This also poses a new complicating dimension to the differential diagnosis of human malaria. Further, the concept that *Babesia* spp. are host-specific is no longer tenable. The first case of human babesiosis was a farmer (Skrabalo and Deanovic, 1957). The patient developed fever, severe anemia, jaundice, and hemoglobinuria. *Babesia bovis,* a common cattle pathogen, was demonstrated in his blood smear. His cattle were later shown to have been infected with the same organism. Many other cases of human babesiosis of non-bovine origin have since been documented (Ristic and Smith, 1974; Healy, 1979). Given the level of tick infestation, the degree of interaction between man and cattle in the tropics where babesiosis is endemic, one would suspect many cases of human babesiosis misdiagnosed as *Plasmodium falciparum* malaria. Trophozoites of these two protozoa are remarkably similar. Specific immunodiagnostic tests must be developed to ascertain such diagnoses and extend the epidemiologic studies in populations at risk, particularly in the areas with heavy cattle and tick populations. As of June, 1979, a world-wide total of 23 human babesiosis cases had been documented (Healy, 1979). Table 2 summarizes some of the human cases confirmed as babesiosis.

Substantial progress has been made in specific immunodiagnosis, isolation in laboratory animals and ultrastructural differentiation between *Babesia* spp. and *P. falciparum* (see chapter 35 on Babesiosis). A typical clinical situation is illustrated by the isolation of the Gray strain of *Babesia* from a woman (Ristic

Table 2. Babesiosis in human beings.

Number clinical form	*Status spleen*	*Tick bite*	*Outcome of the disease*	*Geographic regions*
19 acute	4 without spleen	All with history of tick bite	3 died 1 recovered	Yugoslavia Ireland U.S.A.
	15 with spleen	Most with history of tick bite	All recovered	France U.S.A.
4 asymptomatic	All with spleen	3 with history of tick bite	No further information of illness	Mexico U.S.A.

Babesia species thus far diagnosed or isolated: *Babesia bovis, B. divergens, B. equi, B. microti.* Additional clinically proven cases of *B. microti* infection reported since the above table was prepared were as follows: 7 Nantucket Island and 2 Martha's Vineyard Island, MA, 2 Shelter Island and 1 Long Island, New York (Ristic and Healy, 1981).

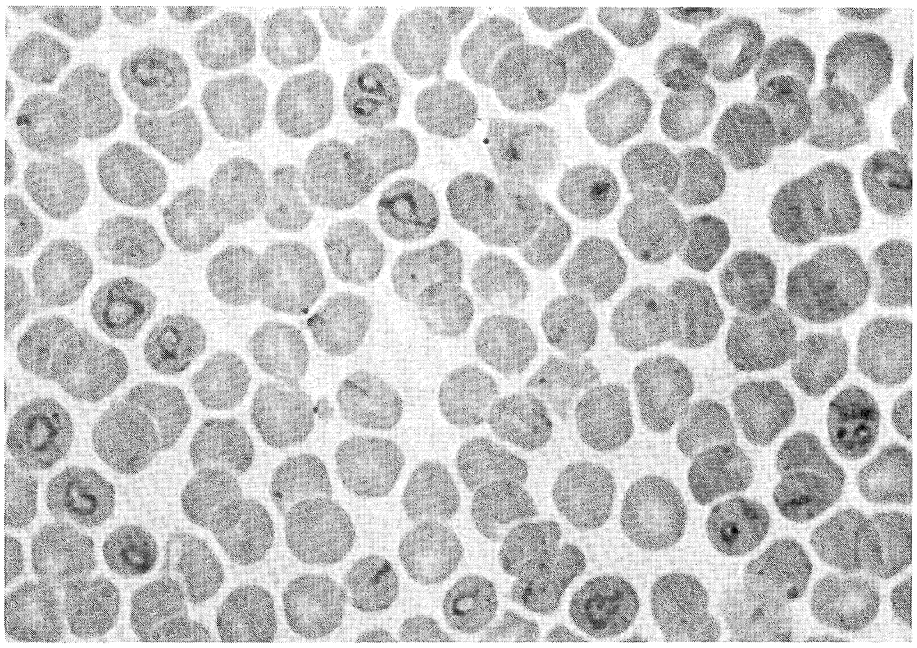

Fig. 1. Hamster erythrocytes heavily infected with the *Babesia microti* (Gray) strain isolated from a clinical case of human babesiosis. Giemsa stain. ×1100

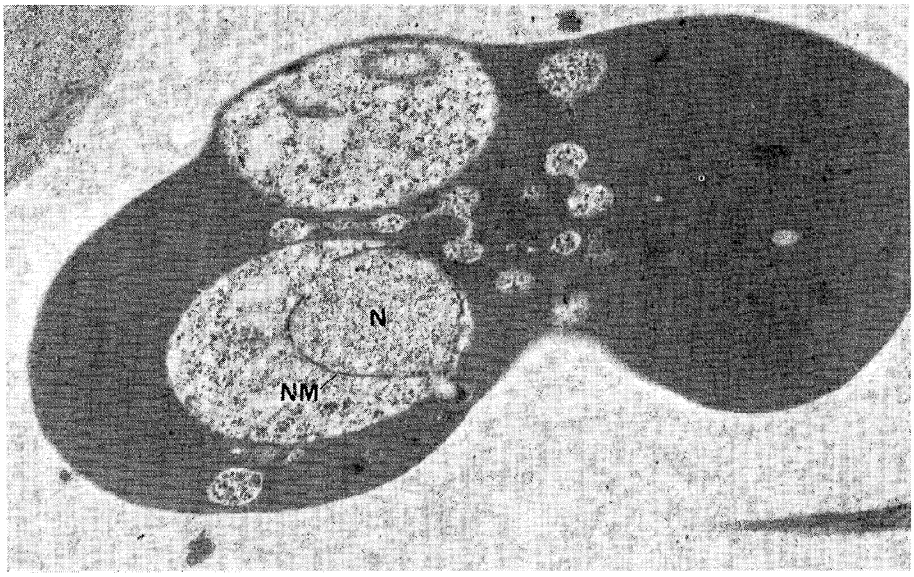

Fig. 2. Electron micrograph of two intraerythrocytic *Babesia microti* (Gray) organisms of human origin from an experimentally-infected hamster. Note the numerous polysomes, nucleus (N) surrounded by a nuclear membrane (NM). Parasite-derived material represented as bud-like structures joined by strands and islets is also shown between the two organisms. ×23 500

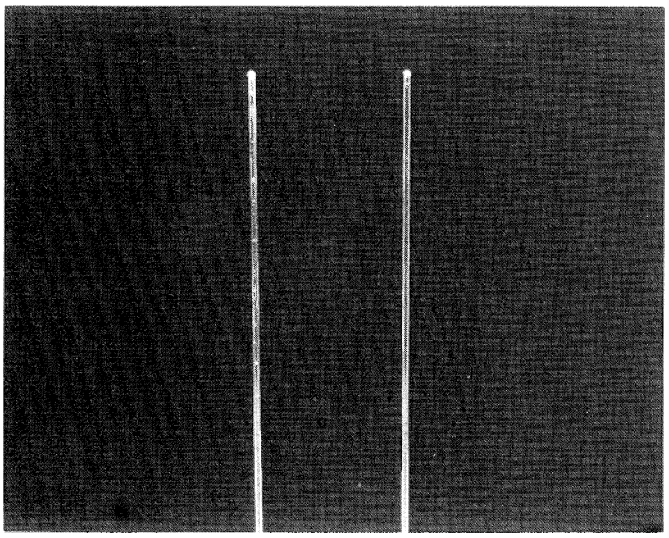

Fig. 3. A typical result of the capillary tube agglutination reaction of paired human serum samples. Right: sample collected during the acute phase of babesiosis (negative); left: sample obtained approximately 3 weeks later (positive).

et al., 1971). These investigators demonstrated that monkeys were susceptible to the Gray strain. Similarly, erythrocytes from inoculated hamsters showed numerous forms of the organism (Fig. 1). The protozoal nature of the isolate was confirmed by electron microscopy (Fig. 2). The organisms contained numerous polysomes, food vacuoles, double membrane vacuoles, and a typical nucleus bounded by a nuclear membrane. It was also shown that the patient's serum contained antibodies capable of reacting with the isolated organism. The result of the capillary tube agglutination test is shown in Fig. 3 and this result was supported by an indirect fluorescent antibody test. Isolation procedures and diagnosis will become relatively easier if the current research on *in vitro* propagation of *Babesia* spp. (see chapter 35 on Babesiosis) becomes readily available thereby obviating reliance on living animals for isolation procedures.

African Trypanosomiasis (sleeping sickness). This form of sleeping sickness must be distinguished from American trypanosomiasis (Chaga's disease). The tsetse fly transmits African sleeping sickness caused by *Trypanosoma brucei, T. rhodesiense* and *T. brucei gambiense* most frequently during the day and away from home. American trypanosomiasis, on the other hand, is contracted at night in a man's home through the feeding of reduvid bugs.

In African trypanosomiasis *Palpalis* group flies which do not ordinarily feed on game animals transmit wild animal trypanosomiasis to man and domestic

animals (cattle), which were earlier infected by *Glossina* spp. feeding on both wild and domestic animals. Cattle appear to be incidental reservoirs (Ristic and Smith, 1974) but may be a very significant source of trypanosomiasis, particularly where wild animal populations have been significantly reduced as in some areas of Eastern Africa.

Conclusions and recommendations

The above account has attempted to highlight the sources of actual or potential zoonoses perpetuated by the close ties between human beings and cattle under frequently sub-standard living conditions in the tropics. The authors do not pretend to have any immediate panacea to these problems but feel that control efforts should be concentrated initially in three broad spectrum fronts of attack: (1) mass education, (2) improved communication facilities and (3) specific control measures (e.g., vector control, immunoprophylaxis).

The majority of the people residing in the tropics cannot read or write and many have not been exposed to routinely accepted standards of hygiene. Even if sewage disposal facilities, running piped water, excellent modern medical facilities were suddenly introduced over all the tropics, the major problems would be contained but not satisfactorily resolved. The cost of such an undertaking would be prohibitive and the solution would be only transient. The best investment of modern technology in the tropics should be aimed at teaching the people the simple practices of personal hygiene which minimize contamination of water and food supplies with unhealthy animals and human products. This can be achieved by boosting the existing maternity centers, rural medicine endeavors (e.g., the Flying Doctor Service of East Africa) and laying extra emphasis on public health and rural development curricula in pre-college schools. A mass educational campaign would discourage situations of meat handling like those described in Guatemala (Schwabe, 1969), for example, where cattle are slaughtered anywhere and meat is handled under improper facilities, without refrigeration, minimal water supply, no sewage disposal systems, massive infestation with flies and rodents, and no trained meat inspectors. Kaplan (1957) and Mann (1963) have discussed these problems in detail within particular country contexts. This problem is so acute that developing countries ought to train as many certified meat inspectors as possible and also attempt to establish creditation procedures for butchers, milk technologists and producers of meat and milk by-products.

The above endeavors would be greatly accelerated by improved mass communication; today there are areas in the world where it takes many hours to communicate by telephone within a 10 to 20 mile radius. Similarly, travel is

extremely difficult in some areas where there are no reliable all-weather roads or where travelers depend on unsafe improvized canoes. This makes pooling of medical services very difficult, especially transportation of emergency cases to appropriate hospitals.

Measures of the nature outlined above would have a tremendous impact on direct zoonoses (e.g., anthrax); cyclozoonosis (e.g., taeniasis) and sapro-zoonoses (e.g., visceral larva migrans) but they may not have a great effect on metazoonoses (e.g., babesiosis and malaria). For these situations, emphasis should be placed on specific immunoprophylaxis and vector control. Experience gained from successful vaccination against other deadly diseases such as rabies, anthrax, various clostridial infections, and a limited number of hemotropic and helminth diseases has provided unequivocal evidence in support of immunoprophylaxis as the most rational and economical means of combating infectious diseases. However, a successful vector control and vaccination program will require trained personnel, an enlightened general public and reliable facilities for research and communication. These conditions would facilitate disease monitoring programs and improved methods of gathering and documentation of epidemiologic data. It must be noted that many excellent facilities are available in various countries of the third world. There is an urgent need to enhance interregional cooperation over and above the political differences and boundaries to optimize the implementation of disease control measures.

Finally, the very nature of zoonoses, by definition, demands a concerted effort between workers in human and animal diseases. Such cooperation would be boosted by encouraging more cooperative training programs for physicians, veterinarians and para-professional personnel.

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5. EPIDEMIOLOGICAL APPROACHES TO DISEASE CONTROL

Michael J. Burridge

Abstract. Many host characteristics, specific agents and environmental factors act as determinants of disease, influencing directly or indirectly the frequency of occurrence and the distribution of any given disease. These determinants, together with some of their important interrelationships such as modes of transmission of infectious agents, will determine the patterns of disease in animal populations. Measures for successful disease control must be based, therefore, on an understanding of the relative importance of each disease determinant. This can be accomplished by application of analytical statistical procedures and mathematical modelling techniques to epidemiological data. Such studies often suggest a number of alternative control strategies. Economic analysis is then required to help determine the optimal control strategy, selecting the approach that offers the greatest social and economic benefits at an acceptable cost level.

Epidemiology (or epizootiology) is the study of the relationships between the various factors that determine the frequency and distribution of diseases in animal populations. The ultimate goal of the epidemiologist is to prevent and control diseases through application of knowledge gained from studies of variables that influence disease frequency and distribution. Epidemiology, therefore, is the basic discipline for a rational approach to the prevention and control of disease. In this chapter, epidemiological methods used to develop and evaluate disease control programs will be discussed.

The need for disease control

One of the most serious problems facing scientists today is provision of adequate supplies of food to an ever-increasing human population. The role of animals in production of that food has been stressed by many authors (Byerly, 1966, 1977; Hodgson, 1971; Hodgson, 1976; Schwabe and Ruppanner, 1972; Pimentel *et al.*, 1975; Wedin *et al.*, 1975). In all parts of the world, disease is an important constraint to increased production of animal food for human

consumption. Efforts to increase the world production of animal protein, therefore, must involve improvement in the productivity of livestock through intensified research activity in disease control as well as in nutrition and genetics. In the absence of effective disease control, an expanded population of unproductive animals is created which cannot fulfill its genetic potential in the utilization of feed for growth and reproduction.

Epidemic disease control is the highest priority requirement for the veterinary services of the economically developing world. The major epidemic diseases of cattle include contagious bovine pleuropneumonia, East Coast fever, foot-and-mouth disease, rinderpest, and trypanosomiasis. Today, they exert their most devastating effects primarily in the tropical areas of Africa, Asia and Latin America. These diseases limit, and in some instances even preclude, the development of viable animal industries wherever they occur. For example, development of the large cattle industry in the southern United States was possible only after successful control of babesiosis. Another hemoprotozoan disease, trypanosomiasis, renders approximately 10 million square kilometers of Africa, capable of supporting some 125 million cattle, unsuitable for livestock production.

The epidemiology of many of the epidemic diseases is relatively straightforward. In most instances, the etiological agents are single or a few related species of pathogenic organisms which are fairly stable genetically and which have a narrow host-range. In addition, diagnostic tests which can be applied to screen affected animal populations, are available for the majority of these infections. Most epidemic diseases, therefore, lend themselves to proven methods of control which involve identification and treatment of cases and carriers, removal of reservoirs of infection, quarantine and sanitary measures, and mass immunization programs. Nevertheless, some of these disease have not been controlled successfully in developing countries because of inadequate veterinary services, political instability, lack of support of the local population, and insufficient financial and technological assistance.

Adequate methods of control do not exist at present for some epidemic diseases whose epidemiology is both more complex and poorly understood. Examples include East Coast fever and trypanosomiasis, both of which cause great losses to bovine productivity in tropical Africa.

The worldwide trend towards urbanization of human populations has led to the acceptance of animal management systems that handle large numbers of animals with the minimum of labor. This trend, together with the successful control of many epidemic diseases, have made possible the development of intensive systems of animal husbandry that utilize genetically improved breeds capable of high levels of production. Profit margins in these operations tend to be so narrow that subclinical endemic infections, such as mastitis and

gastrointestinal parasitism, assume major significance. Disease control is of paramount importance in these intensive systems if animal production is to be maintained at profitable levels. However, rational control measures can be developed only from a clear understanding of the underlying disease processes. Many endemic diseases are epidemiologically complex, with multiple factors influencing the prevalence and incidence of infection in a given herd. Moreover, the epidemiological pattern of a disease can vary markedly between regions or even between farms. Consequently, the unit of concern for epidemiological study must be the whole farm or population of animals rather than the individual animal.

Some economically developing nations have the potential to expand their cattle industries through export of animals and animal products. However, all too often this potential is not realized due to disease. For example, the United States and the countries of the European Economic Community have directed that domestic ruminants and swine cannot be imported from any country in which foot-and-mouth disease or rinderpest exists. The latter countries additionally prohibit the importation of meat from animals showing evidence of tuberculosis or cysticercosis. It is evident, therefore, that disease is also a serious constraint to the expansion of international trade in animals and animal products.

Multiple causality of disease

Epidemiology is concerned primarily with the relationships between: (1) populations of animal hosts; (2) potentially harmful agents which they or the environment may harbor; (3) the environment in which the hosts and agents occur and interact; and (4) time. The visible products of these relationships are health and disease. Many variables directly or indirectly influence the frequency of occurrence and the distribution of any given disease. Such variables are known as determinants of disease, and they include host characteristics, specific agents and their properties, and environmental factors. These disease determinants, together with some of their important interrelationships such as modes of transmission of infectious agents, will determine the patterns of diseases in animal populations. Since they must be considered in any epidemiological approach to control of disease, the more common host, agent and environmental determinants of disease will be outlined.

The occurrence of many diseases is strongly associated with particular host variables. These variables influence an animal's susceptibility or resistance to disease. They can be divided into two groups, the intrinsic host determinants concerned with the nature of the animal itself and the extrinsic host determi-

nants imposed upon the animal by the environment. Intrinsic host determinants include species, breed, sex, age, genetic characteristics (e.g., hereditary defects), immunological state, and physiological state (e.g., pregnant or lactating). Extrinsic host determinants include level of nutrition, composition of diet, functional use of animal (analog of occupation for man), and animal husbandry practices such as the maintenance of open or closed herds and the presence or absence of separation of susceptible from infected animals.

Many of the events that specific agents trigger in animal hosts, such as the development of pathological lesions and clinical signs, are primarily under host control. Nevertheless, several properties of living agents, including infectivity, pathogenicity, antigenicity, immunogenicity, and antigenic variability, also influence the pattern of diseases in animal populations. Antigenic variability is of particular importance in epidemiology, and is seen in the evolution of serotypes (e.g., in *Salmonella* and *Leptospira* bacteria), in antigenic drift and antigenic shift (e.g., minor and major changes, respectively, in influenza viruses), and in antigenic variation. The ability of several species of African trypanosomes to undergo antigenic variation is of profound significance in the epidemiology of bovine trypanosomiasis because it allows the parasites to evade the immune response of the host (Cross, 1978), permitting maintenance of debilitating chronic infections. Properties of agents not directly associated with their animal hosts are also important determinants of disease. Examples include the development of resistant free-living forms, such as the spores of anthrax bacilli, the oocysts of coccidia, and the eggs of nematodes and cestodes, that can withstand environmental stresses.

There are numerous environmental determinants of disease that impinge upon both host and agent. Physical determinants include climate and soil. Various climatic factors will influence host and vector populations and will affect the free-living stages of infectious agents. Neutral and alkaline soils, for example, will favor propagation of anthrax bacilli, whereas soil porosity can affect the distribution of nematode and cestode eggs. Other environmental determinants relate to plants and animals. Of particular importance are animals that serve as reservoirs or vectors of infectious agents. Also, human variables relating to cultural practices and socioeconomic status often can have pronounced effects on disease patterns.

Modes of disease transmission

The modes of transmission of infectious agents will influence disease behavior in animal populations and, therefore, must be taken into consideration in any rational approach to the control of infectious diseases. Two schemes exist

for the classification of modes of transmission of infections; they are vertical versus horizontal transmission and direct versus indirect transmission. Both are important epidemiologically.

Vertical transmission of an agent occur from an individual to its offspring, such as infection of fetuses *in utero,* of neonates via colostrum, or of vectors transovarially. Horizontal transmission, on the other hand, occurs from an infected individual to a susceptible contemporary.

Direct transmission of infectious agents requires close proximity of infected and susceptible animals. It can occur either by physical contact, as in bite-transmitted rabies and venereal diseases, or via airborne droplets, as in infectious bovine rhinotracheitis. Airborne droplets are large infectious particles that are propelled only short distances to the mucous membranes of other animals, such as during coughing or sneezing.

Indirect transmission by vectors, vehicles or droplet nuclei can occur over considerable distances. Vectors are invertebrates which transmit infectious agents from infected to susceptible animals (e.g., ticks are the vectors of *Babesia* species). On the other hand, vehicles are inanimate substances by which or upon which infectious agents pass from infected to susceptible animals. Vehicles, therefore, act as important sources of infection and they include food, water, milk, dust, fomites such as blankets or instruments, and biologics such as serum, blood or plasma. Specific examples are the transmission of *Leptospira* species in water and of bovine leukemia virus on contaminated needles. Droplet nuclei are solid residues of evaporated droplets that are formed when expiratory droplets from infected hosts evaporate quickly in unsaturated atmospheres; these small particles remain suspended in the air as a cloud of droplet nuclei that can travel considerable distances in wind. Foot-and-mouth disease virus and *Mycobacterium bovis* can be transmitted in this manner.

The mode of transmission of infectious agents will influence the dynamics of particular disease events in a population. Diseases spread by physical contact exhibit a sporadic pattern of spread except when there is a common source of infection for a group of animals. Airborne spread, either in droplets or droplet nuclei, is usually rapid with well-defined outbreaks occurring if a sufficient population of susceptible animals is available. Vector-borne diseases commonly show a gradual build-up of cases over a period of time. Diseases transmitted by vehicles show two basic patterns of spread: those acquired through ingestion of contaminated water or food exhibit an explosive pattern (i.e., a point epidemic) reflecting simultaneous exposure to a common source of infection, whereas the pattern of spread tends to be sporadic when dust or fomites are the vehicle.

Analytical methods in epidemiology

Since diseases in populations do have multiple determinants, measures for their control must be based on an understanding of the relative importance of those determinants. Development of the computer has added an invaluable dimension to the epidemiological study of many animal diseases. With this technology, powerful analytical methods such as multiple regression, least square analysis of variance, discriminant analysis, and factor analysis have been applied to epidemiological data to investigate the relationships between the numerous variables that influence the patterns of many diseases. The value of these statistical procedures in epidemiological research has been demonstrated in studies of bovine brucellosis (Kellar *et al.*, 1976), bovine leukemia virus infection (Burridge *et al.*, 1979), dairy calf mortality (Martin *et al.*, 1975a, b, c), and calf diarrhea (Franti *et al.*, 1974). However, these procedures have the important limitation that they do not interpret causal relationships. In contrast, another analytical method, path analysis, does approach the problem of causal interpretation through application of multiple regression procedures to a linear model which represents the causal processes assumed to operate among the variables in nature. A recent study has indicated that path analysis, by permitting interaction between epidemiological theory and statistical analysis, is a valuable additional tool to epidemiologists (Burridge *et al.*, 1977). Path analysis is particularly useful in the identification of important causal pathways in complex biological systems.

Mathematical modelling is finding increasing utility in epidemiology. A major objective of epidemiological modelling is to provide insight into the biomedical mechanisms underlying dynamic disease processes. The disease problem under study is reduced to its essential elements and, using existing data from the field and laboratory, a model is constructed. To be of practical value, the model should behave in a biologically and mathematically reasonable way, must be sensitive to important factors and insensitive to unimportant factors, and should mimic real-life situations (Schwabe *et al.*, 1977). The model can be used to study mathematically the relationships among its components, thus identifying the major determinants of the disease problem.

The likely outcomes of various control strategies can be investigated using models that simulate disease systems. For example, it may be possible to attach probabilities to the occurrence of each event in the model so that the sequence of events that are thought to operate in nature are reflected in numerical terms. From such a model, it is possible to predict infection prevalence trends or likely control measures such as obligatory vaccination or elimination of infected animals by compulsory slaughter. A major advantage

of simulation studies is that the parameters can be altered at any stage in the model, helping the investigator to arrive at some optimal decision regarding control of the disease. An example is a model of bovine brucellosis, designed partially to explore responses to novel control procedures (Hugh-Jones *et al.*, 1975). Path analysis, as introduced above, also provides a method whereby the consequences of realistic manipulation of variables in a linear causal model can be visualized in numerical terms, forming a rational basis for designing a disease control program. It must be emphasized, however, that conclusions made from these models are only as good as the quality of assumptions and data on which they are based.

Economic aspects of disease control

The results of epidemiological studies often indicate that a number of control strategies are available for a given disease problem. In these situations economic analysis is required to help determine the optimal control strategy. The economic principles involved in such an analysis have been outlined (Morris, 1969). It is necessary to demonstrate that in any anticipated disease control program the effects of control measures will result in increased profits to producers. This is essential if the farming community is to be persuaded to cooperate and invest in disease control activities. At the national level, the funding agencies increasingly are demanding benefit-cost analyses to justify their approval of disease control programs. These analyses involve not only comparison of the potential benefits of control against no action, but also evaluation of alternative control strategies. Such estimates require a clear understanding of the epidemiology and economic impact of the disease under consideration.

The economic impact of animal diseases is experienced not only by the producer but also by the community. The total economic impact consists of the actual losses plus the costs of control, prevention and eradication measures. Economic losses can be classified into direct and consequential losses. Direct losses are those suffered by producers and they result from the direct physical effects of disease. They may be subdivided into visible losses (e.g., mortality, abortion, condemnation at meat inspection, damaged hides), invisible losses (e.g., impaired productivity, infertility), and immediate consequences (e.g., loss of production following death of animals, herd replacement costs). Consequential losses are those suffered by the community as a result of animal diseases. They may be subdivided into visible consequences (e.g., effects of zoonotic diseases on human health) and invisible consequences (e.g., loss of foreign markets). The direct losses from animal diseases are well

appreciated and their monetary value is relatively easy to assess. Consequential losses are often more difficult to estimate, can by very high, and frequently are the most costly effect of a disease. For example, the total compensation paid for slaughter of infected animals and contacts during the 1952 foot-and-mouth disease epidemic in Canada was $ 1 million, whereas the resultant closure of foreign markets to Canadian products was responsible for consequential losses of $ 724 million (Wells, 1970). The indirect consequences of animal disease can have even farther-reaching effects. The prime example is African trypanosomiasis which prevents development of livestock production in the 10 million square kilometers of Africa infested by the tsetse fly vectors.

Determination of the optimal control program for a given disease will involve analysis of both the costs and benefits of each control strategy. Benefits are much harder to estimate than costs. They are of two types, financial and social, and both must be taken into account if the full impact of a control program is to be measured. Financial benefits will include costs that can be avoided, such as costs of treatment and prophylaxis, as well as increased profits accruing from reduced production losses. Social, or nonfinancial, benefits are difficult to estimate in monetary terms. They will include lack of suffering, prestige of freedom from disease, avoidance of inconveniences such as treatment of sick animals that disrupt management routines, and increased human productivity in the case of zoonotic infections.

Many disease control programs are of long duration, especially when the disease is endemic. If such programs are to be successful, both public and private sectors must recognize favorable benefit-cost ratios. Generally speaking, private benefits, such as those of producers, must be seen to exceed private costs within a fairly short period of time if farmers are to continue to support the program. In contrast, the community as a whole usually will accept a longer time-lag before benefits exceed costs. The allocation of costs to private and public sectors, therefore, must be carefully balanced to reflect these considerations.

There are few published reports on the benefit-cost evaluation of alternative control policies for animal diseases. One of the few studies published considered the control of foot-and-mouth disease in the United Kingdom following the 1967-68 epidemic which cost that country $ 22 million in direct eradication costs and $ 111 million in consequential losses (Power and Harris, 1973). The traditional slaughter policy (eradication program) was compared with a proposed vaccination policy (control program). Although it was shown that both policies would yield very large net benefits, the eradication program was found to be less costly to the community and, therefore, was the approach of choice for foot-and-mouth disease in the United Kingdom. Another British

study assessed the bovine brucellosis eradication program in England and Wales (Hugh-Jones *et al.*, 1975). It found sufficient justification for brucellosis eradication with benefits only limited to removal of direct losses and routine costs. Inclusion of social benefits markedly increased the benefit-cost ratio of the program.

Methods of disease control

The control of infectious diseases implies reduction in the number of cases or in the opportunities for transmission to levels where infections no longer exist as major health or economic problems and, ultimately, to levels of little or no consequence. An obvious disadvantage of control over eradication as a goal is that a continuing and essentially undiminished effort is required. However, in many instances control is the only scientifically realistic or economically feasible goal. In others, control may be the forerunner to an eventual disease eradication program.

The approach to control of a specific disease is dependent upon an understanding of its epidemiology. Some non-infectious diseases may be brought under control relatively simply by identifying and attempting to eliminate their causes. For example, the prevalence of epidemic bovine hyperkeratosis in the United States was reduced virtually to nil when the toxic lubricating oil additives which caused it were discovered and removed from farm markets. The control of infectious diseases is usually more difficult. Factors that complicate their control include (1) wide host ranges for the etiological agent, especially when many species act as reservoirs or as vectors; (2) the involvement of highly mobile hosts such as birds or bats in transmission and perpetuation of infection; and (3) well-established cultural practices that favor transmission.

Methods used in disease control include the following:

(a) Quarantine – Sick animals are physically separated from healthy ones, and restraints are placed on the movements of infected and exposed animals as well as on items that may have been contaminated.

(b) Selective slaughter – Infected animals are detected through mass surveys, are removed from the population, and are subjected to premature slaughter. This method is dependent upon the availability of a simple testing technique for mass diagnosis. Principal disadvantages are the high initial costs of operation and compensation for animals sent to slaughter, and the difficulties commonly experienced in "selling" this approach to livestock owners. Selective slaughter is not feasible when prevalence of infection is very high,

when replacement animals are not available, or when it would be otherwise disruptive to the local economy. When only high prevalence of infection is a limiting factor, mass immunization or mass treatment can be used to reduce prevalence to a level whereby selective slaughter can be introduced as a control measure.

(c) Mass immunization – Its advantages as a method of disease control include its long-lasting effects and the ability of immunized animals to move about freely. However, mass immunization does have its disadvantages, particularly due to the imperfect protection afforded by many vaccines (e.g., leptospiral bacterins) and the public health dangers of others (e.g., Strain 19 *Brucella abortus* vaccine).

(d) Mass treatment – It can be carried out as a blanket control measure either in emergency situations, as with a sudden rise in disease incidence, or when disease prevalence is very high. This method is dependent upon the availability of safe and cheap therapeutic agents, and finds use in the control of gastrointestinal parasitism.

(e) Vector control – Insecticides and acaricides have played an invaluable role in the control of diseases transmitted by invertebrate vectors. However, the development by arthropods of resistance to an ever-increasing number of these chemicals has posed a major problem for effective vector control. Recent work has indicated that some cattle can acquire resistance to ticks and that this resistance is inherited to a degree whereby selective breeding could become a useful means of tick control (Hewetson, 1972).

(f) Reservoir control – It is only applicable when a population of expendable animals acts as a reservoir of infection. Poison baiting, trapping and shooting are among the most commonly employed techniques for control of reservoir hosts. The first two methods, however, have potential dangers for children, pets and other animals.

(g) Genetic measures – Where genetic factors are major determinants of a disease, control is aimed at reducing the defective gene pool by selective neutering or selective breeding.

(h) Environmental measures – They include disinfection, heating, freezing, vegetation clearance, and improvements in housing. Bush clearance, for example, has been employed as a measure to control the tsetse fly vector of African trypanosomiasis.

(i) Education – This is essential to the success of disease control programs, especially where human cultural practices have to be overcome or modified. Education must precede the beginning of a disease control program based on selective slaughter. The educational program should be aimed at gaining the confidence of livestock owners by demonstration of the importance for control of the disease and of the necessity for using the selective slaughter

approach. In addition, educational campaigns should be designed to motivate the public to do something positive about controlling disease.

Epidemiological studies will indicate which of these methods are applicable for the control of a particular disease. Furthermore, they will lead to the development of a number of alternative control strategies. Socioeconomic analyses must then be carried out on each control strategy to demonstrate the advantages and disadvantages of each. From these analyses a decision will be made to select the approach that offers the greatest social and economic benefits at an acceptable cost level.

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PART II

INFECTIOUS DISEASES

6. BOVINE PAPULAR STOMATITIS

Robert A. Crandell

Abstract. Bovine papular stomatitis (BPS) is a mild disease affecting primarily the oral mucosa of cattle. The disease is caused by a virus belonging to the poxvirus group. Cattle are the natural host and man acquires the infection by handling infected cattle. Calves probably become infected early in life and some remain as carriers. The disease is worldwide in distribution. Although primary infections of BPS are of minor economic significance, they are of considerable importance in the differential diagnosis of all diseases affecting the oral cavity of cattle. The lesions range from hyperplasia to erosions and ulcerations. Lesions of different stages of development and regression are found in the same animal. Intracytoplasmic inclusion bodies are a characteristic feature of the histopathology. In the uncomplicated disease, the lesion regresses without treatment. Recrudescence occurs when the animal is stressed. Virus isolation, electronmicroscopy and histopathology are helpful laboratory procedures in obtaining a diagnosis.

Synonyms: stomatitis papulosa, stomatitis papulosa bovis specifica.

Introduction

Bovine papular stomatitis (BPS), a mild disease caused by a poxvirus, affects principally the oral and perioral mucocutaneous tissues of cattle. The disease is generally considered to be of little economic importance. However, it is important in the differential diagnosis of any bovine disease affecting the oral cavity.

During the last two decades of the 19th century, several accounts of stomatitis in cattle were reported from Belgium and Germany. In Germany, Ostertag and Bugge (1906) described a stomatitis of cattle which they reproduced experimentally and suggested that the etiologic agent was a virus. The name stomatitis papulosa bovis specifica was proposed for the disease.

Additional cases were reported from Germany, and Shaaf *et al.* (1940) described the occurrence of cytoplasmic inclusion bodies in stained tissue

sections. The agent was later shown by electron microscopy to be a member of the poxvirus group (Reczko, 1957). Plowright and Ferris (1959a) first reported the cultivation of the virus in cell cultures.

Etiology

The bovine papular stomatitis virus belongs to the family Poxviridae and genus parapoxvirus. In ultrathin tissue sections from cattle infected with BPS virus, Reczko (1957) described round, incomplete virus particles (207 nm in diameter) and more developed particles (215 nm × 105 nm). Negative stained cell culture propagated virus particles are oval to cylindrical in shape. The mature virion measures 260 nm × 140 nm and has a crisscross surface pattern of tubular fibrils (Fig. 1).

The virus contains DNA, is heat resistant (50 °C for 30 min) and is sensitive to ether and chloroform. Neither hemagglutination nor hemadsorption have been demonstrated using erythrocytes from cattle, sheep, guinea pig, chicken or pig (Liebermann and Urbaneck, 1966).

The common laboratory animals and embryonating eggs are not susceptible to infection. Strains of the virus have been isolated in cell cultures of bovine or sheep testicles, bovine embryonic kidney, monkey kidney or primary human amnion cells.

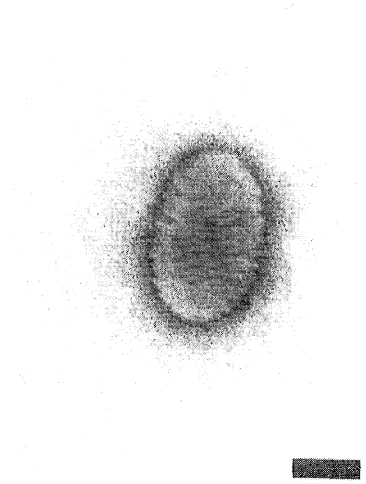

Fig. 1. Bovine papular stomatitis virus particle propagated in primary bovine embryonic cell cultures. Phosphotungstate negative stain. Bar = 100 nm.

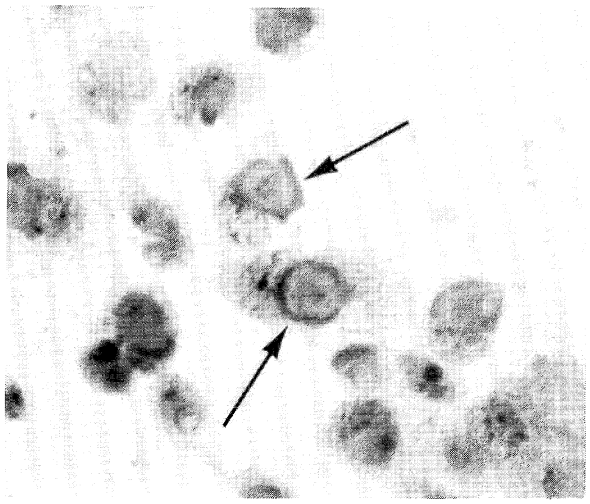

Fig. 2. Intracytoplasmic inclusion bodies (arrows) in primary bovine embryonic kidney cell culture infected with bovine papular stomatitis virus (courtesy of the American Association of Veterinary Laboratory Diagnosticians). H & E stain ×400

There is no evidence to suggest that more than one serotype exists. Antigenic similarities between isolates were demonstrated in calves by cross-protection tests (Plowright and Ferris, 1959b). The serologic relationship of BPS to other members of the poxvirus group has received little attention.

The initial cytopathic change observed in infected cell cultures is a clumping or piling of the cells. As cell degeneration progresses, the cytoplasm becomes granular and the cells are released from the vessel surface leaving spaces or holes in the monolayer. The characteristic feature of the cytopathology in stained cultures is the presence of cytoplasmic inclusion bodies (Plowright and Ferris, 1959a; Lieberman and Urbaneck, 1966) (Fig. 2). Two types of inclusion bodies are described: (1) Type A which is small, eosinophilic and highly refractile, and (2) Type B which is large, basophilic and crescent or horseshoe shaped. Virus particles have not been demonstrated in Type A inclusions, but Type B inclusions contain may virus particles of various developmental stages and appear to be the site of viral maturation (Okada and Fujumoto, 1975).

Distribution

Bovine papular stomatitis has been known for many years and has worldwide distribution. The disease has been described in Belgium, Germany, Poland, Holland, Great Britain, Switzerland, Italy, East Africa, Asia, Japan, Australia, New Zealand, United States, Canada and Mexico.

The disease has been reported to be enzootic in Germany. Pallaske (1955) studied a number of cases of the disease in cattle in Central Germany. The infections involved the mucous membranes of the gums, tongue and lip and occasionally the epithelium of the esophagus and rumen.

The disease was diagnosed in cattle with severe cutaneous streptothricosis and proliferative stomatitis in the Plateau Province, N. Nigeria, by histological examination of tissues collected in 1954-55. Lesions identical to those described for BPS were frequently observed among many hundreds of 2- to 3-year-old research cattle in Kenya. Between April and July in 1958, two outbreaks of the disease occurred in calves. The virus was isolated in cell culture from lesions collected from a 2-year-old ox and a young calf (Plowright and Ferris, 1959a).

The disease is widespread in the United States. It has been reported in cattle from at least 13 states. Although BPS was first recognized as a disease entity in cattle in the North Central region of the United States (Griesemer and Cole, 1960), the disease appears to be more prevalent in the southern states. In recent years, the disease has been observed predominantly in feedlot cattle. It is now believed that the condition called "proliferative stomatitis" was due to the virus of BPS. Proliferative stomatitis was seen in bovine cases of hyperkeratosis (X disease) and occurred in widely scattered areas of the United States during the 1940s and early 1950s. Oral lesions similar to those found in BPS were observed in bovine cases of chlorinated naphthalene poisoning (Olson and Palionis, 1953). Humans associated with the bovine disease became infected, thus supporting the belief that proliferative stomatitis was due to the virus now known as BPS.

The incidence of BPS was determined in 10 groups of cattle located in the Central District of Victoria in Australia (Snowdon and French, 1961). The incidence in three groups of Hereford cattle 3 to 16 months old was found to range from 88% to 100% by repeated examinations of the mouth for lesions. In seven other groups composed of dairy beef and mixed breeds of younger cattle, the incidence of infection was found to range from 10% to 91% in one examination. These variations in the level of infection within the groups were not related to any specific season of the year. There were no predisposing factors identified with the appearance of the disease.

The disease was first described in a 4-month-old Jersey calf in the Manawatu District of New Zealand in 1966. Although BPS had not previously been recorded in New Zealand, erosive lesions in the oral cavity of calves are quite common (Jolly and Daniel, 1966).

Oral and muzzle lesions similar to those described for BPS by Ostertag and Bugge (1906) were found in 28 of 304 calves examined on 15 farms in Dorset, Great Britain (Nagington *et al.*, 1967). Because of incidence, the disease was

considered to be common in Dorset. Similar lesions were observed in cattle in Scotland, and the viruses isolated were believed to be the same as BPS. Pseudocowpox has been reported to be endemic in the United Kingdom, and occasionally pseudocowpox and BPS will occur in the same calf.

The disease was first recognized in the northern part of Japan during the summers of 1969 and 1970 (Kumagai *et al.*, 1976). The infection occurred in a pasture in Aomori Prefecture where 82, 3-year-old beef cattle and 52, 5-month-old calves were grazing. Six of 12 adult cows and all of the 12 calves examined had lesions. Lesions were observed in animals on two of 15 neighboring pastures. The disease was reported in cattle on a total of nine pastures from July to September 1970. When observed monthly between May and July, 75% of the 354 animals had oral lesions. By the end of November the morbidity had reached 96% in the calves over 12 months of age. The outbreaks during each year began between 1 and 2 months after the cattle were turned out to pasture.

Studies on the occurrence of BPS in Switzerland showed that about 3% of the calves in the canton of Berne are clinically infected (Dunant *et al.*, 1975). In an abattoir survey 25 (2.25%) of 1112 calves had lesions which were shown by electron microscopy to contain poxvirus. The percentage of diseased calves on breeding farms was found to be 2.1% with the infection on two of the 20 farms visited. In contrast, the disease was present on seven of nine fattening farms with some having an infection rate of 16.5%. In 1972, 11.5% of the calves entering the large animal clinic in Berne were visibly infected.

In 1974 the disease appeared in Italy 19-20 days after the importation of cattle from Canada (Loda *et al.*, 1974). The cattle recovered without secondary complications in 10 to 15 days and virus transmission to animals held 10 to 15 m from the infected animals did not occur.

The disease was first recognized in 2- to 8-month-old calves located in Ajuchitlan, Queretaro, Mexico in 1977. Lesions were present in 31 (25.8%) of 120 calves examined. Virus was isolated in cell culture and was identified as BPS by neutralization with specific antiserum.

Epidemiology

Cattle are the natural host and man is an accidental host of BPS. Human infections are acquired by handling infected cattle. The hands and arms are the most common site of infection in man. The virus is transmitted in cattle saliva to man by direct contact. The virus enters the broken skin and papules

develop at the site of contact within 7 days. In man, the lesions normally regress within 3 to 4 weeks without treatment.

The disease is observed more often in younger animals, but cattle of all ages are susceptible. There are no known differences in the susceptibility of breeds and sexes.

The BPS virus is transmitted from animal to animal by direct contact. The nasal secretions, saliva and lesions from infected cattle are sources of virus. Although the exact mechanism of transmission is not clear, it is believed that young calves contact the virus early in life from their dams. The disease has been diagnosed in a calf from a dam that had characteristic lesions of BPS and unusual teat lesions. Since the virus is relatively stable, virus contaminated objects in the environment are potential sources of infection.

The disease usually has a high morbidity rate, and in uncomplicated infections the mortality rate is low. Close examinations of herds have revealed clinical infections in 100% of the animals. The disease is endemic throughout the year; however, management practices and mixed infections are believed to influence its recognition (Crandell, 1978). Pallaske (1955) suggested that BPS virus may remain in the host as a latent infection and when the animal's resistance is lowered, lesions will reappear. In the United States the recent outbreaks of severe clinical disease are usually associated with unfavorable environmental factors and infectious or toxic agents.

The stress of transport and moving of cattle appear to be predisposing factors to clinical disease. The disease was first recognized in cattle in Japan 1 to 2 months after release to pasture (Kamagai *et al.*, 1976). BPS occurred in cattle 19-20 days after importation to Italy from Canada by air transport (Loda *et al.*, 1974). In contrast, in Australia where the natural disease was studied over a 3-year period, variations in incidence differed in 3 groups of animals with relationship to time of year and without obvious predisposing factors (Snowdon and French, 1961).

There is no evidence that insects or rodents play a role in the epidemiology of this disease.

Clinical signs

In the uncomplicated infection, the clinical disease may be so mild that oral lesions may go unnoticed unless the animals are examined closely; however, diarrhea, salivation and a slight fever have been reported in primary infections of BPS. Lesions in various stages may be observed on the muzzle, nostrils, lips, oral papillae, gums, hard and soft palate, ventral and lateral surfaces of the tongue and epiglottis. Clinically ill animals with BPS lesions have usually

been stressed or exposed to some chemical toxicosis or other infectious agents. The disease has been associated with such conditions as parasitism, "rat tail" syndrome, infectious bovine rhinotracheitis, pasteurellosis, bovine hyperkeratosis (chlorinated naphthalene poisoning), cutaneous streptothricosis and rinderpest.

The incubation period varies in experimentally infected calves. Lesions occur as early as 2 days and as late as 2 weeks after inoculation of virus in the submucosa of the dental pad or gums. A lesion usually appears at the site of inoculation and occasionally will occur at another site. Secondary lesions may occur over a period of several months. In natural infections, the reoccurrence of lesions is common, and the duration of lesions in individual animals is variable. Although individual lesions may regress within 2 weeks in some animals, secondary lesions have persisted in different areas of the mouth for as long as 227 days (Snowdon and French, 1961).

Pathology

Lesions similar to those found in the oral cavity are often observed in the epithelial surface of the esophagus, omasum, rumen and reticulum at necropsy (Griesemer and Cole, 1961). The lesions occur as single or multiple defects and in various stages of development or regression. Some differences exist in the morphology of lesions according to their location.

The first sign of infection on the muzzle is the appearance of red raised foci which soon become circular, depressed and later appear as brown scabs. Erosion followed by healing occurs at the center of the lesion and extends peripherally, creating circular, reddish to brown rings. A typical lesion on the lip and tongue appears first as a hyperemic focus which develops rapidly into a circular or oblong papule. The surface of lesions occurring on the lip is smooth and glistens, giving the appearance of a vesicle, but they do not contain fluid. Lesions on the tongue are duller and gray in color. The centers become necrotic and erode, leaving a depression. In severe cases, lesions are numerous and coalesce, resulting in massive proliferative areas covered with a yellow necrotic material.

The lesions on the floor of the mouth and cheeks are usually observed as pinkish eroded oval areas that heal rapidly. When involved, the buccal papillae are blunt and covered with a brownish scale. Lesions on the dental pad are common, where they become irregular in size and shape and have a roughened surface (Fig. 3). The erosions on the dental pad and gums become brown in color before healing and may easily be confused with lesions caused

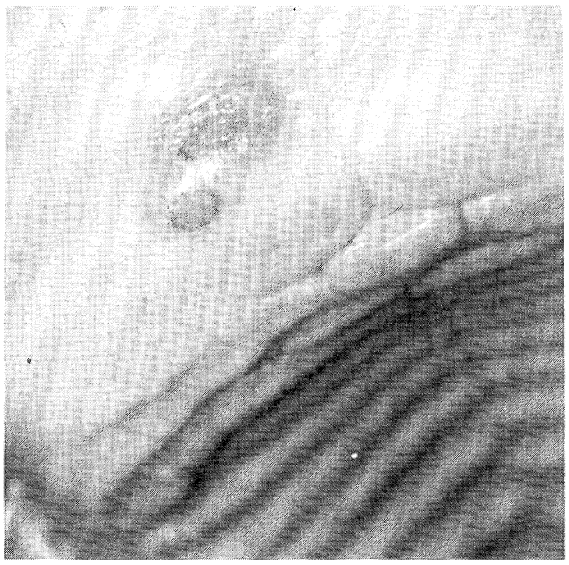

Fig. 3. Erosions on the mucosa of dental pad of calf experimentally infected with bovine papular stomatitis virus.

by other agents. In dark pigmented areas the lesions are more difficult to detect.

The lesions on the soft palate are circular, umbilicated with necrotic centers and an elevated gray periphery (Fig. 4). These are surrounded by reddish-gray to purple concentric rings. The lesions on the hard palate are more irregular in shape and tend to follow the structure of the palatine ridges. Multiple irregularly shaped erosions varying in size may be found in the esophagus.

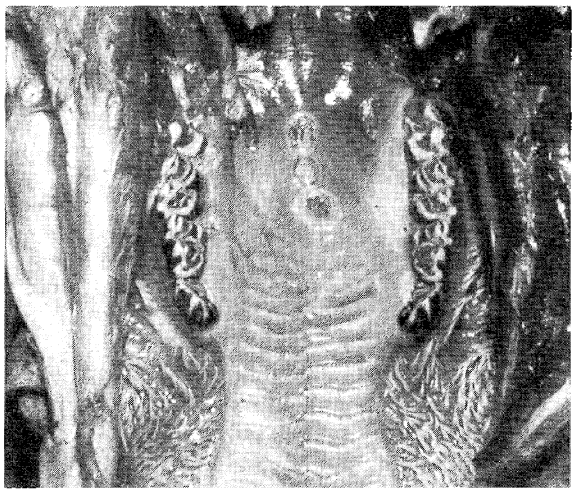

Fig. 4. Typical circular lesions of bovine papular stomatitis on the palate of a naturally infected feeder calf.

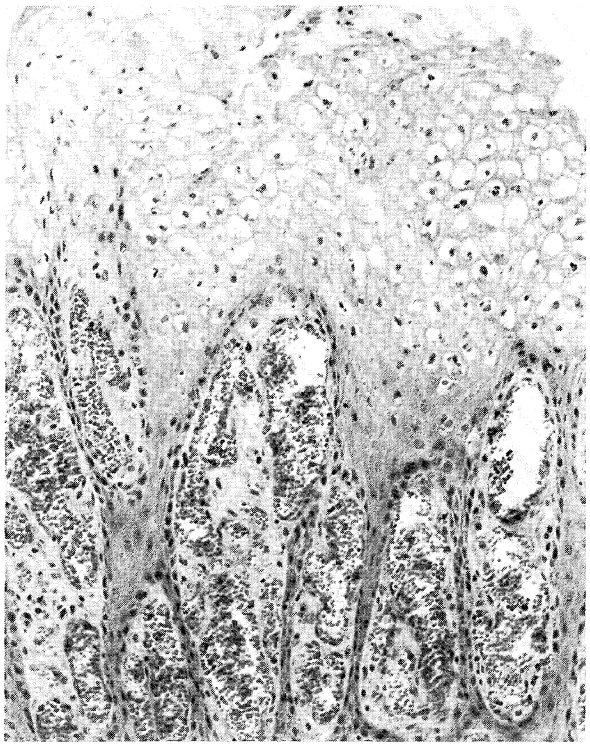

Fig. 5. Lesion in the epithelium of the esophagus of feeder calf with naturally occurring bovine papular stomatitis. Notice ballooning degeneration of epithelial cells and increased vascularity. H & E stain ×100

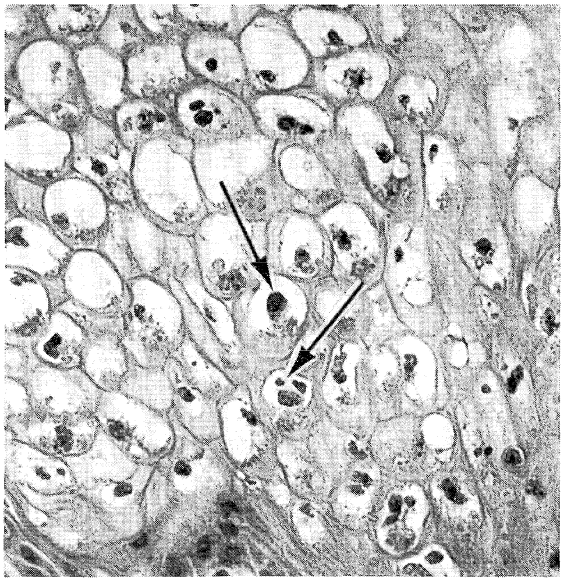

Fig. 6. Higher magnification of Fig. 5 demonstrating intracytoplasmic inclusion bodies (arrows). H & E stain ×600

Histologically, the epithelium may be markedly thickened with the superficial layers frequently eroded. In the eroded areas the epithelial surface may contain sequestered neutrophils and bacteria. Ballooning degeneration of the epithelial cells is a characteristic feature (Fig. 5). The cytoplasm of these cells are clear and the nuclei are often shrunken and misplaced. Within the cytoplasm numerous round, dense, acidophilic inclusion bodies of various sizes can be found (Fig. 6). In the more proliferative lesions, elongated rete pegs are formed, and there is an increased vascularity of the lamina propria. Ulcerated lesions may contain a heavy infiltration of polymorphonuclear cells.

Immune response

The immune response is reported to be of short duration. Although Plowright and Ferris (1959b) demonstrated resistance in calves to reinfection between 24 and 39 days post infection, they were unable to demonstrate significant levels of neutralizing antibodies. Recrudescence of the lesions is a common feature of latent infections and is associated with poor immunity. Snowdon and French (1961) reported recurrent disease of 15%, 33% and 53% in three different groups of cattle examined periodically for a period of several years.

Laboratory aids to diagnosis

Since the lesions of BPS are often mistaken for those of other diseases affecting the oral cavity, the diagnosis should be confirmed by laboratory methods. Several infectious diseases which need to be considered in the differential diagnosis are: bovine virus diarrhea, malignant catarrah fever, foot-and-mouth disease, vesicular stomatitis, rinderpest and mycotic stomatitis.

Virus isolation, electron microscopy and histopathology are useful laboratory procedures for establishing a diagnosis. Although virus has been recovered from saliva and nasal secretions, the recommended specimens for laboratory examination are the lesions (scabs or scrapings) collected either by biopsy or at necropsy.

Virus isolation is accomplished by the inoculation of primary cell cultures of bovine origin with suspensions of the lesions and the virus is identified by serum neutralization or by the fluorescent antibody tests. The application of electron microscopy by the phosphotungstate negative staining technique and

the examination of ultrathin sections is useful for the demonstration of virus particles in the lesions and infected cell cultures (Crandell and Conroy, 1974). The characteristic histologic feature of the lesion is the presence of intracytoplasmic inclusion bodies.

Prevention and control

There are no specific preventive measures or treatments for BPS. Daily rinsing of the oral cavity with 2% potassium permanganate has been used with reported recovery in 10 days. In uncomplicated cases, the lesions regress without therapy. In mixed infections, supportive therapy and treatment with antibiotics may be indicated.

Since recrudescence occurs with stress, good management practices are recommended. These should include balanced rations, parasite control and disease prevention programs and protection against environmental stresses.

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7. MALIGNANT CATARRHAL FEVER

I.E. Selman

Abstract. Malignant catarrhal fever (MCF) is an almost invariably fatal disease in cattle, characterized by fever, depression, profuse nasal and ocular discharge and encrustation, drooling of saliva, photophobia, keratitis, erosion and diphtheresis of oral membranes, generalized lymphodenopathy, skin lesions and, occasionally, cystitis and central nervous involvement. The pathological features, which affect all organs and tissues are necrotizing vasculitis, marked cellular infiltration and superficial necrosis of both epithelial and mucous surfaces. The pathogenesis of the disease is unknown but it has been suggested that it is a virus-associated lymphoproliferative auto-reactive disease.

In certain parts of east, central and southern Africa, the disease arises as the result of cattle acquiring infection from clinically-normal wildebeests. The causal agent in this situation is a cell-associated, polykaryon-forming herpesvirus (*bovid herpesvirus 3*). In other parts of Africa and elsewhere in the world, the etiology of MCF has not yet been established. The reservoir host is assumed to be the sheep but there is little definitive evidence to support this view.

Diagnosis of MCF is usually based on its distinctive clinical and pathological features. On occasions (particularly with the wildebeest-derived infections) transmission studies may help to confirm the presence of the disease. The use of virus-isolation techniques is limited to the latter form of MCF. There is no effective treatment for MCF and, at present, prevention depends upon avoidance of contact with known or suspected reservoir hosts.

Etiology

The etiological agent of wildebeest-derived malignant catarrhal fever (MCF) is a cell-associated, polykaryon-forming herpesvirus (*bovid herpesvirus 3*). For further details of this organism and its behavior in tissue cultures and on animal passage, the reader is referred to Plowright (1968).

It is generally assumed that the agent responsible for the (presumed) sheep-derived form of MCF will prove to be a similar type of virus to the wildebeest-derived MCF. So far, however, all efforts to confirm this have proved fruitless. Nevertheless, serial (i.e., cattle-to-cattle) transmission of this form of

the disease is possible using whole blood (Blood *et al.*, 1961; Pierson *et al.*, 1974; Selman *et al.*, 1974, 1978, and Liggitt *et al.*, 1978) and density gradient-isolated mononuclear cells (Liggitt *et al.*, 1978) from clinically-affected animals.

Pathogenesis

Since the causal agent of the wildebeest-associated form of MCF was first defined as a herpesvirus, a great deal of knowledge has accumulated regarding the characteristics of the virus and its behavior and final effects in susceptible animals and tissue culture (Plowright, 1968). Similar progress has not been made, however, towards understanding the fundamental pathogenic mechanisms which are involved in the disease process. In view of the prolonged incubation period and also the nature and widespread distribution of the histopathological changes which are consistently observed, it seems obvious that the disease does not arise simply as the result of viral infection alone. Indeed, these very considerations have prompted several groups of workers to suggest that the disease process might well be dependent upon an interplay of various immune reactions. In particular, the vascular lesions might represent a type-III (Arthus) reaction and the marked mononuclear accumulations may be a widespread cell-mediated hypersensitivity reaction.

The most recent hypothesis (Liggitt *et al.*, 1978) is based upon the belief that the histopathological features of MCF represent the final effects of a virus-associated, lymphoproliferative, autoreactive disease. The proponents claim the hypothesis is supported by the fact that lymphocytes from affected cattle can be infected by the virus. They also claim that certain characteristics of the virus in culture support their hypothesis, particularly its limited host cell range and its lack of cytolytic activity in tissue culture. Minor clinical and pathological changes, typical of MCF, have been detected during the "incubation period" (i.e., long before the *unmistakable* syndrome arises) and support the concept of a progressively escalating immune reaction, starting almost from the time of infection. Also, graft vs host reactions in species other than cattle support the theory that MCF is an autoreactive disease.

The basic concept of this hypothesis is that the virus of MCF infects a certain population of lymphocytes selectively. It is possible that the population in question normally exerts a suppressor effect on autoaggressive lymphocytes. Alternatively, the virus might infect and activate the autoreactive lymphocytes themselves; furthermore, if this is true and clonal proliferation of activated autoaggressive lymphocytes takes place, the effects may be such that the animal dies of MCF long before the more usual signs of lymphoproliferative disease arises.

Distribution

Malignant catarrhal fever has a worldwide distribution but is usually only a sporadic problem. On a few occasions, however, the disease has been encountered in epidemic proportions in North America (Murray and Blood, 1961; Pierson *et al.*, 1973; Liggitt *et al.*, 1978) and in New Zealand (James *et al.*, 1975). It is generally accepted that high morbidity incidents are much more a frequent feature of the disease in east, central and southern Africa but even in these regions the disease is often low in prevalence; it may, nevertheless, assume considerable significance as a problem due to regular seasonal recurrence.

Epidemiology

Irrespective of where it occurs, MCF in cattle is a very dramatic disease with highly consistent clinical and pathological features. At least two epidemiologically-distinct forms exist in cattle and it is essential that the form of the disease under consideration is properly defined. Basically, the wildebeest-associated form of the disease has received the most attention (Plowright, 1968). The causal organism was established as a herpesvirus almost 20 years ago and proved to be a relatively easy organism to work with in the laboratory and in experimental animals; this has enabled several groups of workers to describe many of the epidemiological features of that particular infection. Extension of these studies has shown that certain other wild ruminant species in east, central and southern Africa may also harbor the virus; while it seems the wildebeest is the major source of infection for cattle in these regions, it is likely that problems may occasionally arise due to infection from an alternative reservoir host.

A disease identical to MCF also occurs in other parts of the world where the responsible agent is not yet known. Transmission studies often appear to be less consistent than the studies with the wildebeest-derived infection. As a result, less progress has been made towards the full understanding of the epidemiology of this form of the disease. For many years it has been claimed that sheep may act as an alternative reservoir host in the absence of wildebeests and other wild ruminants. It has become acceptable to refer to the wildebeest-associated disease as "African MCF", but the presumed sheep-derived infection has also occurred in several different parts of Africa as well as elsewhere in the world (Piercy, 1954). Each form of the disease will be discussed in turn.

In certain parts of east, central and southern Africa, cattle may acquire

infection from either the black or the blue wildebeest (*Connochaetes gnu* or *C. taurinus*). Although it is probable that the infection rate varies markedly between different wildebeest populations, relatively high infection rates occur in wildebeest calves under 3 months of age. Such animals become infected either congenitally or during early neonatal life due to contact with other infected calves. They develop a viremia which may persist for up to 3 months, during which they are highly infective for cattle of all ages.

The exact mode of transmission of MCF virus from wildebeest calves to cattle is not known. Close contact between infected and viremic wildebeest calves housed with cattle has regularly resulted in the development of the disease (Plowright, 1968). Malignant catarrhal fever virus may be found in the nasal secretions of infected wildebeests (Rweyemamu *et al.*, 1974), and young domestic cattle have been experimentally infected by intranasal inoculation of cell-free virus or lymph node suspension of the virus (Plowright, 1968). This may account for the spread of the disease under artificial conditions involving mixed housing and close proximity, however, field observations indicate that domestic cattle become infected as the result of grazing areas recently vacated by newly-calved wildebeests or their calves. This may arise from infected placental membranes and uterine fluids contaminating herbage which is subsequently ingested. Another theory is that infection of grazing areas occurs when the wildebeest calves shed their birth-cloaks at around 3 to 4 months of age (Plowright, 1965). To date, neither hypothesis has been convincingly confirmed. It is definitely established, however, that direct cattle-to-cattle transmission does not occur under natural conditions, probably because of the lack of cell-free virus in cattle. On the other hand, the offspring of clinically normal adult cattle have survived experimental infection regardless of congenital infection. Thus, under certain circumstances, MCF virus seems capable of assuming latent infection, a common feature of other herpesvirus infections.

The above factors tend to make MCF (wildebeest-origin) a seasonal event which occurs soon after the wildebeest calving season (Plowright, 1965). Since in certain areas these animals are not only migratory but also calve while migrating, it is quite common for the disease to arise in cattle grazing in areas where wildebeest herds have passed. Under such conditions, the morbidity rate varies but may sometimes reach epidemic proportions. As already stated, almost all affected animals die.

It is generally accepted that in other areas (even from time to time in east, central and southern Africa), MCF results when cattle are infected from subclinically-affected sheep. The evidence, while entirely circumstantial, is nevertheless convincing. Often the link between affected cattle and sheep involves either parturient ewes or young lambs. In several particularly severe

epidemics, it has been emphasized that lambing ewes and young lambs have been in proximity to intensively-managed cattle and that the lambs have been able to gain entry to hay barns and feed bunkers. Thus, it is tempting to suggest that distinct parallels exist between the wildebeest and the sheep vectors. Usually, the presumed sheep-associated incidence are of sporadic occurrence and involve only single animals or a few cattle over a period of weeks. As already stated, however, severe epidemics have sometimes been described (Piercy, 1954; Murray and Blood, 1961; Pierson *et al.*, 1973). Most cases tend to occur at, or soon after, lambing time, but occasional incidents have been recorded in association with older (weaned) sheep. On a few occasions certain flocks of sheep have been recognized as being of particular danger to cattle (Piercy, 1954).

Malignant catarrhal fever is also a disease of domestic buffaloes in which the clinical, pathological and epidemiological features are as described for cattle. In addition, there have been several well-documented incidents among other types of buffalo, bison, various species of deer, chamois and caribou, usually in zoological collections but occasionally under natural conditions. While the origin of these outbreaks has not been firmly established, it is impossible to ignore the possibility that the affected animals had grazed the same or adjacent paddocks to subclinically infected wildebeests or other broadly-similar captive ruminants.

Clinical signs

Traditionally, clinicians have subdivided MCF into several different syndromes, i.e., the peracute, "head-and-eye"), alimentary and mild (or inapparent) forms of the disease. However, the most recent and detailed clinical descriptions of the confirmed disease as it occurs in Africa (Plowright, 1968), North America (Pierson *et al.*, 1973) and Europe (Selman *et al.*, 1974, 1978) strongly suggest that the subdivisions are an artificial classification of little or no practical value. Such a classification is probably based on either the major presenting sign(s) at one particular stage of the disease (e.g., at the time of the first, or only, clinical examination) or the veterinarian's special interest(s) and/or blind spots. One of the most intriguing aspects of MCF is that, irrespective of the circumstances under which it arises, it is a remarkably consistent clinical entity; the progress of the disease is very predictable and involves most, if not all, of the tissues and organs of the body. Thus, the traditional classification is not only meaningless since it infers a static concept of the disease but it is also misleading. The following description will concentrate on the disease syndrome as it is usually encountered and relevant details

will be drawn from both the naturally occurring (field) disease and experimentally-induced infections, since the effects appear to be identical (Selman *et al.*, 1978).

Experimental studies have shown that the incubation period of MCF is variable, ranging from a little under 3 weeks to more than 2 months with the mean figure taken as approximately 30 days. The disease is almost invariably fatal and once clinical signs are established, there is a predictable deterioration with death usually within 7–10 days. Survival for more than 2 weeks is extremely rare.

Under experimental conditions, the first clinical sign is a transient enlargement of all superficial lymph nodes, starting at around 1 week postinfection; this is usually overlooked, however, and the first sign is generally fever. In the majority of experimental infections, this precedes the onset of other signs. In experimental and field cases, high fever (106 °F) is almost a constant finding, persisting up until the terminal stages of the disease.

Dullness and anorexia appear early in the course of the disease and are soon followed by ocular and nasal discharge of variable severity and signs of oral discomfort (e.g., frequent swallowing, lip smacking and drooling of saliva). The characteristic changes that occur *within* the eye, and which are referable to a progressive panophthalmitis, never precede the appearance of mouth lesions. Skin lesions are also common and occasionally other signs arise due to severe pathological changes in the brain, lower respiratory tract and bladder.

The earliest lesions in the mouth consist of a generalized congestion of non-pigmented areas, particularly the gums, beneath the tongue, the hard palate and the oral papillae. In most instances this quickly progresses to localized congestion and blotchiness, later (particularly behind the incisor arcade) the epithelium becomes swollen and of a granular appearance. Marked features include intense congestion, petechiation and small single or multiple erosions on the tips of the oral papillae in localized areas in the cheeks. Finally, there is widespread oral necrosis and diphtheresis giving rise to marked halitosis. At this stage, examination of the mouth becomes difficult due to the copious amounts of saliva which are present; the mouth is extremely painful and persistent efforts to examine it tend to split the fragile mucosa and give rise to considerable hemorrhage.

At the time the early lesions are arising within the mouth, the upper lips become stiff and swollen. Within a day or two, the muzzle becomes dry and a sero-mucoid nasal discharge appears. At the same time, the animal develops a fairly mild conjunctivitis and an ocular discharge arises. Subsequently, the nasal and ocular discharges become more profuse and purulent. The nasal secretions dry around the muzzle (which by this time has become hard and

cracked) and the nostrils, and results in loud snuffling noises during inspiration and expiration. The ocular discharge runs down the face and forms mats of dried exudate on the face and eyelashes. Eventually there is intense inflammation, sometimes with hemorrhage, within the nostrils and severe conjunctivitis and blepharitis. In the terminal stages of the disease, the muzzle is matted with exudate and various other forms of debris, and in some animals there is complete loss of the integument in this area.

Lesions elsewhere on the skin are common but are often overlooked because of the dramatic nature of the other changes. In the early stages, there may be painful thickening of the scrotal skin; similar effects may be seen on the vulva, sometimes accompanied by petechiation within the vagina. Later, there is often a moist reddening of the skin, particularly in the region of the axilla and groin and infrequently the skin of the teats becomes swollen, congested and hard to the touch. Diffuse congestion may also be found in the areas behind the pastern joints, on the coronets and around the accessory digits; in the terminal stages this gives way to a bluish discoloration of the skin which is extremely fragile and separation may occur with only gentle manipulation of accessory digits. Rarely, hooves and horns may be sloughed.

As already stated, dramatic ocular lesions arise after the onset of oral changes, but the delay may vary from one day to a week. It is rare for cattle to die of MCF without developing obvious ocular abnormalities.

The first obvious ocular problem is photophobia, but its onset is unrelated to that of the ocular discharge. Studies on experimentally-infected cattle have shown that, initially, photophobia is often unilateral although it becomes bilateral within 24 h. Its onset coincides with the onset of meiosis. Soon after the onset of meiosis, the iris becomes swollen and granular; hypopyon sometimes occurs simultaneously. By this stage, the animal is blind.

In early cases, corneal opacity can only be seen with the aid of oblique illumination. It usually starts in the area of the lateral canthus; later, it spreads around the peripheral parts of the cornea and finally it progresses to involve the whole structure. Corneal ulceration is not a clinical feature of MCF. When cattle survive for more than a few days after the onset of corneal opacity, particularly when it involves the entire cornea, a ring of vascularization arises at the corneo-scleral junction and spreads towards the anterior pole of the eye. It should be emphasized, however, that these latter changes usually occur only in animals with a prolonged survival, and many cases of MCF succumb without development of either total corneal opacity or a ring of vascularization.

The other consistent clinical feature of MCF is a marked enlargement of all superficial lymph nodes. In many cases this is so extreme that it is obvious

some distance away from an affected animal. This severe enlargement occurs early in the clinical stages and persists until death.

Respiratory signs such as tachypnoea and coughing are common and arise as the result of a concomitant pneumonia. Severe respiratory distress sometimes develops in the latter stages of the disease due to the accumulation of large amounts of pus and tissue debris within the major airways.

Cystitis is a common feature of MCF and can easily be recognized by frequent tail-swishing, micturition and prolonged straining or posturing; occasionally the lesions in the bladder are severe enough to give rise to marked hematuria. These signs are more obvious in female cattle.

In most cases, nervous signs are not dramatic and it is frequently not possible to separate them from other signs of marked depression, weakness, blindness and extreme discomfort. Nevertheless, affected animals are often dull and exhibit persistent muscular tremors; ataxia is common and some animals show behavioral changes such as continual head-pressing, abnormal biting or sucking activities, or nystagmus. More severe signs, such as mania and convulsions, have been described but would seem to be relatively uncommon.

Pathology

The gross and histopathological features of naturally occurring and experimental MCF have been described in detail by Selman *et al.* (1974) and Liggitt *et al.* (1978).

On post-mortem examination, the lesions which can be seen and characterized in life can be found extending back into the nasal cavities and oropharynx where there is usually widespread congestion, exudation, erosion, necrosis and diphtheresis. Occasionally, small vesicles (up to 5 mm diameter) have been described in the oropharynx. Erosions are sometimes found in the esophagus and fore-stomachs but the rest of the alimentary tract is usually macroscopically normal. The respiratory tract is often involved at all levels and pneumonia is commonly present. In most cases, greyish foci (up to 5 mm in diameter) are found in the kidney cortex and multiple hemorrhagic infarcts are common. Severe hemorrhagic cystitis is also usually present. The carcase lymph nodes are all markedly enlarged, congested and edematous; the cortical areas are expanded with localized areas of necrosis and hemorrhage. Usually the brain appears macroscopically normal. Examination of joint cavities may reveal excessive synovial fluid with the formation of many fibrin clots.

Histopathological examination reveals lesions in all tissues and organs.

These are often quite striking, even in areas such as the small intestine, which appear to be normal on gross examination. These generalized lesions are basically cellular infiltration and vasculitis.

The cellular infiltration, which tends to be arranged in 'cuffs' around blood vessels is composed of lymphoid cells, macrophages and, less frequently, plasma cells. The lymphoid cells range from small lymphocytes to large lymphoblasts. The vascular lesions consist of a severe necrotizing vasculitis; endothelial proliferation is found in both large and small blood vessels.

The above changes, plus widespread microvesicle formation and superficial necrosis, from the basis of the lesions affecting the upper respiratory and alimentary tracts and the skin. Microvesicle formation does not arise in the respiratory tract although all other changes may be found. The greyish foci found on gross examination of kidneys is also due to mononuclear cellular infiltration. The vascular changes in the renal blood vessels also give rise to arterial thrombosis and infarction. Similar changes are the basis of the cystitis.

There is marked congestion, edema and vasculitis within lymph nodes in which there are clear indications of increased activity of the mononuclear phagocytic system. In the majority of cases, while the outer cortical areas are narrow and usually lacking in follicles and germinal centers, the thymus-dependent, paracortical areas are usually broad and active.

The corneal opacity is due to an interstitial keratitis and minute corneal ulcers may be seen in some cases. Hypopyon and irridocyclitis may also be found. Diffuse encephalitis, characterized by marked perivascular mononuclear and polymorphonuclear cellular accumulations is also a consistent finding. Occasionally, similar changes are found in the spinal cord.

Immune response

In studying immunity to infection with the wildebeest-derived virus, Plowright (1968) showed that the small number of cattle which survive experimental challenge subsequently becomes solidly immune to further challenge. This immunity may persist for many years and is irrespective of the strain of challenge virus. On occasion, latent infections have been demonstrated in cattle which have survived experimental infection and which are resistent to further experimental challenge.

Cattle which recover from experimental infection develop serum neutralizing antibodies, although it may take up to 8 weeks before these are detected. Antibodies of this type cannot be detected in the terminal stages of experimental MCF, even when it is known that they have been viremic for more

than 2 weeks. Studies carried out using an indirect fluorescent antibody test (Straver and van Bekkum, 1979) have detected antibodies in titers varying from 1/30 to 1/3000 just prior to death in approximately 30% of cases. The significance of this observation is doubtful, however, in view of the relative lack of specificity of this technique as compared to the serum neutralization test. These comments may be applied to work by the same authors where antibody titers vary from 1/30 to 1/100 in experimentally infected sheep.

"Passive" absorption of serum neutralizing antibodies may occur when young calves suckle dams which are resistent and have recovered from experimental infection. However, this does not protect the calves against virus proliferation. A similar situation occurs in wildebeests in that these calves may develop a persistent viremia despite having absorbed specific antibodies from colostrum: however, the young wildebeests do not develop clinical MCF. In one study (Straver and van Bekkum, 1979), a hyperimmune domestic calf with a serum titer of 1/30 000 (as indicated by the indirect immunofluorescent antibody test) developed MCF within 7 days of challenge.

Limited studies on farms where the presumed sheep-associated form of MCF has been a regular, recurrent problem, use of the above test has revealed no convincing evidence of any significant background infection in clinically normal cattle and sheep (Straver and van Bekkum, 1979). It should be emphasized, however, that the test in question was inevitably established with material from a wildebeest-derived infection.

Laboratory aids to diagnosis

Under normal circumstances, laboratory aids to diagnosis of MCF are not necessary since it is an unmistakable clinical entity. If, however, final confirmation of diagnosis is necessary, then the easiest method is to remove such tissues as brain, oral mucosa, trachea, small intestine, kidney, urinary bladder, skin and lymph node for histopathological examination. All are likely to show the features described above.

Given appropriate facilities, virus isolation and transmission of infection to susceptible cattle, rabbits or tissue-culture can be useful in wildebeest-derived infections. However, in the other (sheep-derived) form of MCF, virus isolation is not possible as yet and the results of attempts at experimental transmission are extremely variable. Thus far, there are no serological or other immunological tests available for general use as aids to diagnosis.

Prevention and control

Theoretically, the control of MCF in the major problem areas of east, central and southern Africa should present little difficulty. Since in these regions MCF virus is often acquired by cattle from subclinically-infected wildebeests, the separation of cattle from areas grazed by these and certain other wild herbivores should result in a major reduction in the prevalence of the disease. In areas where this has proved possible, the desired results have been achieved, but so far, there is little possibility of separating grazing areas. Similarly, the prevention of the presumed sheep-derived infection could be achieved by the rigid separation of sheep from cattle. However, this is frequently neither possible nor desirable for other reasons and, since MCF is often merely a sporadic event in single animals, little or no attempt is made to prevent the disease throughout Africa. Under certain circumstances, when MCF does become an epidemic or recurrent problem, steps should be taken to keep sheep (particularly lambing flocks) away from cattle; this is especially important when the animals are being maintained under intensive conditions. Attempts should also be made to exclude small lambs from feeding bunkers and under no circumstances should cattle food storage areas (e.g., hay barns) be used for the temporary housing of ewes and lambs or other types of sheep. Finally, since the length of time a flock of sheep may harbor infection is not known (and reliable diagnostic tests ae not available), extreme care should be taken with flocks which have been circumstantially linked with cattle epidemics. In short, they must be looked upon as potentially infective, at least for several years.

There is no MCF vaccine available for general use and the treatment of clinical cases is pointless; irrespective of whether they are treated or not, almost all cases of MCF die.

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8. PSEUDORABIES

Robert A. Crandell

Abstract. Pseudorabies (Aujeszky's disease) is an acute infectious disease affecting many species of animals. The disease is caused by a virus belonging to the herpesvirus group. Swine are the natural host in which the virus persists as a latent infection, and they serve as a source of virus for other animal species. The disease is distributed throughout the world. In recent years there has been a dramatic increase in the number of reported cases. Outbreaks of porcine pseudorabies have caused considerable economic losses in Eastern Europe, Scandinavian countries, United States and Mexico. The virus has been introduced into new areas by the movement of carrier swine. There has also been a change in the clinical nature of the disease in swine and cattle. In the past the disease has been considered to be one primarily of the very young pig. The frequency of clinical disease and death losses have increased in feeder pigs and breeding animals. Cases of bovine pseudorabies occur frequently without the classical "mad itch" syndrome. Vaccination of swine has reduced losses but has not eliminated the virus from infected herds. The diagnosis of pseudorabies infection should always be confirmed by laboratory methods.

Synonyms: Aujeszky's disease, mad itch, infectious bulbar paralysis.

History

Pseudorabies was first described as a new disease entity of domestic animals by Aujeszky (1902) who observed the condition in Hungary as a naturally occurring fatal illness in cattle, cats and dogs. The causative agent was experimentally transmitted to rabbits and Aujeszky differentiated the virus from that of rabies by its behavior in the rabbit. The name pseudorabies was given to the disease because of its similarity to clinical rabies. The term "mad itch" apparently originated in the early American literature over a century ago describing a condition in cattle and dogs which resembled in all aspects the disease as it is now known (Hanson, 1954). "Infectious bulbar paralysis" was used to describe the disease in the rabbit.

After Aujeszky's original description of the disease in cattle, pseudorabies was later observed as a naturally occurring disease of swine in Hungary and in other European countries. In 1930, the cause of "mad itch" occurring in cattle in the United States was shown to be caused by a virus identical to Aujeszky's pseudorabies virus. Later it was shown by serology that pseudorabies was highly prevalent as an unrecognized infection in swine in the middle western states (Shope, 1935b). In 1943, pseudorabies was observed as a clinical disease in swine in the United States.

The virus was first propagated *in vitro* in minced cultures of fragments of chick embryos, rabbit and guinea pig testicles.

Etiology

Pseudorabies virus belongs to the family of virus, Herpesviridae, genus, *Herpesvirus* and species, *suis*. The average particle size is 186 nm in diameter (Kaplan and Vetter, 1959). Structure of the virion has been studied by electron microscopy using the negative staining and ultrathin techniques. The virus particle consists of three main components – the core, capsid and an outer membrane (Fig. 1). The core or center of the virion is hexagonal in shape and contains the viral nucleic acid DNA. The capsid surrounding the core is composed of subunits called capsomeres. The capsids with their regularly

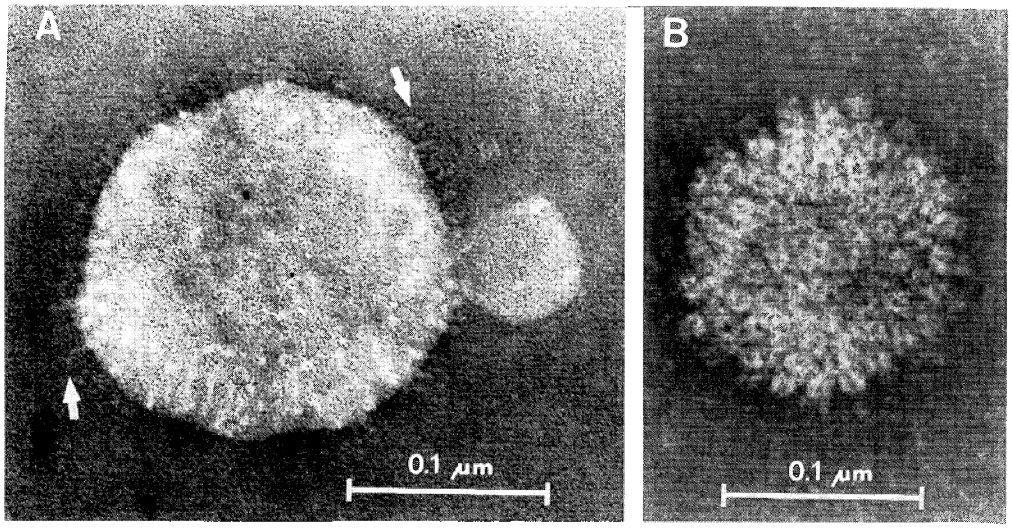

Fig. 1. Pseudorabies virus in phosphotungstate negative stain. A. Intact virion with envelope bleb and surface spikes (arrows). B. Single capsid with hollow capsomeres and hexagonal profile. (Courtesy of A. E. Ritchie.).

arranged capsomeres possess 5:3:2 axial symmetry with an icosahedral shape. The outer membrane or envelope encloses the capsid and varies in shape and size. There is only one antigenic serotype but some virus strains differ in their virulence and biological characteristics.

Chemical analysis of purified pseudorabies virus has shown that the virus contains a double stranded DNA. Pseudorabies virus is sensitive to ether and chloroform. The virus is inactivated by trypsin and chymotrypsin. The virus is inactivated by heat at 37 °C, half-life 7 h, formaldehyde and ultraviolet irradiation. The virus has been reported to survive on hay for 30 days during the summer and for 46 days in the winter. It survives drying with subsequent storage. The virus remains viable for at least 3 years in 50% glycerol when stored in the cold.

Geographic distribution

Pseudorabies is found in most parts of the world and in a large range of climates. After the recognition of the disease in Hungary, it was reported to be present in many countries of the world. The disease has been reported in all countries of Europe. Presently, the disease is of particular economic importance in countries of Central and Eastern Europe including areas of Czechoslovakia, Hungary, Yugoslavia, Romania, Poland and northern Italy.

Porcine pseudorabies was not recognized as a disease problem in the United States until 1962 (Saunders *et al.*, 1963). Thirteen epizootics of pseudorabies occurred in the State of Indiana between January, 1962 and October, 1964. In 10 of these 13 outbreaks, clinical pseudorabies was observed in swine. The other three outbreaks involved cattle in which 20 head died. Pseudorabies was diagnosed in sheep and dogs dying on the farms in which the disease was found in the swine. Of these 13 outbreaks, only two were on the same premises, occurring in 1962 and again in 1964.

Bovine pseudorabies was first demonstrated in the United Kingdom in 1939 when the disease occurred in Northern Ireland. Since then Aujeszky's virus has become well established in the porcine population of Northern Ireland. The disease is of economical importance in the enzootic areas. Sporadic cases occur throughout scattered areas in England and Wales where the prevalence has increased in recent years. The disease appears to be absent or of little significance in Scotland.

In the earlier years pseudorabies outbreaks of economic importance was limited to swine and cattle; however, in 1961 extensive epizootics in sheep were reported in the Balkans where the disease is widespread. Another epizootic occurred in Germany in which 75 sheep in one flock died. The disease

was diagnosed occasionally in sheep in other countries where sheep were allowed to mix with swine.

The disease was diagnosed in Denmark for the first time in cattle in 1931. Pseudorabies infection was first recognized in cattle and young pigs in Sweden in 1965 and since that time has occurred with greater frequency. The geographic distribution of pseudorabies infections in France has increased since 1971 and cattle infections have been observed more frequently. In Brittany, in particular, the disease has caused considerable economic losses. In 1977, 90.2% of all outbreaks were reported in Brittany. In 1977, an epidemiological relationship was established between bovine cases and clinical and subclinical pseudorabies-infected pigs. There were 234 outbreaks recorded in that year.

Bovine pseudorabies was first diagnosed clinically in the state of Veracruz, Mexico in 1943. The disease was reported again in cattle in the state of Guanajuato in 1945, and in 1970 cattle in the state of Guerrero became infected while in contact with imported pigs. The first serious outbreak in swine occurred in 1973, and it was associated with the importation of swine. By 1975 the disease had spread into the largest pig-raising area of Mexico, infecting hundreds of sows and killing 20 000 piglets.

Pseudorabies "Peste de Cocar" was described for the first time in the state of Sao Paulo, Brazil in 1912. In 1922, the virus was isolated from the nervous tissue of a cow from the state of Minas Gerais. During the period of 1936 to 1950, pseudorabies virus was isolated from 38 cattle, one sheep and one swine. Today the disease is widespread throughout Brazil, being reported in at least five states. The increase in incidence observed on other continents, however, is not apparent in South America.

Epizootiology

Natural infections of pseudorabies have been observed in cattle, sheep, goats, pigs, dogs, cats, mink and a number of wildlife species. Infected swine are believed to be the reservoir host and the primary source of infection for other animal species. Swine have long been recognized as the source of infection for cattle (Shope, 1935a) and for sheep (Kojnok, 1962), yet severe losses in these species continue. The disease is more prevalent in areas with a high swine density.

In endemic areas the disease occurs in swine throughout the year. Disease outbreaks are generally associated with the addition of new animals to a herd and to farrowing times. Outbreaks in cattle and sheep occur sporadically. The introduction of infected swine into a pen with cattle will often initiate a

clinical outbreak. Since the immune carrier pig is asymptomatic, there is no clinical evidence of infection. The first indication of pseudorabies may be the appearance of illness in the cattle. Clinical disease usually occurs within 10 to 14 days after the addition of the infected pigs.

The source of infection in closed swine herds without histories of recent additions is still unresolved. Attention has been directed towards the possible role of wild life animals and birds in the natural transmission of pseudorabies. Although pseudorabies virus has been isolated from a number of naturally infected wild animals (skunks, raccoons, opossums, badgers, rats, foxes and coyotes), their exact role in transmission is not known. Evidence to indicate that they play a significant role is lacking. It is postulated that some infected animals may transmit the virus if they were consumed by pigs or some feral animal. It has been suggested that oronasal secretion containing virus could contaminate the feed or water. Because foxes and coyotes are more likely to travel longer distances than skunks or rats before dying, they may initiate an infection several kilometers from where they consumed infected pigs.

The practice of feeding uncooked swine offal from abattoirs has resulted in serious outbreaks of pseudorabies in dog kennels and mink farms. Outbreaks of Aujeszky's disease in mink associated with the feeding of offal contaminated with pseudorabies virus have occurred in Greece, USSR, Czechoslovakia, Poland, Holland, Belgium, Germany and Yugoslavia.

Pathogenesis

Swine

The pathogenesis of pseudorabies in the natural host is important as it relates directly to the epizootiology and control of the disease. Pseudorabies has been recognized for years as a contagious disease of swine but noncontagious in other species of domestic and laboratory animals.

Swine are susceptible to infection when experimentally exposed to the virus by subcutaneous, intracerebral, intranasal, intratracheal, intragastric, oral and intramuscular routes of inoculation; however, the response elicited by the various routes of exposure is variable. A rapid, fatal illness is produced in pigs inoculated intracerebrally and the brains of those animals contain a high concentration of virus. A response similar to the natural infection is produced when animals are ioculated intranasally. In pigs, the natural infection spreads by the respiratory route. Transplacental infections occur as the virus crosses the placental barrier (Csontos *et al.*, 1962). Paralysis and death occur in some animals following exposure by the intramuscular route. Subcutaneous and

intratracheal exposure produces a mild disease similar to that observed in the natural infections. Pigs given the virus intragastrically or orally are the least susceptible to infection.

Cattle

McFerran and Dow (1964) studied the susceptibility of calves to different routes of inoculation and the distribution of virus within the body. Fatal illness of short duration was induced in 2- to 9-month-old calves infected by the oral, nasal and cutaneous route. The incubation period ranged from 90 to 168 h, and duration of the illness varied between <6 and 72 h. The distribution of the virus in the body was shown to be strictly neurotropic with a close relationship to the site of entry and its location in the central nervous system.

Virus was recovered from blood, adrenal, salivary gland, heart, kidney and pharynx in addition to brain and spinal cord in animals inoculated intravenously. In calves inoculated intramuscularly, pseudorabies virus was isolated from local muscles, adrenals, lymph nodes and various nervous tissues (Dow and McFerran, 1966).

The recent demonstration of virus from lungs of naturally infected cattle with and without signs of pruritis is suggestive of an infection by the respiratory route. Reports of fatal infections occurring in sheep and cattle without direct contact with infected swine support this view. Bitsch (1975c) isolated virus from the nasal, tonsillar and pharyngeal mucosal surface of a cow, thus suggesting the possibility of virus excretion by the bovine species.

The duration of clinical disease and site of virus recovery in the central nervous system (CNS) is related to the area of pruritis. Animals with pruritis on the anterior part of their body have a shorter clinical illness than those with pruritis on the posterior part of the body (Bitsch, 1975a). In general, virus localization is greater from brain tissue (medulla oblongata specifically) and thoracic cord in animals with pruritis involving anterior parts of the body. In cases of pruritis on posterior parts of the body, virus is found more consistently in the lumbar cord.

Bitsch (1975b) demonstrated virus in the vagina of three calves with pruritis on the posterior part of the body. Although the three calves were associated with pigs, the actual mode of infection was not determined; however, man to calf infection was considered in one case.

Placental transmission has been reported in a cow that was ill for 8 days (Silva and Giovine, 1966). Pseudorabies virus was isolated from the blood and spinal cord of the cow, and virus was recovered from the spleen, liver and brain of a 9-month old fetus removed from the uterus.

Clinical signs

Swine

Swine of all ages and breeds are susceptible to infection; however, morbidity and mortality is greatest in the newborn pig. Porcine pseudorabies occurs as both a clinical and subclinical infection. In the young pig from a few days to 3 to 4 weeks of age, the onset is sudden and death may occur within 48 h. The disease is manifested by temperatures of 41 °C or higher, accompanied by anoxia, dullness and muscular tremors. As the disease progresses, the nervous signs develop. The animals may become ataxic, circle and stagger; death is preceded by convulsions and prostration. Animals with nervous signs do not recover.

Clinical disease in feeder operations is usually observed one week to a month after the addition of pigs to the feed pens. The clinical signs are similar to thsoe seen in younger pigs but persist longer, and the death losses are less severe; however, in some outbreaks the feeder pig losses have been significant.

Clinical illness in older swine has become a recognized feature in recent years of pseudorabies infection in some areas of the world where the disease previously was considered to be subclinical. Deaths in older animals, fetal deaths and abortions are observed with greater frequency. Some clinical signs of affected sows are anorexia, pyrexia, agalactia, weakness and vomition. Infected sows may abort or farrow stillbirths, mummified fetuses or weak infected pigs which usually die within a few days.

Pruritis is not a feature of porcine pseudorabies.

Cattle

"Mad itch" is perhaps the best known description for clinical bovine pseudorabies in which pruritis is a cardinal sign of the disease, however, in recent years bovine pseudorabies has occurred without pruritis. Pruritis characterized by biting, licking or rubbing is generally seen unilaterally (Fig. 2). When present on the anterior portion of the body, it is usually observed on the nose, around the eyes, below the ear, lower jaw or on the chest. Posteriorly, the affected site is more often seen in the perineal region but occurs at various sites on the udder, limbs and flank. Animals usually die between 1 and 3 days after the appearance of pruritis. The duration of illness is related to the area of CNS involvement. In cattle with pruritis on the head, the course of illness would be shorter compared to those animals with pruritis located on

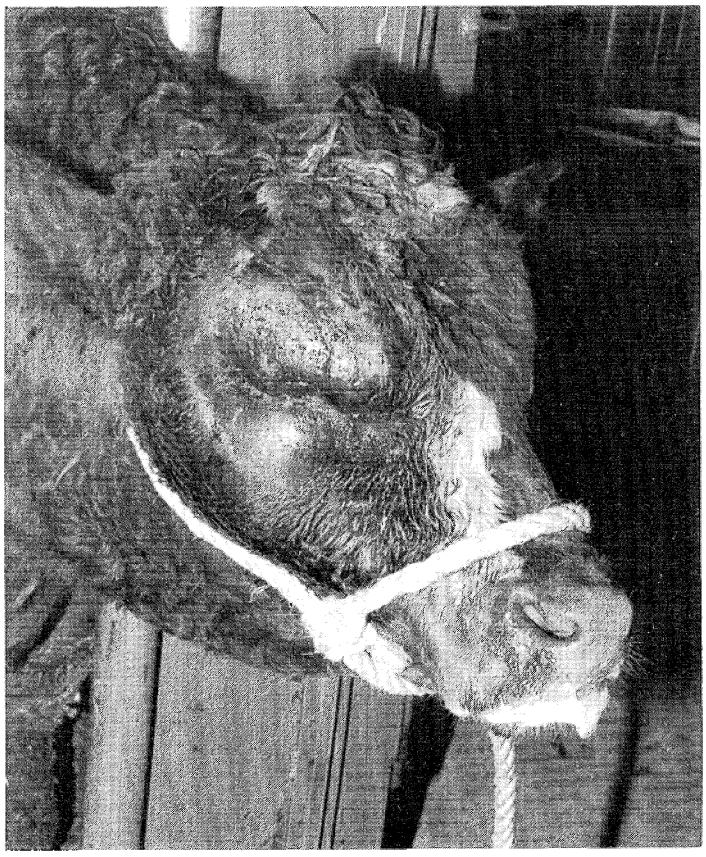

Fig. 2. Pseudorabies in a steer. Note swelling and abrasions about the right oribital region and excessive salivation.

the posterior portion of the body or those without pruritis.

Rectal temperatures may vary and go as high as 43 °C. Continuous or intermittent chewing movements with salivation may occur. The animal may become completely anorectic and there is a marked drop in milk production in lactating cows. The affected animal may appear excited and exhibit signs of restlessness by bellowing, getting up and down frequently and stamping of the feet. Nervous signs often observed are staggering aimlessly, aggressiveness and circling in one direction. In some major bovine outbreaks, clinical signs have been limited to rumenal atony and sudden death in a few affected animals.

Infections in cattle have always been considered fatal; however, two accounts of nonfatal, clinical bovine pseudorabies have recently been documented. Hagemoser *et al.* (1978) described the recovery in a 4-year-old beef cow in Iowa (USA) and Toma and Gilet (1978) reported the recovery of two cows in an outbreak in Brittany (France). The animals all exhibited a variety

of clinical signs, and serum neutralizing antibodies for pseudorabies virus were demonstrated in two of the three cows.

Sheep, Dogs and Cats

The clinical syndrome in sheep is similar to that observed in affected cattle. Pseudorabies infection of dogs and cats is a rapidly fatal disease with death occurring within 2 days of onset of clinical signs. The animals scratch or bite at the area of intense pruritis with the head and face most commonly affected. The face may be swollen with open wounds or badly bruised from scratching and clawing. The affected animal may salivate, vomit or retch as if something was stuck in its throat. After onset of signs, they refuse food and water and become depressed. The lesions seem to be painful; the animals convulse and die. Pruritis is not always present.

Pathological features

Swine

Baskerville *et al.* (1973) recently reviewed the pathologic changes of porcine pseudorabies in detail. The lesions observed in swine vary considerably with the strain of virus. Gross lesions in swine are limited and inconsistent. Saunders *et al.* (1963) reported congestion of the meninges and lymph nodes are the most constant lesions in feeder pigs. The cerebrum and cerebellum are more severely affected than the brain stem in the gray matter of the cerebral and cerebellar cortices. The lesions of the spinal cord are mild and occur mainly in the cervical and anterior thoracic regions. These lesions consist of a diffuse, nonsuppurative meningoencephalomyelitis and ganglioneuritis.

Lesions in the lung vary from a mild cellular infiltration and edema to widespread necrosis and hemorrhage. Intranuclear inclusion bodies may occur in epithelial cells of the airways, alveoli and other cell types. In some outbreaks, focal areas of necrosis are found in the liver and spleen. Intranuclear inclusion bodies, when present, are most often seen in the hepatocytes at the periphery of necrotic foci (Fig. 3).

Cattle

The lesions in the CNS of naturally infected animals are very similar to those observed by Dow and McFerran (1962) in experimental calves inoculated intradermally and subcutaneously in the hips. There is an increase in the amount of cerebrospinal fluid (CSF), and the meninges over the area of the

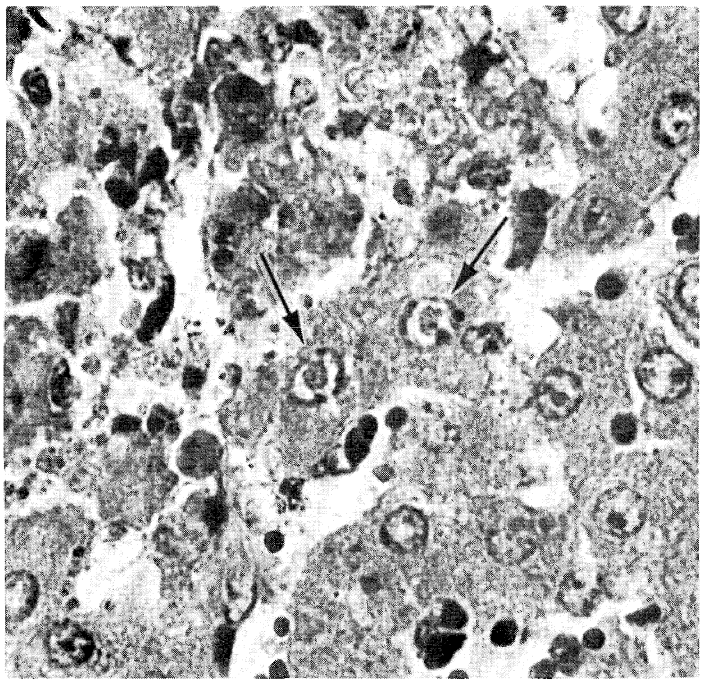

Fig. 3. Intranuclear inclusion bodies (arrows) in hepatocytes in a pig naturally infected with pseudorabies virus. H & E stain 600×.

affected cord may be congested.

The skin over the area of pruritis is thickened, edematous and may be covered with a serosanguinous exudate. The degree of edema, hemorrhage and necrosis in the subcutaneous tissues and associated muscles is related to the amount of trauma. Moderate edema and congestion may be present in the lungs.

The histologic changes in the CNS may be confined to the spinal cord in association with the dorsal root ganglia of the affected side; however, in some cases the lesions may be more widespread and extend up the cord. The lesions consist of acute neuronal degeneration and inflammatory cellular infiltration of the ganglia and dorsal horns.

Focal hemorrhages may occur and characteristic inclusion bodies may be present in the affected nerve cells and in neuroglia. The lesions in the cord and brain are localized and easily missed. The perivascular cuffs are generally thinner and less numerous than those seen in porcine pseudorabies (Fig. 4). Microscopic lesions in the lungs consisting of congestion, edema and focal areas of necrosis may be present (Fig. 5).

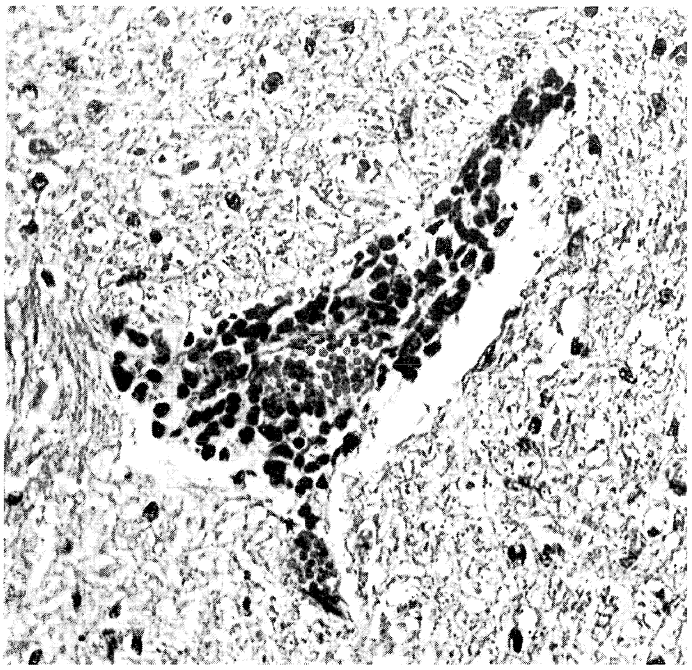

Fig. 4. Lymphocytic perivascular cuffing in brain of a steer naturally infected with pseudorabies virus. H & E. stain, 240×.

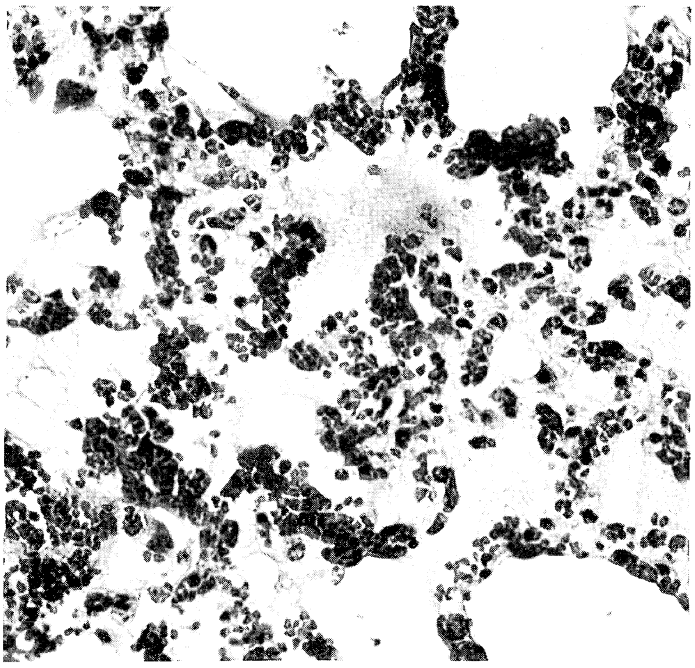

Fig. 5. Congestion and edema fluid in lung of a steer naturally infected with pseudorabies virus. H & E. stain, 240×.

Sheep, Dogs and Cats

Inoculation of sheep by sacrification of the skin resulted in depilation and excoriation with subsequent thickening and edema. Regardless of the route of inoculation, the reginal lymph nodes are swollen, congested and some are edematous. The lesions of the CNS are characterized by severe neuronal degeneration, cellular infiltration and sometimes by the presence of intranuclear inclusion bodies. The distribution of the lesions within the CNS is similar to that in cattle.

The main gross lesions in dogs and cats is the severely traumatized area of the skin generally located about the head and face. The viscera may be congested. The presence of porcine fetal tissue in the gastric contents of canines and felines necropsied after a sudden death suggests possible exposure to pseudorabies virus. Saunders *et al.* (1963) described marked microscopic lesions in the CNS of dogs similar to those described in swine.

Immune response

There is an increase in resistance to the disease with increasing age of swine. Neutralizing antibody is first detected in the serum of pigs 7 days after infection. The optimal level of antibody occurs between 2 and 4 weeks and persists for long periods of time. Although immune, some animals become latent carriers and the disease may recur in the same herd. Immune sows secrete antibodies in their colostrum and milk at each gestation. The persistence of the passive immunity is reported to vary between 3 and 14 weeks in the offspring. The presence of passive antibody in baby pigs does not prevent the excretion and dissemination of the virus.

The cell-mediated immune (CMI) response has been demonstrated, but its role in immunity is not known. The CMI was shown to appear prior to the humoral immunity.

Although most infections are fatal in all species except swine, serum neutralizing antibodies have been demonstrated in cattle and dogs surviving the infection.

Laboratory aids to diagnosis

A history of contact with pigs, clinical signs and outcome of the illness are helpful in making a preliminary diagnosis of bovine pseudorabies. A clinical diagnosis must be confirmed by laboratory methods because the clinical signs

of pseudorabies are similar to other diseases.

A definite diagnosis of pseudorabies is obtained by the isolation and identification of the virus and by the fluorescent antibody (FA) test (Hill *et al.*, 1977). The recommended specimens for virus isolation from suspected cattle are brain tissues collected from different levels of the brain, lung and spinal cord. When pruritis or paralysis is present on the anterior or posterior part of the body, tissues are best selected from the thoracic cord or the lumbar cord, respectively. The tonsils and portions of the olfactory lobes, pons and medulla from pigs are the recommended tissues for virus isolation. The virus may also be recovered from other porcine tissues such as liver, spleen and lung. The tonsil is the choice tissue for FA testing.

Pseudorabies virus is infective and cytopathic for a wide variety of cell cultures derived from different animal species (Fig. 6); therefore, the virus may be readily isolated from infected tissue in many primary and established cell culture lines. The virus is identified either by the serum-virus neutralization (SVN) test or the FA method. In the past the rabbit was commonly used for confirmation of pseudorabies infection.

The histopathologic findings are helpful in the diagnostic process. Serology is not recommended in determining the cause of acute pseudorabies infections because the neutralizing antibody is not demonstrable early in the course of the disease. The SVN test is very useful in conducting surveys and control programs.

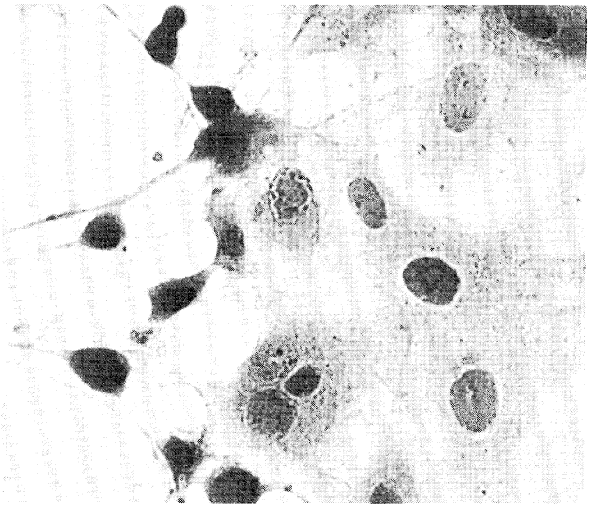

Fig. 6. Intranuclear inclusion bodies in bovine embryonic kidney cell culture infected with pseudorabies virus. H & E. stain, 240.

A standard procedure for the microimmunodiffusion (MID) test for detecting pseudorabies virus antibody has been recommended for use in determining the immune status of swine herds (Crandell *et al.*, 1978). The MID is less sensitive than the SVN test in detecting antibody.

A skin test based on a delayed hypersensitivity reaction to pseudorabies virus antigens has been described for evaluating the immune status of herds. The test is not recommended for determining the immune status of individual pigs.

Diseases from which bovine pseudorabies must be differentiated are listeriosis, rabies and toxicosis. Pseudorabies infections in swine should be distinguished from enterovirus infecions, rabies, water deprivation and hog cholera.

Prevention and control

The subject of how to prevent and control pseudorabies infections is much debated. In the prevention and control of any viral disease, questions concerning carrier, reservoir hosts, quarantine, vaccination and eradication are in the forefront. Although it is not known exactly how infection gains entrance into some swine herds, the source of infection is usually attributed to a carrier pig. Therefore, an obvious goal in any program is to prevent the introduction of an infected animal to a susceptible herd. To achieve this goal, various serological testing programs have been conceived to eliminate the potential carrier animal. Specific regulations have been issued by some local and federal governments to restrict the movement of infected swine. A program of serological testing of all breeding replacement animals for pseudorabies antibodies has been recommended with subsequent quarantine and retest before adding animals to a herd. Some regulations now require swine to be negative to the SVN test prior to exhibition and importation.

The most obvious and effective preventive measure to protect cattle and sheep against pseudorabies is to isolate them from swine. Fatal infections in dogs and cats can be avoided by restricting their exposure to swine or infected carcasses. The practice of feeding uncooked offal to fur-bearing or other carnivores should be stopped.

There is some disagreement among professionals as to the effectiveness of vaccination programs in controlling the disease. Vaccines are widely used to control losses in swine and some vaccines are used in cattle. Although live modified viral vaccines are safe for swine, some are dangerous to other animal species. The use of a live Aujeszky's disease vaccine is prohibited in some countries. Vaccines have been successful in reducing the economical

losses in swine, but their use has not eliminated the disease nor do they prevent pigs from becoming virus carriers and shedders (Skoda, 1976). Pseudorabies virus remains endemic in large areas of Central and Eastern Europe after a decade of extensive vaccination.

An early diagnosis of the disease is important in controlling the spread of the virus. All sick animals should be isolated from the rest of the herd. Pigs and cattle should be separated immediately to prevent any further virus transmission. In some control programs, the farm is quarantined. Dogs, cats and wildlife animals should not be allowed to feed upon dead animals. All dead animals should be buried or burned.

In the few bovine cases in which cattle survived, electrolytes and calcium and magnesium glutamate were administered as supportive therapy. Additional therapy consisted of a mixture of vitamins, amino acids, oxytetracycline and dexamethasone.

The use of hyperimmune serum and specific gammaglobulin in exposed animals has been effective in controlling death losses; however, they have no therapeutic value in pigs showing clinical signs. The use of immune serum does not eliminate animal carriers.

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9. BOVINE RABIES

Richard E. Dierks

Abstract. Rabies is a fatal disease affecting the central nervous system of all warm blooded animals. The disease is caused by a virus belonging to the family Rhabdoviradae and genus *Lyssavirus*. Carnivores, bats, and other wildlife species are the vector hosts of the disease with cattle and man normally acquiring the infection through bite transmission from an infected vector species. The disease is worldwide in distribution and is of major economic and public health significance in many countries. The disease produces clinical signs that must be differentiated from other diseases affecting the central nervous sytem. The lesions observed are minimal in most cattle. Intracytoplasmic inclusions (Negri bodies) are a characteristic and pathognomonic feature of the disease. Fluorescent antibody procedures are routinely used to diagnose the disease. Control mechanisms include vaccination and reduction of numbers of specific vector species and vaccination of cattle. Inactivated nervous tissue, modified live tissue culture, and modified live chicken embryo origin vaccines are used to protect cattle from rabies.

Disease

Synonyms: rabies, lyssa, lytta, habhos, tollwut, rage, rabere, hydrophobia, derriengue.

Rabies is an acute viral infection of the nervous system with progressive paralysis leading to death of the host. The disease has been known and described since ancient history and continues to be a problem in many species of domestic and wild animals throughout most of the world. The major tropical areas currently free of rabies are Australia, New Zealand, Hawaii and other isolated Pacific islands, and some Caribbean islands.

All warm-blood animals are susceptible to rabies virus infection; however, the degree of susceptibility varies widely among the different species. Carnivores (dogs, cats, foxes, wolves, jackals, skunks, mongooses) are the primary vector species in the majority of the tropical areas of the world. Vampire bats also play an important role in transmission of the disease in Central and

South America. Cattle and other domestic animals become infected following bites from endemically or epidemically infected vector species. Cattle are normally dead-end hosts in that the disease is usually not transmitted directly to other species. The disease causes major economic losses of cattle in tropical and temperate areas of the world. Exposure of man to infected cattle also creates many public health problems. No other zoonotic disease creates such widespread fear in man.

Etiology

Rabies virus belongs to the family Rhabdoviridae and genus *Lyssavirus*. Rhabdoviruses constitute a large group of viral agents with a bullet-like or bacilliform morphology that infect plants, insects, and both homeothermic and poikilothermic animals.

Almost all of the physicochemical properties of rabies virus have been described since 1960. Virus particles have been described by negative contrast and thin section electron microscopy as cylindrical with one rounded or conical end and one planar or concave end. The particles are approximately 180 nm in length and 75 nm in diameter with surface spikes or projections of 9 nm (Murphy, 1975; Hummeler *et al.*, 1967; Matsumoto, 1962, 1963).

The virion has surface or coat antigens and a helical ribonucleoprotein capsid internally. The virus contains a surface glycoprotein, at least two membrane proteins, and a ribonucleoprotein that are each capable of stimulating antibody production (Schneider *et al.*, 1973; Sokol, 1975). The virus contains RNA, is heat susceptible, and sensitive to ether, chloroform, and various detergents.

Rabies virus had been considered to be a single antigenic group until recently. This concept was challenged when it was shown that a group of related viruses could be distinguished serologically from rabies and from each other (Shope *et al.*, 1970). A series of viruses were isolated in Africa that were related to rabies virus but were antigenically distinct enough to be classified as separate serotypes. Lagos bat, Mokola, and Duvenhage viruses, along with rabies virus, constitute the genus *Lyssavirus* (Shope, 1975; WHO, 1977). Two additonal viruses isolated from insect vectors produce lesser antigenic cross-reactions with the *Lyssavirus* group. Obodhiang virus and Kotonkan virus were isolated in Africa from mosquitoes and midges, respectively (Schmidt *et al.*, 1965; Kemp *et al.*, 1973). Kotonkan virus was also shown serologically to naturally infect cattle, sheep, horses, and rodents in Nigeria. The characteristics of these separate serotypes of *Lyssavirus* are given in Table 1. The rabies-related viruses have not been found naturally outside Africa.

Table 1. Characteristics of *Lyssavirus* genus.

Virus name and serotype	*Source of isolates*	*Geographic distribution*	*Disease in nature (experimental)*	*Ultrastructural characteristics*			
				Length of virus particles, nm	*Shape of virus particles*	*In vivo site of budding*	*Inclusion bodies*
Rabies	Man,bats, carnivores etc.	Worldwide with known exceptions	Rabies	180	Bullet	Endoplasmic reticulum, plasma membrane	Prominent
Lagos bat	Bats	Nigeria, Central African Republic	(Encephalitis)	180	Bullet	Endoplasmic reticulum, plasma membrane	Prominent
Mokola	Man, shrews	Nigeria, Cameroon	Rabies-like disease (encephalitis)	180	Bullet	Endoplasmic reticulum, plasma membrane	Prominent
Duvenhage	Man (bat)	South Africa	Rabies-like Disease (encephalitis)	180	Bullet	Endoplasmic reticulum, plasma membrane	Prominent
Kotonkan *	(Cattle) Culicoides	Nigeria	Ephemeral fever-like disease (encephalitis)	180	Conical	Plasma membrane	Not too prominent
Obodhiang *	Mosquitoes	Sudan	(Encephalitis)	180	Conical	Plasma membrane	Not too prominent

* Not recognized in *Lyssavirus* genus, but related serologically (WHO, 1977).

A number of other rabies viruses which have unusual biological properties have been studied. In serological tests, there are minor antigenic differences that can be detected; but they have not been demonstrated to have large enough variations to be listed as serotypes as are those viruses listed in Table 1. Previous studies utilizing purified antigens have shown the nucleoprotein to be a common antigen within the group, while variations were noted in the glycoprotein antigens. A new method of producing monoclonal antibodies against rabies virus antigens has been described (Wiktor and Kaprowski, 1978). This technique, using hybrid cells resulting from immunized mouse spleen cells fused with mouse myeloma cells, is providing monospecific antibodies that can distinguish minor antigenic variations between two isolates. Initial studies utilizing these hybridoma techniques have shown some significant variations between street rabies isolates. This methodology will be extremely valuable in determining whether some vaccine failures demonstrated in various parts of the world are due to antigenic variations or other immunological factors.

Distribution

Rabies virus has worldwide distribution with different principal animal vectors in different countries or regions of the world responsible for transmission of the disease to cattle and other food animal species (WHO, 1979). The cat, dog, vampire bat, and insectivorous bats serve as vector species in the tropic countries of the Americas. The specific role of insectivorous bats in transmission of rabies to cattle is poorly understood; while the vampire bat, cat, and dog each play a major role in different countries in Central and South America.

Rabies is endemic in almost all countries of Africa with the cat, dog, jackal, and mongoose serving as primary vectors.

Dogs and wolves are the primary vector animals for transmission fo rabies to cattle in the Middle East, while the cat and dog are the principal carnivorous animals infected as one progresses eastward through Asia.

Specific areas of Oceania (Australia, New Zealand, Papua New Guinea, New Caledonia, Fiji, and Guam) are currently free of rabies. Many of the Caribbean Islands are also free of rabies. Jamaica, Bahamas, Turks, and Caicos Islands followed by a half.circle through the Leeward and Windward Islands are all currently free of rabies virus, while larger islands such as Haiti and the Dominican Republic currently have rabies infections. Small areas of Asia (Hong Kong, Singapore, Brunei, and Bahrein) are also free of rabies.

The prevalence of rabies infection will follow cyclic patterns in the primary

vector species for given countries or regions. The number of infected cattle and human exposures follow the same cyclic patterns when rabies prevalence is followed over a number of years.

Epidemiology

The epidemiology of rabies will vary depending on the vector species that is predominant in a given country or area of the world. The epidemiology can even vary a great deal within a given country, depending on the vector species, rates of infection, and interaction between the vector species, cattle, and man. Cattle are quite susceptible to rabies infections as are foxes. Cats, dogs, and man are intermediate in susceptibility to rabies. Age is also an important factor in the susceptibility of all species to rabies with younger animals being more susceptible than older animals.

The natural epidemiological spread of rabies depends on the classical bite-wound chain of transmission. Aberrant routes such as ingestion and inhalation probably play no significant role in the natural spread of rabies. Carnivorous species will usually have an average incubationary period of from 3 to 8 weeks but can vary a great deal on either side of that time frame. Extremely long incubation periods or latent infections in vector species are believed to play a role in the long-term maintenance of rabies in a given area or region, but these aspects of the epidemiology of rabies are not presently well established or understood. Rabies virus often accumulates and is excreted in relatively high titers in the saliva of vector species. As a general rule, most carnivores will not excrete virus in their saliva sooner than 5 days before showing clinical signs of disease.

Bovine rabies as a result of exposure to rabid vampire bats is enzootic in most of the countries of the Americas from Mexico to Argentina. As with carnivorous vectors, epizootic foci will occur in various endemic areas. It is estimated that between 500 000 and 1 million cattle die of rabies each year following infection from rabid vampire bats.

European breeds of cattle appear to be more susceptible to rabies than the thicker skinned Brahman or Zebu breeds. In many parts of the tropical world where vampire bats play a role in transmission, *Desmodus rotundus* is by far the most common species. *Desmodus* also have a preference for bovine blood in their feeding habits, thus making the incidence of exposure of cattle quite high. The apparent susceptibility of European breeds of cattle may also be the result of feeding preferences of vampire bats.

Clinical signs

The first sign of rabies in cattle is usually a generalized depression that is seen with many viral or bacterial infectious disease processes. Loss of appetite and sudden drop in milk production in lactating animals are seen early in the disease. As the disease progresses, a depraved appetite may be seen with the animal observed to be chewing on or attempting to eat wood, stones, and other nondigestible objects.

Trembling or fasciculation of muscle bundles and twitching of the animal's ears may be observed. Paralysis of muscles of the throat with excessive salivation and grinding of teeth is a common occurrence. The cow will tend to slobber and have difficulty drinking water, but in contrast to man, there is no hydrophobia or fear of water. The diagnosis of choke or esophageal foreign body is often made. Cattle will often bellow incessantly in an altered characteristic low-pitched voice because of paralysis of vocal cords. There is increased sexual excitement in both male and female animals. Some of the animals will develop a furious stage and attack other animals, man, or inanimate objects such as trees, posts, or buildings. Cattle seldom bite during this excitement stage but may butt or charge moving targets. During this period, external stimuli such as bright lights, loud noises, or sudden movements may cause violent reactions, convulsions, and collapse. The animal may groan consistently and stare at its flank with apparent colic. Constipation is usually seen early followed by diarrhea as the disease progresses. Weakness of the hind quarters, a swaying gait, and "knuckling over" may be observed as the disease progresses. Progressive paralysis of muscles will follow. The animal will then collapse and eventually become comatose. Paddling and neural rigidity are often observed after the animal is down. Death can occur rapidly, but the disease usually progresses through various stages to complete paralysis and death over a period of from 4 to 7 days after the first signs are observed.

Schneurrenberger *et al.* (1970) recorded the signs of 97 cattle that died of rabies on Illinois farms during the 1960s. Twenty-one of the cattle developed furious stages of the disease, 37 progressed quietly to a paralyzed state, while 39 could not be clearly classified as either but exhibited some signs of excitement. The most common signs recorded in this field study were bellowing and excessive salivation. Twenty-one of the cattle developed paralysis of the hind quarters, while 20 had paralysis of the neck and throat and appeared to choke.

The clinical signs of rabies must be differentiated from an esophageal foreign body and other conditions that may affect the peripheral or central nervous system. Acetomia in dairy cattle and hypomagnesemia will produce

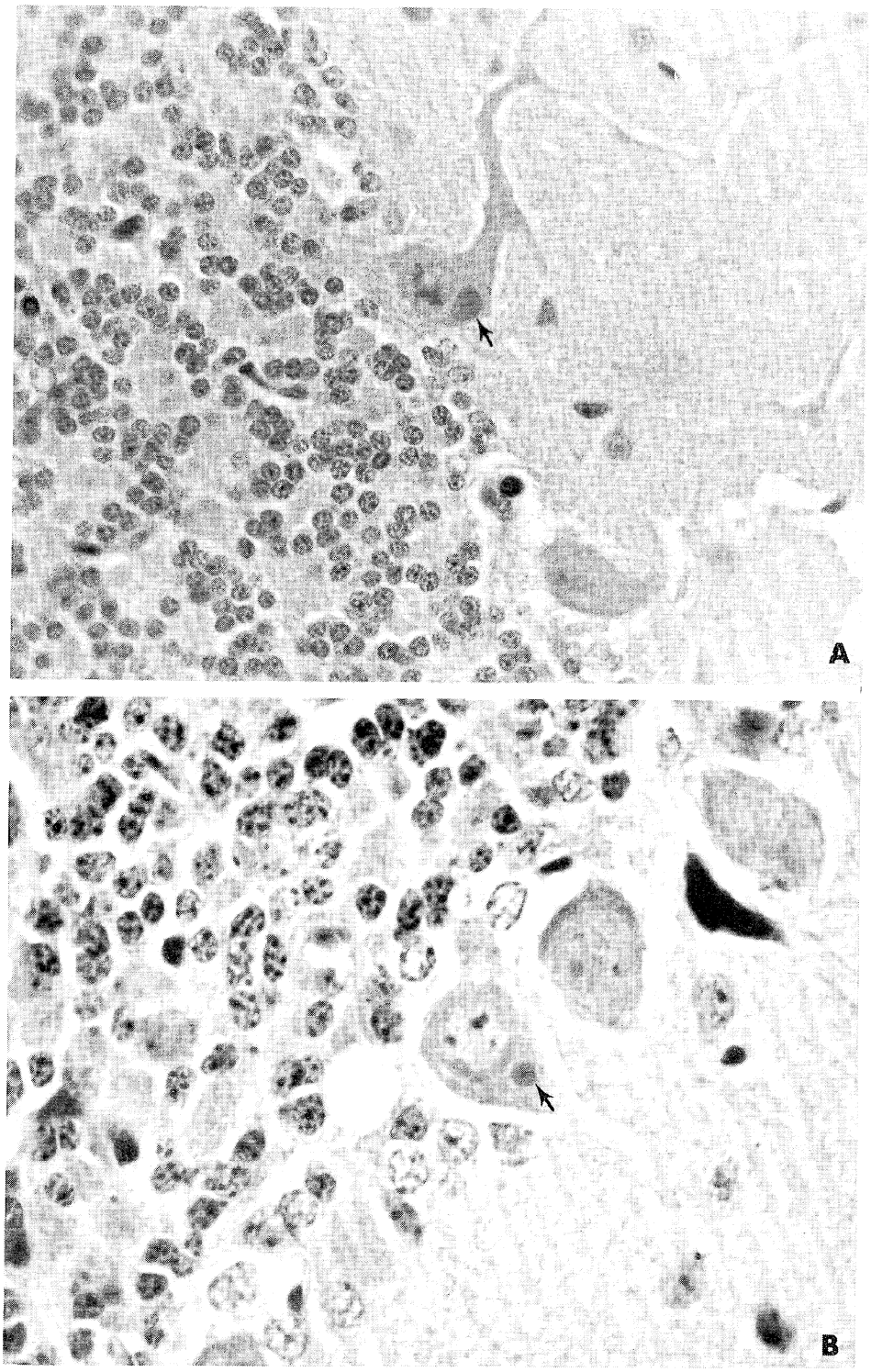

Fig. 1. a and b (arrows): Cytoplasmic Negri body in neuron of cow.

some of the signs obvserved in an animal with rabies. Polioencephalomalacia, pseudorabies, neural infectious bovine rhinotracheitis, listeriosis, thromboembolic meningoencephalitis, and other viral encephalitic diseases will produce many of the signs seen in rabid cattle.

Public health consideration

Even though cattle may butt or attack man, they will seldom bite. Man is often exposed, however, because of the diagnosis of choke or esophageal obstruction with subsequent exposure because the farmer or veterinarian attempts to relieve the condition. The saliva of infected animals will often contain virus 3–5 days before clinical signs appear. Violent movements of the animal's head during examination may result in abrasions or cuts of the examiner's hands on teeth or objects being placed in the mouth. Contamination of these fresh wounds with rabies virus then constitutes a serious exposure. Because help is usually required in treating these animals, multiple exposures of large numbers of people from a single animal often occur.

Rabies should be a serious consideration in any animal with a suspected esophageal obstruction. Rubber gloves should be worn when examining the mouth, and hands should be washed immediately with a quaternary ammonia solution after examination. This is particularly important if the skin or any open wounds are contaminated with saliva.

Precautions must be taken to protect both field and laboratory personnel when dealing with animals suspected of dying of rabies. Thick protective gloves, goggles, and protective clothing should be worn when opening the skull and spinal column. Rubber or strong plastic aprons that can be easily disinfected should be worn. Aerosols produced by the use of mechanical saws, high speed mixing, and centrifugation procedures must be considered. All procedures that might cause aerosols of infected tissue should be carried out in closed containers or negative draught hoods that can easily be disinfected.

Quaternary ammonium disinfectants in 1:500 dilution, 45–70% alcohol, 1% soap solution, or 5–7% iodine solutions kill rabies virus rapidly and are also indicated for the treatment of any wounds. All glassware, plasticware, and instruments used to handle tissues should be autoclaved while carcasses and animal tissues should be placed in plastic bags and incinerated.

Any wounds contaminated with tissues or fluids of rabies infected animals should be washed immediately and thoroughly for several minutes with soap and water. Washing is a very important procedure in preventing infection. The washing should be gentle or prevent further traumatizing of tissues. All

soap should be removed before applying any of the chemical disinfectants mentioned above. Puncture wounds should be probed gently with a chemical disinfectant, and suturing of any gaping wounds should be delayed. Local infiltration of wounds with antirabies serum or human origin antirabies immunoglobulin should be carried out in additon to the washing and disinfection procedure. Additional wound care procedures such as tetanus toxoid or antisera administration should be employed where indicated. Laboratory workers, veterinarians, and others with a high exposure potential to rabies virus should have pre-exposure immunization. The antibody response to pre-exposure immunization should be determined to make certain that an adequate antibody level has been elicited. With a known pre-existing antibody titer and the known rapid anamnestic response to a subsequent booster dose of vaccine, recommended post-exposure procedures for immunized individuals can be employed with reasonable confidence. Exposed individuals who have not received pre-exposure immunization should be treated according to the recommendations of the WHO Expert Committee on Rabies for post-exposure treatment.

Pathogenesis and pathology

Following inoculation of the virus, there is a local replication of the virus in epithelial cells or myocytes (Murphy *et al.*, 1973b). This will occur at the initial site of entry of the virus into the host. Virions will cross neuromuscular and neurotendinal spindles and move centripetally to the central nervous system via nervous pathways as the infection progresses (Johnson, 1965). Peripheral nerve axons support viral replication as it moves via dorsal root ganglia and the spinal cord toward the brain. The virus is spread centrifically from the central nervous system along axons of the trigeminal, facial, olfactory, and glossopharyngeal peripheral nerves into the salivary glands, taste buds, and olfactory cells, and from there into oral and nasal secretions (Murphy *et al.*, 1973a). In infected salivary glands, there may be acute degeneration of the acinar epithelial cells and infiltration of the interstitium with plasmacytes and lymphocytes (Dierks *et al.*, 1969). This is much more prominent in some carnivorous species, such as the fox, than in domesticated livestock.

In the brains of cattle, the virus has a predilection for the brain stem and cerebellum. The microscopic lesions of infected animals are characterized by neuronal degeneration, perivascular lymphoid infiltration, and gliosis with glial nodule formation. The medulla, cerebellum, basal ganglia, spinal cord, and dorsal root ganglia may show various levels of histopathology (Cheville,

1975). Neurons infected with rabies virus may undergo cell swelling, but the cytopathic changes seen are often minimal despite large accumulations of viral antigen as evidenced by fluorescent antibody procedures or electron microscopy. Negri bodies, as seen by light microscopy, are developed as a result of the accumulation of large aggregates of dense granual viroplasm in the cytoplasm of infected cells.

Immune response

The immune response in cattle has been determined by following serum neutralization titers of vaccinated cattle, as well as challenge with street virus to determine the degree of protection following vaccination. Inactivated vaccines and modified live vaccines are both utilized in cattle and will result in differing immune responses. The level of immune response and duration of protection will vary with the type and potency of vaccine used. Modified live virus vaccines will normally produce an immunity of relatively long duration (3 years or more), whereas vaccination with most inactivated vaccines will have to be repeated annually. Calves will receive temporary parenteral immunity via colostral antibody that will interfere with active immunization for as long as 6 months after birth. Calves of immune dams vaccinated with a modified live vaccine (ERA) up to 4 months of age usually died of rabies when challenged at a later date (Abelseth, 1975). It is essential, therefore, to consider the status of the dam when vaccinating calves.

Both humoral and cellular immunity are elicited when an animal is exposed to rabies virus. Although a cellular immune response has not been demonstrated specifically in cattle, it has been studied in laboratory animals. Mice vaccinated intradermally with modified live ERA virus produced specifically sensitized lymphocytes harvested from lymph nodes as early as 3 days after vaccination with a peak cellular response present by day 6. Neutralizing antibody reached its peak level in serum 12 days after infection. The ability of sensitized lymphocytes to respond to viral antigen in an *in vitro* test was transient. The cellular response disappeared by day 20 postinoculation, but neutralizing antibody levels remained high. Reinfection 30 days after the primary infection resulted in a sharp increase in responsiveness of lymphocytes to viral antigens as well as a sharp increase in neutralizing antibody levels (Hill, 1976). The peak cellular response to secondary stimulation was seen in 2 days and fell again to a baseline level by 16 days after challenge while circulating antibody levels remained at high levels.

Live virus produced a stronger primary cellular response than ultraviolet light inactivated virus. Similar serological responses have been observed in

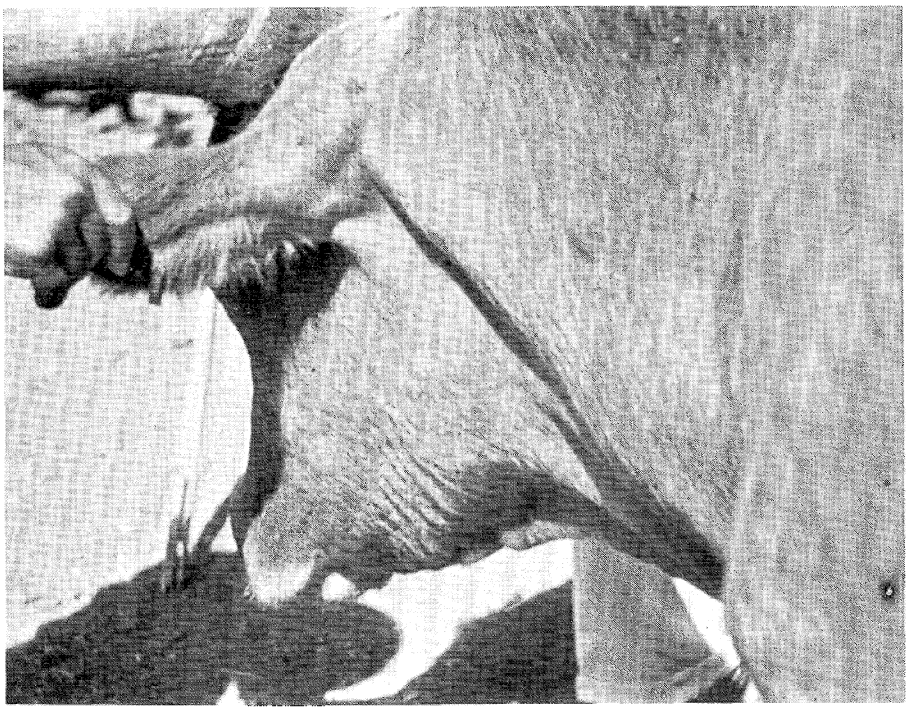

Fig. 2. Vampire bat (*Desmodus rotundus*) wounds on ear of a cow.

cattle. The immune response produced by inactivated vaccines is dependent on the antigenic mass given, so a relatively large dose is required to stimulate an effective immune response. Modified live virus vaccines rely on replication of the virus in the inoculated host to produce the desired immunological response.

Laboratory aids to diagnosis

The laboratory diagnosis of rabies is routinely carried out in most countries of the world utilizing histological, fluorescent antibody, and animal inoculation procedures to confirm the presence of rabies virus. Negri bodies can be detected in impression smears or tissue sections following staining with Sellers, Giemsa, or Mann stains. The Sellers stain combines simplicity and a rapid easy identification of Negri bodies. Fluorescent antibody (FA) procedures are routinely carried out on tissues for the detection of rabies antigen. This test has the advantage of being rapid and specific. It has replaced histological examination in many countries. The indirect FA test and fluorescent focus inhibition test are also utilized to measure antibody levels.

Mouse or other laboratory animal inoculation is also employed for rabies diagnosis. This procedure should be utilized in cases of suspected rabies where Negri bodies are not found and specimens are FA negative. Intercerebral inoculation of mice is also used as a test system to determine the virus neutralization index of serum and for vaccine potency tests. Other tests such as complement fixation, hemagglutination, passive hemagglutination, gel diffusion, and radioimmunoassay have been utilized in rabies studies but are not generally utilized for routine diagnostic procedures.

The brain is the routine tissue used to diagnose rabies in cattle. If the animal is decapitated in the field, the head should be cooled promptly and kept cold. It should be delivered to the laboratory by messenger, but if that is not possible, the head should be packed for shipment by express travel. It should be placed in a tin, heavy gauge plastic, or other water-tight container that can be sealed for shipment. Any sharp protruding bones should be wrapped with layers of newspaper to prevent any tears or punctures in the container. It is best not to freeze brain tissue if it can be preserved by cooling with wet ice during the transportation procedures.

The brain and submaxillary salivary glands should be removed from the head in the laboratory for further examination. Negri bodies and viral antigen, when present, are most readily demonstrated in Ammon's horn (hippocampus major), in the pyramidal cells of the cerebral cortex, Purkinje cells of the cerebellum, and brain stem. Tissue from those portions of the brain should be utilized for histological or fluorescent antibody examinations. Details on these procedures can be obtained from many texts and laboratory manuals. A number of additional specialized diagnostic and research techniques are also used in well-equipped laboratories to study rabies infections and various parameters of the virus itself (Kaplan and Koprowski, 1973).

Prevention and control

Bovine rabies is prevented by immunization of the species as well as control directed at immunization of cats and dogs and population reduction of cats, dogs, terrestrial wildlife, and vampire bats. Attempts to vaccinate species of wild animals that serve as rabies vectors have not been very successful in controlling the disease in either the vector species or cattle. Reductions in wildlife populations have been successfully carried out by trapping, hunting, gassing, and poisoning. Reduction in numbers of a vector species has been demonstrated to effectively stop a rabies epidemic and lower the number of cases of rabies in cattle but are temporary in nature.

Control of vampire bat rabies has been divided into two categories (Con-

stantine, 1970). Nonlethal use of repellents, physical barriers, and roost destruction have been utilized to temporarily relieve a major problem. The use of lights, bat proofing animal quarters, dynamiting roosts, cutting roost trees, and other mechanisms have been utilized. Lethal population reduction techniques have been more successful in effectively reducing vampire bat rabies in cattle. Various poisons, rodenticides, insecticides, and herbicides have been used to destroy large numbers of vampire bats. Most recently, vampire bats have been controlled by using the anticoagulant, diphenadiane. The drug is administered either as a petroleum jelly paste on the back of captured bats or as a liquid form of the chemical injected into the rumen of cattle (Turner, 1975). The topical application method makes use of the fact that vampire bats spend much time grooming themselves and each other in the roost. The anticoagulant jelly is applied to the backs of bats captured in mist nets. The bats are then released and returned to their colony where they and their roostmates ingest the anticoagulant through grooming. Spontaneous hermorrhages occur in the wing webs and in the digestive tract, and the bats die of internal bleeding. With the intrarumenal injection technique, a dose is administered that will not harm cattle but will kill a vampire bat feeding on blood in which the anticoagulant is circulating.

Effective rabies vaccines have also been developed for use in cattle. Inactivated tissue vaccines have been utilized with varying success. A suckling mouse brain vaccine used in most South American countries (Fuenzalida *et al.*, 1969) has been shown to effectively protect cattle for over one year. Other nervous tissue origin inactivated rabies vaccines are used to vaccinate cattle in Africa and Asia.

Flury Low Egg Passage Chick Embryo Origin (LEP) vaccine has been shown to cause vaccine deaths in cattle and should not be used for immunization in that species (da Salina and das Passas, 1966). Flury High Egg Passage Chick Embryo Origin (HEP-CEO) vaccine was shown to give reasonable protection when used properly in relatively high doses, but protection with a single dose of vaccine was relatively short-lived. HEP-CEO is currently being used to vaccinate cattle in several countries (WHO, 1979). A tissue culture origin vaccine (ERA) has been developed for use in domestic animals (Abelseth, 1975). This porcine tissue culture origin modified live vaccine is approved for use in cattle, horses, sheep, and goats, as well as dogs.

Three factors must be considered when selecting a vaccine for use in cattle. It should have the ability to protect animals against exposure, it should be inexpensive, and it should produce a long duration of immunity. Inactive vaccines are used throughout the world. Large doses are often required because of low potency, and vaccination must be repeated annually. If HEP-CEO vaccine is to be used, it must be obtained from a manufacturer who has

met the established standards for potency, safety, and purity. Since it is grown in embryonated eggs, it is a difficult vaccine to prepare properly. It may be manditory to vaccinate 2 to 3 times per year if HEP-CEO is used to protect against vampire bat rabies.

ERA strain vaccine has been proven to be effective in rather extensive field and laboratory trials (Abelseth, 1975). It is currently being used widely in Mexico to vaccinate cattle in the prevention of rabies from vapire bats.

Both inactivated nervous tissue (mouse, goat, sheep, rabbit, calf) and modified live (chicken embryo, tissue culture) vaccines are available that will effectively prevent rabies in cattle. Incidence, vector species, and other epidemiological factors that exist in a given area or country must be taken into account in establishing a vaccination program that will effectively control rabies in cattle.

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10. FOOT-AND-MOUTH DISEASE

J. B. Brooksby

Abstract. Foot-and-mouth disease affects cloven-footed animals, both domestic and wild, in most regions of the world except North and Central America, Australia, New Zealand and Japan.

The virus is a member of the Picornaviridae. Seven main serotypes are known and include a large number of subtypes which pose a significant problem in vaccinating against the disease. Spread takes place very readily by direct contact between animals and by the airborne route. Internationally, trade in meat and other animal products has led to spread to clean areas.

Diagnosis is by clinical observation backed by serological tests for differentiation from other vesicular conditions of livestock such as vesicular stomatitis and swine vesicular disease.

Control in areas suffering sporadic infection is best achieved by slaughter, destruction of infected carcases and disinfection. Where the disease is endemic, vaccination campaigns reduce the incidence and, in the absence of reimportation of virus, can lead to eradication.

Synonym: apthous fever.

Foot-and-mouth disease is an acute febrile disease which affects cloven-footed animals. It is characterized by vesicle formation on the tongue, in the mouth and on the feet, particularly at the margin of the hoof. Vesicles also occur on the teat and mammary gland and in the rumen. In young animals degenerative changes occur in muscle, and extensive involvement of the cardiac muscle can result in death. Human infection is recorded but is rare.

Etiology

The disease is caused by a small virus about 25 nm in diameter. The virion contains about 30% RNA enveloped in a protein coat. There are four major polypeptides with molecular weights ranging from 30 000 to 10 000. The RNA is a single.stranded continuous segment with a molecular weight of

2.6×10^6. RNA sequence homology studies by hybridization reveal differences between all seven antigenic types. The greatest differences are between the group of types O, A and C and Asia 1 on the one hand and SAT 1, SAT 2 and SAT 3 on the other (Robson *et al.*, 1977).

The virus is most stable between pH 7.4 and 7.6 and rapidly destroyed by acid or alkali. It survives well below 4 °C and may be stored for many years at temperatures below freezing. Resistance to heat varies with the strain; most field strains are inactivated by heating at 56 °C for 30 min but a number of laboratory strains have shown an ability to resist even 80 °C for several hours.

There are seven serological types: O and A (Vallée), C (Waldmann), SAT 1, SAT 2 and SAT 3 (Southern African Territories), and Asia 1. These types are antigenically distinct and recovery from infection with one confers no immunity against subsequent infection with another. Within the main antigenic types there are sub-divisions which can be sufficiently important to require the selection of strains for the preparation of vaccines for the regions where they occur. These subtype variations range from those which are detectable experimentally but have no epidemiological significance to differences which are almost as great as that between the separate antigenic types. Thus, the A_{22} strain which spread through the Middle East into Russia and Turkey in 1965-66 is so different from the classical European A strains that the immunity of recovered animals can be broken in about 5% of cases.

Distribution of the disease

The disease is widely distributed throughout the world and has been reported in almost all countries, with the exception of New Zealand and Japan (FAO/WHO/OIE Yearbook, 1977). Some countries, however, have only occasional outbreaks and by using strict import controls on possible sources of infected material manage to maintain freedom for many years. The disease has not occurred, for example, in Australia since 1872, the United States have been free since 1929 and Canada has suffered only one outbreak – in 1952. The O, A and C types are widely distributed in Europe, South America and Asia, types O and A occurring more frequently than type C. In the last two decades types O, A and C have spread to affect a wider area in Africa. Formerly they were restricted to countries north of the Equator but they have now appeared as far south as Angola, Namibia and Mozambique. The three SAT types also appear to have extended their range though, as surveillance in many countries is of recent date, the data recorded may reflect the detection of a situation which has existed for some years. SAT 1 and SAT 2 are more common than

SAT 3, which has been confined to a few areas in southern Africa. From time to time the prevalence of SAT 1 or SAT 2 appears to increase in several countries more or less simultaneously but major waves of the disease are not generally obvious, in contrast to the situation in Europe during 1951-52. When type SAT 1 spread through the Middle East from the end of 1961 onward it moved extremely rapidly, reaching European Turkey in the autumn of 1962, probably because this type had gained entry to a totally susceptible population. The seventh type, Asia 1, was first observed in Pakistan but has a wide distribution from Hong Kong and Thailand in eastern Asia to the eastern Mediterranean countries. It spread into Turkey in 1973 but would now seem to have been eradicated from that country.

Surveillance of foot-and-mouth disease in tropical and sub-tropical countries is rarely good enough to give a true indication of the level of endemicity. The general picture which emerges is one of long periods when cases of the disease are relatively few and far between and the irregular occurrence of more severe spreading disease affecting possibly up to 80% of the animals in herds which are attacked. The situation probably results from the interplay of host immunity and virus, complicated by the existence of the different antigenic types and subtypes. Among the domestic animals cattle, pigs and sheep are most commonly affected but, although the signs are less obvious in goats, they may be important as a reservoir of infection. Many wild species of ungulate have been reported to have been affected by the disease. Important observations have been made on the behaviour of the disease in the Cape buffalo, which acts as a carrier animal (Hedger, 1972). Overt signs of the disease are infrequent but the virus is maintained in herds over many years and can be recovered from the pharynx. Young animals regularly become infected at six months to one year of age and appear to remain carriers for several years. Transmission from buffalo back to cattle has not been affected under strictly controlled conditions but circumstantial evidence suggests that it may take place from time to time when ecological conditions are appropriate. Other wild ruminants may occasionally carry virus and waves of infection have been seen in eland and other animals, including smaller antelopes such as impala. From such outbreaks of disease cattle may have become infected but it is probably equally common for game animals to have become infected through contact with diseased cattle. The susceptibility of deer varies from species to species (Forman, 1974) but they do not appear to have played a major role in the spread of the disease. Many other species of small animal have been infected experimentally and, in one instance, infected hedgehogs were found to be associated with outbreaks in Britain. Some birds, including domestic fowls, can be infected experimentally but this has not so far occurred in the field.

Epidemiology

The disease spreads extremely readily. When an atypical type of virus appears in a region which it has not hitherto affected, spread proceeds very rapidly. In tropical countries it is likely that spread takes place mainly by direct contact between animals. All fomites are infective and contaminate the environment of an infected animal. Airborne spread can take place in temperate countries over considerable distances (Report – Committee of Inquiry on Foot-and-Mouth Disease, 1969). In tropical regions airborne spread is likely to be limited to the nighttime when humidity is high. Where accurate records exist, the incidence of the disease is found to peak in the cool season – probably linked to the greater facility of spread in damper air. Since the virus can survive on material commonly found close to animals – such as hay and straw – movement of the materials can lead to spread of the disease.

The milk of infected cows may contain high titers of virus and provides another source of indirect infection (Report – Committee of Inquiry on Foot-and-Mouth Disease, 1969). The virus will also survive in the tissues of animals slaughtered for meat, although it is inactivated in the musculature following rigor mortis (Henderson and Brooksby, 1948). Lymph nodes, offals and bone marrow, however, will remain infective and may be responsible for the movement of virus on an international scale. This is probably an infrequent occurrence in tropical countries but may in the future have an important effect on prospects of establishing trade in carcase meat from tropical areas to more temperate regions and countries where the disease has been controlled.

Clinical signs

The disease is generally not fatal but can vary widely in severity in accordance with the strain of virus involved and the immune status of the population. It is more serious in highly bred animals and those in good condition. In tropical countries among native stock the disease is usually mild and may be limited to lesions in the mouth without spread to other areas. Falconer (1972) in Botswana has described an "occult" form in which small and insignificant lesions are found in a few cattle in the herd and there appears to be little tendency to spread to other animals. Nevertheless, the disease continues to be present in the herd and may appear in a more severe form at any time. The mildness of disease between major outbreaks is probably the result of partial immunity derived from previous contact with virus and the susceptibility of native breeds of animals. The virus itself is still fully virulent, as was shown

when it was recovered from animals showing mild disease in southern Africa in the 1930s and passaged in European cattle. The disease which resulted was as severe as that produced by strains recovered in temperate climates. Severe lesions sometimes occur in native breeds, most probably when there is little immunity in the population to a new strain which has arisen or been imported into the region.

In severe disease the typical picture is of rapidly developing vesicular lesions in the mouth, especially on the tongue and around the muzzle, on the coronary band and interdigital space of the feet, on the udder and (on post mortem examination) in the rumen and sometimes other parts of the alimentary tract. The epithelium of the vesicles is lost and there may be secondary bacterial infection of exposed areas. Foot lesions of this kind result in severe lameness which may be complicated by the loss of the horny covering of the foot. Mastitis is seen in dairy animals and frequently leads to loss of the mammary function in the quarters concerned. There is marked loss of condition and, together with lameness, this may produce serious economic sequelae in the case of animals used for draught purposes. In young animals the disease can take a more generalized form, with lesions in the musculature and especially in the myocardium. This may lead to death of calves and piglets. In India, a syndrome called "panting" has been described; it is suggested that this is linked to pituitary lesions and loss of thermal regulation.

Pathology

Lesions begin with pycnosis of the nuclei of the cells of the basal layer of stratified epithelium, most frequently on the tongue or in the epithelium of the coronary band of the foot. Virus recovery studies suggest that the initial site of infection after exposure is in cells of the dorsal surface of the soft palate or on the lateral walls of the pharynx but there is little sign of the development of the characteristic lesions at these sites. On the more obvious sites in the mouth and on the feet, vesicles develop rapidly and loss of the epithelial covering takes place within 12–24 h. In the absence of secondary infection, the resulting ulcers heal quickly. Scarring takes place when secondary infection occurs and bacterial invasion of the lesions on the feet leads to severe damage and sometimes loss of the horny covering of the feet. Primary mastitis may occur, apparently due to virus multiplication in the mammary gland, but the changes are not characteristic of foot-and-mouth disease and usually progress with bacterial involvement. In muscle, especially in the heart, there is acute degeneration of the cell fibers. The characteristic sign of infection at this site in the calf is a striped appearance of the musculature

which has been described as "tiger heart". In the young animal death may follow through muscular involvement without the characteristic vesicular lesions.

Less typical lesions of the disease may result in necrosis of the epithelium without true vesicle formation. This is frequently seen in indigenous cattle in tropical regions and may result in no more than the loss of the superficial layers of epithelium as brown necrotic material, with rapid healing. In these circumstances, differential diagnosis may be difficult and it may be necessary to passage material into an obviously healthy animal to assist in diagnosis. In such a case difficulty may arise where the animal chosen has had previous contact with the disease and so fails to react.

In endemic areas the disease may be confused with rinderpest and possibly with bovine viral diarrhea. The typical mouth lesions in foot-and-mouth disease, however, involve the epithelium to a greater depth and the ulcer has characteristic underrun edges.

Other vesicular diseases of animals which are sometimes extremely difficult to differentiate from foot-and-mouth disease are vesicular stomatitis, vesicular exanthema of swine and swine vesicular disease. While all three differ in their epidemiological characteristics, clinical differentiation in the case of a single animal showing lesions can be well nigh impossible. Diagnosis then depends on the laboratory examination of material by serological methods, by electron microscopy and by studies of the host range of the virus involved.

Immune response

Approximately 3 days after the appearance of lesions, antibody can be detected in the serum and the antibody titer increases rapidly to a peak at from 21 to 28 days. The initial class of antibody is IgM and the specificity of this is less than that of the IgG which follows it. With declining titers, antibody will persist for 2 to 4 years or even longer. Immunity to the homologous strain of virus depends on the route of infection chosen for challenge of the recovered animals. Lesions can be produced by inoculation of virus into the susceptible tissue of the tongue within six months in some cases but contact infection is unlikely within 2 to 4 years. There is no cross immunity against virus of other types. The duration of immunity against a heterologous strain or subtype is difficult to predict since it depends on the antigenic relationship between the new strain and the original infecting strain. After vaccination, the immunity reflects the efficiency of the vaccine used but is in all cases less than that following recovery from infection. Where vaccination campaigns are being carried out it is necessary to revaccinate at

intervals which depend on the exposure to infection which animals are likely to encounter. In some cases, annual vaccination has been satisfactory but in general repeated inoculations at six-monthly intervals have been necessary. If there is risk of exposure to heterologous strains, the need for repeated vaccination becomes even greater.

Laboratory aids to diagnosis

In countries where the disease is endemic, little difficulty is usually encountered in deciding that the condition met with is or is not foot-and-mouth disease. If laboratory confirmation is required, it is usually given by complement fixation test in which antisera against all seven types are used. If good antigenic material has been collected from lesions in the field, this test will provide an answer within 2–3 h of receipt of the material in the laboratory. If the material is poor in virus or viral antigen, however, it may be necessary to passage it in tissue culture or in experimental animals such as mice. The time taken to provide a diagnosis will then be lengthened to 2 to 3 days. If a new strain is encountered – indeed a new antigenic type – it may be necessary to carry out extensive investigations involving cattle and proceed to a full virus characterization by physical and chemical methods.

The tests referred to also differentiate foot-and-mouth disease from vesicular stomatitis, vesicular exanthema of swine and swine vesicular disease. Vesicular stomatitis occurs in the United States and Central America and in some parts of South America. Vesicular exanthema occurred in parts of the United States from 1930 to 1956 but has apparently been eradicated. Swine vesicular disease has occurred in Hong Kong and Japan and possibly in some other countries of the Far East. It has also been seen in a number of European countries but may well have extended its range to tropical or sub-tropical regions. Inclusion of the appropriate sera in a complement fixation test or the employment of the electron microscope, if one is available, can give rapid differentiation of these viruses.

Prevention and control

The policy which should be adopted by a country in relation to foot-and-mouth disease is dictated by its initial status. If the country is free from the disease, the policy must be directed at preventing its importation and, in addition, defining the measures which would have to be taken should the disease be imported. First of all, the control of importation must cover the

introduction of live animals from areas where the disease exists. If imports of such animals are essential for schemes of livestock improvement or for other purposes, the importation should only be undertaken under strict regulations covering tests on the animals for carrier virus and for the presence of antibody in the serum and with appropriate quarantine both at the country of origin and on arrival in the importing country. If possible, imports should be limited to young stock which have not been vaccinated so that if antibody is found, there is evidence of exposure to virus and more rigid tests can be applied in the search for virus.Unvaccinated animals are also preferred as they are less likely to be in a state of inapparent infection.

Prohibition of importation of animal products is the next measure to be applied. The principal risk attaches to carcases and offals. The risk from boned and prepacked meat and cooked meat is minimal. Pork and mutton represent a slightly greater hazard than beef and are less likely to be imported as prepacked products. In the tropical zone these regulations are most important to countries of Central America, since in Africa and Asia meat trade to free areas is small in volume. In countries where the disease is limited to a certain region (and this is rare), spread to free areas must be controlled in somewhat similar ways to those described in relation to whole countries. With control programs involving the creation of "free areas" from which meat might be exported, rules are being drawn up to govern the protection of such areas from surrounding areas where the disease may be present.

Turning to control measures for countries in which the disease occurs, even infrequently, a decision must be taken as to the economic practicability of dealing with the disease. This demands some assessment of the cost benefit from a control program and the results of such a study will influence the kind of program which can be practiced. If outbreaks are sporadic and limited to relatively small units, it may be possible to proceed directly to a slaughter policy. If, however, the disease is more widespread and if there is no very obvious barrier to importation from neighboring countries, the policy is likely to be one of prophylactic vaccination in an attempt to bring the disease under control and in the end achieve eradication, when a slaughter policy may become the most cost-effective method.

Vaccines against foot-and-mouth disease have been of two kinds: living modified strains and inactivated vaccine. The living modified strain vaccines, although satisfactory in some conditions, have not proved as safe and reliable as might be desired and the agents at present in use are inactivated vaccines prepared by the growth of virus in tissue culture and its inactivation by a variety of agents. The resultant product is then formulated with an adjuvant for inoculation.

As has been referred to above, successful prophylactic campaigns must be

based on repeated vaccinations and the organization of such campaigns depends on attention to many details so that adequate coverage of the population is maintained. Young stock must be vaccinated, preferably within the first six months of life and again before a year. Because of the difficulty of covering the population against all possible variant subtype strains, a vaccination campaign should be supplemented by movement controls to guard against importation of virus into the vaccinated areas and also against the development of new variant strains at the borders of the area.

The vaccination of cattle and sheep presents relatively little difficulty. Pigs are more difficult to immunize successfully and, although in the last few years an oil adjuvant has been employed, there are still difficulties in the prophylaxis of the disease in a pig population. Frequently, if pigs represent only a small fraction of the domestic animals in the country, it would seem to be adequate to vaccinate cattle and possibly sheep and deal with any outbreak in pigs by ring vaccination as and when it occurred. Little can be done to deal with the prevention of infection in the wild ungulate but experience in East Africa and in Botswana suggests that if the disease is kept under control in the domestic animal population, it will also be reduced among the free-living wild animals with the possible exception of the Cape buffalo. Information is still lacking on the possibility of eradicating the disease from such areas where the wild animal reservoir exists and it may be that continuing vaccination will be necessary until some other approach to control is found.

Problems also exist in dealing with immunization in mixed populations of domestic stock where cattle improvement schemes have taken place and there exist nuclei of high-yielding animals. Immunization of the population at large may not be economically possible since the value of the individual animal will not be sufficiently high to justify the cost of vaccine. With the vaccines at present available it is difficult to guarantee the continuing protection of the high-grade animal against constant risk of exposure to viruses which may be circulating in the indigenous stock. Once again, improvement in vaccine or the discovery of alternative methods would seem to be the only solution to this problem. Living vaccines have been suggested in this connection but the difficulty has arisen that, with the multiplicity of strains against which vaccines would be required, the task of providing well-controlled, safe and immunogenic vaccines is expensive and difficult. There is still need for field investigations on the methods most suitable for immunoprophylaxis in many tropical countries.

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11. RINDERPEST

Hugh W. Reid

Abstract. The principal features of rinderpest virus infection of cattle are described. Following a brief historical introduction, the current classification and physical characteristics of the virion are detailed. The characteristics of epizootic and enzootic occurrences of rinderpest in cattle and the role of other species in the transmission of the disease are elucidated. Clinical signs and pathogenesis are considered with reference to the course of viral replication and excretion. The role of active and passive humoral immunity in protection against the disease and the development of live and killed immunogens capable of inducing protective immunity are discussed. Methods of diagnosing rinderpest and the diseases likely to create differential diagnostic problems are considered. It is concluded that the application of control procedures, which includes systematic vaccination with tissue culture propagated virus, could ensure the global eradication of rinderpest.

Synonym: Cattle plague.

Rinderpest or cattle plague is an acute or subacute contagious viral disease characterized by necrotic lesions in the mouth and gastrointestinal tract and destruction of lymphocytes. All species of Artiodactyla (even-toed ungulates) are probably susceptible but it is in cattle that the disease is of greatest importance. Its distribution now is restricted to North Africa, the Near East, the Indian subcontinent and parts of Asia, but in the last hundred years it has ravaged cattle populations throughout the world.

The earliest descriptions which unambiguously identify rinderpest date from the European epizootic of 376–386 AD. Thereafter, epizootics in Europe regularly succeeded the movements of armies with their trek oxen. The need to control the disease was instrumental in establishing veterinary colleges and state veterinary services during the 19th century.

Trade cattle brought rinderpest to Great Britain in 1865 and 420 000 cattle perished before its eradication was achieved by quarantine and slaughter. The application of similar legislation in other European countries ensured that rinderpest was controlled by the latter part of the 19th century. Subsequent

introduction of the disease to Belgium in 1920, Italy in 1951 and 1954, Brazil in 1920 and Australia in 1923 was rapidly stamped out.

Self limiting outbreaks in North Africa in 1805, 1828 and 1865 were followed in the 1890s by a devastating Panafrican epizootic believed to have originated from the importation of Indian cattle to Somaliland in 1889. The toll of domestic livestock and wildlife was immense. Between 80% and 95% of all cattle, buffalo, eland, giraffe, wildebeest, kudu and other antelope died; the social and economic effects were profound (Mack, 1970).

In South Africa, at that time the only African country with an extant veterinary service, an estimated $2\frac{1}{2}$ million cattle died before the disease was finally eradicated in 1905. Control in the rest of Africa was slow, national programs seldom achieved more than temporary respite and it was not until coordinated international programs were instigated in the 1960s that the goal of total eradication came close to being realized. Political vagaries and lack of attention to conservatory measures contribute to the persistence of rinderpest in northern Africa which continues to threaten the rest of the continent. As in Europe, the development of African national veterinary services was largely in response to the need for rinderpest control. Rinderpest absorbed the greater part of veterinary research and development while other livestock diseases were neglected. Only now are the other diseases being given the attention they deserve.

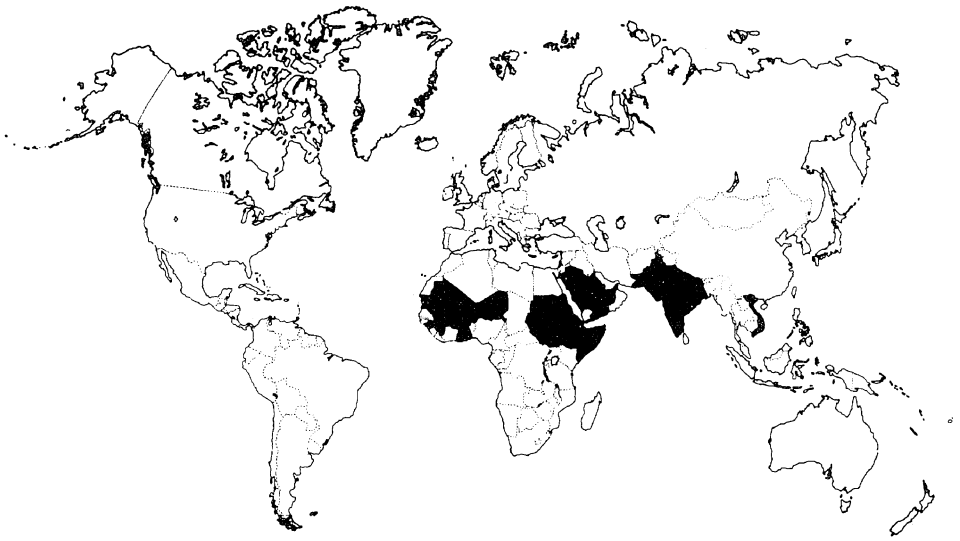

Fig. 1. Distribution of rinderpest recorded in 1977. The shaded countries are those reporting the presence of rinderpest.

Rinderpest persists in the Near East, periodically breaking out in epizootics, reaching as far west as Turkey in 1970. National control programs so far have failed to eradicate the disease from India, Pakistan or Nepal. In the rest of Asia, eradication programs have progressively eliminated the disease though it was still present in Vietnam in 1977. The distribution of rinderpest as reported in the 1977 statistics of the Office International des Epizooties is shown in Fig. 1.

Etiology

Rinderpest virus is an enveloped RNA virus belonging to the genus *Morbillivirus* (medieval Latin morbili = measles) of the family Paramyxoviridae. The roughly spherical virions have a diameter of 100–150 nm but larger filamentous forms also occur. All isolates are antigenically identical and related to the other morbilliviruses comprised of measles, canine distemper, and peste des petits ruminants (PPR) viruses. The latter causes a rinderpest-like syndrome of sheep and goats in West Africa (Fig. 2). Despite this close antigenic relationship, hemagglutination has been demonstrated only with measles virus although rinderpest infected cattle do produce antibody which inhibits measles virus hemagglutination (Kingsburn *et al.*, 1978).

Infectivity of the virus is rapidly destroyed by ultraviolet light, more slowly by diffuse daylight and it is heat sensitive. Lyophilized virus is, however, relatively stable with a half life of approximately 9 weeks when stored at +4 °C and >5 years at −20 °C. Viral stability is optimal at pH 7.2–8.6 and minimal below pH 3 or above pH 11. Chemical inactivation of infectivity is achieved by lipid solvents, ß-propiolactone, glycerol, formalin and phenol

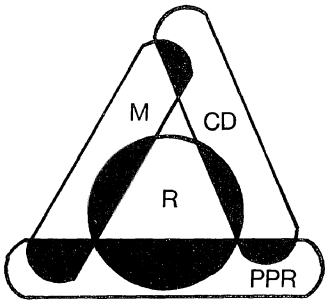

Fig. 2. Diagramatic representation of the antigenic relationship between rinderpest virus (R) and measles virus (M), canine distemper virus (CD) and the virus of peste des petits ruminants (PPR). The shaded areas indicate the extent of cross reactivity.

disinfectants. The tremendous potential of tissue culture techniques was first exploited by Plowright and Ferris (1959, 1962) who found that rinderpest virus could replicate and produce readily recognizable cytopathic changes in monolayer cultures of bovine kidney cells. It has subsequently been shown that rinderpest replicates with the production of cytopathic effects in a wide variety of primary and continuous cell cultures from cattle, buffalo, sheep, goats, dog, rabbit, hamster, humans, monkey and chick embryo. Primary and low passage bovine calf kidney cell cultures have been most extensively used although continuous cell lines are becoming increasingly popular. All strains of rinderpest virus have been found to replicate in tissue culture although considerable difficulty was experienced in adapting strains that had been serially passaged many times in goats or rabbits.

The cytopathic changes observed in monolayer cultures are characterized by the formation of small foci of rounded refractile cells, stellate cells and small syncytia which may progress to extensively involve the monolayer. In stained preparations, eosinophilic intranuclear and basophilic intracytoplasmic inclusions can be seen.

Epizootiology

Epizootic and enzootic rinderpest present two distinct patterns. Epizootics of rinderpest are typified by high mortality, frequently in excess of 90%, affecting all ages and spreading rapidly, particularly along cattle trade routes. In contrast, where rinderpest is enzootic, the disease is generally of a milder nature primarily affecting one- to two-year-old animals while the adult population is immune from previous exposure and the calves are protected by colostral antibody.

Rinderpest epizootics in areas where infection has been absent have almost invariably arisen due to the movement of live infected animals; imported meat or animal products have seldom been implicated. Nomadic migration of livestock over long distances, particularly in Northern Africa, and movement of cattle for trade purposes with inadequate quarantine supervision continue to perpetuate cattle plague.

The principal route of natural transmission of rinderpest is by aerosol. Experimental infection by the oral route only irregularly succeeds whereas infection can regularly be established by intranasal inoculation or aerosolization. Nasal excretion of virus occurs in a greater proportion of infected animals over a longer period of time and at higher titers than by any other route. It is probable that this portal of entry and shedding is of predominant importance in transmission of the disease. Close contact is necessary, howev-

er, for experimental transmission of virus from infected to susceptible cattle and transmission does not occur over distances greater than a few meters. The probable explanation for this limitation is that infection is generally spread by fairly large droplets which are rapidly deposited, and that the concentration of virus in small droplets, capable of moving greater distances, is insufficient to establish infection (Hyslop, 1972).

Aerosol dissemination should not, however, be regarded as the sole mode of virus transmission. Paddocks devoid of vegetation from which sick animals had been removed remained infective to susceptible cattle for 6 to 8 h in sunlit areas and for 19 to 24 h in shaded areas. Infectivity can be retained on grass for 24 to 36 h and in buildings for up to 3 to 5 days after removal of sick animals. Thus, although close contact of infected animals is generally required to ensure transmission, infection of susceptible animals can occur by less direct routes.

A possible method of transmission by infected meat or animal products has attracted considerable attention. This has been of particular concern to rinderpest-free countries when assessing the desirability of importing meat from areas where rinderpest may be present. Furthermore, pigs can be infected by the oral route and can transmit virus to cattle. The danger of importing infected meat which subsequently might be fed to pigs in inadequately sterilized swill is obvious.

Meat will retain infectivity at $+2\,^{\circ}C$ to $+7\,^{\circ}C$ for up to 7 days while in lymph nodes this period is 55 days. Infectivity in frozen meat is likely to survive indefinitely but will not survive in meat that has become putrid or autolytic. There is a definite risk, therefore, of importing rinderpest into disease-free areas in meat, particularly if it is frozen. However, meat was imported into Europe without disease incidence in 1917 and during the 1940-45 war from areas where rinderpest was enzootic. Thus, although there is a theoretical risk of transmission by infected meat, the relative fragility of rinderpest virus makes this means of transmission of little importance (Anon, 1976).

Apart from cattle and water buffaloes, all species of Artiodactyla are probably susceptible to rinderpest and some have been shown to have important epizootiological roles. Small domestic ruminants have generally been considered unimportant in the epidemiology of rinderpest. In India, however, there has been an increasing incidence of disease in these species. It has been suggested that this may have been due to the adaptation of virus to goats in the preparation of vaccine. Goats imported from India to feed troops were responsible for introducing the disease into Malaya and Ceylon. Furthermore, as national and international cattle vaccination programs have progressed, infection of sheep and goats has been a cause for increasing concern. Inci-

dents of confirmed outbreaks in sheep in Africa and India have often been severe and in the Sudan it was considered necessary to include small ruminants in the vaccination campaign. Small ruminants are therefore capable of playing a role in the maintenance and dissemination of rinderpest.

Except in India and Asia, natural infection of pigs has not been recorded. In Asia, infection of pigs has frequently been reported, especially in the southeast where this species may be important in the maintenance and dissemination of infection to cattle and buffalo.

Many wild animal species have been shown to become infected with rinderpest virus. This has been of particular concern in Africa where vast herds of antelope and buffalo are present. There can be little doubt that these great herds had an important role in the rapid and extensive spread of rinderpest during the African panzootic at the end of the 19th century when a large proportion of these wild animals died. With the eradication of rinderpest from among domestic animals, an increasing proportion of the wild animal population has become susceptible and there are now few immune animals. Were rinderpest to be reintroduced, not only could one anticipate a high mortality in these species but they would contribute to the dissemination of virus to susceptible domestic livestock.

Clinical signs and pathogenesis

The severity of the clinical response of cattle to infection with rinderpest virus varies greatly, depending on the virulence of the virus strain and the innate resistance of the cattle. Some strains of virus will produce a rapidly fatal disease in a high proportion of cattle of all breeds while at the other end of the scale there are strains which produce only a mild disease with few fatalities in the more resistant breeds of cattle. These less virulent strains have generally been associated with enzootic foci. Similarly, cattle breeds show a wide spectrum of innate susceptibility; thus the use of goat-adapted virus vaccine which proved satisfactory in Indian plains cattle produced a severe reaction in Himalayan cattle. Similarly, a rabbit-adapted virus vaccine can safely be inoculated into most breeds of cattle but causes a severe clinical response and 30% mortality in Japanese cattle. European breeds are generally much more susceptible than zebu cattle and among them Channel Island breeds are the most susceptible.

It is therefore clear that rinderpest may present a wide spectrum of clinical features depending on virus and host characteristics. Classically, however, the clinical picture presents four phases: incubation, prodromal, mucosal and convalescent (Table 1). These four phases and their relationship to viral

infection have been established in the classic studies by Plowright (1964, 1965) and his colleagues (Liess and Plowright, 1964).

Incubation phase

This period lasts from the time of infection to the start of the febrile response and has been shown to vary according to the route of administration, dose and strain of virus used, but is generally accepted to be 2 to 9 days. Following contact, however, precise limits are not readily defined as it is not possible to establish exactly when infection occurs, but the prodromal phase may not commence for up to 15 days after exposure to virus excreting cattle. Natural infection is probably via the mucosae of the upper respiratory tract and initial viral replication occurs in the pharyngeal lymph node. Hematogenous spread thereafter disseminates virus to the spleen and other lymphopoietic tissues. Further dissemination rapidly follows to the lung, bone marrow and mucosal membranes of the whole respiratory and alimentary tracts. High titers of virus may be present in nasal secretions and affected tissues prior to the development of a febrile response.

Prodromal phase

This period is defined as extending from the initial febrile response to the appearance of mucosal lesions in the mouth and by definition lacks any specific clinical signs of diagnostic value. It generally lasts for 3 days but can be as short as 1 day or as long as 6 days and is characterized by the rapid onset of fever reaching peak temperatures of 40–41.7 °C by the second or third day. Initially, no other clinical signs can be observed although a loss of

Table 1. Principal clinical features of rinderpest.

	Stage			
	Incubation	*Prodromal*	*Mucosal*	*Convalescent*
Duration	2 to 15 days	1 to 6 days	3 to 5 days	2 to 6 days
Fever				
Mucosal lesions				
Diarrhea				
Death				
Virus excretion in:				
Nasal secretions				
Urine				
Feces				

milk yield in dairy animals may occur. During the second and third days animals become increasingly depressed, restless and anorexic. The muzzle becomes dry and there may be a serous nasal discharge which tends to become increasingly profuse and mucopurulent. Excessive lacrimation may also be present. Visible mucosal membranes are hyperemic and may be abnormally dry. Rumination is depressed and animals are constipated and pass firm dry feces coated with mucus which may be streaked with blood.

Viral titers in all tissues increase exponentially and plateau during this phase. Highest titers are found in the lymphoid and lymphoepithelial tissues where they can reach 10^7 to 10^8 infectious units per gram in the cephalic lymph nodes. Virus may be present in nasal secretions but titers are generally low at this stage. It should be kept in mind that cattle can excrete virus for up to 6 days before the appearance of mucosal lesions.

Mucosal phase

This is the period when the specific clinical signs of rinderpest are observed. Initially small foci of necrosis and erosion appear in the mucosal membrane of the oral cavity followed by the development of diarrhea. This phase ends in death or the onset of convalescence and may last for 3 to 5 days.

External symptoms may become increasingly severe. Respiration is accelerated and shallow, frequently of an abdominal type which in the terminal stages becomes labored and may be accompanied by expiratory grunting. The muzzle becomes dry and cracked and the nasal discharges may be streaked with blood. Historical descriptions of rinderpest frequently mention skin lesions but contemporary reports seldom refer to them.

The first oral lesions are small (1 to 2 mm in diameter) greyish areas of necrotic epithelum surrounded by a zone of hyperemia affecting the lower lip and adjacent gum. These necrotic foci detach leaving hemorrhagic erosions They are generally first seen on the lips and gums, often extending to affect the tongue, floor of the mouth, buccal papillae, dental pad and ridges of the hard palate. The dorsum of the tongue may develop deep yellowish necrotic plaques. The areas of necrosis become extensive and the epithelium is sloughed leaving hemorrhagic erosions. Frequently there is excessive salivation. The pharynx may also become involved and in the severely affected animals the areas of necrosis extend to the entire epithelium of the buccal cavity with widespread thick yellowish accumulations. The nasal cavity may be extensively necrotic involving the nares and turbinates. In the severely affected animals, exhaled air has a repulsive fetid odor. The mucosa of the vulva and vagina may become affected.

Diarrhea usually does not appear until one or two days after the develop-

ment of lesions in the buccal cavity. Initially, large volumes of watery feces are voided which often contain excessive mucus streaked with blood; the feces become increasingly blood stained and may contain fragments of necrotic epithelium and blood clots. Animals become progressively dehydrated and may die shortly thereafter. Death generally occurs 3 to 9 days after clinical signs develop.

At the beginning of the mucosal phase, tissue virus titers are still maximal but rapidly decline as the disease progresses. The highest titers of virus in body excretions are reached during this period, consistently being greatest in nasal discharges where titers in the region of 10^6 infective units per ml may be present and less frequently and at lower titers in urine and feces. Virus titers first fall in the lymphopoietic tissues which support the initial cycles of viral replication and persist longest in the epithelial cells of the respiratory and alimentary tracts. The decline in virus titer is associated with the exhaustion of cells capable of supporting virus replication and the appearance of neutralizing antibody. By the time death occurs, little infective virus can be detected in the majority of animals.

Convalescent phase

Resolution of mucosal lesions normally ensues 3 to 5 days after the first appearance of lesions and generally progresses with dramatic speed. The integrity of mucosa is often restored in as little as 48 h; diarrhea may persist for some time after the mucosal lesions have healed, and recovery of body condition takes several weeks. Any residual virus in the body tissues and excretions rapidly disappears.

Pathology

The most marked changes are in the alimentary tract. Necrosis of the oral mucosae (described above) often extends to the anterior third of the esophagus. Lesions are seldom apparent in the rumen, reticulum or omasum while the abomasum, particularly the pyloric region, is constantly affected. Necrotic foci (<1 mm diameter) of the epithelium are initially present and are accompanied by bright red or brown hemorrhage in the lamina propria. These foci frequently progress to involve a large part of the abomasal epithelium which sloughs leaving irregularly shaped erosions from which blood oozes. Severe edema of the submucosa may be present in the fundus.

Generally the small intestine is less markedly involved. Most frequently, lesions are restricted to the anterior portion of the duodenum and terminal

portion of the ileum, where a varying degree of hyperemia, hemorrhage and erosion may be present. Peyer's patches are frequently entirely necrotic and may be sloughed.

The most frequently affected areas of the large intestine are the mucosa adjacent to the ileo-cecal valve, the junction of the cecum and colon, and the crests of the mucosal folds. Lesions consist of erosions of the mucosa. Hyperemia and hemorrhage of varying intensity may blacken the mucosa, giving rise to the so-called "zebra striping" along the crests of the folds. The terminal portion of the rectum is frequently similarly affected. Hemorrhages varying from petechiae to diffuse blotches are often present in the mucosa of the gall bladder.

Lesions in the respiratory tract are generally limited to the petechiae in the turbinates and larynx where small (1 to 2 mm diameter) erosions of the mucosa may be present. Longitudinal hemorrhages are frequently seen in the anterior portion of the trachea; gross changes of other organs are generally not observed.

The striking microscopic feature is the severe necrosis of lymphocytes in the absence of hemorrhage or inflammation in the lymphatic tissues. In the lymph nodes, necrosis commences in the germinal centers and progresses to affect all lymphocytes in the follicle leaving a reticulum mesh. This severe depletion of lymphocytes is associated with a reticular cell hyperplasia and the formation of multinucleate syncytia. Similar changes occur in the hemolymph nodes, spleen and Peyer's patches. Mucosal lesions consist of necrosis and desquamation of the mucosa, hemorrhage of the lamina propria and edema of the submucosa. Syncytia, intranuclear and intracytoplasmic inclusion bodies occur in both epithelial and lymphoid tissue and are most frequently seen in the tonsillar tissue. These cytopathological changes are associated with the accumulation of viral nucleoprotein or nucleocapsid material.

Immune responses and protection

Active protection

There is complete cross-protection between all strains of rinderpest virus. Following recovery from natural infection or after vaccination, neutralizing antibody appears which is associated with solid and longlasting protection from virus challenge. All live vaccine strains of virus confer protection prior to the appearance of neutralizing antibody. This phase of protection is generally attributed to the interference phenomenon. Following inoculation with large doses of tissue culture-adapted virus, cattle are protected from virulent challenge by the 3rd or 4th day while smaller doses of virus do not protect

until day 4 or 5. This correlates with the widespread dissemination of attenuated virus in the tissues of vaccinated cattle and is responsible for the initial resistance. Titers of attenuated virus remain high for 3 or 4 days and thereafter fall rapidly as neutralizing antibody begins to appear. Thus, immunologically mediated resistance commences approximately 7 days after the administration of tissue culture attenuated virus. With more virulent strains of virus this time scale may be shorter as cattle given goat-adapted virus are resistant to fully virulent rinderpest virus within 48 hours of inoculation.

Subsequent to the development of immunity, resistance to clinical rinderpest is life-long. Cattle vaccinated once with goat attenuated or tissue culture attenuated strains, and maintained in areas free from rinderpest, have been shown to be completely protected from clinical disease after $12\frac{1}{2}$ years. In some animals with low levels of antibody, however, limited viral replication may occur after parenteral inoculation with virulent virus (Rweyemamu *et al.*, 1974).

Passive protection

The protective properties of serum from recovered cattle was established in 1893 and was exploited extensively in Africa as a means of protecting cattle from infection. Serum prophylaxis has no role in modern control of the disease. Passively acquired colostral antibody is, however, of great importance (Brown, 1958). Calves responding irregularly to infection or vaccination were reported in 1915 and later it was found to be due to calves acquiring colostral antibody. Approximately 30 to 48 h after suckling, calves of immune mothers generally have neutralizing antibody titers significantly greater than those of their dams. The half life of this passive antibody is approximately 40 days. Passively protected calves are solidly refractory to infection or vaccination for at least 3 months and thereafter an increasing proportion can be infected, all being susceptible after 8 months of age. It is essential that allowance for this is made in vaccination campaigns, and it is advisable not to record any animals as vaccinated under 12 months of age.

Antibodies

1. *Neutralizing antibody.* After infection or vaccination, cattle acquire neutralizing antibody. The dose of virus given determines the time at which antibody can first be detected. With large doses, this may be as early as 5 to 6 days but with minimal infective doses, seroconversion can take up to 4 weeks. Titers peak in 2 to 3 weeks after which there may be a small decline and thereafter titers may be maintained relatively constant for up to 11 years.

In some animals titers rapidly decline to just detectable levels. During the initial 3 to 4 days of an antibody response, IgM is the principal class of immunoglobulin but thereafter IgM is progressively replaced by IgG and can not be detected after the 3rd week. Tissue culture systems are used extensively for detection of neutralizing antibody in epidemiological studies and vaccine evaluation.

2. *Complement fixing antibody.* Several workers have used complement fixation (CF) tests for detecting antibody in bovine serum. The presence of CF antibody following infection cannot be consistently detected and its titers are low and of short duration. These disadvantages, compounded with the inherent difficulties of performing CF tests with bovine serum, have rendered this technique of limited value.

3. *Precipitating antibody.* The agar gel diffusion technique for detection of antibody in cattle is of limited value. However, an indirect method has been developed in which precipitating antigen is mixed with test sera prior to reacting with hyperimmune rabbit serum. Agar gel precipitation tests have proven of greatest value in detecting antigen in tissues of suspected cases of rinderpest (see section on Diagnosis).

4. *Hemagglutination inhibiting antibody.* It has not proved possible to obtain hemagglutinin from rinderpest virus; however, measles virus anti-hemagglutinin can be demonstrated in serum from convalescent cattle. Following inoculation with virulent or vaccine strains of virus, antibody may be detected in 9 to 21 days, reaching maximum titers of 1/32 to 1/64 which tend to decline rapidly. Such tests have limited value.

5. *Indirect hemagglutination antibody.* The use of tannic acid-treated goat erythrocytes coated with boiled rinderpest antigen have been used to detect antibody in an indirect hemagglutination test. It has been reported that in buffalo calves inoculated with virulent rinderpest virus, indirect hemagglutinating antibody may be detected in serum after 6 to 7 days and in nasal discharges and saliva after 3 to 7 days.

Laboratory aids to diagnosis

Only provisional diagnosis of rinderpest can be made on the basis of clinical, post-mortem findings and history. Rinderpest like diseases, particularly bovine virus diarrhea (BVD), may present indistinguishable signs and serious differential diagnostic problems (see chapter 15 on BVD). This is particularly

acute in areas bordering rinderpest enzootic regions or those which import live animals from such regions. Should rinderpest be suspected on the basis of clinical signs, post mortem findings and herd history, it should be treated as such until proven otherwise in the laboratory. Rapid and accurate laboratory confirmation of rinderpest outbreaks is of paramount importance in the control of the disease and may be accomplished in one or more of the following ways: Isolation and identification of virus, detection of virus specific antigen, detection of altered antibody status, and detection of virus specific histological lesions.

1. *Isolation and identification of virus.* Previously this was achieved by inoculating rinderpest susceptible and immune cattle or goats with blood or tissues from clinically affected or dead animals. Currently the virus is being isolated by use of culture methods.

For the isolation of virus in tissue culture, the material of choice is washed buffy coat from febrile animals. Virus titers fall progressively with the onset of diarrhea and may be present at minimal levels by the time of death. Thus, in a suspected outbreak, 15 to 20 ml of blood should be collected into anticoagulant (EDTA) from several febrile animals and transported rapidly on ice to the laboratory. Specimens should not be placed on gel packs or frozen as it is impossible to separate buffy coat from such samples. The buffy coat is separated by centrifugation, washed, resuspended in tissue culture medium and inoculated into culture tubes of preformed monolayers of primary bovine kidney cells. Cell monolayers are examined daily for the development of typical cytopathic effect (CPE).

Confirmation that the CPE is due to rinderpest virus may be sought in a number of ways. Virus neuralization can be demontrated if additional tubes inoculated with the original material together with rinderpest antiserum fail to develop CPE. When CPE is well advanced, complement fixing or precipitating antigen may be detected in the cells disrupted by ultrasonic treatment or 3 cycles of freezing and thawing. Alternatively, antigen may be detected using the fluorescent antibody method.

2. *Detection of virus specific antigen.* Antigen may be detected rapidly and with relatively simple equipment using either CF or gel precipitation tests. Precipitating antigens cannot be detected early in the course of infection nor is either test effective after antibody has appeared. Therefore, specimens should be collected during the acute phase of disease before diarrhea develops. The sensitivity of the two tests is inferior to virus isolation technique and may fail to detect animals infected with relatively mild enzootic strains of virus.

Precipitinogen may be prepared from any lymph node of a freshly dead animal. If the carcass has decomposed, only the lymph nodes should be collected and kept on ice or frozen until tested. The fat and the capsule are removed and the chopped lymph nodes are ground in a mortar and pestle with some sand. Following centrifugation, the supernatant is used as antigen. Alternatively, biopsy material may be collected using a 14 gauge needle to aspirate tissue from the prescapular lymph node. These antigens are then reacted against hyperimmune rabbit serum in an agar gel precipitin test (Scott and Brown, 1961).

Antigen for CF may be prepared in a similar fashion to precipitinogen except that the fluid extract from lymph nodes is diluted $\frac{1}{4}$ in veronal buffered saline. Although this test is more sensitive than the agar gel precipitin test it lacks the simplicity of the latter test.

Indirect hemagglutination tests in which terazotized benzidine treated sheep erythrocytes are coated with antibody to rinderpest virus have also been used to detect antigen in tissue extracts, nasal secretion and saliva. Interpretation of such tests tends to be confounded by non-specific activity.

3. *Detection of altered antibody status.* To confirm a diagnosis of rinderpest, serum collected in the acute phase of the disease and another collected 2 weeks later are required. A four-fold or greater rise in titer during the 2 weeks, detected by any of the tests detailed in the section on immunity, confirms the presence of rinderpest. Of the tests available, however, neutralization is generally considered the most suitable for this purpose. As this entails at least a 3-week delay, these tests have little application in the diagnosis of rinderpest, however, confirmation of rinderpest can be made if neutralizing antibody associated with IgM immunoglobulin can be demonstrated. Such antibody is only present for 3 weeks after infection. For this, a single serum collected during the acute phase of the disease is adequate and markedly reduces the interval for confirmation of disease. To demonstrate IgM antibody, fractions of sera from sucrose gradient zone ultracentrifugation may be tested for neutralizing antibody or more simply, the titers of whole serum may be compared with serum heated to 65 °C for 30 min or treated with 0.2 M 2-mercaptoethanol for 18 h at 23 °C, reduction in titer in the treated samples indicating that IgM antibody is present.

4. *Detection of virus specific histological lesions.* Several authors have suggested that diagnosis of rinderpest may be made on the basis of detecting syncytia with intracytoplasmic and/or intranuclear inclusions. For such tests a variety of tissues are collected including tonsil, mucosa of the pharynx, oral cavity, vulva, prepuce, conjunctiva and nares. They are rapidly fixed, sec-

tioned and stained for histological examination. Generally, the presence of virus-induced lesions would not be considered adequate confirmation of rinderpest and would serve only as an adjunct to other more specific tests such as the detection of viral antigen by fluorescent antibody.

Differential diagnosis

A wide variety of conditions have been considered to produce clinical signs which may be confused with rinderpest, undoubtedly the most important of which is bovine virus diarrhea (BVD), the clinical and pathological signs of which can closely resemble that of rinderpest (see chapter 15 on BVD). Papular stomatitis, malignant catarrh, foot-and-mouth disease, infectious bovine rhinotracheitis, and Allerton-type herpes virus infection are the other principal virus diseases which may cause confusion with rinderpest. In addition, acute enteritis through poisoning, parasitic infestation or salmonellosis may also have clinical similarities with rinderpest. At post-mortem examination, trypanosomiasis and theileriasis may also present pathological features similar to those of rinderpest. Finally, in small ruminants bluetongue, Nairobi sheep disease and sheep pox have created problems of differentiation and PPR presents identical clinical signs.

Prevention and control

General

Of all the major diseases of domestic livestock, rinderpest is considered to be the most readily controlled. Even in 1714 it was known that following the introduction of rinderpest into disease-free countries, effective control could be achieved by a policy of slaughter and quarantine. The strategy for controlling rinderpest in disease-free countries depends on whether they are geographically remote from or bordering enzootic regions. In either case, the most probable route of introduction is by the movement of live infected animals. In countries remote from enzootic areas, it is generally the policy to ban the importation of live animals and all unprocessed animal products from rinderpest infected countries with the exception of animals destined for zoological parks. Should such a policy not be practical for sociological or economic reasons, rigid quarantine measures must be applied. All animals must be held in isolation in an area of the exporting country which is free from rinderpest for a period of not less than 3 weeks and for a similar period of isolation on

arrival. If there is a breakdown, it is essential that the national veterinary departments must have legislative authority to immediately slaughter sick and in-contact ruminants and pigs and to destroy or bury the carcasses. Also, animal houses and enclosures in the infected zone must be isolated and disinfected and the movement of all livestock, meat or hides prohibited. In high risk countries, it is customary either to immunize the whole cattle population or to create a barrier zone of immunized cattle in regions adjacent to countries where rinderpest is enzootic. In these areas all cattle are vaccinated followed by annual vaccination of the previous year's calf crop.

The principal strategy for control in enzootic regions is by mass vaccination, which has been greatly facilitated by the availability of cheap and effective vaccines. Increasingly, national and international campaigns based on vaccination have brought this disease under control. In these campaigns it has generally been the policy to vaccinate all cattle each year for three consecutive years. Following the initial phase of annual vaccination, it is essential to maintain the annual vaccination of the previous year's calf crop. Adequate quarantine and surveillance must also be maintained.

Prophylaxis

During the 20th century prophylactic procedures have progressively improved through application of attenuated virus vaccines. A variety of attenuated virus strains have been developed, including caprinized, lapinized, and avianized viruses. The most effective and most widely used are tissue culture-derived attenuated vaccines. Protection induced by these latter vaccines is rapid and durable; and, all species of animal including cattle, water buffalo, sheep and goats can be immunized without any side reaction to vaccination.

Development of prophylactic measures

The credit for the first practical method of immunization goes to Koch who in 1897 advocated the use of bile from infected animals to immunize cattle. This method was employed extensively to control rinderpest in southern Africa. In 1893 it was shown that serum from recovered animals was protective and for many years serum prophylaxis was the principal method of control in India and parts of Africa. However, serum alone could provide only transitory protection; a more durable immunity could be provided by the administration of serum followed by virulent virus. From the 1930s onward these methods of protecting cattle were increasingly superseded by inactivated and later attenuated vaccines.

Inactivated vaccines

Inactivated vaccines were first prepared from virus containing cattle tissues. Glycerine, formalin and chloroform have all been found to inactivate the virus satisfactorily without destroying antigenicity but the duration of immunity was limited unless several inoculations were made. This could be overcome by administering virulent virus after the inactivated vaccine in which case immunity lasted for over 5 years; the attendant risks rendered this method of little use. Alternatively, durable immunity could be stimulated when adjuvants were incorporated, however, lesions following the use of Freund's complete adjuvant were unacceptable. Inactivated vaccines prepared from chick embryos were ineffective. Attempts to produce inactivated vaccines from tissue culture propagated virus has had limited success. Recently it was found that virus inactivated with merthiolate (sodium ethylenmercurithiosalicylate) or ultraviolet light and absorbed onto aluminum hydroxide or emulsified with incomplete Freund's adjuvant induced immunity lasted for at least 2 years (Mirchamsy *et al.*, 1974).

Despite safety and stability advantages, the expense of inactivated vaccine production compared to live attenuated vaccines has limited their application. Certain rinderpest-free countries, however, have expressed concern at the possibility of importing rinderpest virus in meat from cattle immunized with live vaccine.

Attenuated vaccines

1. *Caprinized.* To overcome the risk of transferring bovine infectious agents through vaccination by the serum-virulent virus method, goat spleens have been used as a source of virus. This was attempted initally by Koch who reported in 1897 that virus serially passed in goats had a reduced pathogenicity for cattle. In a field trial, no mortalities were reported to occur in 7,216 vaccinated cattle; however, in field trials in East Africa, 18% of zebu cattle died following inoculation, hence a strain of greater attenuation was required for African use. This was achieved in Kenya by passaging the virulent Kabete "O" strain of virus through goats. This strain, referred to as the KAG strain, became progressively attenuated for cattle and by the 250th passage only 2% of East African zebu cattle died while 95% still developed a febrile reaction. The strain was considered suitable for use as a vaccine in East African native cattle, but was still too virulent for the more susceptible Asian and European cattle or their crosses. This passage of KAG was also found to be too virulent for use in native cattle in Nigeria and Egypt where further passages were necessary before an acceptable level of attenuation was achieved.

Caprinized vaccines which induced lifelong immunity represented a major step forward in rinderpest control. Batches of caprinized vaccines were, however, frequently contaminated with bacteria (*Bacillus anthracis, Salmonella* spp. *Brucella melitensis*) and viruses such as foot-and-mouth disease. Also, the response of goats to infection was variable, necessitating the use of large numbers of goats to ensure potency. Furthermore, only crude virus titrations were possible in goats rendering quality control of caprinized vaccines difficult (Edward, 1928; Kesteven, 1949; Anon, 1970).

2. *Lapinized.* The earliest evidence of the replication of rinderpest virus in rabbits was produced in Japan in 1910. In the late 1930's a strain fully virulent for rabbits but attenuated even for moderately susceptible cattle was developed. This strain, which had undergone 795 serial passes prior to distribution, became widely used for vaccination in China, Asia, India and Africa. Vaccine was produced from homogenates of lymphoid tissue from infected rabbits and where rabbits were difficult to procure such as Southeast Asia, infected pig tissues were used. Generally it was lyophilized for field use where it induced immunity comparable to that produced by caprinized vaccines. The lower virulence for cattle compared to the caprinized strains resulted in its use in the more susceptible breeds of cattle. It still possessed all the inherent disadvantages of a vaccine produced from infected animal tissues and was too virulent for use in hypersusceptible cattle.

3. *Avianized.* Under the fear of rinderpest being introduced during the 1939-1945 world war, workers in Japan and the United States independently developed avianized vaccines which were found suitable even for vaccinating hypersusceptible Japanese black cattle. The American avianized vaccine strain was developed from virus isolated from cattle, whereas in Japan, Nakamura and Miyamoto (1953) adapted the lapinized virus to eggs. This latter strain, referred to as LA, was the more attenuated and was used after back passage in rabbits (LAL strain) to immunize cattle in Korea and Vietnam, where it was reported to induce immunity in approximately 90% of vaccinated cattle.

In Africa the LA strain and other avianized strains were tested experimentally and were generally found to induce high titers of antibody and confer protection. The responses tended to be irregular, however, and despite their economy and simplicity of production, they never superseded the use of caprinized and lapinized vaccine strains (Nakamura and Miyamoto, 1953; Watanabe, 1970).

4. *Tissue culture.* Control of rinderpest by vaccination is now largely dependent on the production of attenuated rinderpest virus in tissue culture. The

strain of virus which has acquired virtually universal acceptance was derived from the laboratory strain of virus Kabete O which had been maintained by serial needle passage in cattle and had lost its contagious capacity. The virus was adapted to growth in primary bovine kidney cell cultures. After 45 passages in this cell culture the virus could be inoculated into all categories of cattle without inducing a febrile response, and when serially back passaged 5 times in cattle no evidence of reversion to virulence was observed. Studies of the virus after 95 passages demonstrated that following inoculation of cattle, widespread dissemination of virus occurred in the body and titers of 10^4 to 10^5 $TCID_{50}/g$ were detected in lymph nodes, however, virus did not replicate in the mucosa and it was inferred that the virus had become "exclusively lymphotropic." It was further concluded that this pattern of virus replication explained the lack of pathogenicity and inability to spread by contact, yet the virus has retained the capacity to induce consistently strong and durable immunity.

Virus of the 90th to 100th passage in primary kidney cell cultures is used for vaccine production. Virus harvests regularly contain in excess of 10^5 $TCID_{50}$ per ml and the recommended cattle dose contains 10^2 $TCID_{50}$.

Storage of the lyophilized virus at $-20\,°C$ ensures that there is virtually no loss of infectivity. There is, however, a decline of infectivity when stored at higher temperatures. Vaccine is generally issued to the field in vials which are reconstituted to give 50 or 100 cattle doses of 2 ml each. Preferably vaccine is recontituted in sterile saline in dark bottles, kept on ice and inoculated within 2 h.

The acceptance of international standards for the manufacture and administration of this vaccine, together with adequate surveillance and conservatory programs could ensure global eradication of rinderpest in the near future. Political and social factors may, however, conspire to render this goal elusive (Plowright, 1965, 1968, 1972; Scott, 1964).

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12. RIFT VALLEY FEVER

F. G. Davies

Abstract. Rift Valley fever is a zoonosis caused by an insect-borne virus which until recently had only been recorded in the sub-Saharan region of the African continent. It is transmitted by mosquitoes of several genera and produces abortions in pregnant animals and deaths in the very young. Cattle, sheep and goats are the principal disease hosts. Affected animals show characteristic widespread liver necrosis. The disease tends to occur in epizootics which follow periods of heavy and prolonged rainfall. These may be separated by intervals of up to 15 years.

The virus also affects humans causing fatalities. The disease is characterized by a diphasic temperature rise accompanied by signs related to the hepatic necrqsis. Retinitis and encephalitis are common complications; hemorrhagic complications are generally fatal.

Rift Valley fever (RVF) is an acute arthropod-borne virus infection with a wide range of vertebrate disease hosts. Until recently, its natural range has been confined to the Ethiopian faunal region of Africa. The virus is not related serologically to other arboviruses, but tentatively has been classified as a member of the Bunyamwera group on the basis of its physical charactistics. Daubney *et al.* (1931) first recognized the disease entity in the Rift Valley of Kenya, although there may have been earlier outbreaks. Mortality in young lambs was over 90% with widespread abortions in pregnant ewes. While the initial problem was recognized in sheep, it became evident that cattle also were affected. The mortality in calves was not as great as in young lambs, but many calves died and there were abortions in pregnant cattle. Liver lesions were obvious in the affected animals. An interesting observation was made that humans associated with the animals suffered a severe influenza-like disease. The original workers showed that the causative agent was a filterable virus transmitted by mosquitoes.

Subsequently, the disease manifested itself in epizootic form in many tropical and subtropical African countries. Specific antibody to RVF virus was demonstrated in sera from humans and domestic animals in many areas

where the clinical disease itself had not been recognized. Recently, a significant development occurred in which an epizootic of RVF produced dramatic and significant losses in both human and animal populations of Egypt. This is an extension from the natural ecological range for this virus which apparently has now established a maintenance cycle in the irrigated lands alongside the Nile river. Since RVF is one of the most dangerous zoonoses known to man, this extension is of major importance.

Natural Ecological Range

In Africa south of the Sahara, there have been periodic epizootics of disease in Kenya, Uganda, Tanzania, Sudan, Zambia, Malawi, Mozambique, Rhodesia, South Africa and South-West Africa. Epizootics of RVF have occurred at intervals of 4 to 15 years, and interestingly it is in these countries that the greatest development of the livestock industries has been achieved with introductions of exotic animals. There is evidence to show that exotic breeds are much more susceptible to RVF than indigenous cattle, sheep and goats. Some of the native breeds are relatively insusceptible. The epizootics of RVF appear to be precipitated by favorable climatic factors such as persistent and heavy seasonal rains. It is thought that foci for the maintenance of the virus in interepizootic periods occur in most of these countries. Favorable conditions allow the overlap of a forest or bushed savanna maintenance cycle, and a new epizootic cycle involving different mosquito species is established in the grasslands. The wet climatic conditions cause dramatic increases in populations of the vector species, many of which breed as opportunists in the temporary water pans.

In other African countries such as West and Central Africa and possibly Botswana, there is evidence of enzootic maintenance of RVF indicated by the presence of specific antibody in the human and domestic animal populations. There was a limited outbreak of RVF in Nigeria in imported sheep, however, indigenous varieties appear to be relatively resistant to the disease. It is probable that many of the west and central African countries with heavy tropical forest areas have enzootic cycles for RVF. Improvements in animal production by upgrading indigenous breeds may increase the numbers of susceptible disease hosts and RVF may manifest itself in these countries.

The countries north of the Sahara are likely to be free of RVF. While the disease or antibody to RVF has not been recognized in Ethiopia, it may occur there. Epizootics have occurred in the Sudan and there are reports of its occurrence in Somalia. The extension of epizootic RVF in Egypt may have occurred directly along the Nile from the Sudan and aided by the movement

of camels for trade purposes. The establishment of virus maintenance cycles in the dry irrigated areas of Egypt is significant since similar irrigation practices are prevalent throughout the eastern Mediteranean region and north Africa. Rift Valley Fever may extend its range considerably in this new ecological niche.

Epidemiology

The epidemiology of RVF is at present incompletely understood. The virus and disease disappear from the worst affected areas where widespread losses occur during the epizootics (Davies, 1975). The enzootic maintenance cycle occurs in certain tropical and temperate forests in East Africa (Smithburn *et al.*, 1948; Haig *et al.*, 1957; Davies, 1975). This type of forest may be found across central to western Africa, in parts of East Africa, and down the Rift Valley. Coastal forest is also a suitable enzootic focus for the virus. Such forest may be found along the eastern coast of the continent through Mozambique to South Africa. While enzootic cycles have not been identified in countries such as Angola, the flora and fauna there are identical with forest areas in other parts of Africa where the virus is maintained.

The forest maintenance cycle has not been clearly defined despite efforts made in many countries (Smithburn *et al.*, 1948; Scott *et al.*, 1956; Scott and Heisch, 1959; Henderson *et al.*, 1972; Davies, 1975; Davies and Onyango, 1978). Rodents which are highly susceptible to RVF are not involved in the enzootic nor the epizootic cycles of RVF activity. Initial studies of viremia in various common rodents suggested they might be amplifying hosts although field work does not support this hypothesis. Monkeys were found to be susceptible to RVF in early work but the field studies in Uganda and Kenya, in an enzootic forest area show that monkeys do not play any part in the maintenance cycle. Baboons (*Papio anubis*) are common in many epidemic areas of RVF. Experimentally, it was found that they developed a transient viremia and an antibody response, however, a survey in Kenya a year after an epizootic of RVF showed no evidence of baboon exposure. Three of 176 birds trapped in an endemic area in Uganda may have had a low level specific antibody to RVF. These were weavers of the family Ploceidae. The suggestion has been made that the Sudan dioch, a small seed-eating bird found in large numbers in Africa, may carry the virus in its north to south movements along the Rift Valley. It is not known whether this bird will sustain a viremia with RVF. Birds are generally refractory to the virus. Further work is necessary to determine whether the dioch does play any role. Bat sera contained antibody to RVF when examined by hemagglutination inhibition tests (Addy

and Tukei, 1975). Bats may be the key vertebrate hosts for the maintenance of RVF in the forest cycle.

Except for bats, the only other animals found to contain antibody to RVF during the interepizootic periods were bovids, both domestic and wild. Davies (1975) showed that 1 to 3% of cattle born after a previous epidemic of RVF living in forest and forest-edge situations were seropositive to RVF. Similar results were reported from a forest area in South Africa (Haig *et al.*, 1953). Forest ruminants may also be involved (Maurice and Provost, 1969; Davies, 1975).

In the forest cycles, various mosquito species of the genera Eretmopodites and Aedes found to contain RVF virus were considered to be the vector species in such situations. The species involved in epizootics in South Africa are *Culex theileri, Aedes dentatus* and *A. caballus, A. lineatopennis* and *Anopheles coustani* (MacIntosh, 1972). Davies and Highton (1980) recently isolated RVF virus from *A. lineatopennis* and *An. coustani* during an epizootic in Kenya. There may well be other species capable of transmitting the virus. It is thought that there is an overlap between the forest maintenance cycle and the feeding cycles of the large mosquito populations in the grassland habitat.

Domestic cattle, sheep and goats play a major role in the amplification of RVF during periods of epizootic spread. Wild ruminants are susceptible and may be significant where they are numerous. Camels are also challenged with the virus, presumably at water holes, and there is some evidence that they suffer abortions (Scott *et al.*, 1963). Horses and donkeys are not clinically affected but develop antibody after a transient viremia. Many other species of animals known to be susceptible to RVF, such as dogs and cats, are not normally present in any significant numbers to play a role in the amplification of virus in epizootic areas. Pigs are relatively insusceptible (Scott, 1964).

The species of mosquitoes which transmit RVF in most African countries feed upon both domestic animals and humans, however, most human cases of RVF in Africa have been related to direct and indirect contact with diseased or dead domestic stock. Infection is considered primarily from aerosols or wound contamination. *Culex pipiens* may be the principal species of mosquito transmitting the virus in Egypt.

The post-endemic level of immunity in cattle, sheep and goats is very high (Davies, 1975). No additional clinical cases appear and further amplification of the virus cannot be demonstrated by sero-conversions in newly-born animals. The range contracts to the forest maintenance areas until conditions are ripe for epidemic spread of the disease once more.

Large numbers of Culicoides have been trapped in areas where activity is high during an epidemic. The isolation of RVF has been recorded from engorged specimens of Culicoides. During a recent epidemic, Davies and

Highton (1980) isolated RVF virus from a pool of Culicoides of mixed species from which all insects showing any trace of blood had been removed. The role of Culicoides in epidemics of RVF needs further evaluation.

Clinical Signs

Rift Valley fever is more pathogenic for sheep and goats than it is for cattle. In very young lambs or kids the mortality approaches 100% of those affected. The mortality in sheep is generally greater than that encountered with cattle populations. Simultaneous disease in sheep, goats, and cattle suggests that RVF is the cause. The mortality rate in calves varies from 10% to 70%. It is considered that the exotic breeds introduced to Africa are more susceptible.

Calves are highly susceptible to RVF and they may die within 19 h with few premonitary signs. Clinical signs include anorexia, blood-stained nasal and lachrymal discharges, raised respiratory rate and temperature up to 106 °F. These may be the only signs seen before the animal suffers total prostration, generally lying on its side with opisthotony and severe respiratory distress before death. The course of the disease is generally rapid, taking only 1 to 4 days.

Slightly older calves show the high temperature reaction, raised respiratory rate and slight nasal and lachrymal discharges; while they may not suffer the collapse seen in younger animals, they remain very sick for several days. During this time they may show abdominal pain and a profuse diarrhea which is usually fetid or hemorrhagic. There may be respiratory signs with a moist cough and stertorious respiration. The superficial lymph nodes may become slightly enlarged. A feature of the clinical disease is a sharp fall in the total white cell count, often below 2000 cells per mm^3. The temperature may be raised for a period of 4 to 7 days. Some of the affected calves will have jaundice and if this is severe, the animals generally die.

Older cattle show a variable clinical response to natural challenge with RVF. The temperature reaction may be brief with some nasal and lachrymal discharges. There will be a sharp fall in the milk yield in dairy cattle and often a profuse watery diarrhea, which is usually accompanied by abdominal pains. Respiratory signs are a common complication. The temperature reaction usually lasts from 1 to 5 days during which time there is a viremia. The leukopenia is also marked. Deaths may occur in adult animals at this stage. A relatively high mortality rate has been encountered in adult cattle with severe liver fluke infestation. Abortion is the principal economic significance of RVF in adult cattle apart from the dysgalactia. A herd of some 1200 dairy cattle suffered nearly 500 abortions over a 2-month period during a 1968 epidemic

of RVF in Kenya. Abortions may occur during the period of viremia and up to 5 weeks later. The virus is readily isolated from fetal tissues and the characteristic liver necrosis may be seen in the aborted fetus. Complications from the abortions, notably pyometritis, are another cause of mortality indirectly due to RVF and observed in about 10% of the aborting cattle. In extensively managed range cattle, the only evidence of RVF may be the large number of abortions.

A fall in milk yield, a high temperature, followed by abortions, and mortality in calves with few specific clinical signs, other than complete prostration after the sudden onset of a febrile disease, should create suspicions of RVF. Other helpful observations are the simultaneous disease in sheep and goats with a particularly high mortality in the very young, and the occurrence of a febrile disease in people in contact with the sick animals. Common signs in humans are a temperature of 104 to 106 °F, a raised respiratory rate, muscle and joint pains, headache, anorexia, vomiting and diarrhea, photophobia, hepatitis and complications such as retinitis or mycocarditis. There is a marked leukopenia. The temperature reaction in humans is often diphasic. Fatal cases are associated with a hemorrhagic syndrome which is thought to be a consequence of severe hepatic necrosis, or encephalitis. Jaundice is a common complication of the disease in humans, and is frequently encountered in the fatal cases.

Gross Pathology

In the hyperacute cases in young calves, the carcass will show various non-specific signs associated with viremia. There may be petechial and ecchymotic hemorrhages subcutaneously and on the serous surfaces of the abdominal cavities and organs. There may be blood-stained fluid in the abdomen and pericardial sac. The principal lesions are found in the liver which is generally enlarged with rounded edges and may vary from deep red to yellow depending upon the course of the disease in the particular animal. A large swollen liver contains 1 to 3 mm foci of a white-yellow necrosis, areas of necrosis may occur which are much larger. Sometimes the liver presents a bronzed appearance with areas of necrosis and bile stained tissue, producing a mottled effect. The surface of the liver may be further mottled with subcapsular hemorrhage. In other cases the liver may be extremely yellow. In all livers, areas of white-yellow necrosis may be found. The gall bladder shows edema with hemorrhages on the surface and its attachments to the liver. The bowel surface may be covered with subserosal hemorrhage and the lumen may be filled with blood. The abomasal folds may be edematous with an

excess of mucous and the lumen often contains blood. The kidneys are congested with small hemorrhages visible on close examination of the cortex. There may be small hemorrhages on the wall of the bladder. The lymph nodes throughout the carcass are enlarged and edematous with many small areas of hemorrhage and necrosis in the cortex and medulla. Similar lesions are present in the spleen which is not usually notably enlarged. There may be edema and obvious jaundice in the fat deposits throughout the carcass.

Histopathology

The most characteristic lesions of RVF are found in the liver. In calves there are small foci of coagulation necrosis together with much larger areas of massive acute necrosis. The smaller foci have no specific distribution in relation to the lobular structure of the liver. They may be centrilobular, midzonal or paracentral. There is a cellular infiltration with lymphocytes, neutrophils and histiocytes, many of which show degenerative changes. A massive pannecrosis occurs, destroying the entire architecture of the liver, leaving only a reticular infrastructure. Other characteristics of the RVF liver lesions are bile stasis and mineral deposition in the necrotic parenchymal cells. Intranuclear inclusions are of a distinctive sickle shape and probably of a degenerative nature. No viral antigen can be demonstrated in the nucleus by fluorescent antibody methods.

The gross changes observed in the gall bladder may be confirmed histologically. The spleen shows some evidence of hemorrhages with focally necrotic lymphoid cells in the follicles. This necrosis is also obvious in the lymph nodes together with the evidence of edema and hemorrhage observed macroscopically. In the kidney there are foci of degeneration in the glomeruli and tubules, some of which contain casts.

Immune Response

Natural or needle propagated infection with RVF virus is followed by the formation of antibody which persists for many years and can be demonstrated by a variety of serological methods. The presence of antibody is correlated with resistance to infection; animals recovering from RVF are immune for life.

Various serological tests have been employed for the study of the antibody response to RVF. These include complement fixation, indirect fluorescent antibody, hemagglutination, and serum neutralization. The antibody re-

sponses and immunity to both pantropic and attenuated strains of RVF virus in cattle were studied by Coakley *et al.* (1967b) and Pini *et al.* (1973). The antibody responses in cattle to the Smithburn neurotropic strain of virus are poor when assayed by serum neutralization. The cattle, however, are resistant to challenge with pantropic virus up to 28 months after vaccination. The serum neutralization index for 30 heifers on primary vaccination with the Smithburn strain was 1.3 ($\log_{10}$). The response to pantropic virus is normally very good with neutralizing indices of 4.5 to 5.5 ($\log_{10}$). These are greater than any found with other virus diseases of domestic animals. The antibody persists and may be detected by serum neutralization or by indirect fluorescent antibody tests up to five years after infection.

Neutralizing antibody has frequently been found in the serum of humans recovered from RVF after periods of 20 years or more. The serum neutralization tests have been used to measure the antibody responses to both attenuated and inactivated virus antigens used for the prophylaxis of RVF. More recently further use has been made of the hemagglutination inhibition tests.

Laboratory Aids to Diagnosis

A field investigation of a disease condition characterized by high fever and the presence of some of the following features should lead to a tentative diagnosis of RVF: 1) a high mortality in lambs or kids, and calves; 2) illness with some deaths on older sheep, goats and cattle; 3) a febrile disease with abortions in sheep, goats and cattle; 4) the presence of liver lesions in dead animals; 5) an acute febrile illness in humans associated in any way with the sick animals.

Laboratory Confirmation of Infection

Virus isolation

Liver, spleen or blood are the tissues of choice for primary isolation of RVF virus. Blood taken at the febrile stage is highly infective and high titering virus is present in the plasma. It is recommended that dilutions of 1/10 to 1/1000 be made from the original tissue or plasma for primary isolation. Frequently the virus concentration is so great that infected tissue culture monolayers are completely destroyed in less than 24 h.

A variety of tissue culture may be employed for the primary virus isolation. Primary or secondary monolayers of kidney, lung, thyroid or testis of bovine,

caprine or ovine origin are particularly suitable. A variety of continuous cell lines have also been found to be sensitive to the virus (Easterday, 1965). Those recommended for routine laboratory isolation of virus are the BHK 21 C 13 cells, Vero cells, WI 38 diploid cells or L 929 cells. The virus produces a cytopathic effect characterized by focal degenerative changes which rapidly spread to involve the entire monolayer. These effects will be found in most cultures from 24 to 72 h after inoculation.

The virus may be identified in the cell cultures within 6 to 12 h by staining the monolayers with fluorescein labeled specific antibody. This is the recommended method for the identification of the virus whenever tissue culture facilities exist. Alternatively, the cell culture supernatant fluid taken at a time before the cytopathic effects become apparent contains a high titer of virus which may be used as antigen in complement fixation tests or for serum neutralization.

High titers of RVF virus grow in eggs and cause embryonic death. The optimal inoculation age of embryonated eggs was one day followed by incubation at 37°C for 48 to 72 h. The end point may be used to identify the virus by serum neutralization or as a source of virus antigen for complement fixation.

Laboratory Animals

Laboratory mice of most varieties are susceptible to infection with RVF virus by intracerebral or intraperitoneal inoculation. Mice 4 to 6 weeks of age have been found to be uniformly susceptible and are extremely useful for the primary isolation of virus when inoculated by the intraperitoneal route. Death occurs 3 to 6 days after inoculation and the characteristic liver lesions may be observed macroscopically or microscopically. Infant mice may be used and are inoculated by the intracerebral route. Deaths occur in 3 to 8 days when primary isolation virus is used. The incubation period is rapidly reduced to 2 to 3 days when virus is used following passage *in vivo*. The virus may be identified in liver or brain by fluorescent antibody methods (Pini *et al.*, 1970). The virus may also serve as a source of antigen for the inoculation of cell cultures or to carry out complement fixation tests. Serum neutralization tests were used for many years in African laboratories for the identification of RVF viral strains; the assay is carried out in 6-week-old mice.

Histopathological Diagnosis

The presence of the focal coagulative necrosis in the liver, cholestasis, mineralization and presence of intra-nuclear inclusions help to confirm the diagno-

sis of RVF infections. This is possible with field material, fetuses or livers from a laboratory animal.

Serological Confirmation of Infection

Serological testing is especially valuable when dealing with the disease in cattle when abortions may be the only sign. Paired serum samples may be assayed in a variety of ways. Complement fixation tests, indirect fluorescent antibody and serum neutralization tests (Pini *et al.*, 1973) or hemagglutination inhibition tests (Mims and Mason, 1956) are all useful diagnostic aids. Rising RVF antibody titers in samples from affected herds provide a good indication of recent infection.

Prevention and Control

Management

The movement of affected flocks of sheep to higher altitudes above the disease-infected Rift Valley reduced losses to a minimum according to Daubney *et al.* (1931). At these higher altitudes, cases of RVF stopped within a few days. Such a move took the sheep from the epizootic areas to an environment not suitable for the disease vector. Control in this manner is not often possible.

Vector Control

Methods to control mosquito populations might be valuable when their breeding sites can be defined and application of insecticide can be employed. The cost of carring out vector controls, however, may be too great and inapplicable in the face of disease outbreaks in domestic stock.

Prophylactic Vaccination

Attenuated Smithburn Neurotropic Strain. The Smithburn strain of RVF was produced by the serial intracerebral mouse brain passage of virus and is attenuated for domestic animals. Inoculation of sheep or goats with this strain is followed by a significant antibody response (Coackley *et al.*, 1967a) although cattle show a much lower antibody response using the serum neutralization test method. Animals with low titers of neutralizing antibody, however, are protected during epizootics of the disease. This strain is

extremely valuable for the prophylaxis of RVF. The antibody remains detectable in sheep for up to 3 years and while it falls more rapidly in cattle, it is though to be valid for at least one year after vaccination. Annual revaccination is recommended for cattle. This vaccination provides a valuable means for reduction of the economic losses incurred in an epizootic of RVF.

The vaccination of sheep in the early stages of pregnancy is not recommended because fetal abnormalities have been attributed to the vaccine. Pregnant ewes may abort after vaccination with the Smithburn strain, although this is not a common sequel. Lambs born after immunization may show fatal neurological abnormalities and grossly obvious developmental deformations. These, however, occur in only 1 to 2% of the vaccinated ewes and such hazards may be acceptable in the face of epidemic losses. Cattle do not seem to be affected in a similar manner. Fetal abnormalities and abortions were not observed in large numbers of pregnant cattle inoculated with the Smithburn strain of virus.

Inactivated Vaccines

The high titer virus grown in cell culture and inactivated with formalin is a potent immunogen. Several inoculations with this antigen produce a good antibody response in humans. The tissue culture antigen may be used for the prophylaxis of the disease in domestic animals. A killed vaccine may be more acceptable as opposed to the possibility of introducing live vaccine virus and creating a new foci of infection. Live attenuated vaccine has been used for many years in the interepizootic periods in Kenya (several million doses) and no focus of disease has been attributed to its use.

The killed viral vaccines require more than one inoculation to produce a satisfactory antibody response and are more costly than attenuated vaccines. The principal advantage for killed vaccines is that the danger of accidental infection of operators with the live virus can be eliminated. This is important since RVF can be a fatal infection in humans.

Passive Immunity

Calves and lambs or kids born to mothers recovered from a RVF infection or vaccinated with the Smithburn strain of virus generally have neutralizing antibody to the virus in their sera. Maternal antibody is passed through the colostrum in the case of calves (Coakley *et al.*, 1967b) and apparently *in utero* in sheep since some lambs born to vaccinated sheep have antibody before suckling (Weiss, 1962).

The use of hyperimmune serum to protect exposed animals may be successful if the serum is given soon after exposure. Such procedures are not possible on a large scale but may be used for very valuable animals.

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13. LUMPY SKIN DISEASE AND PSEUDO-LUMPY SKIN DISEASE

W. B. Martin

Abstract. Lumpy skin disease (LSD) is a poxvirus infection of cattle in which cutaneous nodules and lymphadenitis develop. The disease occurs sporadically in cattle in African countries. Generally the prevalence is low but serious epidemics may develop at intervals. Much of the epidemiological information suggests that transmission is indirect and that insects play a part in the spread of LSD.

The main clinical signs are those of fever, the development of multiple, painful skin nodules and lymphadenitis. As lesions progress the center of the cutaneous lesions becomes hard, necrotic and difficult to remove (so-called "sitfasts"). On pathological examination lesions may be present in internal organs. Histology of the lesions shows the presence of intracytoplasmic inclusion bodies in epithelial and mononuclear cells.

A clinically similar disease caused by bovid herpesvirus 2 (BHV2) is known as pseudo-lumpy skin disease (pseudo LSD). Bovid herpesvirus 2 is also associated with another clinical syndrome, that of mammillitis. Pseudo-LSD disease has been reported mainly from Africa and mammillitis from more temperate regions.

In both syndromes skin nodules develop. Those on the teat break down readily into ulcers. On the hairy skin, nodules of the superficial layers become necrotic and, with the exudate, form a dry scab which eventually separates, leaving a hairless pigmented area.

Histopathology of early lesions shows syncytial formation and eosinophilic intranuclear inclusion bodies.

Confirmation of LSD and pseudo-LSD is most accurately obtained by virus isolation.

In 1929 a disease called "pseudo-urticaria" was recognized for the first time in Northern Rhodesia (now Zambia). In the following decade the same disease was reported in other areas of Zambia and in the 1940s it was described in South Africa where it became known as "Ngamiland cattle disease." Since then this disease, now called lympy skin disease (LSD), has been recognized in East Africa in 1957 and subsequently in more northern countries of Africa.

Confusion existed between true LSD and a somewhat milder disease with similar characteristics now known as pseudo-lumpy skin disease (pseudo-

LSD). The separation into two diseases with different causal agents was made by Alexander *et al.* (1957). It is now recognized that LSD is caused by a poxvirus and pseudo-LSD by a herpesvirus. In this chapter the two virus infections are described separately.

Lumpy skin disease

Lumpy skin disease (LSD) is a poxvirus infection of cattle in which cutaneous nodules, lymphadenitis and other associated signs develop.

Although initially LSD was not thought to be infectious, the way in which the disease spread during the 1940s suggested an infectious condition. In 1945 it was shown to be transmissible to cattle by the inoculation of nodule suspension. A few years later a virus, sometimes referred to as van den Ende virus, was isolated and considered the cause of LSD. Since that time the van den Ende virus has been identified as a picornavirus unrelated to LSD.

Etiology

The LSD virus is a poxvirus belonging to the genus *Capripoxvirus* and has been termed the lumpy skin disease or "Neethling" virus. Neethling was the name of the farm where the virus was first isolated from cattle. Lumpy skin disease virus has physical characteristics similar to the orthopoxviruses, such as vaccinia, with large rectangular virions measuring 350×300 nm. The external coat of the virion contains lipid and tubular protein structures which cover lateral bodies and a DNA genome. The virus is ether and chloroform sensitive and produces no hemagglutinin. It is antigenically related to the viruses of sheep and goat pox but probably differs biologically because the geographical distribution of LSD differs from sheep pox.

The LSD virus replicates in calf and lamb kidney or testical cell cultures and produces cytopathic changes. The virus also replicates in sheep embryo cells, rabbit and chick cells. Lumpy skin disease virus produces plaques on the chorioallantoic membrane of developing chick embryos. On primary isolation cytopathic changes appear after about 10 to 14 days and progress slowly. With repeated passage, cell degeneration appears some days earlier. The cytopathic effect in infected monolayers includes small discrete groups of refractive round cells with a few elongated cells, giving each focus of infected cells a star-like appearance. As the cytopathic changes progress the monolayer becomes fenestrated with clear areas surrounded by strands of cells with long pointed extremities.

In monolayers stained with hematoxylin and eosin intracytoplasmic inclu-

sion bodies are often seen close to one pole of the nucleus. These are round, oval or crescent shaped and are usually surrounded by a clear halo. Initially inclusions are somewhat basophilic but as they increase in size they become acidophilic with basophilic granules ("inner bodies"). As cells die the cytoplasm becomes eosinophilic and the nuclei degenerate. Much of the LSD virus in cell cultures appears to remain cell bound. Like other poxviruses, the LSD virus is very stable. There is no reduction in infectivity in 5 days at 37 °C, or at pH 6.6 to 8.6 (Weiss, 1963). The virus will also survive for 33 days in lesions on salted hides and for 18 days on air dried hides at room temperature.

Distribution

Lumpy skin disease has been reported from most African countries but in many it has a low, sporadic or seasonal incidence. It has been reported from South Africa, Zambia (1929), East Africa (1957), Zaire, Sudan (1971), Chad (1973), Niger (1973) and Nigeria (1974). Countries in the near East and in Asia, with the exception of Bhutan, appear to be free of the disease. Lumpy skin disease does not occur in North or South America or in Europe.

Epidemiology

Lumpy skin disease affects all breeds of cattle, indigenous native as well as exotic breeds. In South Africa where sheep pox does not occur, LSD in cattle could not be transmitted to sheep. Sheep have, however, been blamed for introducing the disease into cattle in Kenya. Repeated passage of one isolate of LSD virus (Londiani strain) failed to infect goats but appeared to infect sheep successfully in Kenya. Uninoculated sheep from the same group, however, had a skin condition clinically and pathologically like sheep pox (Capstick, 1959). The Isiolo sheep strain (probably sheep pox) initially caused lesions on intradermal inoculation into cattle but only for two successive passages. These cattle were immune when challenged with LSD virus.

It seems probable that LSD occurs naturally only in cattle, and sheep are unlikely to be involved in its transmission.

In South Africa the prevalence of LSD is low but occasional outbreaks of epidemic proportions have occurred at irregular intervals, apparently when there is a build-up of a susceptible cattle population. In some epidemics large numbers of cattle are involved. For example, in one serious epidemic in South Africa it was estimated that approximately 8 million cattle were infected with LSD; in Nigeria, where the disease appeared in 1974, about 2000 cattle were believed to be affected. In contrast with the initial outbreak

in Kenya, movement of the disease was slow and few herds of cattle developed the clinical disease.

Lumpy skin disease can occur during any period of the year but outbreaks in South Africa were more prevalent during the summer months and died out after the first frost. In Nigeria the epidemic of 1974 occurred during the rainy season and ceased during the dry period. In some epidemics, cases of LSD occur with greater frequency in moist low areas. These observations suggest that LSD may be spread by arthropods. Furthermore, in 1957 during an outbreak of LSD in Kenya, exceptionally large numbers of *Culex* and *Aedes* mosquitoes were found breeding along the shores of the saline lakes. Outbreaks occurred on farms some distance from infected cattle and appeared to spread along cattle routes beside water courses. Quarantine measures have failed to control the spread of LSD. Lumpy skin disease poxvirus has been recovered from *Biomyia fasciata* and *Stomoxys calcitrans* flies feeding on affected cattle, but the actual method of transmission of LSD is still unknown. It is recognized that transmission does not take place directly between cattle although virus is present in lesions, blood and saliva of infected cattle. Intradermal and subcutaneous inoculation of virus seems to be a more effective route of transmission than intravenous inoculation. Transmission to suckling calves through the milk has been claimed.

It has been suggested that one outbreak in Kenya followed the introduction of sheep to the farm, however, this association has not been confirmed elsewhere in East or South Africa. The disease is known to occur in cattle on farms close to the Kruger National Park in South Africa. Giraffe and impala can be infected experimentally, resulting in typical lesions and death but wildebeest and buffalo are refractory. The role of game animals in the maintenance and spread of LSD is uncertain and needs further investigation.

The herd morbidity rate of LSD is variable, ranging between 5 and 100%. Mortality is generally negligible but may reach 10%; Haig (1957) reported losses of up to 75% on some farms. It is likely that serious mortality occurs only when there are secondary bacterial invaders or other complications.

Clinical Signs

The incubation period of the natural disease is not known but LSD has occurred 2 to 5 weeks after exposure to infection. Following the inoculation of infective blood or tissue suspensions, lesions appear within 4 to 14 days and usually about day 6 or 7.

Fever (40–41 °C) occurs early in many cases and may persist for up to 14 days accompanied by ocular and nasal discharge and salivation. Raised, firm, circumscribed nodules of varying size and number appear on the skin and

subcutaneous tissue over which the hair stands erect. Some lesions may be as large as 5 cm in diameter. Crops of lesions occur on the muzzle, neck, brisket, back, perineum and thighs and the associated lymph nodes are markedly enlarged. White, flat or raised necrotic foci and erosions occur in the mouth where they cause excessive salivation; those in the mucosa of the nostrils result in snoring if large enough to obstruct the nasal passages. Other lesions may be seen on the vulva and pharynx. Conjunctivitis and keratitis have been observed, as has edema of the brisket and one or more limbs with no lameness. Marked loss of weight and cessation of milk production can result. Abortion has been reported with skin lesions on the fetus. In many cases, however, there may be little systemic disturbance.

In the early stages lesions are painful and exude a little serous fluid. Sometimes skin lesions regress but usually separation from the surrounding skin occurs. The center of the lesion becomes dry, hard and necrotic – the so-called "sitfast". After 3 to 4 weeks this separates completely leaving a granulating area of tissue which heals after 2 to 3 weeks unless complicated by pyogenic bacteria (Capstick, 1959).

Pathology

Skin lesions extend through all layers to the subcutis. On section, nodules are firm, grey-white and circumscribed. The subcutaneous tissue beneath lesions usually contains a quantity of reddish serous fluid. Sometimes greyish nodules are present in the subcutis or in the muscles. Where edema of a limb is present, a yellow serous infiltrate is evident. Apart from the skin lesions the most striking feature is lymphadenitis. Some nodes are 4 to 5 times the normal size and petechial hemorrhages may be present.

Lesions in the oral cavity are fairly common and may be present on the tongue, cheek, lips, palate and behind the incisor teeth. The raised grey-yellow plaques or nodules are covered with soft necrotic tissue which, on separation, leaves erosions and ulcers. Similar lesions can occur throughout the respiratory tract from the nasal passages to the lungs. The mucosa over the turbinate bones can be grossly thickened. Congestion and lesions in the lungs may be accompanied by patches of pneumonia. Scattered petechiae are frequent on the surface of the liver, spleen and kidney. The liver may be paler than normal and the liver and spleen may be enlarged.

Histopathological examination of skin nodules shows severe inflammatory changes. The epidermis and sebaceous glands are greatly swollen, due mainly to enlargement of epithelial cells many of which contain large vacuoles. There is edema of the corium and dermal papillae, perivascular infiltration by mononuclear cells and some polymorphs, and thrombosis of small vessels. Acido-

philic, oval or round intracytoplasmic inclusion bodies occur in mononuclear and epithelial cells and occasionally in proliferating fibroblasts. Most inclusions are surrounded by a clear halo and become more basophilic as lesions advance. Many cells with inclusions and some without show nuclear degenerative changes similar to those present in sheep pox (Burdin, 1959).

Lesions of the skin at the sitfast stage show coagulative necrosis of the surface layers which appears as lamellated hyaline material raised above the surrounding skin and covering the vestiges of the epidermis. In the subcutis there is venous thrombosis and perivascular accumulation of cells. At the edge of the lesion intracytoplasmic inclusions may be recognized in the epidermal and mononuclear cells.

Immunity

It is generally assumed that immunity to LSD is likely to persist for several years. The value of some early reports on the shortness of immunity to LSD is doubtful because of the more recently discovered condition of pseudo-LSD.

Neutralizing antibodies to LSD virus are detectable for at least 5 years (Weiss, 1963). Capstick (1959) noted that focal skin thickening and pain lasted for several weeks when cattle were reinfected by intradermal inoculation up to 9 weeks after initial infection.

Laboratory Aids to Diagnosis

Diagnosis in the past depended on histological confirmation of lesions and the presence of eosinophilic intracytoplasmic inclusions in cells in the lesions. Virus isolation in appropriate cell cultures is now advisable. The fluorescent antibody technique will confirm a diagnosis 48 h after inoculation of lamb testes cell cultures with suspected material. Vaccinia-type particles can also be identified by direct electron microscopic examination of phosphotungstic acid-stained preparations from biopsy material, crusts or fluid from lesions (Davies *et al.*, 1971).

Prevention and Control

Restricting the movement of stock and dipping do not appear to be very effective in controlling outbreaks. The slaughter of clinical cases and insecticide spraying in addition to movement restrictions appeared, however, to confine the disease when it broke out in Kenya.

The prophylactic use of vaccines is likely to be the only successful means

of preventing and controlling epidemics. Attenuated LSD virus has been used in South Africa. This vaccine, injected subcutaneously, causes a temporary local reaction in about 50% of vaccinated cattle and stimulates high titers of antibody. Antibodies are difficult to detect in cattle not developing a local reaction, however, all are immune when challenged. Production of this vaccine is described by Weiss (1963).

Vaccination of cattle with sheep pox virus has been used in Kenya to control the disease but this vaccine can be used only where sheep pox exists.

Pseudo-lumpy skin disease

Bovine mammillitis, bovine herpes mammillitis and bovine ulcerative mammillitis are synonymous with pseudo-lumpy skin disease (pseudo-LSD).

The confusion between lumpy skin disease (LSD) and pseudo-LSD was clarified by the work of Alexander *et al.* (1957) who showed these to be two diseases caused by different viruses.

Pseudo-LSD is the name given to the herpesvirus infection of cattle associated with skin nodules. This herpes virus is also known to cause lesions on the teats and udders of cattle, therefore, the terms bovine mammillitis, bovine herpes mammillitis and bovine ulcerative mammillitis virus have also been applied to the disease.

Pseudo-LSD virus was originally isolated in South Africa from cattle on a farm called Allerton. The virus was termed "Allerton" and is sometimes still referred to by this name. The herpesviruses are not yet grouped officially but it has been suggested that the pseudo-LSD herpesvirus should be classified as bovid herpesvirus 2 (BHV2). In this article the virus will be called by this term to avoid confusion over the several names which have been applied. Isolates of BHV2 have been given titles depending on their origin such as Allerton (South Africa), bovine mammillitis (Britain), 69/1LO (Italy) and dermopathic herpesvirus (USA). A useful review of the BHV2 diseases is given by Cilli and Castrucci (1976).

Etiology

Bovid herpesvirus 2 is an enveloped particle with a double stranded DNA nucleocapsid of cubic symmetry with hollow capsomeres. The particle has a diameter variously estimated as 80 to 150 nm. Like other herpesviruses it replicates initially in the nucleus of infected cells and acquires a lipoprotein membrane during passage through the nuclear membrane.

The virus grows readily in bovine and lamb cells and in cat, rabbit and some human cell cultures. The virus will also grow in several cell lines such as BHK21 and MDBK. Cytopathic changes generally appear within one day to one week, depending on factors such as the concentration of virus. Large multinucleated cells develop in infected monolayers. Acidophilic Cowdry type A inclusions can be seen when stained with hemotoxylin and eosin.

Bovid herpesvirus 2 is inactivated at pH 3 and by treatment with ether and chloroform. It is sensitive to heat at 50 °C for 30 min and is destroyed by iodophore disinfectant at a dilution of 1/320 within 20 s.

Distribution

For many years infection with BHV2 virus was known only on the Continent of Africa, having been reported from South Africa, Kenya, Tanzania and Ruanda-Urundi. In 1963 the virus was isolated from cows in Britain and has since been found in other European and African countries, the United States and Australia. It is probable that BHV2 infection of cattle is worldwide.

Outbreaks of the disease are generally limited in extent and in the number of animals involved, though occasional outbreaks have included many herds within an area. Ten herds in one west district of Scotland were affected in one year, involving an average of 57% of cattle (Martin *et al.*, 1966).

Epidemiology

All ages of cattle (*Bos taurus* and *Bos indicus*) and both sexes are susceptible to infection with BHV2. With the exception of buffalo (*Syncerus caffer*) other animals do not appear to be naturally affected with the disease. Experimentally it is possible to infect rabbits and guinea-pigs which develop local skin lesions on intradermal or subcutaneous inoculation. Mice and rats up to 5 days of age will develop inflamed lesions of the skin, particularly of the extremities when inoculated with virus and many will die. It is doubtful if sheep can be infected but goats develop lesions from which virus can be reisolated; pigs also show skin lesions and fever.

Cattle skin lesions contain virus which in the serous fluid titers at $\log_{10}$ 6 to 7 per ml. Virus is also present in saliva, nasal discharge and milk, and has been recovered from leukocytes and occasionally from urine, feces and semen.

The transmission of peudo-LSD is unknown but fly-borne infection has been suggested. Because only intravenous inoculation results in widespread nodules, it is possible that an arthropod vector produces hematogenous infection. Virus was isolated from nymphs and adults of the tick *Rhipicephalus*

appendiculatus which were fed on buffalo experimentally infected with BHV2. Little additional information is available to explain the mammillitis form of disease which occurs mainly in dairy cows. In affected herds, lesions frequently appear first in primiparous cows (heifers) within about 3 days of calving. It was unlikely that infection occurred following calving and it was suggested that cattle were infected several weeks previously but failed to develop recognizable lesions. Depression of cell mediated immunity at the time of parturition may permit virus replication and the development of lesions. Supporting this theory, BHV2 was recently shown capable of latent infection for several months (Martin and Scott, 1979).

Spread of infection within herds may follow two patterns. In some herds few cows become infected, in other herds with many susceptible cows, most animals develop lesions. Mechanical spread appears to occur sometimes, presumably during the process of milking.

Transmission between herds is not readily explained because the disease may break out even in closed herds. Biting flies may act as vectors of virus even in temperate regions. *Stomoxys calcitrans* can be infected experimentally by allowing the fly to feed on infected fluids but transmission to cattle has not yet been proven.

Clinical Signs

Two clinical forms of BHV2 virus infection are known, multiple skin nodules (pseudo-LSD) and plaques and ulcers on the teats and mammary glands of

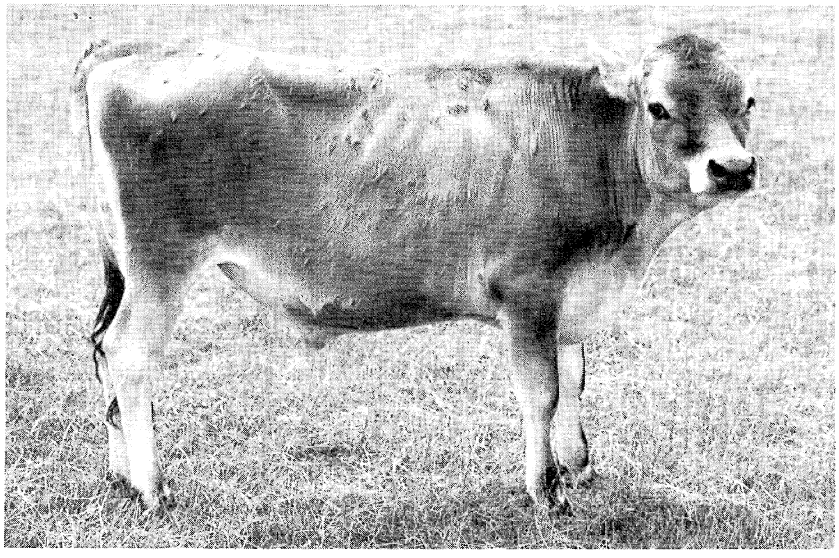

Fig. 1. Widespread lesions of experimental pseudo-lumpy skin disease.

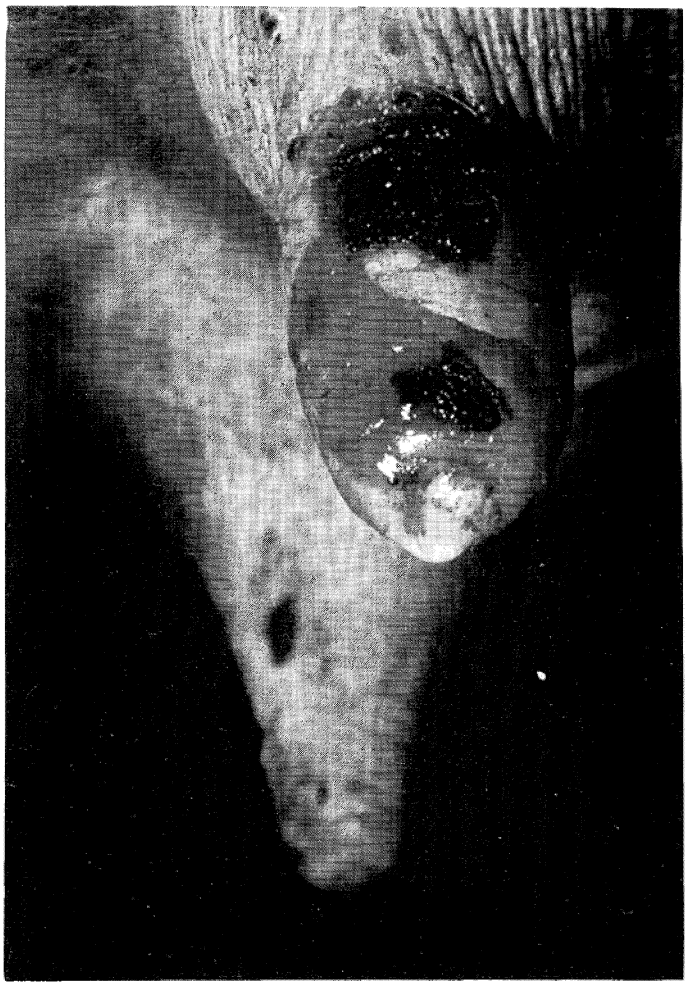

Fig. 2. Lesions of herpes mammillitis on the teats of a dairy cow.

cows (herpes mammillitis) (Figs. 1 and 2). The former is seen mainly in tropical regions, and may reflect a difference in the method of transmission, however, both skin nodules and mammary lesions have been noted in some outbreaks in Africa. All isolates of BHV2 virus will produce pseudo-LSD on intravenous inoculation. Intradermal or subcutaneous inoculation on the teat or other areas of skin generally results in the development of a local lesion only.

The incubation period following experimental inoculation of virus is 5 to 8 days for pseudo-LSD (Capstick, 1959) and 2 to 9 days for herpes mammillitis (Martin, 1973).

In the generalized form of the disease firm, round, raised nodules 1 to 3 cm in diameter with flat surfaces and slightly depressed centers appear on the skin of affected cattle. Some animals may have few nodules but others have widely distributed multiple lesions. The sites most frequently affected are the head, neck, back and perineum. The mammillitis form affects the teats, mammae and perineum. Occasionally a vesicle is seen in the early stages but usually the initial lesion is a raised circumscribed plaque involving the wall of the teat, sometimes causing distortion of its contour. The diameter of such lesions is usually 1 to 2 cm.

In the early stages of lesions, whether on the hairy skin or teat, some exudate occurs in which the content of virus is high. Necrosis of the superficial layers of the epidermis results in the formation of ulcers with irregular borders on the teat probably due to trauma from milking or sucking. The lesions are extremely sensitive and heal slowly with scab formation. Udder lesions are often more extensive and have large areas of necrotic skin coated with dried serum covering the udder. When this separates, large strips of dead skin peel off giving rise to the term "skin gangrene" of the udder.

On the hairy skin the exudate and necrotic superficial layers of skin form a dry scab covering each lesion. In time this scab separates with the dead hair, leaving a hairless pigmented scar in which the hair slowly regrows.

Lesions are seen on the muzzle and in the mouths of calves in infected herds. These generally form circular areas of necrosis or ulceration on the mucosa of the lips or rhinarium. Experimentally such lesions, as well as others in the nostrils, are seen in cattle with generalized infection. Bovid herpesvirus 2 ulcers are also found on the scrotum and vulvas of experimentally-infected cattle.

Pathology

In the early stages of lesion development, histological changes include severe inflammation of the epidermis and formation of syncytia. Eosinophilic or slightly basophilic intranuclear inclusion bodies are found in the syncytia and in cells of the strata germinitivum and spinosum. Severe epidermal necrosis rapidly follows with heavy infiltration of polymorphonuclear leukocytes, affecting hair follicles and sebaceous glands in the skin. Many cells contain inclusions which fill the nucleus. Within a few days these inclusion bodies become much less numerous, marked necrosis of the epidermis is still evident and there is ulceration of the dermis. About 10 days after the initial reaction much of the epidermis seems normal but patches of debris composed of dead epidermis, leukocytes and old hemorrhage are evident. By about day 30 most

of the epithelium appears normal; however, slight edema and inflammatory reaction persists in the dermis.

In the early lesions virus particles with single membranes pack the nuclei of infected cells. Virus with double membranes are seen in the cytoplasm.

Immune Response

In cattle infected experimentally, virus-neutralizing antibodies are generally detectable about 3 to 7 days, and titers continue to rise until about 3 to 4 weeks after the initial appearance of lesions. Peak titers are generally low ($<1/100$) and some studies indicate these decline within 2 to 4 months. Other workers claim that in infected herds antibodies are still present after 4 years. The difference in these findings may be explained by infection recycling within a herd or latent infection in certain cows with undetected episodes of disease.

Precipitin and complement-fixing antibodies are usually present 6 to 10 days after infection. Complement-fixing antibodies usually peak by 3 weeks then decline. Precipitin antibodies remain undiminished in intensity for at least one month. No information is available on cell-mediated responses in BHV2 infection.

It is likely that cattle are immune at least for several months after infection, although intradermal inoculation of virus in convalescent cattle may produce a transient local thickening of the skin.

Laboratory Diagnosis

Virus isolation can be used to confirm a diagnosis of BHV2 infection. For recovery of virus the exudate from early lesions is valuable. This may be obtained by swabbing or sucking the fluid into a syringe and needle, then placing it in virus transport medium which is kept cool during transportation to a laboratory. Scabs from older lesions are of less value than scrapings or biopsy samples.

Histological examination may confirm the presence of lesions typical of BHV2 infection. A rise in serum antibodies may be helpful in establishing evidence of infection but single serum samples are of little value except to confirm that the disease has been present in cattle.

Prevention and Control

No commercially produced vaccines are available. Live virus vaccines have shown protection against challenge infection for at least 8 months (Rweyema-

mu and Johnson, 1973). It should be emphasized, however, that latent infections with BHV2 do occur and live virus vaccines should be used only in infected herds.

Because the mode of transmission is unknown, the application of control measures is not possible. In dairy herds with BHV2 mammillitis, transmission throughout the herd may be controlled by hygienic measures such as milking affected cows last and dipping teat cups in iodophore disinfectant after milking each cow.

Treatment of affected cattle is difficult. Systemic antibiotics, corticosteroids and antihistamines have no apparent effect. Light applications of emollients prior to milking have been recommended to reduce trauma. Preparations of halogenated deoxyuridines, e.g., bromo-deoxyuridine (BUDR), have a specific inhibitory effect on the replication of herpesviruses and may be tried.

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14. EPHEMERAL FEVER

W. B. Martin

Abstract. Bovine ephemeral fever is a non-contagious arthropod-borne virus disease which affects cattle in many tropical and subtropical countries. The disease is short and generally lasts only a few days. Signs are fever and lameness. Epidemic outbreaks of the disease occur in Africa and Australia. Few characteristic lesions are evident at necropsy. Vaccines have been produced which will protect, but the protective immunity does not seem to endure.

Disease

Bovine ephemeral fever is an arthropod-borne virus disease of cattle characterized by a short acute febrile illness with rapid recovery and seasonal occurrence. Because of the brevity of the clinical illness, it is also known as "three-day sickness". Other synonyms are stiff sickness and in Japan, bovine epizootic fever.

Etiology

The virus of ephemeral fever is bullet or cone-shaped, measuring about 70 by 175 nm. Features similar to the rabies virus place it in the family Rhabdoviridae (Della-Porta and Brown, 1979). Morphologically, Australian and Japanese varieties of the virus appear to be bullet-shaped in contrast to the South African ones which are cone-shaped; antigenically they are related (Lecatsas *et al.*, 1969).

The virus can be adapted to grow in the brain of unweaned mice or hamsters where it develops by budding from the marginal membranes of infected neurons. Several passages in mice may be necessary before uniform illness and death occur. Virus will also replicate and produce cytopathic effects in baby hamster kidney (BHK21) and monkey (Vero and MS) cell lines. Ephemeral fever virus will form plaques in Vero cells under an agar

overlay. Cell culture systems do not appear to be more sensitive than mouse inoculation for the isolation of virus (Snowdon, 1970).

The virus contains RNA and is sensitive to ether and desoxycholate treatment. Virus infectivity is lost in 18 h at 37 °C or 120 h at 25 °C and within 19 min at pH 2.5, 60 min at pH 5.1 and 90 min at pH 9.1 (Heuschele, 1970).

Isolates of ephemeral fever virus appear to vary in their virulence for cattle following experimental inoculation (Tzipori *et al.*, 1975).

Distribution

The disease was first recorded in Central Africa in 1867 and is now known to be widely distributed in tropical and subtropical parts of the old world; it has been reported from many African countries, India, Pakistan, Palestine, Indonesia, Japan and Australia. Prior to 1936, ephemeral fever was unknown in Australia but since then there have been several major epidemics with lengthy intervening periods during which the disease was subclinical or only sporadic cases were seen. Similarly in Kenya, where a major outbreak was noted in 1968, epidemics occur only periodically.

Epidemiology

An excellent early account of many features of ephemeral fever is given by Mackerras *et al.* (1940). Ephemeral fever is recognized as a naturally occurring clinical disease only in cattle (*Bos taurus* and *B. indicus*) and domestic water buffalo (*Bubalus bubalis*). Though the precise arthropod vector is uncertain, minute, biting sandflies belonging to the family Ceratopogoridae are abundant in areas of Australia where the disease occurs and have been suggested as carriers of the virus (Mackerras *et al.*, 1940). More recently, ephemeral fever virus has been isolated from five species of the *Culicoides* midge. There is epidemiological evidence from Kenya that the distribution of antibody in cattle and game animals is similar to that *Culicoides* (Davies *et al.*, 1975). Ephemeral fever virus will multiply in mosquitoes of *Culex* and *Culicoides* after feeding on infected blood. Attempts to transmit ephemeral fever cyclically by means of the tick *Rhipicephalus appendiculatus* or by the interrupted bites of *Stomoxys calcitrans* have not been successful.

Outbreaks of the disease are known to occur a few weeks after rainy periods; in Kenya an association between epidemics and greater than average rainfall has been noted (Davies *et al.*, 1975). Vectors of the disease may

spread rapidly by wind; spread of the disease over a 3000 mile range within a 5-month period has been recorded.

Following infection, virus is present in the blood of cattle for about 5 days where it is readily available to blood-sucking insects. Direct intravenous inoculation of volumes of blood as small as 0.002 ml will result in disease in susceptible cattle, whereas subcutaneous or intradermal routes are less successful. Blood is infective immediately before, during and for about 4 days after the fever, and the virus seems to be associated with the leukocyte-platelet fraction. With repeated cattle passages the infectivity of blood appears to be reduced. Nasal discharge is occasionally found to contain virus as are spleen and lymph node, but the infectivity is low. It is unlikely that infection could be transmitted directly by nasal discharge.

Antibodies have been found in several species of game animals in Kenya, particularly buffalo and waterbuck, indicating that these animals as well as domestic cattle can act as reservoirs of the virus. Antibody present in game animals is believed to be specific for ephemeral fever virus. Illness in these animals has not been reported during major epidemics, suggesting that the disease is subclinical. As many extensive ranching systems in Africa carry wild game on their premises, it is possible that these act as amplifying or reservoir hosts of the virus. Sentinel herds in Australia have shown that virus can persist in herds without apparent disease.

Ephemeral fever does not produce disease in sheep, goats, horses, pigs, dogs, guinea pigs or chick embryos (Mackerras *et al.*, 1940). Day-old chicks cannot be infected. Antibodies do not appear to develop in sheep or pigs after experimental inoculation of virus.

Clinical signs

Following experimental inoculation, the incubation period is generally 2 to 4 days but can range from 2 to 10 days. Clinical signs start with a sudden fever reaching 103 to 108 °F, followed by nasal discharge, anorexia, stiffness, muscle twtchings, shivering, shifting lameness and even recumbency. Generally these signs are of short duration and recovery takes place within a few days. On the day the fever is highest, affected cattle are often completely anorectic, drink little though they appear to be thirsty and may dip their muzzles in water, and are extremely depressed so they stand with head down and ears flat against the neck. Sometimes a small trickle of saliva will run from the mouth and there is often a serous or sero-mucous nasal discharge. The respiratory rate is usually increased. On the second day of fever, stiffness and marked lameness can develop which may shift quickly so that lameness

occurs in different legs within a few hours. Occasionally lameness may persist for several weeks after other signs have disappeared. About 50% of affected cattle become recumbent for 8 to 24 hours but some remain down for days and may die or have to be killed. Older and heavier cattle may show the disease more obviously than younger ones and morbidity is usually greater in adult cattle than in calves. Swelling of the joints may be evident and milk cattle generally cease production, at least temporarily. There may be discomfort during milking and the milk may be watery and contain blood. Subcutaneous emphysema has been described as an exceptional complication and excitability and even aggression have been noted. A rapidly developing neutrophil leukocytosis has been described which coincides with the onset of fever (Mackerras *et al.*, 1940).

In epidemics 1 to 80% of a herd may develop signs of the disease and loss of milk production can be significant. During the 1955 epidemic in Australia 78% of 27 240 dairy cattle were affected in 394 herds. Approximately 90% of the cows and heifers but only 25% of the calves showed clinical signs (Spradbrow and Francis, 1969). Despite the alarming signs of illness, the disease is generally benign and recovery occurs rapidly with little mortality. Where mortality does occur it is usually in less than 2% of the affected animals and may result from prolonged recumbency, apparently due to motor paralysis and the resulting secondary infection or lack of water and dehydration. Colostrum-deprived newborn calves inoculated intravenously with virus develop severe clinical signs and viremia (Tzipori, 1975). There is no evidence that ephemeral fever virus causes abortion or damage to the fetus following either natural or experimental infection. Generally the sudden onset of symptoms shown by several cattle about the same time allows a diagnosis to be made. Confirmation may be obtained by virus isolation, the sub-inoculation of blood or in some instances the development of antibody.

Pathology

Since most cattle recover rapidly, few are available for necropsy and much of the pathologic information comes from experimental studies. Pathological changes are not always definite. There may be swelling and edema of the carcase lymph nodes, inflammation of the abomasal mucosa, enlargement of the spleen which appears reactive with the Malpighian corpuscles pouting from the cut surface, renal engorgement and a fibrinous exudate on serosae. A serofibrinous synovitis is often present with increased fluid, sometimes containing deposits of fibrin and a roughened synovia. Around the affected joints there is a periarthritis. The nasal mucosa may be intensely inflamed

and the lungs congested. There is edema of the brain and even hemorrhage on the surface of the brain and in the meninges. Focal necrosis of skeletal muscle and local necrosis of the skin have been described.

Histological examination of cases of ephemeral fever shows that vascular changes occur which vary in distribution and severity (Basson *et al.*, 1969). These changes consist of swelling and hyperplasia of endothelium, necrosis of the muscle coat of small arteries, perivascular cellular reaction and fibroplasia. Occasionally thrombosis of vessels in the muscle tissue occurs. Mackerras *et al.* (1940) noted in all cases engorgement of the lung, swelling of alveolar walls, collapse of and exudate in alveoli and bronchial and bronchiolar exudation. Sometimes these changes may be extensive. The lymph nodes throughout the body show dilatation of channels and evidence of activity in the germinal centers.

Immune response

Evidence regarding the duration of immunity following infection is conflicting. Some reports suggest that immunity is solid whereas other, particularly field observations from Africa, indicate that protective immunity may be as short as 6 weeks. Experimental challenge infection with the homologous strain of virus has proved that immunity can last for up to 2 years (Mackerras *et al.*, 1940) and antibodies may be present for long periods (Tzipori, 1974). An apparent correlation between the presence of neutralizing antibody and resistance to challenge infection has been noted. Some isolates of ephemeral fever virus show reduced virulence for cattle and do not multiply readily or produce a lasting antibody response.

At present there is no clear evidence that strains of virus from cattle differ antigenically, but isolates from mosquitoes in Australia were found to be antigenically different (St. George and Standfast, 1976). Recovered animals show complement-fixing and neutralizing antibodies in their serum.

Laboratory aids

Virus isolation may be attempted either by intra-cerebral inoculation of mice or by use of baby hamster (BHK21) or monkey kidney cell lines. The primary isolation of ephemeral fever virus is difficult; several virus passages may be needed before it becomes adapted to growth in cell cultures or mice.

Antibodies may be detected by serum neutralization or complement-fixation tests. Virus antigen can be detected in the cytoplasm of leukocytes in

smears from the infected blood of cattle by means of fluorescent antibody tests. The test is claimed to be a sensitive and accurate method for diagnosing the disease and could be used under field conditions (Theodorides, 1969). A hemagglutination test has not been described.

Prevention and control

Vaccines of different types have been used to immunize cattle against ephemeral fever. The rapid loss of pathogenicity for cattle following passage in mice or cell cultures has allowed the development of avirulent strains of the virus as possible attenuated vaccines. Most of these strains are still under test and some have given satisfactory results but as yet are not available commercially. In preparation of vaccines, high virus titers cannot always be produced and the antibody which results following vaccination may not be enduring. Field trials of virus passaged by serial transfer between calves and Vero cells have been undertaken (Tzipori, 1974). Viremia probably does not occur with many attenuated vaccines and spread by arthropod vectors seems unlikely.

Formalin-inactivated vaccines prepared from virus grown on cell cultures and adsorbed onto aluminum hydroxide gel have also been examined. Attenuated virus has also been adsorbed directly onto aluminum hydroxide. This process seems to inactivate over 90% of the virus (Tzipori, 1974). Two doses of vaccine stimulated higher levels of neutralizing antibodies and gave better protection against experimental challenge than a single dose. Vaccinal antibody responses, however, wane faster than those resulting from infection with virulent virus (Spradbrow, 1975). Control of ephemeral fever by means other than vaccination is not possible.

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15. BOVINE VIRAL DIARRHEA

Robert F. Kahrs

Abstract. Bovine viral diarrhea (BVD) is an endemic infection of cattle caused by an RNA virus of the genus pestivirus of the family Togaviridae. Infection is usually inapparent but can result in abortion or congenital anomalies, respiratory signs or a frequently fatal systemic disease (mucosal disease) characterized by diarrhea, necrosis, and erosions of the alimentary tract.

The infection can be diagnosed serologically or by viral isolation and the disease can be recognized by clinical signs and specific lesions.

Control is based on use of modified live virus vaccines.

The disease

Bovine viral diarrhea (BVD), sometimes called bovine viral diarrhea-mucosal disease (BVD-MD) or mucosal disease, was first recognized in the USA (Olafson *et al.*, 1946) when herd outbreaks of an acute, frequently fatal, rinderpest-like syndrome with ulcerations of the alimentary mucosa and diarrhea occurred. Concurrently, similar cases with variations in severity, chronicity and sporadicity were described as mucosal disease (Ramsey and Chivers, 1953). For some time it was thought that several different infections existed. The term "mucosal disease complex" describes a gamut of acute or chronic syndromes with crusting of the muzzle and ulcertaion, erosion, necrosis or hemorrhage in mucosa throughout the bovine alimentary tract. As diagnostic technology developed, it became evident that diseases diagnosed as mucosal disease have many causes including the viruses of BVD, malignant catarrhal fever, rinderpest, bluetongue, occasionally infectious bovine rhinotracheitis, and possibly other microorganisms.

The majority of BVD infections are non-clinical with biphasic temperature elevation and leucopenia followed by a specific immune response measurable by neutralization tests on serum.

Etiology

Bovine viral diarrhea is an endemic infection of cattle caused by an RNA virus classified as a pestvirus of the family Togaviridae (Andrews *et al.*, 1978). Some strains of BVD virus are easily isolated because of distinctive cytophatic effects (CPE). Non-cytopathogenic strains require use of indirect techniques, or immunofluorescent techniques for identification. The virus is immunologically related to hog cholera (swine fever).

Distribution

Serologic surveys indicate worldwide distribution of BVD virus. Antibody prevalences ranging from zero to 100% of adult cattle tested (with averages around 50%) have been reported in Africa, Europe and North America and the Middle East (Abraham and Barzilai, 1972). The virus replicates in swine and sheep (Ward, 1971) but these hosts are probably not highly significant in its transmission or perpetuation in the nature.

Epidemiology

Epidemiologically, BVD is characterized by widespread distribution, easy transmission, high antibody prevalence, frequent inapparent or undiagnosed infections, an irregular incubation period, and some chronic persistent infections.

Sheep and swine can be infected. Antibodies have been detected in deer and other wild ruminants. Cattle are probably the principle reservoir. Cow to cow contact probably explains most transmission. The presence of persistently infected (usually unthrifty) cattle probably aids perpetuation of the virus in populations. Continued addition of new susceptible animals to a herd, however, is equally important. A true latent persistent infection, capable of reactivation by corticosteroids, has not been demonstrated. On the other hand, steroid inoculation prior to exposure seems to intensify the clinical manifestations (Bittle and House, 1973).

Clinical signs

There is a variety of clinical disease patterns attributed to BVD. These vary in severity from inapparent non-clinical infection or mild febrile disease (often

suspected as being a respiratory disorder) to an acute fatal syndrome. A chronic debilitating infection can also occur. Sometimes losses occur in epidemic proportions but are usually sporadic and affect less than 1% of exposed populations. Abortion and congenital anomalies (Kahrs, 1973) may be the major economic impact of BVD. When totally susceptible populations are infected, however, the morbidity and mortality can be impressive.

In populations in which BVD is endemic, there is sporadic occurrence of clinical cases of a rinderpest-like disease characterized by fever, leucopenia, erosions of the alimentary mucosa, and an intractable diarrhea with dehydration preceding death. Such cases are usually 6 to 24 months of age and in contact with clinically normal herdmates undergoing inapparent infection. If carefully examined, these may be found to have sparse erosion or ulceration of the oral mucosa or epithelial sloughing of cheek papilla. Occasionally, as was the case in the first reported outbreaks, adult cattle may manifest a similar syndrome. When fatal cases occur among mature cattle, however, it usually involves a totally susceptible population with fatalities occurring in cows under stress of recent parturition or other disease. This suggests that clinical BVD should be considered a complicating syndrome associated with the animal's inability to resist an otherwise mild infection.

The chronic form of BVD develops as a sequel to either non-clinical infection or non-fatal clinical cases. These cattle manifest continuous or intermittent diarrhea, nasal discharge and sometimes a severly crusted muzzle. If examined repeatedly, these cattle can be seen to have oral necrosis and ulceration which heals rapidly and then recurs. Usually such cattle are unthrifty and unproductive and are culled.

Infections of intermediate severity are frequently diagnosed as nonspecific respiratory disease because elevated temperature, excess nasal discharge and rapid breathing are observed.

When it occurs simultaneously with other infections in feedyards or other intensive husbandry situations, BVD contributes to the bovine respiratory disease complex. When occurring singularly in non-intensive conditions, however, it is usually a mild endemic infection. Under these conditions, its economic signficance lies in its effects on the developing fetus.

If a pregnant cow is susceptible to BVD at the time of primary infection (clinical or non-clinical), a variety of fetal abnormalities can result (Kahrs, 1973). These include abortion (Carsaro *et al.*, 1971; Kendrick, 1971), fetal mummification (Kendrick, 1971; Scott *et al.*, 1973) and cerebellar and ocular disorders (Scott *et al.*, 1973). Many fetuses survive maternal infection and the nature of fetal disease appears related to gestational age at the time of fetal infection. Abortion and fetal mummification appears most commonly in fetuses infected in the first trimester. Congenital anomalies appear most

common in fetuses affected in the second trimester. Birth of weak or normal calves with humoral antibody produced *in utero* can occur in calves infected during the last trimester of gestation.

Pathology

The BVD virus has an affinity for lymphocytes, causing leucopenia and lymphoid depletion in lymph nodes and Peyers patches. It has been suggested the BVD infection has an immunosuppressive or immunodepleting effect (Johnson and Muscoplat, 1973) which contributes to its clinical role and its ability to participate as one component of multiple-cause syndromes (Heuschele, 1978). The lesions consist of hyperemia, necrosis, ulcerration and erosions of alimentary tract mucosa (Ramsey and Chivers, 1953). The classic lesions are found in the mouth (ulcers), esophagus (linear erosions), and Peyers patches (hemorrhage, necrosis or lymphoid depletion). In many cases classic lesions are sparce.

Immune response

Following primary infection, serum antibody, measurable by the virus neutralization and complement fixation tests, appears. Humoral neutralizing antibody is related to immunity (Robsen *et al.*, 1960; Shope *et al.*, 1978) and appears to be protective. Lack of detectable antibody has been associated with persistent viremia and chronic unthriftiness (Malmquist, 1968) and with altered responsiveness of humoral lymphocytes (Johnson and Muscoplat, 1973).

Colostrally acquired passive antibody affords partial protection which persists for 2 to 11 months, depending on initial titer (Kahrs *et al.*, 1966; Malmquist, 1968). Fetuses infected *in utero* can be born with active immunity (Braun *et al.*, 1973).

Laboratory aids to diagnosis

Virus isolation can be accomplished from nasal or conjunctival swabs from live animals or from spleen or lymph nodes harvested at necropsy. Cytopathogenic strains are readily detected in cell cultures and noncytopathogenic strains can be detected by immunofluoerescence or an interference test (Malmquist, 1978; Carbrey *et al.*, 1971).

Serologic diagnosis requires detecting development of antibody (seroconversion) or a significant rise in titer by comparing serum collected early in the course of infection and again 2 to 3 weeks later. Use of serology is limited because many clinically affected cattle die before developing neutralizing antibody and because chronic cases can persistently be infected and lack detectable antibody (Coria and McClurkin, 1978). Because of wide-spread antibody prevalence resulting from non-clinical infection, vaccination or colostral acquisition by neonates, testing single serum specimens has limited diagnostic value except as a retrospective means of eliminating BVD in herd problems.

Prevention and control

The ubiquity of endemic BVD infection and its ease of transmission make it unreasonable to attempt control by isolation of cattle.

A modified live virus (MLV) vaccine has been developed. Although controversial, it has widespread use. The vaccine should be administered after 6 months of age, by which time most colostrally-acquired maternal antibody has dissipated. Because of its abortifacient properties, it should not be administered to pregnant cattle. At one time, BVD vaccine-induced immunity was regarded as being relatively solid and affording protection for the lifespan of most cattle. This concept is currently challenged (Heuschele, 1978).

Aside from contraindications for pregnant cattle, the other safety concern associated with BVD vaccine has been developement of "post-vaccination mucosal disease" (Chennekatu *et al.*, 1967). This condition, resembling the clinical disease, appears within 3 weeks of vaccination. It is frequently blamed on vaccine and has been stated to be more prevalent when BVD vaccine is used in combination with other MLV vaccines. Actually, some so-called post vaccination disease is probably natural disease occurring in cattle already exposed at the time of vaccination.

It has been shown that calves treated with corticosteroids prior to vaccination have a higher probability of "post-vaccination mucosal disease" (Bittle and House, 1973). It has been speculated that clinical expression of the otherwise mild infection may require a certain immunologic ineptness on the part of the infected individual. Inactivated vaccines have been studied experimentally (McClurkin *et al.*, 1975).

Therapy of clinical cases is of limited value. Mild infections disappear spontaneously and severe cases frequently succumb despite use of antidiarrheals, antibiotics for control of secondary infections, and various other reme-

dies. Massive blood transfusion may prolong survival time but severe cases usually succumb when transfusions are discontinued.

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16. INFECTIOUS BOVINE RHINOTRACHEITIS

Robert F. Kahrs

Abstract. Infectious bovine rhinotracheitis (IBR) is a herpesvirus infection with diverse consequences including non-clinical inapparent infection, upper respiratory disease, conjunctivitis, lesions of the mucous membranes of the male and female reproductive tract, abortion, and occasionally encephalitis. The virus is readily transmitted and has worldwide distribution. It is perpetuated in populations by latent persistent infections which can be reactivated by stress.

The disease can tentatively be diagnosed on the basis of clinical signs and distinctive tissue lesions. Definitive diagnosis requires laboratory tests of which a variety are available.

The control of IBR is based on vaccination with inactivated or modified live virus vaccines.

The disease

Infectious bovine rhinotracheitis (IBR, necrotic rhinitis, rednose, infectious necrotic rhinotracheitis) was first described in 1955 as a new respiratory disease of feedlot cattle in western USA. Isolation of the virus was accomplished soon thereafter (Madin *et al.*, 1956). It was soon recognized in dairy cattle and later associated with infectious pustular vulvovaginitis (IPV) and abortion. Infectious pustular vulvovaginitis was present in Europe for years predating reports of IBR. McKercher (1963) speculated that IPV gave rise to viral strains with respiratory predilections and these evolved and thrived in feedlots.

Etiology

The etiologic agent, bovid herpesvirus 1 (Smith, 1976), or bovine herpesvirus 1 (Gibbs, 1977) is a member of genus *Herpesvirus* of the family Herperviridae (Andrewes *et al.*, 1978). Sometimes called the IBR-IPV virus, it has all

properties of herpesviruses and grows readily in a wide variety of cell cultures producing distinctive cytopathologic changes which are readily neutralized by specific antisera. It is a comparatively easy agent to isolate and identify.

Most IBR and IPV isolates are similar immunologically although the wide variety of clinical manifestations suggest that biotypes with affinity for various tissues may occur. Slight strain differences can be detected by immunologic methods (Potgeiter and Mare, 1974).

Distribution

The IBR virus is widely distributed. The virus has been isolated or antibody detected from cattle in all parts of the world (Gibbs, 1977). In addition to cattle, goats, swine, and water buffalo (Kahrs, 1977), the virus has been reported in a variety of wild ruminants throughout the world (Gibbs, 1977).

Epidemiology

Because of the ease of transmission and capacity for perpetuation, IBR is regarded as virtually ubiquitous among cattle populations.

The incubation period varies from 2 to 6 days depending on the dose, the route of inoculation and the criteria for indicating the onset of disease.

The infection is easily transmitted because large quantities of IBR virus are exteriorized in respiratory, ocular and reproductive secretions of infected cattle. The virus is perpetuated in populations by direct contact between infected cattle and by occasional reactivation of latent infections, resulting in a renewed shedding of the virus.

Feedlot cattle seem to have higher attack rates, more severe disease and higher fatality rates than range cattle or dairy cattle. These differences probably result from shipment, aggregation, social acclimatization, exposure to multiple pathogens and other stresses.

Clinical signs

A wide variety of clinical manifestations are associated with IBR. In addition to respiratory and reproductive tract infections, the virus can cause ocular and neurologic disease. The outcome of natural infection probably has many determinants including the biotype of virus, the dose and route of exposure or

inoculation, the immunologic status of the exposed animal and environmental influences.

In the respiratory form of IBR, cattle have elevated temperatures, reduced appetite, rapid respiration and upper respiratory dyspnea. Occasionally, blockage of the upper airways results in gasping for air. Affected cattle usually have profuse nasal discharge which is clear in the early stages and later becomes mucopurulent. Hyperemia and reddening of the nasal turbinates and muzzle occur. The visible portions of the nasal mucosa frequently have white necrotic plaques which result from the coalescence of pustules. These lesions are very suggestive of IBR.

The case fatality rate in respiratory IBR is low unless secondary bacterial infections, superimposed viral infections, or other complications occur. These conditions are more likely to occur in feedlots than in dairy herds or among cattle on range. When respiratory IBR occurs in pregnant susceptible cattle, abortions may occur immediately or up to 100 days postinfection.

Inflammation of the conjunctiva often accompanies the classic respiratory form of IBR. Sometimes there are outbreaks in which conjunctivitis, ocular discharge and occasionally corneal opacity are the principle manifestations of IBR infection (Rebhun *et al.*, 1978). Occasionally in young cattle, IBR infection casuses a non-purulent leptomeningitis and encephalitis characterized by incordination, occasionally circling or licking at the flanks, recumbency and death. This manifestation is extremely rare.

Infectious pustular vulvovaginitis (infection of the vaginal and vulva mucosa with IBR virus), is manifested by pustules and mucopurulent discharge. This disease, also known as blaschenausschlag, was reported in Europe many years before the recognition of respiratory IBR (McKercher, 1963). It occurs sporadically and occasionally as epizootics.

When the IPV infection is mild, it may go unobserved. If severe, it is manifested by failure of the cow to return the tail to the normal position after defecation or urination, indicating pain in the perineal area. There may be vulvar edema or mucopurulent discharge, white necrotic pustules on the mucosa of the vulva and vagina and pools of mucopurulent, usually odorless material on the floor of the vagina. It can be transmitted by natural breeding, by sniffing cattle, or by dogs licking the vulvas of cattle.

Bulls which breed cows with IPV can become infected and may develop a severe balanoposthitis with lesions similar to IPV. Virus from these lesions can contaminate semen and constitute a hazard in natural breeding or artificial insemination (White and Snowden, 1973). If semen is frozen, the virus may be preserved. There has been concern over IBR contamination of semen from healthy seropositive bulls with reactivated latent infections. Compared with likelihood of transmission by other means, this risk is minimal especially

if semen is collected from healthy bulls maintained in commercial studs with a rigorous veterinary program (Kahrs, 1977). "Insemination of susceptible cattle with semen containing IBR virus can produce endometritis, shortened estrous periods, and marked reduction in conception rates. On a herd basis, the seeding of hitherto uninfected herds with IBR is a concern, but its significance is unknown and may be comparable to the effects of vaccinating single individuals with live virus vaccines" (Kahrs, 1977).

The systemic form of IBR in neonatal calves frequently terminates fatally. These calves have respiratory distress and may have white necrotic lesions on the mucosa of the mouth, tongue, esophagus, and all four stomach compartments.

Pregnant cattle may abort following non-clinical infections or clinical manifestations. For abortion to occur, the female must be both pregnant and susceptible at the time of primary infection. Most fetuses aborted due to IBR are expelled in the last third of gestation, but fetuses exposed at any stage of gestation can be aborted.

Pathology

The infection produces a variety of lesions. The characteristic lesion is adherent whitish necrotic material raised above mucosal surfaces (Baker *et al.*, 1960). These lesions are frequently referred to as plaques. They result from coalescence of descrete pustules and consist of leucocytes, fibrin and necrotic epithelial cells. Intranuclear inclusion bodies are a histologic feature (Bruner and Gillespie, 1973).

Lesions may appear at the site of inoculation or at other target organs after systemic viral distribution by macrophages. In the respiratory tract, they are common in the trachea and nasal passages and cause upper respiratory disease. In addition to pustular lesions, the tracheal mucosa may be congested or may contain petechial or echymotic hemorrhages and mucopurulent material. On mucosal surfaces of the reproductive tract, the same lesion is called IPV in the female and infectious balanoposthitis in the male. In the parenchyma of organs, focal necrosis is the classic lesion. Fetuses and newborn calves are more likely to suffer serious systemic effects than mature animals (Baker *et al.*, 1960).

Immunity

Following natural IBR infection or vaccination with live IBR vaccines, cell mediated and humoral components of the immune system are activated (Davies and Carmichael, 1973).

The humoral response, usually measured by serum neutralization tests, has traditionally served as an indicator of past infection and as an indirect measure of resistance. Evidence has been accumulated suggesting that resistance following recovery from early IBR infection involves development of local tissue cell mediated immune response (Rouse and Babuik, 1974). Thus, presence of neutralizing antibody in serum is probably not an accurate indicator of immunity. Nevertheless, until superior alternatives to serology gain acceptance, neutralizing antibody will remain the basis for immunologic consideration of IBR. Thus, cattle with actively induced serum antibody can be regarded as partially immune. This partial immunity may be stimulated by natural infection or successful vaccination. It can persist for long periods but to be maintained at detectable levels, it may require occasional restimulation by exogenous exposure or endogenous viral release. Chow (1972) contended that partial immunity persisted for six years after experimental infection with a field strain virus.

Partially immune cattle can experience superficial infections of mucosal surfaces but are less likely to develop a severe systemic disease than susceptible cattle undergoing a primary infection. They are resistant to IBR abortion if immunization took place prior to pregnancy (Saunders *et al.*1972). Cellular immunity probably plays a dominant role in protection because cattle vaccinated with some inactivated vaccines do not withstand virulent challenge even though they have demonstrable antibodies (Schultz *et al.*, 1976).

Colostrally acquired antibody appears in serum of calves nursing immune dams. This passive immunity diminishes rapidly via metabolic degradation and its persistence varies from calf to calf in relation to the amount of colostrum ingested and the efficiency of intestinal absorption. Some calves lose their maternal antibody as early as a month of age and some have detectable antibody at six months of age.

The protective nature of maternal antibody is subject to some controversy. Controlled laboratory studies indicated its protective value (Langer, 1960), however, field observations indicate this protection may be overridden by severe challenge. It has been suggested that some vaccines (particularly intranasally administered products) are adequate to actively immunize calves with colostrally acquired passive immunity. There is no assurance, however, that vaccination in the face of maternal antibody will be successful. Therefore, calves vaccinated prior to four months of age should be revaccinated if long-term protection is desired.

Clinical and laboratory diagnosis

Clinical diagnosis of the respiratory form of IBR must be made with caution (Kahrs, 1977). The plaques (if seen), must be distinguished from mucopurulent material which is free-flowing or temporarily lodged in the nose, conjunctiva or vulva. This determination can be made by manually dislodging the material to see if it is an adherent lesion removable only by peeling off a layer of mucosa. The white necrotic lesions must be distinguished from the red punched-out ulcers of bovine viral diarrhea, rinderpest, and malignant catarrhal fever. These diseases can all resemble IBR and careful search must be made for the distinguishing features of each disease. Pulmonic pasteurellosis may be a sequel to IBR and the two diseases may occur simultaneously. Care must be taken, however, to avoid incriminating IBR as the principle culprit in pasteurellosis because this disease has multifactorial etiology and parainfluenza-3 virus and other viruses are equally important.

Laboratory confirmation of suspected IBR infection can be obtained by serologic tests, virus isolation, or fluorescent antibody (FA) staining of tissues. It is sometimes easier to diagnose by virus isolation or direct FA techniques because carefully timed paired serum are needed for serologic diagnosis. Care is essential in laboratory identification of virus isolates because other herpesviruses have been found in cattle (Smith, 1976).

Caution is recommended in ascribing primary etiologic roles to IBR virus isolates because of the occurrence of latent infections capable of reactivation. Therefore, for purposes of publication or legal testimony, an iron-clad diagnosis requires both virus isolation and a change in serum titer from negative to positive. This combination of diagnostic criteria indicates a primary infection and if clinical signs and lesions are compatible, diagnosis of IBR will stand the most rigorous criticism.

Specimens for the laboratory

The most appropriate specimen is dictated by the form and outcome of the disease. For the living patient with the respiratory IBR, the ideal specimen for virus isolation is a nasal swab which must be frozen or placed in viral transport media. Best results are obtained from early febrile cases in which nasal discharge is serous rather than mucopurulent. The mucopurulent discharge usually appears late in the disease after development of local and serum antibodies. In addition, mucopurulent material is frequently heavily contaminated. Specimens collected for virus isolation are inoculated into cell

cultures which are observed for cytopathic changes. Isolates can then be identified by serologic or FA procedures.

The diagnosis of IBR conjunctivitis utilizes the same techniques with swabs being collected from the conjunctiva rather than the nasal mucosa. In identifying IBR as the cause of encephalitis, a post-mortem specimen is necessary. Virus can be isolated from brain tissue by standard isolation procedures or identified by FA techniques. Isolation of IBR from the milk of mastitis cases can also be accomplished, however, milk is toxic for cell cultures and special techniques are required to eliminate toxicity.

Infectious pustular vulvovaginitis is diagnosed by similar laboratory techniques using swabs from vulvar or vaginal mucosa. The fatal septicemic form can be diagnosed by nasal or conjunctival swabs collected before the animal succumbs. When the gross lesions are observed at necropsy, however, spleen, lymph node, liver, brain, or forestomachs can be inoculated into tissue cultures. Necropsy materials are also highly amendable to FA technology using smears or thin frozen tissue sections for detecting virus. In addition, histopathologic examination of liver, or any other organ in which gross lesions are present, frequently reveals intranuclear inclusion bodies which support the diagnosis when lesions and signs are consistent with IBR.

The diagnosis of an abortion due to IBR is made by virus isolation or FA techniques (Shimizu *et al.*, 1972) using fetal liver, brain and spleen. Fetuses aborted from IBR rarely have focal necrosis of the mucosa of the forestomachs. Focal necrosis is usually not evident grossly but may be observed microscopically in liver and adrenals of fetuses. Whenever specimens are taken from a live animal, such as an aborting cow, it is essential that serum be tested. It is sometimes difficult to demonstrate a significant rise in titer. Unlike bovine viral diarrhea, IBR aborted fetuses rarely have measurable IBR antibody.

Prevention and control

Prevention of the disease by isolation of newly introduced cattle is usually ineffective; most control efforts are therefore based on use of vaccines which are available in a variety of forms and are used under a variety of circumstances. The modified live virus (MLV) vaccine for intramuscular inoculation (the first IBR vaccine developed) is still in use. It is easy to administer and is available in combination with other vaccines. It is contraindicated for use on pregnant cattle. Intranasally administered MLV IBR vaccines have gained widespread acceptance. Their major advantage is that they can be used safely on pregnant animals. Additional advantages are rapid protection due to inter-

feron production and rapid induction of secretory antibody at mucosal surfaces. An activated IBR vaccine combined with pasteurella bacterin and killed parainfluenza-3 vaccine is available in some parts of the world.

In most countries, the question of eradicating IBR is usually not considered seriously among disease control priorities because of economic considerations and lack of pathogenicity for man. Any nation embarking on eradication programs must suspect that other countries would probably not cooperate. They should ascertain that the virus is not present, limit imports to seronegative cattle, have a 90-day quarantine period for imported cattle, carefully screen semen imports, and blood test all cattle and slaughter any which are seropositive. The potential of reactivation of latent persistent infections and the potential of non-bovine reservoirs make IBR a difficult and expensive disease to eradicate from enzootic areas.

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17. LEPTOSPIROSIS DISEASES OF CATTLE IN THE TROPICS

Lyle E. Hanson

Abstract. Bovine leptospirosis is a disease of cattle caused by at least 23 serologically distinct serovars (serotypes) of *Leptospira interrogans*. Leptospires also cause disease in other domestic animals, wildlife and man.

Acute leptospiral infections in cattle are clinically recognized by fever, icterus, hemoglobinuria and agalactia. The signs vary extensively in incidence and intensity. Pregnant cattle infected during the last third of gestation frequently either abort or give birth to dead or weak calves. Chronic leptospirosis has been associated with infertility.

Diagnosis of leptospirosis is usually dependent upon serologic evidence correlated with an evaluation of clinical signs. Isolation of the leptospires from urine of recently affected cattle can often be accomplished with the use of special media or inoculation of laboratory animals such as the weanling hamster.

Cattle generally respond to treatment with streptomycin or tetracyclines if the administration occurs before lesions are advanced. Vaccination alone or in combination with antibiotic therapy can reduce spread of the disease when administered early in a herd infection. Vaccination with polyvalent leptospiral bacterins provides clinical protection if antigens of the homologous serovars of the region are contained in the bacterin.

Preventive measures should also include separation of the cattle from other livestock and fencing off pasture areas where surface waters have been contaminated by urine from other potential carrier hosts.

Disease

Leptospirosis is a major zoonotic disease which affects all domestic animals, wildlife and man. The disease is caused by a large number of related spirochetes belonging to the genus *Leptospira* (Amatredjo and Campbell, 1975; Johnson, 1956; Sullivan, 1974). As leptospires are often transmitted during occupational activities, synonyms such as rice field worker's disease, swine herder's disease and sugar cane worker's disease have been applied to human infections. Cattle infections are usually referred to as leptospirosis regardless of the causative strain (Michin and Azhinovi, 1935). In the tropics leptospiro-

sis has had a major impact on the health of man, domestic animals, and the cattle industry. Dependence on manual labor in agriculture, the abundance of rainfall and the absence of climatic variables have allowed the agent to propagate widely in tropical hosts and their environment (Johnson, 1956; Szyfres, 1976). A high incidence resulting from the widespread distribution of leptospires in a large variety of carrier hosts (Babudieri, 1958), and in the abundant surface waters in the environment have been complicated by the lack of available methods for diagnosis and control. Also in many tropical countries there has not been adequate information concerning host reservoir and modes of transmission (Szyfres, 1976).

Etiology

Leptospirosis is caused by over 100 leptospiral serologically distinct serovars (serotypes) which affect most mammals. The leptospiral serovars have been classified under 18 serogroups (Galton, 1975; Johnson, 1956; Sulzer, 1975). Twenty-three serovars from 11 serogroups have been isolated from cattle (Table 1). Nineteen of the serovars have been isolated from cattle in tropical or subtropical regions of the world thus indicating the significance of leptospirosis to cattle production in the topics. Furthermore, serologic evidence indicates additional serovars may be present in regions where laboratory facilities have not been adequate for isolation studies (Szyfres, 1976).

Leptospires, members of the genus *Leptospira* and the order Spirochetales, are long, very thin, filamentous organisms varying in length from 4 to 10 μm and 0.1 to 0.24 μm in width (Johnson, 1956) provided with closely wound spirals. Most leptospires have a hook at one or both ends and move by spinning on the long axis. The organisms are wound around an axial filament which is coated by a slime layer and an outer envelope. Leptospiral identification is based upon morphological and serological characteristics established by microscopic agglutination techniques utilizing either known antigens or antisera. All pathogenic leptospires are classified as serovars under the genus and species *Leptospira interrogans*. The saprophytic leptospires are grouped in the genus and species *Leptospira biflexa*.

Classification of leptospires has been based entirely on serologic differentiation. Other classical techniques such as biochemical reactions and conventional staining procedures do not provide differentiation. Serovars in each serogroup have common antigens but also contain additional antigenic characteristics which have been utilized for differentiation based upon degree of the agglutination absorption patterns.

Table 1. Leptospiral isolations made from cattle.

Serogroups (11)	*Serovars (23)*	*Countries (19) from tropics*
Icterohaemorrhagiae	icterohaemorrhagiae	Brazil, New Zealand, United Kingdom, U.S.A.
	copenhageni	Peru
Canicola	canicola	Argentina, Israel, U.S.A.
	galtoni	Argentina, Colombia
Pyrogenes	pyrogenes	Peru
Autumnalis	autumnalis	Japan, China
Australis	australis	Philippines
	peruveana	Peru, Japan
Pomona	pomona	Peru, Thailand, New Zealand, U.S.A., Australia, Argentina, Brazil, Chile
Grippotyphosa	grippotyphosa	Kenya, USSR, U.S.A.
Hebdomadis	hebdomadis	Japan
	balcanica	New Zealand, USSR
	gaicurue	Brazil
	hardjo	Canada, Italy, Australia, New Zealand, Argentina, Peru, United Kingdom, U.S.A.
	kremastos	Japan
	sejroe	Belgium
	szwajizak	Australia, U.S.A.
	wolffi	Brazil
Bataviae	argentiniensis	Argentina
	bataviae	Peru, China
	paidjan	Argentina
Tarassovi	tarassovi	USSR
Ballum	ballum	Venezuela, New Zealand

Although more than 100 serovars are pathogenic to animals, the degree of pathogenicity varies considerably with the host and the virulence of strains within a serovar. Organisms which have an opportunity for rapid serial transmission in a completely susceptible host species may attain a much greater virulence than similar organisms exposed to less susceptible host populations and environmental stresses.

Epidemiology

Although all pathogenic leptospiral serovars infect more than one host, some serovars such as *pomona* and *grippotyphosa* invade a variety of hosts. Others such as *hardjo* have been isolated primarily from cattle and occasionally from sheep and man. Generally, each leptospiral serovar involves one or more hosts which are the primary carriers. An effective carrier is readily infected without symptoms other than a persistent kidney infection which provide for shedding organisms in the urine during prolonged periods. The rat has been identified as the major carrier host of *icterohaemorrhagiae* in most areas of the world. It is frequently infected and often remains as an asymptomatic host while constantly shedding leptospires in the urine for periods of months to years. The rat is an especially active carrier in the tropics due to the high populations in cane fields and coconut groves which provide abundant food for large rat populations. In time of heavy rains leptospires are washed out of the agricultural fields and the flooding forces the rats out of the fields into nearby housing areas. Likewise, the leptospires existing in domestic animal carriers become important sources for potential exposure to man due to the high rainfall and contaminated surface waters in most tropical regions. Of the

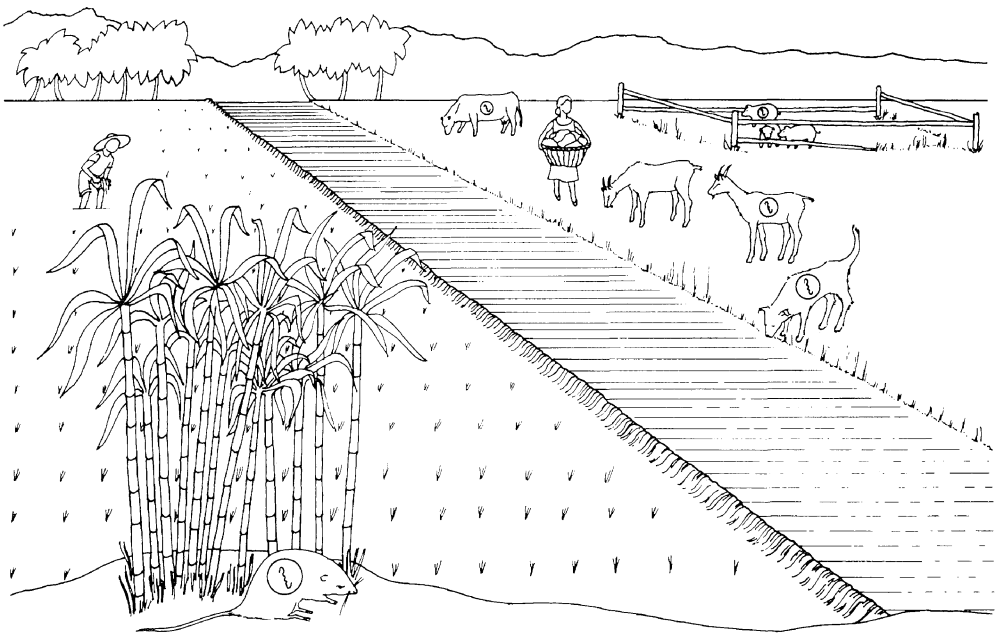

Fig. 1. Modes of transmission of leptospires between wildlife, domestic animals and man.

domestic animals, the pig has a greater potential of transmitting leptospires to cattle due to the large number of organisms which are shed in the urine for extended periods of time. Figure 1 illustrates the usual modes of transmission.

Leptospires appear to have a special affinity for the pregnant animal and the fetus. Leptospiral infections resulting in abortions have been recorded in all domestic animal species, wildlife and man. Leptospires are most likely to pass from the maternal circulation across the placenta late in the gestation period when the fetal attachments are less secure (Fennestad and Borg-Peterson, 1958). Fatal infections generally occur in the last trimester of gestation. Some infected fetuses, however, may survive prenatal infection and reveal it at birth. Serologically demonstrable antibodies have been demonstrated in the serum of newly-born infected calves (Fennestad and Borg-Peterson, 1957).

Infections can be transmitted by bulls either directly by infected semen or indirectly through urine contamination (Sleight and Williams, 1961). Leptospires have only been detected in semen during the acute stages of the disease when venereal transmission can occur. It is unlikely leptospires are transmitted by artificial insemination, although a few organisms may survive freezing and thawing procedures.

Leptospires can be tansmitted through the intake of contaminated foods if some animal consumes carcasses infected with leptospiras (Reilly *et al.*, 1970). However, the infected tissue must enter the stomach in large enough quantities to avoid deleterious effects of hydrochloric acid on the organism. In studies with five species, all but one, the raccoon, could be infected if they consumed leptospira-infected mice. The pig and dog can also be infected by oral route as they swallow large boluses of feed and occasionally by eating rats and mice.

Arthropod transmission is possible, but due to the generally short leptospiremia in most animals, it is not a major mode. If heavy tick infestations occur during an acute stage of leptospirosis, however, blood transmission could initiate a herd outbreak.

Leptospires are shed in the milk during the acute stage. Transmission can occur if the milk is consumed directly by the calf or drank by another host within a few hours of milking. Leptospires are inactivated in undiluted milk within 3 h after collection.

Most leptospires have a broad host range. Cattle frequently affected with a variety of serovars are hosts of such serovars as *pomona, hardjo* and *grippotyphosa*. Other serovars such as *icterohaemorrhagiae, pyrogenes* and *tarassovi* have only occasionally been isolated from cattle. Table 2 lists the host range of leptospires in the tropics.

Leptospiral infections are most extensive in herds which have been pre-

Table 2. Leptospiral serovars isolated from cattle and other animals in the tropics *.

Serogroup	*Serovar*	*Animal hosts*	
		Domestic	*Feral*
Autumnalis	autumnalis	cow, dog, pig	bandicoot [1] (sp.) brown rat [2] black rat [3] bandicoot rat 4
Australis	australis	cow, dog, horse, pig	bandicoot (sp.) short-nosed bandicoot [5] house mouse [6] water rat [7] Muller's rat [8] allied rat [37] brown rat black rat canefield rat [36]
	peruvaeana	cow	none
Bataviae	argentiniensis	cow	hairy armadillo [9]
	bataviae	cow, dog, pig	bandicoot sp. hairy armadillo short-nosed bandicoot house mouse Muller's rat brown rat black rat shrew [10]
	paidjan	cow, dog	hairy armadillo Azara's opossum [11] spiny rat [12]
Ballum	ballum	cow	house mouse Philander opossum [13] Pacific rat [14] brown rat black rat
Canicola	canicola	cow, dog, cat, horse, pig	Asiatic jackal [15] hairy armadillo short-nosed armadillo lesser Indian mongoose [16] Pacific rat brown rat black rat Cherrie's rat [17] Cotton rat [18] gray fox [19]
	galtoni	cow	none

Table 2. (continued)..

Serogroup	*Serovar*	*Animal hosts*	
		Domestic	*Feral*
Grippotyphosa	grippotyphosa	cow, dog, goat, horse, pig, sheep	short-nosed bandicoot guinea pig [20] (*C. pamparum*) hedgehog [32] bandicoot rat mole rat [27] (*B. gracilis*) water rat burrowing mouse [22] rice rat [23] brown rat cane rat [36] Pacific rat Muller's rat whitehead rat [21] shrew South American field mouse [26]
Hebdomadis	balcanica	cow	none
	guaicurie	cow	none
	hardjo	cow, sheep	none
	hebdomindas	cow, dog	brown rat
	kremastos	cow	none
	sejroe	cow, dog	lesser Indian mongoose
	szwajizak	cow	opossum [24] Eurasian hedgehog [26] short-nosed bandicoot spiny rat
	wolffi	cow	South American field mouse black rat
Ictero-haemorrhagiae	ictero-haemorrhagiae	cow, dog, pig, horse	Philander opossum opossum guinea pig (*C. aperea*) nutria [29] chimpanzee [31] Nile rat [30] mole rat (*B. gracilis*) mole rat (*B. malaborica*) [33] grass rat [34] spiny rat Pacific rat Muller's rat brown rat black rat whitehead rat
	copenhageni	cow, dog	brown rat

Table 2. (continued)..

Serogroup	*Serovar*	*Animal hosts*	
		Domestic	*Feral*
Pomona	pomona	cow, cat, dog, goat, horse, pig, sheep	guinea pig (*C. aperea*) guinea pig (*C. pamparum*) Panama spiny pocket mouse spiny rat black rat South American field mouse
Pyrogenes	pyrogenes	cow, dog, pig	hairy armadillo *Philander opossum* opossum house mouse rice rat black rat brown rat palm civet [35]
Tarassovi	tarassovi	pig, waterbuffalo	guinea pig (*C. pamparum*) opossum *Philander opossum* bandicoot rat

KEY FOR SCIENTIFIC NAMES

1. *Bandicoata* (sp)
2. *Rattus norvegicus*
3. *Rattus rattus*
4. *Bandicota indica*
5. *Isoodon macrourus*
6. *Mus musculus*
7. *Nectomys squamipes*
8. *Rattus mulleri*
9. *Chaetophractus villosus*
10. *Suncus luboneus*
11. *Didelphis azarae*
12. *Proechimys semispinosus*
13. *Philander opossum*
14. *Rattus exulans*
15. *Canis aureus*
16. *Herpestes auropunctatus*
17. *Zygodontomys cherriei*
18. *Sigmodon hispidus*
19. *Urocyon cinereoargentus*
20. *Cavia pamparum*
21. *Rattus whiteheadi*
22. *Oxymycterus quaestor*
23. *Oryzomys eliuris*
24. *Didelphis marsupialis*
25. *Erinaceus europaeus*
26. *Akodon arviculoides*
27. *Bandicota gracilus*
28. *Cavia aperea*
29. *Myocastor coypus*
30. *Arvicanthis niloticus*
31. *Pan troglodytes*
32. *Hemiechinus* (species)
33. *Bandicota malabarica*
34. *Arvicanthis nilaticus*
35. *Paradoxurus hermaphroditus*
36. *Rattus conatus*
37. *Rattus assimilis*

* Information compiled from references 5, 13 and 14.

viously free of leptospirosis and have not been vaccinated. Following an acute infection, some cattle recover rapidly while others retain agglutinins for many years. During subsequent exposures, these cattle usually do not exhibit major

clinical signs and often show little laboratory evidence of the disease. Herd immunity is a significant factor in interherd cattle infections as serial transmission in susceptible cattle is apparently necessary for development of highly severe forms of the disease.

Pathogenesis

Leptospires are carried by the blood from the portals of entry to the liver where primary multiplication occurs. During the initial bacteremia the first clinical evidence is a rise in body temperature. At this time a generalized leptospiremia occurs, with leptospires present in most tissues and especially the brain, kidney, and lungs and in the uterus and mammary gland in the cow and the testicles in the bull. Secondary multiplication occurs generally in the kidney and brain tissues where leptospires persist for days to weeks and in some cattle up to 1 year.

The initial kidney infection is characterized by edema, hemorrhages in the glomeruli, and cloudy swelling and necrosis of proximal tubular epithelium. In severe cases, the glomerular and tubular changes are so extensive that renal shut-down can result. The degenerative changes are postulated from the direct action of a toxin or a combination of toxin and bile salts. Deaths in acute leptospiral infections are due primarily to severe kidney lesions although concurrent hepatic lesions can contribute to fatal infections.

As the disease progresses in many animals, the infection becomes subacute with interstitial infiltrations of lymphocytes and plasma cells. The glomerular lesions consist of thickening of the Bowman's capsule wall and infiltration of monocytes, neutrophils and lymphocytes. Casts and cell debris are frequently observed in the distal tubules.

In chronic infections of the kidney, the most prominent lesions are glomerular atrophy and interstitial infiltrations with lymphocytes, plasma cells and fibroblasts. In all stages of infection, the tissue changes do not necessarily correlate with the degree of leptospiral shedding, as in some animals, large numbers of leptospires may be shed in the urine without concurrent significant lesions in the kidneys.

Liver lesions are usually a focal necrosis accompanied by mononuclear cell infiltrations in animals with marked icterus. Bile ducts contain the cell debris. All the acute signs are suggestive of a leptospiremia and toxemia. Some limited studies have suggested that exudation of plasma and erythrocytes into the adjacent tissue may result from capillary damage.

Clinical Signs

The first sign most consistently detected in the disease process is an elevation of body temperature of 2 to 5 °F which usually persists from one to several days. The temperature elevation is often associated with malaise, anorexia, conjunctivitis, and anemia. In the most severe cases, jaundice, pneumonia, and hemoglobinuria occur and encephalitis manifested by rigidity of the head and neck may also be present. Fatal infections are generally due to severe renal degeneration.

During the acute infection in lactating cattle, production of yellow clotted milk, which may be stained, occurs early followed by agalactia which in most cases persists from a few days to 2 weeks. Although most cattle return to a normal level of lactation in 2 to 3 weeks, some fail to reach their full potential during the entire lactation period.

The most commonly associated signs of leptospirosis are abortion and stillbirths which occur 1 to 3 weeks following onset of clinical signs. Interruption of gestation is most frequently associated with infections originating during the last third of the gestation period. Fetal infections can result in fetal antibody responses detectable by the microscopic agglutination tests in either aborted fetuses or stillborn calves.

Infertility has often been associated with the less acute disease most frequently caused by members of the *Hebdomadis* serogroup. Annual vaccination of the entire herd with a homologous serotype bacterin has reduced these infertlity problems. It is postulated that IgG stimulated by vaccination can pass through the tissues into the uterus and aid in controlling the uterine infection.

Encephalitis probably occurs in most infections. Leptospires can constantly be isolated from brain tissue although clinical signs of encephalitis are observed infrequently (Hoag and Bell, 1954). Headache, a frequent symptom recognized in man, cannot be identified in cattle although irrational behavior of some animals has been reported.

Anemia which is more common in ruminants than other animals can be marked along with jaundice in the most severe infections caused by such serotypes as *pomona* and *grippotyphosa*. Hemoglobinuria is also present in many of the same cattle (Michin and Azhinovi, 1935).

Clinical evidence of nephritis occurs in both the acute and chronic forms of the disease. In acute leptospirosis, nephritis is recognized by hematuria and the presence of cellular debris in the urine. Anorexia is indirect evidence of the disease. Usually a decrease of specific gravity of the urine is the only constant evidence of chronic leptospirosis. In severe leptospirosis, however, the urine often contains tissue casts and albumin.

Anuria is a critical sign which is often followed by death. Jaundice, an indication of liver damage, is also frequently present in fatal infections although the renal shut-down is the most critical consequence of the disease.

Pathology

The initial changes occur in the liver, usually in the form of scattered necrosis of hepatic cells, bile retention, and infiltration with mononuclear cells. Although in most severe liver lesions, extensive necrosis is present along with hemorrhage and considerable bile retention due to occlusion of bile ducts with cellular debris.

Kidney lesions generally contain the most critical changes in acute leptospirosis which are concentrated in the glomeruli and proximal convoluted tubules. The lesions consist of cell necrosis, and hemorrhages, which vary from petechial to eccymotic hemorrhages on the cortical surfaces (Fig. 2). Leptospires are most frequently demonstrated in the lumen of the tubules and the blood in the glomeruli. As the disease progresses, the infection often localizes in the kidney producing subacute and chronic lesions which are evident grossly as white foci on the surface. Chronic microscopic lesions

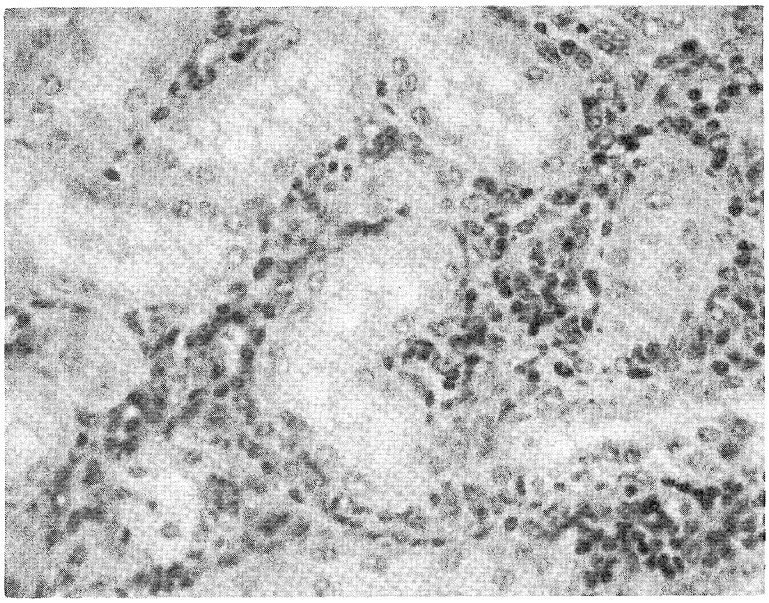

Fig. 2. Kidney section of a calf naturally infected with *L. pomona* showing interstitial nephritis. 300× (D. N. Tripathy).

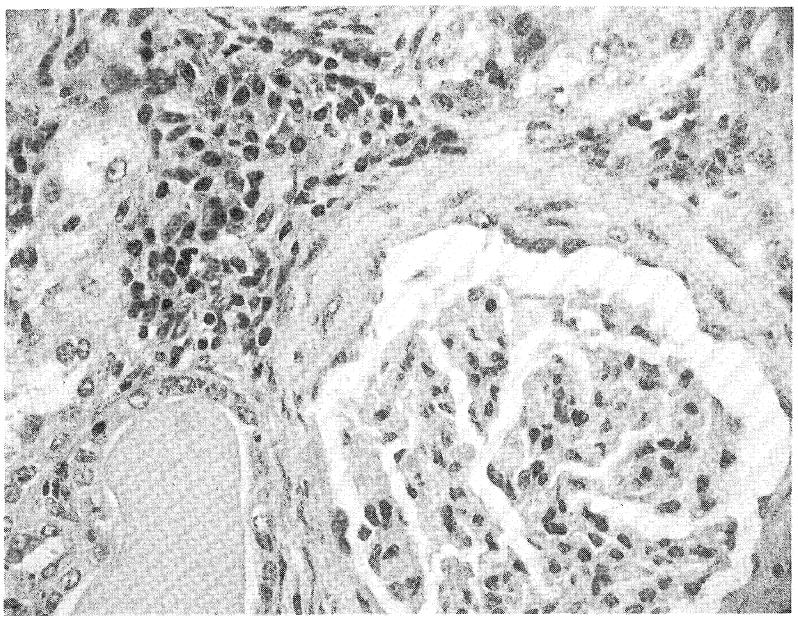

Fig. 3. Kidney section of a cow naturally infected with *L. hardjo* showing chronic glomerular changes and interstitial nephritis characterized by mono-nuclear cell infiltration and hyaline tubular cast. 300× (D. N. Tripathy).

consist of thickening of the Bowman's capsules, atrophy of glomerular tufts, interstitial infiltration with lymphocytes, plasma cells and fibroblasts, casts of cellular debris and dilation and atrophy of some tubules (Fig. 3).

Leptospires actively invade the uterus and fetus during acute infections (Fennestad and Borg-Petersen, 1958). The lesions present in cattle where abortions and stillbirths occur are generally mild with predominantly edema of the placental tissue and some necrosis of the cotyledon tissues. Retained placentas occur rather frequently in cattle infected late in the gestation period, apparently due to local tissue lesions. Infertility associated with chronic leptospiral infections has not been associated with extensive uterine changes although mucosal changes have been demonstrated in the uterus of infected nonpregnant animals.

Production of abnormal milk and agalactia are frequent signs associated with acute leptospiral infections in cattle. Mammalian infections have not been associated with extensive inflammatory reactions although little information is currently available concerning the histologic changes.

Acute pneumonic lesions often occur in experimental infections suggesting similar lesions occur in natural cases. The lesions consist of hemorrhages and inflammatory cell infiltrations. Although lung lesions are usually not extensive, activation of latent bacterial infections may result under field situations.

Immune Response

Antibody response is usually detectable with an agglutination test within 3 to 7 days after the onset of the first clinical signs. The agglutination titers increase rapidly with the greatest response usually detected in 7 to 14 days. The agglutinating titers will then persist from a few weeks to as long as 10 years in a few cattle. The primary agglutinating response is contributed by IgM class of antibodies while the IgG antibody response appears somewhat later. The latter antibodies have a neutralizing ability which can be detected and measured with either a hamster protection test or growth inhibition test. The IgG class of antibodies can be detected in significant concentrations following vaccination with leptospiral bacterins even when no agglutinins are detectable (Tripathy *et al.*, 1975). The IgM or IgG antibodies react to a limited extent with other serovar antigens within serogroups and a trace reaction occurs between members of other serogroups. Only minor cross protection is provided between serovars within a serogroup.

Laboratory Aids to Diagnosis

Diagnosis based only on clinical signs is unreliable as the clinical signs vary extensively. It is recommended, therefore, that a diagnosis involves evaluation of a good history, clinical signs and laboratory findings. A thorough understanding of the pathogenesis of the disease is important in order to select appropriate samples from each stage of the disease. In the acute disease, during the febrile response, leptospires may be isolated from the blood during the first few days either in appropriate liquid or semisolid media or by inoculation of laboratory animals. Special media, usually containing either serum or bovine albumin, should be incubated at 30 °C. Weanling hamsters are susceptible to more serotypes than other laboratory animals. Darkfield examination of serum or plasma is generally misleading due to possible confusion with fibrin particles (Johnson, 1956).

Although leptospires have been isolated from milk, semen, and cerebral spinal fluids during the initial stages of the disease, most routine laboratory attempts to do so are unsuccessful. Urine samples are generally the most appropriate source of leptospires for either culturing or animal inoculations. Leptospires are shed in the urine starting a few days after the onset of disease and either intermittently or continuously for several weeks for as long as a year. Darkfield examination of the urine may be successful when large numbers of the organism are present and their typical motility can be observed. Urine should be collected in sterile containers for isolation techniques and

inoculated into appropriate media or laboratory animals as soon as possible, preferably within an hour of sampling. Leptospires become immotile and are inactivated in less than 3 h in undiluted urine. When urine cannot be cultured immediately upon collection, the urine should be diluted 1 to 10 in a sterile liquid transport medium and held at refrigeration temperatures. Liver, kidney, and brain tissues provide the most consistent source of leptospires from a necropsied animal. In the febrile stage, leptospires can be isolated from blood and most visceral tissues. Tissues should be cultured immediately upon collection although they can be held at refrigeration temperatures for several hours with fair results. In no case should the tissues be frozen. Leptospires are seldom isolated from either aborted or stillborn calves, probably because of advanced tissue putrifaction. Serum of aborted fetuses or stillborn calves, however, may contain agglutinins which are diagnostically significant.

Serologic testing is the laboratory procedure most frequently utilized to confirm clinical diagnosis. Leptospiral antibodies (IgM) appear in the serum within a few days after onset of illness and persist for weeks to months and, in some cases, years. Demonstration of an agglutination response in serum dilutions of 1 : 100 or greater are indicative of exposure to leptospiral antigens. In the absence of vaccination, microscopic agglutination (MA) titers indicate a current or previous leptospiral infection. A rising titer in paired serum samples indicates a current illness when samples of acutely infected and convalescing animals are tested. Serum samples of an acute infection would not be available, however, if the illness was not recognized prior to an abortion. Titers at 1 : 100 may persist for 2 to 3 months after vaccination.

Tissues collected for histologic examination should be stained with a silver stain (Levaditis technique) as analine dyes do not adequately impregnate leptospires. Preparations stained by fluorescein conjugated antibody should be interpreted with caution as nonspecific staining has frequently been observed.

Prevention and Control

An understanding of the host-agent-environment interaction in domestic animals and wildlife of the region is imperative in developing a valid control program. Exposure of cattle to contaminated surface water should be considered in developing this program. In the tropics, extensive distribution of leptospires in domestic animals and wildlife provides many opportunities for transmission of a variety of leptospiral serovars in cattle. Complete confinement is the only procedure which can assure reasonable protection from exposure for a herd of cattle. All ponds and streams are potential sources of

leptospiral exposure. Rodent control is very important due to the high incidence of leptospires in rats and mice (Table 2).

Herds with stable populations that only receive herd replacements after testing, prior to, and following an isolation period can reduce introductions of the hebdomadis serogroup of leptospires. This group is primarily restricted to cattle. All cattle introduced into a cattle herd should be serologically tested for all potential serovars prevalent in the region where the replacements originate. Other livestock, particularly goats, should be considered as potential carriers and therefore be tested for leptospiral antibodies. Clinically normal carrier cattle remain potentially the most likely source of a new infection.

Treatment

As soon as acute leptospiral infections are suspected, the affected animals should be isolated from the herd. All secretions should be suspected of containing leptospires. No milk, unless boiled, should be fed to other animals or consumed by persons. As urine usually contains leptospires from a few days after onset of signs up to several months after onset, owners should be cautious to avoid exposure. Infected animals should be placed in confined areas which provide comfort and isolation.

Usually, initiation of antibotic therapy as soon as possible to exposed animals in a herd will significantly reduce clinical signs and mortality. Administration of either dihydrostreptomycin at a dosage of 25 mg/kg or tetracyclines at 200 mg/lb of body weight daily for 3 to 5 days provides the most effective therapy (Stalheim, 1969). Intramammary infusion of antibiotics has not been shown to affect the severity or length of mammary infections.

As the severity of the disease increases, response to antibiotic therapy decreases due to the lesions in the liver and kidney tissues. Blood transfusion from an animal recovered from the disease will contain adequate antibodies to provide some assistance. Therapy instituted at the time of an abortion will have no effect on the clinical course of the disease because the acute infection and death of the fetus occurred earlier. Administration of antibiotics to affected cows and other cattle in a herd following a leptospiral abortion, however, can reduce spread of the infection, decrease signs of the disease in the remaining cattle and reduce the number of abortions.

Antibiotic therapy administered in chronic infections reduces the shedding but apparently does not reduce the antibody titers of affected animals. Combination of antibiotic therapy with administration of bacterins generally reduces infertility problems. Also, concurrent treatment given to all animals

in the herd during acute infections has been shown to be an effective procedure to reduce abortion losses.

Vaccination

Vaccination of cattle with regional leptospiral serovars has been demonstrated to be an effective control procedure (Hanson *et al.*, 1972). As the protective antibodies induced by leptospiral bacterins are serovar specific, it is important to determine the serovars prevalent in the region. This can be a difficult diagnostic problem if only serologic information is available. Cross agglutination occurs between serogroup members. Leptospiral isolations followed by cross absorption tests for identification of the isolates provides the only effective method of serovar determination. Bacterins should be administered to calves after 3 months of age and annually thereafter. In endemic herds where new herd additions are common, vaccination at 6-month intervals is suggested. Herd immunity is an important factor in leptospirosis control, therefore, the entire herd should be vaccinated. Vaccination can be discontinued when the herd becomes serologically negative but only if the prevalent serovar infection is limited to cattle. In most regions, however, more than one serovar is prevalent and wildlife is usually infected, requiring continuation of annual vaccination as a prophylactic procedure.

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18. BOVINE GENITAL VIBRIOSIS

Alvin B. Hoerlein

Abstract. Bovine genital vibriosis is worldwide in distribution. The disease is transmitted venereally and causes temporary infertility in the female. The pathogenesis is described in detail to form a basis for tentative diagnosis of the disease in the infected herd. The diagnosis must then be confirmed by laboratory methods. Control procedures including exclusive artificial insemination and vaccination are described.

Bovine genital vibriosis is an important cause of temporary infertility of female cattle. The disease is venereal and spread from infected to noninfected animals during breeding.

Vibriosis is caused by *Campylobacter fetus* subsp. *fetus*. This bacterium has been previously known as *Vibrio fetus* var. *venerealis*. The organism is a motile, Gram negative, curved rod which grows under reduced oxygen tension and produces 1 mm smooth colonies on blood agar plates in three to five days when incubated at 37 °C. Growth in semi-solid media is 2 to 4 mm below the surface.

Vibriosis appears to be worldwide in distribution. While most attention has been paid to the disease in North America, Western Europe, Australia, New Zealand, and South Africa, diagnoses have been made wherever the disease has been studied. It is probable that if adequate diagnostic procedures are used the disease will be found wherever there are cattle.

Transmission

Previously noninfected female cattle of all ages and breeds are uniformly susceptible to infection. Vibriosis is spread during the act of breeding and not by other contacts. The infected bull places semen and *C. fetus* into the anterior part of the vagina and the cervix of a susceptible female during breeding. The bacteria multiply in the cervix and progress into the body of the uterus and the oviducts. The fertilized ovum is killed by action of the

bacteria and the female returns to estrus. She is infertile for a variable number of estrual periods up to nine months. Noninfected bulls become infected when breeding an infected female and may remain infected transiently or permanently for years. There is a degree of convalescent immunity in recovered females. This immunity is variable in length and in the absence of reinfection, wanes with time. The female may carry the infection through a successful calving and become the source for the infection of bulls. Under our conditions it is probable that the carrier cow is the main source of continued herd infection year after year.

Clinical Manifestations and Diagnosis

The clinical signs of vibriosis are subtle and easily missed in ordinary management. The infected females return to estrus at regular intervals until recovery from infection. In our experiments more than half of the heifers returned to estrus at the normal interval after initial exposure to infection. A number of others returned to observed estrus at intervals divisible by 21 days, suggesting the first estrus was missed. Irregular estrual cycles are more common in females that have calved previously. There is no exudate since the embryo dies so early after fertilization. There are no clinical signs in the bull except in the herd situation where he rebreeds the females over and over until eventually his breeding capacity is exhausted. After a period of rest the bull will again breed estrual females.

The most useful method of making a diagnosis of vibriosis is a thorough study of the herd history. If cattle are closely observed, the return to estrus is noted. Usually this is not observed and in seasonally bred herds common in our beef cattle herds the first sign is often found when pregnancy examinations are made in the fall. A large number of nonpregnant animals suggests a fertility problem. Due to the variable recovery time of individual animals, a variation of fetal age is observed during pregnancy examinations. If not examined after the breeding season, similar signs will be observed during the calving season. Depending on many different management practices, the calving rates will vary from 10% to 80% and most notable is the prolonged calving season. This results from the variable time that individual females recover from the infection. Ranchers often recall a year when the herd calved all summer. This is then repeated since those cows which calve late will be bred late and calve late the next year.

Where all of the bulls were infected and the heifers all susceptible from the beginning of breeding, 20 to 30% pregnancy resulted in a 60 day breeding

season. This type of situation has not been common in our experience. In the more typical case, the beginning of breeding finds the herd consisting of a population of infected and noninfected bulls and susceptible, carrier, and immune females. Thus, there are problems in arriving at a tentative diagnosis of vibriosis. It is common practice in our beef cattle herds to use young bulls to breed the heifer replacements. Without previous exposure to infection, these breeding groups often have good reproductive performance (Carroll and Hoerlein, 1972). Heifers with their first calf are then usually bred to the older herd bulls in the main cow herd. Since they are still susceptible to infection, the lowest pregnancy rates are seen in the heifers being bred for their second calf. Chronically infected herds often have annual calving rates of 70 to 85% as revealed when prophylactic vaccination was used to prevent the introduction of infection from neighboring infected herds. In these herds the heifers had a short calving season with good pregnancy rates and eventually when the old late-calving cows were culled, the whole herd had a satisfactory calving performance.

The herd problem is little different in dairy cattle when natural breeding is used. In numerous herds artificial insemination is practiced, except that bulls are used for natural service for heifers and cows not pregnant by artificial insemination. The disease is often not suspected until the heifers expected to freshen are found to be nonpregnant. This can cause a serious loss in the expected milk production base.

Pathogenesis

While the pathologic changes in vibriosis are not spectacular, they are basic to the pathogenesis of the infection. They are essential to an understanding of the disease and especially in its diagnosis and control. Following breeding when semen and the bacteria are deposited in the area of the cervix, *C. fetus* multiplies, and by the fifth day may be found in the body of the uterus. The ovum is fertilized, but in 10 to 15 days the organisms are found in the oviducts. The fertilized ovum is apparently killed by direct action of the organisms. There are no signs of disease and no exudate will be noted since the embryo is killed at such an early stage. It is suspected that the pathogenesis of infertility in subsequent breedings may be quite different. Fertilization may not occur due to the mild endometritis and salpingitis which are present. Recently it has been found that *C. fetus* acts on the cilia of the epithelial cells of the oviduct (Stalheim and Gallagher, 1975). There is mild inflammatory infiltration of the endometrium with accumulation of leukocytes in the uterine glands. A neutrophilic response is found in the uterus, cervix, and

vagina by the seventh day. Neutrophiles with phagocytized organisms were found in the deep inguinal lymph nodes by the fifteenth day.

Following exposure to infection, the number of organisms increases sharply. In about half of our experimental heifers, *C. fetus* was recovered from cervicovaginal mucus as early as one week after exposure. From three weeks to three months the growth of *C. fetus* from cervicovaginal mucus was almost confluent on plates, indicating that myriads of organisms were present. Later the number of organisms was reduced until only a few were found. Individual heifers might be negative one week and positive the next. As animals recover from the infection and conceive, the number of positive cultures in the herd decreases.

Some cows will carry the organisms through the entire gestation period, deliver a normal calf, and subsequently be a source of spread to bulls when rebred after calving. *C. fetus* has been recovered for as long as 464 days after exposure and for 229 days after parturition.

Abortion of larger fetuses may occur at any time during gestation. We have found several near full-term dead or weak calves which were positive for *C. fetus*. The number of such abortions appears to be very low in most herds, 3 to 5%. The pathogenesis of late abortions is unknown, but placentitis is the cause of fetal death.

Bulls become transient or permanent carrier of *C. fetus* after breeding infected females. The organisms are superficial contaminants of the bull's external genitalia and produce no pathologic changes. The epithelial crypts of the glans penis and prepuce provide a favorable environment for growth of the organism. Contamination of semen with *C. fetus* in bull studs is due to mechanical removal of the organisms by the artificial vagina during collection. While some bulls have carried *C. fetus* for as long as six years, many are not susceptible to permanent infection. When we tested 47 bulls from heavily infected herds by test-heifer mating we found only five to be permanently infected. The length of time that transiently infected bulls transmit the infection is not known. Because of the high numbers of organisms present during the first months of infection, a bull becomes grossly contaminated when rebreeding an infected female and would be expected to carry the infection to another cow by mechanical means. We have observed infected bulls to cease transmitting the infection after a few weeks of rest.

It has been shown that the permanent carrier state in bulls is age-related and that susceptibility increases with age. Bulls more than five years of age were highly susceptible to permanent infection which may be related to an increase in size and number of epithelial crypts in the penis of older bulls. No inflammatory changes were found in the genital organs.

Immunity

After recovery from infection, cows are resistant to reinfection and usually have a high degree of fertility even if bred to infected bulls. This convalescent immunity is partial and when reexposed to infection, *C. fetus* can be isolated from cervicovaginal mucus for several days or weeks. Conception usually occurs and a normal gestation is the rule. The mechanisms responsible for convalescent immunity are not understood, but it appears to depend on local phenomena. It has been shown that convalescent immunity decreases with the passage of time and that after three years of breeding to negative bulls, these cows are almost completely susceptible to reinfection (Hoerlein and Carroll, 1970). Convalescent immunity appears to be reinforced by reinfection at a subsequent breeding to an infected bull.

Isolation of the Organism and Detection of Agglutinins in Vaginal Mucus

A tentative diagnosis of vibriosis made from a study of the herd history must be confirmed by laboratory means. Isolation of *C. fetus* by bacteriologic examination of an aborted fetus is most efficient if the fetus can be transported to the laboratory in good condition. Abomasal contents are the best material for culture, but other organs may be successful if the abomasum is contaminated. The tissues must be examined no more than 24 to 48 hours after abortion. They are best preserved by freezing. Aborted fetuses are difficult to obtain since late abortions are infrequent and the fetuses are often lost to scavenging animals and birds.

The development of improved methods and equipment for the collection of cervicovaginal mucus has facilitated the diagnosis of vibriosis (Seger and Levey, 1962). The equipment most used is prepared by sterilizing a 12 inches long, 8 mm diameter, pyrex speculum in a pipette envelope. When the specula are cool, plastic artificial insemination pipettes (1.5 mm bore) are removed aseptically from their plastic shipping bags and dropped into the sterile specula. The top of the pipette envelope is closed to maintain sterility. Immediately before use, the top of the pipette envelope is torn off and the insemination pipette is attached to a 2 ml syringe with a short rubber tube.

The sleeved arm is passed into the rectum of restrained cattle to immobilize the cervix and to direct the insemination pipette. The vulva is wiped clean with two or more dry paper towels. With the lips of the vulva parted by an assistant, the speculum containing the retracted insemination pipette is passed into the vagina until near the cervix. The tip of the insemination pipette is then advanced out of the speculum onto the surface of the cervix and mucus

is drawn into the pipette by retracting the syringe plunger. The mucus is quite viscid, especially during diestrus, so that patience is required to allow the mucus to be drawn into the pipette. When the syringe plunger is maximally withdrawn the insemination pipette is eased back through the speculum so that the tip may be observed through the speculum wall to determine the amount of mucus collected. If the amount of mucus is insufficient, the plunger is returned and another attempt can be made without contamination. A mucus sample of one-half to two inches is adequate for bacteriologic examination. The speculum containing the pipette is removed, and after withdrawal of the pipette, it is closed with a plastic "poly-bulb syringe" of the type used for artificial insemination after it has been removed aseptically from its original sterile package. The syringe end of the pipette is similarly closed and the bulb marked with the animal or sample number with a plastic marking pen.

If the mucus can transported to the laboratory within six hours, it need not be frozen, but should be kept cool in hot weather. For longer transport immediate freezing in dry ice has been satisfactory for as long as four days (Hoerlein and Kramer, 1963). A rather complex medium for transporting cervicovaginal mucus and preputial smegma from bulls has been developed by Clark *et al.* (1978). Winter and Caveney (1978) found this transport medium satisfactory in their tests. It appears that this transport medium would be useful where dry ice is difficult to obtain.

Selection of the animals to sample is important when using cervicovaginal mucus for the diagnosis of vibriosis. Almost all susceptible heifers are positive for *C. fetus* from two weeks to three months after exposure to infection. After this time the number of positive cultures decreases so that six to seven months after exposure only 20% of the nonpregnant cattle will be positive. At this time we have collected 20 mucus samples to assure accuracy. The young cows being bred for the first time to old herd bulls are preferred since they do not have convalescent immunity. The sampling of pregnant animals is rarely productive. A single positive culture of *C. fetus* is adequate to establish a diagnosis of vibriosis in the herd.

These culture media have been satisfactory in our laboratory, but others may also be effective. The cervicovaginal mucus is inoculated on cystine heart agar (Bacto) with 10% defibrinated bovine blood. These plates are incubated in an atmosphere of 10% CO_2 at 37°C. After three to four days (some negative plates had colonies at seven days), small suspicious colonies are picked for staining and inoculation into differential media for positive identification of *C. fetus*. Pure cultures are inoculated into semi-solid thiol medium (Bacto) with 0.5% added glutathione in deep stabs in screw-capped tubes for catalase tests. Cystine heart infusion medium is inoculated with lead

acetate paper strips under the screw caps for the determination of H_2S formation. *C. fetus* subsp. *fetus* produces abundant catalase and is H_2S negative (sometimes a trace). This organism will not grow in media containing 3.5% NaCl or 1% glycine.

There are two other closely related bacteria that must be differentiated in the diagnosis of vibriosis. *C. fetus* subsp. *intestinalis* is the cause of epizootic abortion in sheep. It is a common inhabitant of the intestinal tract of sheep and cattle. It has been found rarely in aborted bovine fetuses. It is not spread venereally. The organism can be separated from the venereal organisms by its ability to produce H_2S and growth in 1% glycine media. *C. sputorum* subsp. *bubulus* is nonpathogenic and commonly found in the smegma of bulls. It is catalase negative, produces H_2S, and will grow in 3.5% NaCl and 1% glycine media.

Some cattle have vaginal mucus agglutinins from 60 days to seven months after infection (McEntee *et al.*, 1954). In our hands and in those of others (Clark *et al.*, 1970), the mucus agglutination has not been satisfactory due to false positive reactions. Blood serum agglutination tests have been unreliable for the diagnosis of vibriosis (Plastridge, 1955).

Isolation of the Organism from the Bull

Diagnosis of vibriosis in the bull is most efficiently done by the test-mating of virgin heifers. The bull is allowed to breed a virgin heifer after negative cervicovaginal mucus cultures have been obtained. Approximately half of the heifers have been positive one week after breeding, a few were not positive until three weeks. It is desirable that stud bulls are not used in natural breeding. Smegma from preputial scrapings, preferably, or semen combined with nutrient broth washings of the artificial vagina may be instilled into the cervix of virgin test-heifers. There appears to be no difference in susceptibility between heifers in natural estrus, artificially induced estrus, or not in estrus. Cervicovaginal mucus cultures from the test heifers are used to diagnose infection in the bull.

There are a number of methods used for collection of preputial smegma from bulls. A simple method used in our experiments has been to vigorously massage the sheath with a squeezing motion over the glans toward the fornix. After the loose dirt is removed from the external preputial orifice with a dry paper towel, the secretions are collected with a scraping action into a sterile artificial insemination pipette with a syringe attached for suction. When 2 to 10 cm of smegma are collected in the pipette the contents are washed out into 3 to 5 ml of nutrient broth for use in a test heifer or for bacteriologic examination in the laboratory. Preputial material should not be frozen.

Direct culture of preputial material is difficult because of two main problems. There is gross contamination with saprophytic bacteria which tend to overgrow the more fastidious *C. fetus*. The number of *C. fetus* varies from time to time and when the numbers are low contamination is more of a problem. Preputial material is cultured directly on highly inhibitory antibiotic blood-agar plates or filtered through a 0.65 μm cellulose acetate filter (Millipore) before inoculation on plates. The filter allows *C. fetus* to pass through while retaining most of the larger saprophytic bacteria. The antibiotics selectively inhibit the growth of contaminants. The use of both procedures has been shown to be superior to either alone (Shepler *et al.*, 1963). Colonies found on the plates must be isolated in pure culture and inoculated into confirmatory media to differentiate them from *C. sputorum* subsp. *bubulus* and *C. fetus* subsp. *intestinalis*. These techniques are quite exacting and are best performed by experienced personnel. Positive cultures indicate that the bull is infected, but negative results do no exclude infection. As many as 14 negative cultures have been made from an infected bull before a positive one was obtained (Kendrick, 1963).

Fluorescein-conjugated *C. fetus* antibody for the detection of the organism in preputial material from bulls was studied by Mellick *et al.* (1965). It was possible to differentiate *C. fetus* from *C. sputorum,* but could not distinguish venereal and intestinal strains of *C. fetus*. Unless the examinations are made by an expert technician, it can be expected that false positive diagnoses will result (Taul and Kleckner, 1968).

In all cases vibriosis must be differentiated from trichomoniasis which it closely simulates clinically. The two diseases are not uncommonly found simultaneously in the same herd. Another type of infertility easily confused with vibriosis is due to malnutrition during drought (Carroll and Hoerlein, 1966), on good pastures severly overgrazed during the breeding season, and in lush pastures in high rainfall areas. The problem results from an insufficient intake of energy (Wiltbank *et al.*, 1964). This condition is most frequently seen in heifers nursing their first calf. There is a lack of estrual activity. Dry cows will often be pregnant. Genital examinations made during the breeding season will reveal inactive ovaries. The cows produce milk first, the calves are often in good condition, but there is not enough energy intake to stimulate normal estrual cycles.

In some herds where it was difficult to procure or transport samples to the laboratory, the diagnosis of vibriosis has been made by the use of vaccine. Part of the replacement heifers were vaccinated and identified and allowed to be bred by the old herd bulls. If vibriosis is present the vaccinated heifers should have a pregnancy rate of 90% or higher while the unvaccinated controls will have a much lower pregnancy rate depending on the level of

infection in the herd. If another infection or management problem is the cause of the infertility, the pregnancy rates in the two groups should be similar.

Control Methods

Methods for the control and prevention of vibriosis have been firmly established. The exclusive use of artificial insemination with semen properly treated with antibiotics can effectively control the disease in dairy herds and small beef cattle herds. In most beef cattle herds it is usually necessary to use "clean-up bulls" after artificial insemination to assure satisfactory pregnancy rates. This practice allows the spread of the infection to the nonpregnant cattle and maintains the disease in the herd.

Experiments at Colorado State University led to the development of a highly effective vaccine for the prevention of vibriosis (Hoerlein *et al.*, 1965). A highly virulent and immunogenic strain of *C. fetus* was killed with formalin and a heavy suspension emulsified in an oil adjuvant. A single injection of the bacterin has been shown in field trials and controlled experiments to result in 90% or higher pregnancy rates in infected herds. The efficacy of the vaccine depends on high concentration of antigen, immunogenic strains of *C. fetus,* and oil adjuvant. Bacterins made with other adjuvants require two injections and have been shown by field experience and guinea pig tests (Bryner *et al.*, 1979) to be inferior to oil-adjuvant bacterins.

After more than 15 years of widespread field use, vaccination procedures are well established. In animals not previously vaccinated, the vaccine is injected as a single dose 30 to 120 days prior to breeding and possible exposure. After a diagnosis is made in the herd all female cattle should be vaccinated since convalescent immunity may not be adequate to protect many of the older cows. Annual revaccination is essential for maximal protection since vaccinal, like convalescent, immunity wanes with time (Hoerlein and Carroll, 1970). It has been convenient and satisfactory to revaccinate cows at weaning time when oil-adjuvanted vaccines are used. One should expect 90% or higher pregnancy rates when all female cattle are vaccinated in the absence of other disease or management problems. Heifers vaccinated after infection recovered more quickly than those not vaccinated.

Since bulls have shown no evidence of convalescent immunity, their vaccination has not been recommended in the past. Recent work in Belgium and Australia (Clark *et al.*, 1975) suggests that the carrier state in bulls, especially the transient carrier, may be altered by vaccination. It appears that the vaccines used for bulls must have a higher antigen content than that used for

females. At this time it does not appear that bull vaccination will replace the vaccination of females as a means to assure maximal reproduction performance.

C. fetus is sensitive to streptomycin and several other antibiotics. In well controlled experiments (Seger *et al.*, 1966), it was shown that bulls could be cured of vibriosis. A single injection of dihydrostreptomycin (10 mg per pound of body weight) combined with local application of 5 g of a 50 % solution of the same antibiotic to the glans penis and prepuce was used. When later exposed to *C. fetus* these bulls were susceptible to reinfection. The elimination of infection from stud bulls has been successful only if strict sanitation was carried out to prevent spread from infected to treated bulls. The use of antibiotics does not appear to change the course of the disease in female cattle.

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19. INFECTIOUS KERATOCONJUNCTIVITIS

David E. Hughes

Abstract. Infectious bovine keratoconjuntivitis (IBK) is a disease of cattle occurring perennially in all areas where cattle are raised. It is caused by a Gram-negative diplobacillus, *Moraxella bovis*. The organism is carried by diseased and inapparent carrier cattle. Signs begin with lacrimation, proceed to corneal ulcer formation, corneal opacity, corneal vascularization, and scar formation. Occasionally weakening of the cornea leads to rupture and subsequent loss of the eye.

There is no effective treatment for IBK. Early in the infection, repeated applications of antiseptic solutions containing heavy metals, antibiotic ointments, powders, or sprays are helpful. The problem of maintaining effective therapeutic levels of the drug of choice in the ocular tissues makes repeated applications necessary. Systemic administration of antibiotics or sulfa drugs may be used to maintain effective levels. Such applications may be impractical in large groups of cattle dispersed over wide areas. Immunoprophylaxis has not been developed to a practical tool.

Disease

Infectious bovine keratoconjunctivitis (IBK) is commonly called "pinkeye of cattle." Synonyms for IBK are infectious ophthalmia, infectious keratitis, and keratitis solaris. Pinkeye was described first by Akkerman in 1886 and Schimmel in 1888 in the Netherlands, and by Billings in 1899 in Nebraska. The history of the disease and its relationship to sunlight were reviewed by Hughes *et al.* (1965).

The disease is of considerable economic importance. Losses occur through direct and indirect causes. Indirect causes are attributed to discomfort during the acute stages because cattle with severely diseased eyes tend to protect the affected eye(s) from contact with other cattle as well as objects in their environment. Resulting inactivity tends to reduce feeding time and causes decreased growth and milk production. Treatment necessitates handling the animals and costs may be direct for labor, drugs, facilities, or indirect from weight loss, heat prostration, and injury. Disfigurement may vary from tiny corneal scars to microphthalmia following rupture of the eyeball. Frequently such cattle are sold for less than comparable unblemished animals.

Etiology

The disease is caused by *Moraxella bovis* (Barner, 1952; Bryan *et al.*, 1973; Hughes *et al.*, 1965; Jackson, 1953; Jones and Little, 1924; Pedersen *et al.*, 1972a; Pugh *et al.*, 1968), a Gram-negative nonmotile, bipolar staining nonsporulating diplobacillus. It occurs as short plump rods with rounded ends in pairs or in short chains. Cells are from 0.5 to 1.0 μm in width and from 1.0 to 2.0 um in length. Freshly isolated cultures are usually encapsulated. Colonies are apparent on blood agar after 24 h at 37 °C and are 1.0 to 3.0 mm in diameter with a zone of beta hemolysis about 0.5 to 1.0 mm in width. The colonies are round with entire margins, convex to umbonate, glistening, translucent, grayish, and slightly indented into the medium. These colonies are firm but tend to fragment when moved across the surface of the medium. Nonhemolytic colonies present the same characteristics (Pugh *et al.*, 1968). Suspensions of these smooth colonies autoagglutinate in liquid mediums except for 10% magnesium chloride. After a further incubation at 25 °C the colonies increase to 3.0 to 4.0 mm diameter, appear more flat, and the zone of hemolysis becomes 1.0 to 1.5 mm in width. The dissociated, rough colony forms occur as both hemolytic and nonhemolytic types. The margin of the colony erodes and slumps in varying degrees, and a small portion or the entire border may become involved. Suspensions of rough colony forms do not autoagglutinate. In addition, they will not become established in susceptible eyes and are seldom found on initial isolation from acutely infected eyes.

Factors that have been recognized as enhancing influences in the occurrence of IBK are sunlight, wind, dust, flies, and nutritional deficiencies (Barner, 1952; Hughes *et al.*, 1965). Ultraviolet radiation (UV), particularly that part of the spectrum which causes sunburn (<3200 nm) varies on a global basis according to latitude, altitude, and time of year. Ultraviolet radiation produced by sunlamps has been used to produce experimental IBK in calves confined indoors (Hughes *et al.*, 1965). Flies have been shown to be mechanical vectors for *M. bovis* but apparently do not become infected, simply transporting the organism on their external surface (Brown and Adkins, 1972; Jones and Little, 1924; Steve and Lilly, 1965).

Trauma and other ocular infections may serve as trigger mechanisms for induction of infections with *M. bovis* (Langford and Dorward, 1969; Pugh *et al.*, 1970).

Distribution

The disease has been reported worldwide wherever cattle are raised. Since IBK is not a reportable disease, however, information on the specific inci-

dence and occurrence is not available. The causative agent, *M. bovis,* has not been isolated from non-bovine hosts. Persistence of the infection in a herd may be by an inapparent carrier state in a low percentage of animals or in convalescing cattle.

Epidemiology

Usually IBK is introduced into a noninfected herd by an *M. bovis* infected animal. The animal harboring the organism can be either an inapparent carrier or an animal undergoing acute or chronic stages of the disease.

The occurrence of an IBK epizootic in a chronically infected and diseased herd differs from that in a herd without previous infection or disease. The introduction of susceptible cattle either through movement or by birth results in a new episode of IBK. During periods of quiescence (midwinter in temperate climates), the incidence of carrier animals demonstrated by cultural isolation of *M. bovis* may be as low as 2 to 3%. Therefore, the probability of carriers present in a given herd increases as the number of animals in the herd increases or with the frequency of contact with carrier animals from another herd (Bryan *et al.*, 1973; Hughes and Pugh, 1970).

Transmission of *M. bovis* from animal to animal may be by direct contact with infectious ocular and nasal discharges or indirectly by mechanical vectors such as flies. The persistence of *M. bovis* on or in flies has been studied. It is thought that *M. bovis* does not survive inside the fly but can survive for 24 to 72 hours on the surface of the fly. With this limitation in mind, it would appear that herd to herd transmission by flies would be rare except when the cattle comingle or are in very close contact.

During the course of an acute episode of IBK in an infected herd, the onset of signs of disease can be observed. Less readily observed is the spread of infection with *M. bovis.* Not all infected animals develop disease. Furthermore, the time between onset of infection and onset of disease may vary from 1 to 20 days (Hughes *et al.*, 1970, 1976). Distinct differences between rate of infection and rate of disease in cows and in calves has been observed. The mean infection and disease rates observed in a 5-year study were 75% infection for calves, 63% for cows and 58% disease for calves, 16% for cows. These differences have been interpreted to reflect greater resistance of the adults which was attributed to previous experience with the infection (Hughes *et al.*, 1970). Field studies have shown that the presence or absence of IBK in a calf was not significantly related to the presence or absence of IBK in its dam (Hughes *et al.*, 1970).

Ultraviolet radiation is an important ancillary factor which appears to short-

en incubation periods and increase the number of animals showing signs of disease (Hughes *et al.*, 1965). The disease is seen more frequently during the time of year of greatest solar ultraviolet radiation. Peiods of intense sunshine after periods of cloudy weather seem to precipitate the onset of disease. Reflection of ultraviolet rays from the surface of snow or sandy surfaces with little vegetation may increase the dose incident on the eye and result in the appearance of new cases.

Clinical Signs

Signs of disease may be divided into four periods which provide a convenient framework for discussion. The first stage is exemplified by moist eyes or slight tearing. The second stage is the ulcerative stage concomitant with drooping of the eyelids. The third stage or the period of vascularization is when the corneal repair process predominates. The fourth stage is the time when the scar tissue resolves.

The period of moist eyes is an ambiguous time when the cattle appear to tear readily. It may be caused by other environmental influences such as wind, dust, insects, plant irritation, and the like. In experimental IBK the first signs are usually an increased tear pool. The eye simply seems full. Edema and reddening of the conjunctiva is either nonexistent or so minimal that it cannot be accurately assessed.

The period of ulceration can be recognized when the eyelid begins to droop. At the same time the iris begins to close. Comparison of affected and normal eyes will show these changes as well as the start of photophobia. Careful examination will reveal, either at this time or within a few hours, the initial ulcer. The acute ulcer may be easily stained by a 1% aqueous solution of fluorescein dye. Later, the area around the ulcer will lose its transparency, the eyelid will close more and more, the iris will become spastic and close the pupil completely, and profuse lacrimation will occur. The initial ulcer may be a single spot barely visible (0.1 mm) or several such spots closely grouped. The ulcers usually begin in the central part of the cornea. Some may be located near the edge of the cornea but still will be surrounded by cornea. The opacity is associated with the ulcer and does not originate from a conjunctivitis. At the early ulcer stage conjunctival changes are usually minimal but become evident after formation of the ulcer.

In very severe cases the stroma of the cornea disintegrates in the vicinity of the ulcer. Loss of stroma results in herniation of the inner layers of cornea. Descemets membrane is elastic and cannot resist the pressure of the anterior chamber fluid. Small holes may be plugged by the iris. At such times the

eyeball is at great risk from any sort of trauma. Rupture of the descemetocele usually results in evulsion of the globe contents with subsequent permanent loss of function.

The period of vascularization begins with the organization of vascular elements in the conjunctiva adjacent to the cornea. Capillary loops form in the adjacent avascular cornea in a band 1 to 3 mm wide. The bright red band progresses inward by the same capillary building process until it reaches the center of the cornea 10 to 15 days later. The band coalesces into a central tuft which gradually becomes devoid of blood and turns into an avascular scar. The scar is composed of abandoned vascular elements and fibroblasts which accompanied the ingrowth of capillaries. Repair of the cornea is closely associated with the vascular band and follows it from the conjunctiva to the center of the cornea. The corneal epithelium is repaired in the same way. Transparency is restored in the wake of the repair process and starts at the limb of the cornea and proceeds centrally.

The period of cicatrization has its beginning in the repair process and can be recognized for a long time and in severe cases, for the remainder of the lifetime of the animal. The scar is composed of tissue elements which formed or migrated into the area during repair. Initially, clearing of these scars is fairly rapid. The rate declines slowly so that changes become less perceptible after the first 6 to 8 weeks. Comparison of photographs taken at 6 months or yearly intervals suggest that some scars continue to fade during the second year. Even in eyes with dense central scars over the central cornea, peripheral vision enables most animals to function.

All eyes and cattle are not affected to the same degree so the clinical signs may vary and the healing process may be different, depending on the specific animal, husbandry, and management. In the early stages the ulcer may heal spontaneously, leaving a small scar. In others, a slight loss of corneal substance may leave a flat area or facet associated with some amount of scar formation. Some eyes heal with only a small part of the cornea becoming vascularized while in others only conjunctivitis occurs. During the late healing stages, it is not unusual to see erosion of the skin on the margin of the eyelids. These erosions are ulcers which heal slowly. They may involve one or both canthuses or may be extensive and involve most of the lid margins. In pigmented animals the erosions are most noticeable because of the loss of pigment. After healing, the pigmented cells are replaced and the lid margins appear normal.

The two eyes may be affected simultaneously, sequentially, or in different years. This means that the disease may have quite a different effect on different animals. Those with simultaneous bilateral disease are most affected by loss of sight and may account for 8 to 10% of the affected animals.

Pathology

Moraxella bovis does not invade the tissues, but appears to produce the tissue changes by means of surface phenomena. The most striking change in the cornea is the loss of transparency. The opacity is mainly due to edema. The scars are formed by the ingrowth of blood vessels, fibroblasts which support them, and by the random replacement of the corneal stroma cells. The eyes that rupture may lose the contents of the globe. Loss of the mechanisms that control the internal fluids results in a small, undersized, sightless eye.

Immune Response

When cultures of *M. bovis* are injected into rabbits, antibodies develop indicating that the organism is antigenic, but there is some question about its immunogenicity. However, the difference in the IBK disease response in cattle may indicate to some extent the variation of immune response to the disease (Pedersen and Nansen, 1972b). Disease rates of susceptible animals (calves) may be compared with previously exposed animals (adult cattle) in a herd having a history of IBK. In one herd the 5-year mean infection and disease rates for calves (75/63) were higher than for the adults (58/16) (Hughes *et al.*, 1970). Generally, animals that have the most severe disease develop detectable levels of antibody while those with very mild changes do not. Multiple injections of *M. bovis* suspensions are necessary to cause all cattle to develop antibody (Hughes *et al.*, 1976). Vaccinated cattle are protected to a greater extent against homologous challenge than against heterologous challenge. Only a minimal correlation has been found between the presence of antibody and protection against the disease. Vaccination under field conditions has been less successful, presumably because of the presence of several infecting strains of *M. bovis*. While vaccination appears to be theoretically possible, the effective application has not been worked out due to the multitude of biological variables associated with the disease.

Laboratory Aids to Diagnosis

Cultural isolation of *M. bovis* from diseased eyes is the principal confirmative test. The usual precautions regarding sample collection, transport, and culturing are necessary. Contaminants frequently found around the diseased eyes may overgrow and obscure *M. bovis* colonies. The organism may not survive prolonged transport under unfavorable conditions. The medium of choice is a 5% bovine blood agar. A known culture of *M. bovis* should be available for

comparison and as a control for the culture medium (Henriksen, 1971). Frequently laboratories have been unable to recognize *M. bovis* in routine cultures until they obtained a control organism.

Cultures of *M. bovis* have a marked tendency to autoagglutinate. This characteristic makes the preparation of antigens for plate and tube agglutination tests unsatisfactory. Use of a freeze-thaw antigen in an agar gel diffusion test has made it possible to observe antibody changes in cattle after episodes of IBK and after experimental vaccination (Hughes *et al.*, 1965; Hughes and Pugh, 1976; Pugh *et al.*, 1977a).

Immunofluorescence may be of some help in recognizing the diplobacillus in eye secretions but isolation and culture tests are quite specific. The latter tests require less complex equipment (Pugh *et al.*, 1977b).

Prevention and Control

Infectious bovine keratoconjunctivitis is a contagious disease and most preventive measures used in disease control are useful. Cattle with acute eye disease or *M. bovis* carriers should not be introduced to a herd free from IBK. Because of the frequency of infected herds and carrier animals, it is recommended that cattle be treated with antibiotics or other chemotherapeutic agents before they are added to a herd. Also, cattle may be treated before the calves are born in the spring. Fly control measures, sanitation, sprays, dusts , self oilers, as well as systemic insecticides are recommended.

Once an infected animal is found in a herd, all animals should be treated. Treatment has assumed a wide variety of forms. Eye drops and sprays may afford a direct approach to the infected eyes. Unfortunately, the difficulty of maintaining a therapeutic level of drug in the conjunctival sac necessitates repeated application of the treatment. In addition, the importance of nasal infection in the carrier state, and the effect of ocular treatment on the nasal carrier state has not been determined. It is more important how and when the treatment is given than what is used. The importance of treating all eyes (animals) cannot be overemphasized. Affected eyes should be treated to achieve a prolonged therapeutic level of drug and the treatment should be repeated. Treatment should be applied as early as possible because elimination of *M. bovis* from a diseased eye will not restore the destroyed tissues and cannot be expected to speed the repair process. Examination and treatment during an acute outbreak of IBK should be done at least three times a week. Severely affected animals should be separated, kept in darkened quarters, and provided with suitable clean, fresh feed and water. Treatment should be given at least twice daily.

Antiseptic eye solutions such as boric acid, mercurials, argyrol, silver nitrate, and copper sulfate were used beneficially during the first half of the century. More recently, zinc sulfate in a 1:40 solution and ethidium bromide were found useful (Jones and Little, 1924; Mitter, 1915). Topical application of ointment, dust, or spray preparations containing a variety of antibiotics and sulfonamides have been used (Barner, 1952; Jackson 1953; Pedersen, 1973; Eichler, 1958). All reports stress repeated treatment applied early in the disease to achieve best results.

Systemic administration has not been used extensively. Pedersen (1973) has used sulfadimidine in therapeutically active dose levels to successfully eliminate ocular infection with *M. bovis*. We have used oxytetracycline and tylocin in the same way.

Control of IBK by vaccination of calves has not been successful when done in large herds under controlled conditions (Hughes *et al.*, 1976, 1979). Experimental evidence indicates that the approach has merit and that further research is justified.

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20. BLACKLEG AND MALIGNANT EDEMA

I. E. Selman

A: BLACKLEG

Abstract. Blackleg is a relatively common disease of cattle which generally leads to rapid deterioration and death. It is usually considered to be due to infection by the anaerobic bacterium, *Clostridium chauvoei* although on occasion other organisms such as *Cl. novyi* and *Cl. septicum* may be implicated. Spores of *Cl. chauvoei* occur in the soil in problem areas most frequently as the result of contamination by the decomposing bodies of earlier cases. It is probable that infection takes place by ingestion, but how the organism reaches the musculature and why there may be a sudden stimulus for organismal proliferation is not understood. The pathological lesions arise as the result of the action of highly potent exotoxins which are produced and released by the bacterium. A severe inflammatory reaction takes place rapidly progressing to gangrene. The animal usually dies within a few hours of first becoming ill. Treatment of established cases is usually unsuccessful and prevention rests upon the use of a monovalent or polyvalent vaccine.

Etiology

The cause of classic or "true" blackleg is a strictly anaerobic bacterium, *Clostridium* (*feseri*) *chauvoei*. This organism is a slender (5 μm × 0.5 μm), rod-shaped bacillus with rounded ends. It usually occurs singly or in pairs and is Gram positive and motile. The oval spores may be situated centrally or subterminally, giving rise to lemon-shaped, pear-shaped and tennis racquet forms.

Cl. chauvoei is a somewhat fastidious organisms and grows best on media containing blood, serum, brain, liver, meat or glucose. Other details regarding its cultural and biochemical behavior have been reviewed by Soltys (1963).

Several different exotoxins are produced by this organism including deoxyribonuclease, hyaluronidase and a hemolysin. These toxins are similar but not identical to those of *Cl.septicum* (see Malignant Édema).

Pathogenesis

In classic blackleg, the clinical disease in cattle is rarely if ever associated with previous penetrating wounds or obvious bruising. The organism, found in the

spleen, liver and alimentary tract of apparently normal animals, is most probably acquired by ingestion. From the intestine, the organism invades muscular tissue. The nature of the stimulus which may provoke bacterial proliferation and toxin production is not understood. Some evidence exists suggesting that the metabolic activity of various muscle groups may play a role in the pathogenesis of the disease. The intramuscular injection of calcium chloride following the oral administration of *Cl. chauvoei* spores results in disease even when a period of 25 days has elapsed between the two events (Soltys, 1963). Nevertheless, factors, not yet properly understood, create local conditions which favor invasion and proliferation in the host's musculature. Highly potent toxins are released which give rise to a necrotizing and gangerous myositis and toxemia. The condition is almost invariably fatal.

Distribution

Blackleg, a soil-borne infection, is acquired by cattle as the result of ingesting contaminated food. The bacterium is not considered to have a saprophytic existence outside the animal body. The organisms in soil are probably deposited there following the disintegration of infected carcases. The spores are extremely resistant and may remain infective for many years; for this reason, certain farms, pastures and handling areas may remain potentially dangerous indefinitely.

On some occasions, blackleg may appear as an outbreak where several animals may succumb over a period of days or weeks, but quite often the disease arises in only one or two animals. The disease is most common in grazing animals during the warmer months of the year and in certain countries it is most prevalent during the rainy seasons. Outbreaks have occurred at other times, however, particularly when drainage operations have been carried out.

All ages of cattle may be affected. However, most cases occur from six months to two years suggesting that animals may become infected via breeches in the oral epithelium which arise as deciduous teeth are shed. In almost every instance of blackleg affecting young cattle, it involves only the best nourished individuals in the group. The reasons for this are not clear.

Clinical Signs

In most cases, blackleg results in sudden death; if live cases are encountered, death usually follows within 12–24 h.

In very early cases, there is profound depression and high fever. The most

commonly affected site is the upper limbs which suffer lameness or complete loss of limb function. On closer examination, there will be extensive pain and hot swelling although this quickly changes to a cold, painless stage with a dry, bluish-black skin covering. Latterly, there is distinct edema around the site and emphysematous crackles may be palpated. At this stage, however, the animal is almost always completely recumbent and near death. Other sites involved include the throat, neck and thoracic inlet, the back and loins and, occasionally in newly-calved animals, the vulva, vagina and perineal areas. Rarely is the disease limited to the diaphragmatic area.

Pathology

In cases which are found dead, there is usually little sign of a struggle and when the affected area is a limb it is usually uppermost and held extended. The carcass is often bloated, and blood-stained froth and blood clots are present at the nostrils and anus, respectively.

When a newly-dead carcass is opened, putrefaction will be seen to be rapidly advancing. Considerable quantities of sero-sanguineous fluid are present in the pleural, pericardial and peritoneal cavities and gas-bubbles are often present in the affected muscles, which are swollen and dark, sometimes almost black. On rare occasions such lesions are localized to a relatively small area; usually the lesions are extensive.

Immune Response

It is said that cattle recovering from blackleg are resistant to the infection for the rest of their lives. Proof that acquired immunity arises following mild exposure to the organism is not yet available to explain relative resistance in adult cattle.

Laboratory Aid to Diagnosis

In many instances, use of laboratory aids for confirmation of diagnosis is not necessary since the epidemiological, clinical and pathological features of blackleg are so characteristic. Moreover, if attempts are made to positively identify the causal agent, bacteriological examination must be carried out on tissues and samples from recently deceased animals because rapid putrefaction leads to a massive invasion of many other clostridia from the alimentary tract.

Under optimal conditions, *Cl. chauvoei* can usually be identified from a suitably stained direct smear of affected tissue or edema fluid. In many cases, however, it would appear that blackleg is not simply the result of a pure infection of *Cl. chauvoei.* In many cases a mixed infection with *Cl. septicum* may be involved; in other cases the organism is not found and the agent held responsible is *Cl. novyi* with or without *Cl. septicum.* Clearly, when dealing with fastidious organism such as *Cl. chauvoei,* lack of identification does not necessarily imply that it had no role in the pathogenesis of the condition. The situation does, however, illustrate how complicated laboratory investigations into blackleg may become and how important it is to be prepared to carry out special isolation and identification techniques if a proper attempt to establish a finite diagnosis is made (Williams, 1977).

Prevention and Control

Whenever possible, carcases of animals known or strongly suspected of blackleg infection should be burned or deeply buried. In addition, certain areas on farms known to be endemic for blackleg should be put to alternative agricultural use or grazed only with vaccinated stock. In this context, it should also be emphasized that blackleg is a well-recognized problem in sheep of all ages.

Various *Cl. chauvoei* vaccines are available, most of which are currently prepared from formalin-killed whole cultures of the organism. Immunity is almost entirely antibacterial rather than antitoxic. Vaccination strategy must be varied according to local conditions, but in general it is recommended that the first dose of the vaccine be administered six weeks, and the second dose two weeks prior to the onset of the period of maximum risk. Thereafter, annual vaccination of all cattle is advised if local conditions suggest this is necessary.

When faced with an outbreak, vaccination of all cattle should be carried out immediately and the group at risk should be promptly moved to an alternative grazing area. Since the vaccine does not confer protection for about two weeks, it is often suggested that all cattle should also receive blackleg antiserum or large doses of long-acting penicillin at the time of vaccination. Of these options, penicillin is preferred since it is much more economical.

If clinical cases are encountered and treatment is attempted, the animal should be given massive doses of procaine penicillin intramuscularly and around the infected area in addition to intravenous soluble penicillin. Again, the use of blackleg antiserum is of questionable value.

Finally, blackleg may possibly occur as the result of other (or mixed)

clostridial infections. This must be considered, particularly if a problem persists among stock which have been vaccinated against *Cl. chauvoei*. In view of the practical problems which may arise with detailed diagnostic tests, an alternative vaccination prevention program using a mixed vaccine (*Cl. chauvoei, Cl. novyi, Cl. septicum*) may be the best course of action if mixed clostridial infections are suspected.

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B: MALIGNANT EDEMA

Abstract. Malignant edema is usually due to infection by the anaerobic bacterium, *Clostridium septicum*, although other clostridia may occasionally be involved. The organism is a common inhabitant of the intestinal tract of all herbivores and occurs widely in soil and feces. In most cases it gains entrance to an animal's body through contaminated wounds and thereafter produces a severe local reaction leading to necrosis, edema, gangrene, and profound systemic effects due to toxemia with rapid deterioration and death. Treatment of established cases is only occasionally successful but the disease may be prevented by use of a vaccine.

Etiology

The cause of malignant edema is the strictly anaerobic bacterium, *Clostridium septicum*. It is a large (2.0–9.0 μm×0.4–0.6 μm) rod-shaped organism which closely resembles *Cl. chauvoei* except that it commonly appears in long chains. It is Gram positive and motile. The spores are oval, subterminal and slightly larger in diameter than the bacillus. The organism grows well under anaerobic conditions on most ordinary culture media although it prefers slightly alkaline conditions. For other cultural, biochemical and behavioral characteristics, the reader is referred to the work of Soltys (1963).

A number of different strains of *Cl. septicum* exist but all strains and also *Cl. chauvoei* possess a single common spore antigen. For this reason, it has been suggested that the two organisms should be regarded as two types of a single species (Soltys, 1963). Under suitable circumstances, *Cl. septicum* produces a very potent exotoxin which induces severe liquefactive necrosis. The

exotoxin is made up of two major components, α toxin (hemolysin) and β toxin (desoxyribonuclease) and also hyaluronidase and collagenase (Soltys, 1963).

Pathogenesis

In cattle, infection usually gains entrance to the body through a wound contaminated by dirt or feces. Deep wounds, providing conditions favorable to anaerobic growth, are most often associated with the disease. Proliferation of the organism gives rise to local inflammatory changes which quickly progress to gangrene; usually there is marked toxemia resulting in rapid deterioration and death.

Distribution

Malignant edema occurs widely throughout the world but is subject to local variations in prevalence.

Epidemiology

Clostridium septicum is a soil-borne infection with highly resistant spores. The organism is also a common inhabitant of the alimentary tract of herbivores. The disease in cattle usually arises following contamination of either surgical or accidental wounds with either dirt or feces. Occasionally it may arise following parturition.

The disease occurs sporadically throughout the year and may affect all ages of cattle. It has been suggested that it chiefly involves housed animals (Henning, 1956) although this may well reflect only a local tendency.

Clinical Signs

The incubation period is short (i.e., within one or two days). When the disease is a post-parturient infection, signs are usually seen within a few days of calving. There is marked depression, anorexia and high fever; within a short time affected animals are totally recumbent. The infection site is swollen, hot and painful in the earlier stages. This rapidly progresses to a stage where swelling persists, sometimes with a foul-smelling, sanguineous dis-

charge but pain is not evident. The swollen area eventually becomes cold, discolored and sometimes emphysematous. Occasionally, a syndrome is encountered where the condition is initially limited to the head, particularly the intermandibular space. The inflammatory lesion in such cases quickly tracks down the dewlap, sometimes to extent into the thorax to produce a necrotizing pleurisy. Death usually occurs within a few days; commonly animals are presented as sudden deaths.

Pathology

In most cases, lesions are localized to the area around the infection site and if adjacent musculature is involved it is rarely extensive. There is usually gangrene of affected tissue with a foul, putrefying smell. Gelatinous, blood-stained edema is found around the site and extending subcutaneously; often it also extends along intermuscular septae. In many instances, gas bubbles are present in the surrounding tissues. Further examination usually reveals generalized petechiation and relatively large quantities of serosanguineous fluid in the pleural, pericardial and peritoneal cavities. Associated lymph nodes are usually enlarged, edematous and hemorrhagic; occasionally there will be gas bubbles present.

Immune Response

It is assumed that recovered cases are solidly immune to reinfection.

Laboratory Aids to Diagnosis

Swabs may be taken from infected sites before and after death to establish the identity of the causal organism. As with blackleg, the examination of animals which have been dead for more than a few hours, particularly in warm climates, is likely to produce information of little or no value due to the massive invasion of the body tissues by other clostridia. In recently-deceased animals, it is worthwhile to examine a smear of peritoneum or the liver surface when the typical long chain of *Cl. septicum* may be seen. This organism is easily confused with *Cl. chauvoei* on morphological grounds and for definitive identification it is usually necessary to carry out more detailed studies (Henning, 1956; Williams, 1977). In addition, it has to be recognized that other similar organisms (i.e., *Cl novyi, Cl. perfringens* and *Cl sordelli)* have

also been isolated from lesions similar to those of malignant edema (Blood and Henderson, 1979).

Prevention and Control

In general terms, the proper management of both accidental and surgical wounds, including administration of antibiotics, is often adequate enough to prevent malignant edema arising in cattle. However, it has to be recognized that under certain conditions this is hardly practical. Whenever the disease is considered to be a significant potential hazard, a vaccination regime should be instituted. In view of the possibility that mixed (or other) infection may occur, it is probably wise to use a polyvalent clostridial vaccine in problem areas.

When faced with an outbreak, for example after castration of a large number of cattle, it is probably best to carry out herd vaccination and to administer a large dose of long-acting penicillin to each animal. Again, the at-risk group should be moved to an area known to be free of this disease. The treatment of individual cases is usually unsuccessful but when attempted it should involve massive doses of procaine penicillin, both intramuscularly and around the infection site, together with large, frequently-repeated doses of soluble penicillin administered intravenously. Attention should also be paid to ensuring that the wound is carefully cleaned and drained adequately. As with blackleg, the use of antiserum is probably of little or no value.

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21. CONTAGIOUS BOVINE PLEUROPNEUMONIA

E.P. Lindley

Abstract. A short general account of contagious bovine pleuropneumonia including a brief description of the causal organism *Mycoplasma mycoides* subsp. *mycoides* is given. The history of the epizootic is traced and its economic importance as an obstacle in the mid 19th century to the devlopment of an intensive cattle husbandry, e.g., milking herds, hence its central role in the formation of the veterinary services of many countries is stressed. The relative late development of mycoplasmatology and the early eradication of the disease from developed countries is blamed for the slow advance in disease control in other areas.

Clinical and pathological features of the disease are described as are the laboratory aids to diagnosis. The significant role of the complement fixation test as a diagnostic procedure is mentioned. Since the spread of the disease takes place by contact between infected and susceptible cattle, historical methods of control were based on the application of strict quarantine measures for newly imported cattle and the slaughter of infected animals or whole herds. It was not until improved diagnosis and standardized vaccines were available that a system of control more suitable to the less developed parts of the world was possible. A lyophilized vaccine made from strain T1/44, although it produces a low percentage of post vaccinal subcutaneous reactions, has been demonstrated to be stable and immunogenically efficient. The availability of new potent antibiotics may add a new weapon in the development of control measures.

It is suggested that, given social and political stability in the present enzootic areas and continuous application of modern control measures, the disease could be eliminated from these remaining areas by the turn of the century.

Introduction

Contagious bovine pleuropneumonia (CBPP) is a specific disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides* (Freundt, 1974). The disease produces massive pathological changes in the thoracic cavity. It is of considerable historical importance because the state veterinary services of many countries were established in the 19th centry in an effort to reduce the severe losses caused by the impact of this disease on newly-developing forms of intensive husbandry, e.g., in dairies using improved, more valuable breeds of

cattle. Presently the disease exists only in some countries of Africa and Asia where social or political factors, extensive forms of husbandry and logistic difficulties make control measures difficult to apply.

In the older literature it was known as "lungsickness", "Lungenseuche der Rinder" (German) but usually the name is more explicit. The prefix 'peri' or 'pleuro' is added to characterize the pneumonia and the contagious nature of the disease and the host is indicated, e.g., "péripneumonie contagieuse des bovidés" (French) and "perineumonia contagiosa bovina" (Spanish).

Etiology

For a long time it was disputed whether *Mycoplasma mycoides* subsp. *mycoides,* the organism isolated from lung lesions of affected cattle, was the sole cause of the disease. This was because of the difficulty of reproducing the typical lesions in susceptible cattle by the inoculation of pure cultures of the above organism. When this technical problem was resolved the etiological role of the specific mycoplasm was no longer in doubt.

The name of the organism has been changed several times since it was first described by Nocard and his coworkers in 1898 (Turner, 1959). Originally three similar diseases of unknown etiology were observed occurring in domestic ruminants. These were contagious agalactiae of sheep and goats, contagious caprine pleuropneumonia and contagious bovine pleuropneumonia. The causative agent of the CBPP was the first to be isolated. It was 50 years later that improved laboratory techniques were developed which permitted the extensive study and identification of this organism (Leach, 1967). Based upon results of these studies, the organism was taxonomically placed in the class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae.

The typing of new species within the genus *Mycoplasma* can only be undertaken by specialists in well equipped laboratories (Tully, 1974). *Mycoplasma* subsp. *mycoides* is less fastidious than most mycoplasms, grows quite readily in cell-free media and hence certain cultural procedures are well within the scope of the ordinary diagnostic laboratory or investigation center.

The agent and its properties

The organism lacks the rigid membrane of the bacteria and is pleomorphic. In young broth cultures it appears as branching filaments and in old cultures as small coccal bodies. Globular and asterodiscule bodies also occur but these

forms may be a result of differences in the media, pH, etc. The mycoplasms in these cultures can be seen with the light microscope after staining with Giemsa but the small forms are almost at the limit of a resolution of the light microscope. Dark field microscopy is preferred although some experience is required to differentiate the mycoplasm forms from other particles found in biological fluids.

Chemically defined culture media have been developed, but for routine purposes, a digest broth with 10–20% serum gives satisfactory growth. Penicillin and thallium acetate are usually added to the medium to inhibit growth of possible contaminants. Enrichment additives such as yeast extract, liver digest and glycerol may enhance growth. Twenty-four to forty-eight hours after inoculation, growth can be seen in the form of a light turbidity in the broth.

In the broth media positive cultures appear turbid and after 3 days of incubation a very light deposit may be seen at the bottom of the tube which produces a characteristic swirl when the tube is rotated. Growth on solid media is slower, taking about 4–5 days to produce colonies of about 1 mm in diameter. The colonies are typical of mycoplasms having a raised center, a flattened periphery, and a central projection down into the media. This colonial appearance is referred to as a 'fried egg' colony. In transmitted light the center of the colony appears darker than the periphery. There is some variation in colony form between different strains of *M. mycoides* subsp. *mycoides* which serves as a useful identification marker. The organism requires CO_2, it grows well at 37 °C and at pH 7.4–8.0.

For its growth the organism requires cholesterol. It does not hydrolyze urea and produces acid but not gas in the presence of glucose, fructose, maltose and galactose. Lactose and sucrose are not fermented. Some strains are proteolytic and some produce slight haemolysis on blood agar plates (Leach, 1967; Ernø and Stipkovits, 1973).

Several specific organismal protein and polysaccharide antigens have been identified and studied. The organism is probably exceptional among mycoplasms with regard to the quantity of galactan it produces. Pure cultures are rich in galactan, both diffused in the media galactan F and inside the mycoplasm, galactan C. This galactan is important pathologically in that it interferes with the host defense mechanisms and also may interfere with the specific reaction in some serological tests. Although related polysaccharides occur in other microorganisms, it is sufficiently specific to be used as a precipitinogen in the precipitin test for CBPP.

History and distribution

Because CBPP presents very distinctive signs clinically, pathologically and epizootiologically, its recorded history since the 18th century is quite credible in spite of the rudimentary veterinary services available at the time and the confusion over disease description. The disease originated in Central Europe and spread throughout the continent during the Napoleonic wars. It is known with certainty that it was introduced into England in about 1840, into the United States in 1843, and into Scandinavia in 1847. During the period of colonial expansion, CBPP was taken to South Africa (1854) and to Australia (1858); in both places it passed along cattle routes spreading into the two continents. At the turn of the century the disease was already in several African countries bordering the Sahel but it may have been introduced into this region much earlier via West African ports, e.g., Dakar. In the early decades of this century it was diagnosed in India, China and Japan.

The economic impact of CBPP upon the developing animal industries in Europe and America prompted energetic responses and legislation for the control of animal diseases. Progress towards eradication was continuous except for interruption due to wars. Sweden recorded its last outbreak in 1856, U.S.A. in 1892 and U.K. in 1898 (Anon., 1965). The disease was eradicated from Japan in 1932 and from the U.S.S.R. about 1946. An isolated outbreak was reported on the French-Spanish border in 1967; afterwards Europe became free; Australia reported its last case in 1967.

The disease was of great economic importance. Reliable data (Anon., 1965) indicate that the introduction of the malady into the United States, South Africa and Australia caused losses in certain susceptible herds of up to 50%, resulting in millions of dead cattle in the first years after the introduction.

The disease still exists in Africa and Asia (Table 1). In the Arabian Gulf states, e.g., Kuwait, its presence is associated with the importation of live slaughter cattle. In countries where one still finds the disease, its distribution is limited and localized in remote areas where the methods of animal husbandry, the paucity of veterinary services and logistic difficulties make control difficult.

It has proved extremely difficult to make a rational estimate of the cost of CBPP in the areas where it still exists for methods of animal husbandry in these regions are geared more to survival than to profit. When incapacitated animals are slaughtered the meat is salvaged to avoid a complete loss. The disease presents a major obstacle to development and improvement of animal industries in regions unsuitable for other forms of agriculture. Hence the countries concerned, frequently with the help of international aid agencies, seek to carry out campaigns for the control and eventual eradication of CBPP.

Table 1. Regions of current existence of contagious bovine pleuropneumonia (CBPP).

Countries with the disease *	*Disease status*								
	Suspected	*Exceptional occurrence*	*Low incidence*	*Moderate incidence*	*High incidence*	*Reduced but existent*	*Certain regions only*	*Recently recognized*	*In imported animals*
Mauritania				+					
Sudan			+						
Ethiopia							+		
Djibouti									
Somalia				+					
Kenya						+	+		
Rwanda	+								
Chad				+					
Niger						+	+		
Upper Volta									
Mali					+				
Senegal			+						
Guinea			+						
Sierra Leone			+						
Liberia									+
Ivory Coast					+				
Ghana							+		
Togo							+		
Benin							+		
Nigeria				+			+		
Cameroon							+		
Central African Empire					+		+		
South Africa		+						+	
Angola					+				
El Salvador	+								
Iraq	+								
Bahrein		+							
Kuwait			+						
India							+		
Nepal			+						
Bhutan			+						
Burma			+						
China							+		
Mongolia				+					

* Countries not on the list indicate diseases not recorded.
Source: Animal Health Yearbook FAO/WHO/OIE, 1977.

Some programs are still in progress and results indicate that the incidence and distribution of the disease is being continually reduced. A long-term view is needed to assess the cost-benefit of these programs.

In recent years, where normal services have been disrupted through political or other disturbances, CBPP has reappeared and caused serious losses.

Epizootiology

The causative agent is spread in the expired breath from infected to susceptible cattle in 'close' proximity. There is no evidence that any other means of infection, i.e., by fomites, the urine and other excretes are of practical field importance. The mycoplasma in droplets of the expired air of the infected animal may be carried by currents of air a relatively long distance. Sunlight may affect the viability of these droplets; the relative humidity affects their size and hence the degree of penetration into the respiratory system and the lung of the recipient animal. This may explain the poor results obtained in the earlier attempts at artificial infection with aerosols. In spite of these considerations, the natural disease is transmitted with ease and rapidity in both the humid tropics and semi-desert areas. The closeness of contact between animals seems to be the sole criterion dictating the speed with which the infection would spread in a susceptible herd.

Hudson (1971) carried out an experiment in which healthy susceptible animals were moved into heavily contaminated stables from which infected animals had just previously been evacuated. The atmosphere of the stable was free from air-borne infected droplets. This rotation was carried out daily for 66 days and then another 22 days were allowed to elapse before the test group was slaughtered and examined. It was not possible to demonstrate any evidence of infection in these animals. This experiment demonstrated that the risk of spread of infection other than air-borne is negligible.

Although strains of *M. mycoides* subsp. *mycoides* have been isolated from other animals, e.g., goats, and lesions of CBPP reported in buffaloes and other ruminants, there is no evidence that these animals are of any practical field importance in the spread of CBPP to cattle. The introduction or reintroduction of CBPP (after eradication) into countries or regions has invariably been associated with the arrival of infected cattle.

The absence of a suitable laboratory animal, the difficulty of reliably reproducing the disease in cattle, and the inadequacy of laboratory techniques for dealing with microbiological and serological aspects of mycoplasmology have hampered research progress in CBPP. After CBPP had been eliminated from most of Europe and America, attention was transferred to other veterinary

problems and funds and research on CBPP dried up. In these countries interest was switched to other mycoplasma diseases of man and animal.

Incubation period

The length of the incubation period depends upon the volume of the infective dose, the virulence of the strain, and the immune state of the animal. The organism can remain within the tissues of the host, e.g., retrophyaryngeal lymph gland without causing macroscopic pathological change and the progress of subclinical disease may depend upon extraneous factors. Turner (1959) considered that under natural conditions some animals may have long periods of clinical resistance before succumbing to the disease. These factors may account for the various incubation periods (5 to 207) days reported in the literature. Under the artificial conditions of constant exposure mentioned above, the incubation period varies from 5 to 28 days.

Clinical signs

An early phase of CBPP usually escapes clinical detection (Hudson, 1971). The first clinical sign of CBPP in a dairy herd may be a fall in milk production. In range animals the first sign is the malaise exhibited by an animal with fever, i.e., a standing coat and a reluctance to move. In those animals in which the disease runs a subclinical course, fever may be transient.

The signs in the animal with the acute disease are striking; the animal is in extreme distress with labored respiration, heaving flanks, elbows turned outwards, head poked forward, dilated nostrils, drooling or frothing at the mouth, and the legs 'planted', quite unwilling to move.

Outside the experimental stables such cases are seen infrequently and more usually one is presented with an animal exhibiting the sub-acute stage of a pneumonia or pleuropneumonia, with a standing coat, high temperature and a moist intermittent cough. There may be a discharge from the nostrils and the mouth, the animal stands apart from the herd and moves reluctantly.

Cattle of all ages may contract CBPP but young calves may exhibit an arthritic condition with swollen puffy joints without signs of pneumonia.

Diagnosis

In the event of a single case this can be difficult and will always be tentative. Two factors help considerably. In infected zones, one is on the lookout for CBPP and any pneumonic case is treated as suspect. Secondly, when one is usually examining a group of animals, the whole *herd* is being examined. The herd should be made to move around and trot in a circle. This activity may elicit a cough and also indicate the animal which is reluctant to move, being a suspect of CBPP. This is followed by taking temperatures, examination by percussation and auscultation of suspected cases.

On the basis of the clinical examination where CBPP is suspected, it may be justifiable to slaughter a diseased animal and perform the autopsy. The taking of samples for laboratory examination to confirm diagnosis is always essential. Once the diagnosis has been established, the investigation into the origin of infection is the next important step. At this stage also, the preliminary measures of control must be initiated as CBPP is a reportable disease in nearly all countries.

Pathology

The principal lesions are confined to the thoracic cavity and may be conveniently classified for description purposes according to the degree of change as acute, subacute, or chronic with sequestrum.

1. Acute Form

In the acute case the most striking features on opening the thoracic cavity are the presence of the pleural edema fluid which may amount to several liters. The lungs are swollen as a result of the inflammatory reaction and remain firm even when removed from the chest cavity. There is frequently a thick caseous fibrinous deposit over the visceral and parietal pleura. The fluid is clear, straw-colored, may contain pieces of fibrinous material and large numbers of mycoplasms.

A cut into the solidified lung will demonstrate another classical feature of CBPP, the interlobular septa distended with fluid separating the hepatized lung lobules (Fig. 1). This tissue abnormality is referred to as 'marbling'. This effect is caused not only by the appearance of the thickened septa but also by the different colors of the lobules from deep red to bluish. Frequently the relatively tiny portion of the remaining functional lung tissue is striking which explains the acute respiratory distress of the animal in the terminal

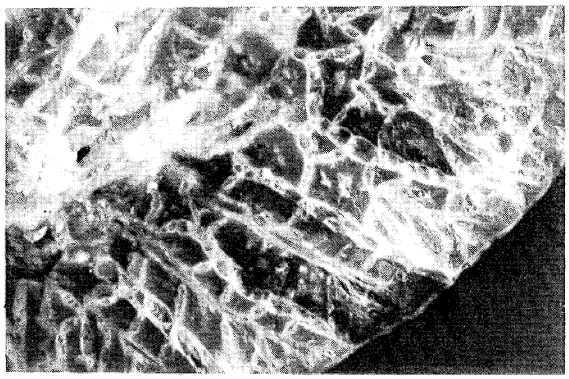

Fig. 1. Incised lung from an acute case of CBPP. The 'marbled' effect and the distended interlobular septa are clearly distinguishable.

stage of the disease. It should be noted that the pulmonary form of hemorrhagic septicemia (pasteurellosis) may occasionally produce lung changes similar to the above. The other features which may be seen such as hemorrhagic infarction, ischemic necrosis, bronchial plugs formed by the clotting of the bronchial exudate and emphysema are not peculiar to CBPP.

2. Subacute Form

This definition refers to those cases in which the lung involvement is less intensive and may not involve the pleura. In this category belongs the early progressive case, as well as those in which the development of the lesion has been arrested by the immune response of the animal. It is appropriate to consider the pathogenesis of the disease. The droplet nuclei reach the alveoli or terminal bonchioles of the recipient animal where they multiply in intimate association with the host cells (but not intracellularly). The mycoplasm spreads peribronchially and endobronchially to involve the entire lobule and along the bronchial pathways to neighboring lobules.

The site becomes edematous and infiltrated by macrophages and polymorphonuclear leukocytes. Serous occlusions occur in the lymphatic drainage channels and there is an extension of the infection along the lymph vessels. The distension of the septa is due to serous exudation via the dilated lymphatics and is a result of the increased vascularity. There is an accumulation of lymphocytes around the lymphatics and the arterioles. Hemorrhagic infarction leads to necrosis but centers of organization are also evident at this stage. One or more lobes may be involved in one or both lungs. All states of this process may be seen in the subacute form of the disease.

3. Chronic Form

Depending on the degree of arterial thrombosis and interference with the blood supply, there may be organization and gradual repair of affected tissues; also, more extensive tissue areas may become necrotic, walled off by fibrous tissue and encapsulate forming a *sequestrum*. The contents of the sequestrum may retain the form of the original tissue and usually remain infective for several months even after the animal has clinically recovered. These animals are the chronic carriers and are highly important in the epizootiology of the disease. When there is a breakdown of the wall of the tissue sequestrum, infective material may escape via bronchial passages and be coughed up to be passed out as droplets which may serve as a source of infection for susceptible animals.

In many cases there are fibrous adhesions between the pleura following fibroblastic reorganization of the fibrinous exudate. There may also be pathological changes in other tissues, for example, the kidneys, heart, pericardium and the joints, especially in the acute case but macroscopically these are not pathognomonic lesions.

Immune response

The immune processes, although differing in detail, parallel those seen in other microbial diseases. After experimental infection with suitable cultures of *M. mycoides* subsp. *mycoides* cattle develop precipitating, agglutinating and complement-fixing antibodies, the level of which can be estimated using appropriate serologic tests (Hudson, 1971). Of practical importance is the protective mechanism(s) by which the animal becomes resistant to natural infection, for example, following successful vaccination. Although the available immunological tests provide information relative to the exposure of the immune system to specific antigen, none is available to indicate the immune state of the animal. This can only be done by natural challenge using cattle. Although circulating antibodies may contribute to protection, the cell mediated immunity (CMI) seems to play the major role in that respect.

Laboratory aids to diagnosis

1. Dark-field Microscopy

After some experience with dark-field microscopy, it is possible to distinguish mycoplasms in the pleural lymph of positive cases but it is always desirable to carry out additional confirmatory tests.

2. Isolation

The isolation and characterization of the etiological agent can be done on selective enriched serum media and in both liquid and on solid media. The organism can be easier manipulated than many other mycoplasms, consequently the isolation can be done in a small laboratory and the organism later dispatched to a reference laboratory (Tully, 1974).

3. Precipitin Test

The agar gel double-diffusion method is suitable for a small diagnostic laboratory. It consists of cutting small wells in agar gel on a petri dish and placing the reactants, in this case, hyperimmune antigalactan serum, in a central well and lung fluid or pieces of macerated lung lesion from the diseased animal in the surrounding wells. After 24 to 72 h, lines of precipitation form if the specific galactan is present. Negative results should be treated with caution and the test must be properly controlled with known negative and positive material.

4. Agglutination Test

A field modification of this test in which a drop of stained antigen and a drop of fresh blood from the ear vein of the suspected animal are mixed on a slide has been adopted as a preliminary field test of suspected herds. Some experience is needed in interpretation of the test as the results on a single animal can be anomalous. The test, however, gives immediate results and it is therefore of practical value in the field. A more refined technique using serum instead of blood can be applied in the laboratory. This is also a useful test for assessing the results of vaccination.

5. Complement-Fixation Test (CFT)

This is the most reliable and widely used serological test for CBPP. It is especially valuable for detecting early cases and the chronic carriers. There are several methods of this test, including a field modification test (Hudson, 1971). Animals positive to this test are either naturally infected or have been vaccinated. Some chronic cases and very early infections may fail to react, thus a repeat performance of the test in one-month intervals is indicated.

Several other serological techniques such as the indirect hemagglutination, the conglutinin test, the intradermal allergic test, and the indirect fluorescent antibody test have been used in the study of CBPP. Growth-inhibition and metabolic-inhibition tests are used, particularly in mycoplasm classification.

Mice and rabbits have frequently been used in studies of certain aspects of CBPP but the lesions in these animals are different from those seen in cattle and correlation presents problems.

Prevention and control

Several factors concerning CBPP need mentioning in order to understand the control measures required. The disease, for all practical purposes, is restricted to the bovine species; the etiological agent is spread to susceptible cattle in the expired breath of an infected animal, i.e., spread of infection depends upon the living animal; the majority of infected animals (at any one time) do not present clinical signs, and a proportion of infected animals become chronically infected and may serve as a source of infection for several months.

Under the circumstances, it is obvious that the spread of the disease from one herd to the next is related to the movement of cattle. If the movement of live infected cattle could be stopped, the disease would die out, hence the first control measure to be applied in the case of an outbreak is a restriction on cattle movement, imposition of quarantine regulations and closing of cattle markets. Since slaughtered animals do not spread infection, the next control measure is to slaughter all infected animals.

Historically, in Europe and America CBPP was eventually eradicated by applying the above measures. Since all infected animals could not be accurately identified, the whole herd must frequently be slaughtered. In order that these drastic measures may be accepted, compensation had to be paid to the cattle owner. In some areas of the world such measures were not implemented for three reasons: physical resources and veterinary police were not sufficient; often the areas involved were vast; compensation, if paid, was not adequate, hence, the measures were not socially acceptable.

Control and eventual eradication of CBPP from the latter regions was associated with two technical developments, namely and foremost, efficient vaccines and availability of serological tests for identifying infected animals, especially the chronic carriers. These tests play an important role in keeping countries or portions of countries free from infection. Imported cattle are tested using the CFT to ensure they are not harboring the organism. Animals are held in quarantine and released only after two negative tests conducted in one month intervals.

The main criteria in deciding which control measures should be used is economic. Quarantine and slaughter remain the surest (and cheapest) methods to adopt in the case of a CBPP outbreak in a disease-free zone or country. There is considerable evidence that the most suitable way to deal with CBPP

in many African countries is by properly designed vaccination campaigns. These should be continued until the incidence is reduced to a level which would permit the creation of disease-free zones and the introduction of other methods. Such an approach must be regional in concept because of the clandestine movement of animals across the long land frontiers. For detailed recommendations on control measures, the reader is referred to the reports of the FAO/OIE/OAU expert panel on CBPP (F.A.O., 1967).

Vaccines

Original vaccines consisted of using infective pleural 'lymph' from a diseased animal perhaps modified in some way, e.g., storage for a few days. Such mixtures were inoculated subcutaneously and frequently resulted in severe post-vaccinal reactions which subsequently had to be treated with a hot iron or drained. In the early part of this century pure cultures of *M. mycoides* subsp. *mycoides* were used instead of natural lymph and this was followed by 50 years of research effort into producing a stable attenuated strain for vaccine production. The difficulty here was that what seemed a suitable strain in one area produced lesions in vaccinated animals in other locales. The difficulty in interpretation of results was compounded by the fact that in the field the broth culture vaccines being used were themselves variable as far as viable contents were concerned, i.e., the dose of live organisms being admin-

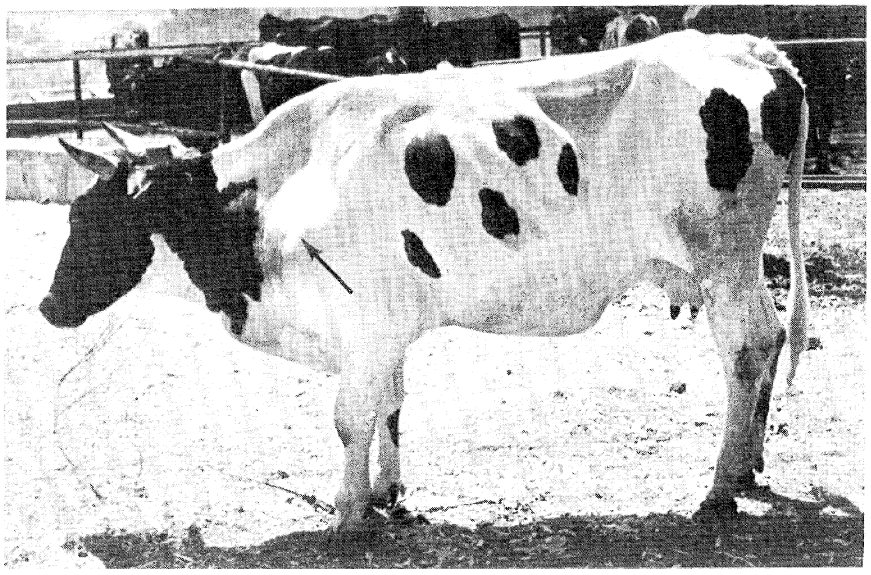

Fig. 2. Local edematous reaction one month after subcutaneous vaccination at the neck region with a culture vaccine. Gravitation of the fluid into dewlap and forelimb is evident (arrow).

istered could easily vary by more or less than 10^2 logs. Although every effort was made to standardize these vaccines, field conditions mitigated achievement of consistent results. A vaccine prepared from a partially virulent strain which can provoke a subcutaneous reaction may also produce lung lesions in a small proportion of susceptible vaccinates (Fig. 2). Lung lesions have also resulted from the use of avianized vaccines. In spite of occasional accidents, culture vaccines provided for many years a most useful control measure against CBPP.

These problems were overcome when it became possible to freeze-dry the vaccine. In Africa the most commonly used strain is the T1/44. It is not completely attenuated and may provoke a subcutaneous post-vaccinal lesion in some animals. In most cases these lesions do not progress and can be treated. The lyophilized T1/44 vaccine is stable and efficient giving a useful protection of about 18 months.

Vaccines have also been prepared from a completely attenuated strain (KH_3J) and also by using a suspension of inactivated mycoplasms with adjuvants. Such products, however, require a much bigger dose of antigenic material and are more costly to produce. The inoculation of concentrated mycoplasm antigens with adjuvants may induce immediate or delayed allergic reactions thus introducing inacceptable complications for use as a vaccine.

Postscript

In conclusion, it is reasonable to ask if 'the end of the chapter' is in sight. From the early chronicles of Veterinary Services, it is apparent that the disease was a great stimulus in their development. Now, given continuation of the present trends, contagious bovine pleuropneumonia itself could well have passed into history by the end of the century.

Acknowledgement

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22. BRUCELLOSIS

David T. Berman

Abstract. Brucellosis of cattle and buffaloes, caused by several biotypes of *Brucella abortus,* has a moderate to high prevalence in most countries throughout the tropics. Information on prevalence is derived primarily by small sample surveys and subject to uncertainty. Abortion more than once in a single cow is unusual and therefore presence of the disease in herds may remain unsuspected. Both antibody, and cell mediated immune responses occur, with diagnosis being based on demonstration of specific antibody. Recent information emphasizes the role of cellular immunity in protection. Vaccinal immunity is engendered by use of attenuated living strain 19 or by the rough killed strain 45/20. For the tropics, control to reduce prevalence by large scale application of whole herd vaccination, with or without serological testing is advocated as the most cost effective program.

Etiology

Bovine brucellosis (Bang's disease, contagious bovine abortion) is caused by organisms of the genus *Brucella,* and *B. abortus* is the species involved in the disease of cattle, including buffaloes, in most cases throughout the world.

Brucella organisms are small, non motile, non spore forming rods or coccobacilli growing singly, in pairs or small groups. They are aerobic, may need added CO_2 for growth, grow poorly on simple media, and do not ferment carbohydrates on ordinary media.

The conventional tests for designation of species of *Brucella* include CO_2 requirement, H_2S production, urease activity, inhibition of growth on medium containing the aniline dyes thionine and basic fuchsin, agglutination in monospecific abortus or melitensis antiserum, and susceptibility to lysis by the reference bacteriophage Tb. Within each species, strains group themselves on the basis of variations in response to one or more of these tests. These sub groups are termed biotypes, and eight biotypes are recognized in *B. abortus.* Details of methods of isolation and characterization have been presented by Alton *et al.* (1975), and are not considered further.

The ability to characterize distinct biotypes of *B. abortus* is important in establishing geographical distribution of infection, in tracing spread among herds, and in establishing the involvement of other host species in the epidemiology of the disease. Although a relatively limited amount of work has been done on isolation and characterization of strains of *Brucella* in most parts of the world, an illustration of the importance of biotyping is the description by Polding (1948) of the two types of *B. abortus,* now recognized as biotypes 1 and 3, infecting, respectively, large organized cattle farms and village cattle in India.

B. melitensis infection in cattle has been reported from many countries, but this occurs in regions in which *B. melitensis* infection of sheep or goats is enzootic. In these situations, cattle infected with *B. melitensis* constitute a serious public health problem. Organisms originally identified as *B. melitensis,* isolated from cattle in countries in which there is no *B. melitensis* infection in sheep or goats, were subsequently shown to be *B. abortus* biotypes 5 or 7 (Meyer and Morgan, 1962).

B. abortus is communicable from infected cattle to sheep in close contact, and the disease is characterized by abortions and by sheep to sheep, or sheep to cow transmission (Luchsinger and Anderson, 1979). In developed countries this apparently occurs sporadically, but its significance in tropics has not been determined.

Brucella organisms isolated from naturally occurring cases of the disease are almost all in the smooth (S) colonial phase, although small numbers of non smooth organisms may be seen on primary isolation (Berman *et al.*, 1955). The major surface antigen of the S organisms is a lipopolysaccharide (LPS) protein complex which carries the A and M antigenic specificities involved in serological identification of species and biotypes (Diaz *et al.*, 1968). S-LPS is also the antigen detected in the standard serological diagnostic tests (Diaz and Levieux, 1972). S-LPS can be extracted from cells by hot phenol-water, and it partitions into the phenol phase. It is an endotoxin with many chemical and biological characteristics similar to the endotoxins of Enterobacteriaceae but also has significant differences (Diaz and Oyeledun, 1977; Moreno *et al.*, 1979).

B. abortus and *B. melitensis* cultures undergo variation during growth in culture. Changes in colonial morphology from the smooth to rough and mucoid forms are associated with loss of virulence and alterations in antigenic specificity. Such dissociation influences typability of cultures and their use in production of vaccines or diagnostic antigens (Alton *et al.*, 1975).

Distribution

The distribution of bovine brucellosis is world wide and parallels the distribution of domesticated cattle. Programs of control have been successful in reducing the general prevalence substantially in such developed countries with large cattle populations as Austria, Australia, Canada, Great Britain, Federal Republic of Germany, Netherlands, Poland, Taiwan, New Zealand and the U.S.A. Eradication has been accomplished in all of Scandinavia, Switzerland, Austria, Czechoslovakia, Romania and Japan.

In most countries in the tropics, resources for organized animal disease control have not been sufficient to extend to brucellosis and information on prevalence has been obtained from surveys. Although surveys which are adequately planned, properly analyzed and reported can give rather precise data, most surveys on brucellosis have not met this ideal. The surveys also lag behind, or give inadequate attention to such factors as increased mobility of human and livestock populations, and the introduction of intensive husbandry methods, often side by side with small dispersed village herds, nomadic herds, or wild susceptible herbivores.

Prevalence, by geographic regions for tropical countries, as reported in the Animal Health Yearbook for 1977 is summarized in Table 1. Many of these officially reported figures are rough estimates, as they may be derived almost entirely from data from government or quasi government farms in countries where programs do not extend to private, village, or nomadic herds. Surveys frequently involve small samples, and the difficulties experienced in obtaining samples from small holdings and village or nomadic herds which may have high infection rates will bias the findings (Banerjee and Bhatty, 1970).

It is difficult to extrapolate from officially reported data to the national situation, even for countries with well developed veterinary services. For example, Venezuela with approximately 8.5 million cattle including 3.6 million dairy cows, reported 306 862 individual serum samples tested in 1976, with approximately 7% positive or suspicious. For individual districts, these combined rates varied from approximately 1.5% of the samples to as high as 12.8%, but there was no breakdown by herds (Ruiz Martinez, 1977). In Table 1 the incidence is described as "moderate". Colombia, with 22.5 million cattle also lists its incidence as "moderate", and it was reported that, "the entire country is considered to be infected", but herd or individual reactor rates were not reported (Manrique, 1976). Where precise data may be available for dairy cattle, there may be little or none for large beef cattle populations.

This type of "open" literature usually does not contain the information necessary to establish prevalence rates. There is also often a body of more

Table 1. Distribution and prevalence of bovine brucellosis in the tropics. Number of countries reporting.

Geographic region	*No Information*	*Not recorded/ Not present*	*Disease present, distribution and prevalence unknown*	*Disease present: incidence*					
				Rare	*Low*	*Moderate*	*High*	*Reduced*	*Limited*
North and East Africa (20) [1]	1	1	1	2	5	8	1		1
Western Africa (20)	4		2	1	5	3	2		3
Southern Africa and South America (19)	1			2	1	10	4		1
Mexico, Central America Caribbean (18)		2		3	4	5	4		
South East Asia, China (21)	6	2		2	7	2	2		
Australasia/Indonesia Oceania (20)	1	7		1	4	2		2	3
Totals	13	12	3	11	26	30	13	2	8

[1] Numbers in parentheses are total number of countries reporting.

definitive data in internal government reports, which, if available can be used to make more precise estimates. Such an intensive examination has made it possible to increase the Animal Health Yearbook estimates on national prevalence to include virtually the entire African continent. An incidence determination key was used to make estimates of national and regional incidence by herds and individual cattle. This key, which stresses the herd as epizootiological unit, extrapolates from herd and individual animal prevalence survey data to the larger population, and has been used to draw important conclusions on the distribution and incidence of bovine brucellosis in Africa (Thimm and Wundt, 1976).

In Africa, the whole South and East as well as Cameroon and Guinea in the West, and Morocco in the North, are considered to have a "moderate degree of incidence", corresponding to 11 to 20% of herds. A "very high degree of incidence" (above 30% of herds) is described for a ring of countries situated within the wet and dry savanna and tropical rain forest zone of West, Central and East Africa. In the high incidence area, 44 to 88% of the herds reacted to the milk ring test (MRT) and 42 to 100% of the villages had brucellosis reactors within their herds.

In both East Africa, with herd prevalence rates of about 15% and in West Africa, with herd prevalence rates close to 30%, individual animal reactor rates were in the 10 to 16% range.

There is a striking similarity in the factors which influence the incidence of bovine brucellosis in India and Africa. Incidence levels tended to be relatively high in organized farms, whether these were indigenous cattle or introduced breeds. Both in Africa and Indo-Pakistan, *B. abortus* biotype 1 is most frequent in this type of herd. In the organized farms, the disease also presents the classical signs of abortion as described in the temperate zones. The incidence and severity of the disease within such herds is also apparently directly related to the rainfall.

Under traditional systems of husbandry, both small dispersed herds of village cattle, and nomadic or semi nomadic cattle herds, biotype 3 has been most frequently isolated (Abdussalam and Fein, 1976). Abortion is relatively uncommon, and in Africa, hygromas and abscesses are the major clinical sign (Ferney and Chantal, 1976). Within herds, the incidence and the abortion rate is apparently more directly related to rainfall than to herd size (Thimm and Wundt, 1976).

The question of the relative susceptibility of imported and indigenous cattle and buffaloes often arises, both as a result of survey data which may show different within herd infection rates among breeds, as well as apparent difference in clinical manifestations. Such survey data suffer from the deficiency that indigenous cattle in traditional husbandry are less likely to be counted if

abortion is not apparent. Imported cattle, especially in organized farms, are more costly and more likely to be under veterinary supervision. Failure to observe abortion may be because of high levels of herd infection occurring prior to first pregnancy, and also to the high frequency of abortions caused by other agents. It is also possible that apparent breed differences in susceptibility may reflect differences in virulence of the two biotypes of *B. abortus* which infect cattle in Africa and Indo-Pakistan. However, differences in breed susceptibility do exist. This is documented in the markedly greater likelihood of persistent infection with vaccine strain 19 in animals of the Channel Island breeds compared with Holsteins (Jones and Berman, 1976).

That brucellosis is an "old" disease in Africa, may be deduced from the fact that there is serological evidence of the presence of disease reservoirs in both domestic and wild ruminants and their predators in some areas before the introduction of exotic breeds (Thimm and Wundt, 1976). Serological evidence of infection among wild animals has also been found in so called "remote areas". Species of herbivores involved include, but are not limited to eland, African buffalo, water buffalo, hippopotamus, zebra, wildebeest, impala, water buck and bush buck. Both *B. abortus* biotypes 1 and 3 and *B. melitensis* biotype 1 have been isolated from wild herbivores. The brucellosis infection cycle in wild fauna is considered be to self-sustained and forming a reservoir for domesticated stock (Thimm and Wundt, 1976).

Sheep and goats are traditionally herded with cattle by the nomadic and seminomadic peoples in various parts of Africa, and in village herds throughout the tropics but data on the distribution of *B. abortus* and *B. melitensis* infection among the three domesticated species is limited.

Pathogenesis and Pathology

Virulent brucellae are highly invasive and initiate infection through mucous membranes of the oropharynx and upper respiratory tract, the conjunctiva, scarified skin and cervix. The major route in natural infections appears to be oropharyngeal. Following localization in the regional lymph nodes, there is a bacteremic phase with seeding of organisms within phagocytic cells in lymph nodes and spleen. Microscopic granulomas without necrosis are seen. In pregnant animals, invasion of the glands and endometrium of the gravid uterus is followed by colonization of the placenta. Multiplication of the organisms is relatively unchecked and very large populations are generated in the placenta, the fetal fluids and the fetal tissues. The placental epithelial cells are packed with brucellae and destruction of the villi is common. Abortion follows, usually during the last trimester of pregnancy, although the incuba-

tion period is inversely proportional to the stage of pregnancy at the time of exposure. Very large numbers of organisms are shed with the fetal fluids and membranes. The numbers shed from the uterus decrease rapidly and usually few brucellae are found in the mucus after 4 to 6 weeks.

Exposure of non pregnant animals results in a similar progression except that colonization in the non gravid uterus is rare. When pregnancy occurs in such an animal, invasion of the uterus is delayed, the bacterial population is smaller and abortion is less likely.

Non pregnant animals exposed to small numbers of organisms may develop self limiting, immunizing infections. This may be responsible for low infection rates in small self contained herds, but this outcome is unpredictable, and some animals become carriers.

Of animals which abort, approximately 80% do so only once and more than two abortions by the same animal is rare. Reinvasion of the uterus occurs in subsequent pregnancies but the placental damage is limited and bacterial populations are reduced. Full term pregnancies are usual but brucellae are shed with the membranes and fluids, serving as a source for further spread within or between herds.

Persistent infection of the mammary glands and supramammary lymph nodes is common, with constant or intermittent shedding of organisms in the milk in succeeding lactations. The inflammatory changes in the infected mammary gland reduce milk production by an estimated 10 percent.

Sexually immature animals are relatively resistant to persistant infection. Most calves borne to infected dams have infection in the lungs and regional lymph nodes. Those fed infected milk have infections of the lymph nodes draining the gastrointestinal tract, and may shed brucellae in the feces while receiving infected milk. Most recover from these infections and are fully susceptible when they reach sexual maturity. A small and undetermined proportion of calves born to infected dams remain latently infected, with clinical signs and serological response becoming apparent only at abortion or parturition in their first pregnancy (Plommet, 1977). While this is rare, it has important implications for control and eradication schemes. Other sites of localization include the carpal and suprascapular bursae where hygromas containing large populations of brucellae may be formed. These may be seen in work oxen as well as cows.

In the male, localization in the testis, epididymis and accessory sex organs is common, and organisms are shed in semen. This may result in infertility, but it is not a major mode of transmission to cows.

Immune Response

Both antibody mediated and cell mediated immune (CMI) responses are seen. As the major diagnostic tests for the disease are designed for the detection of antibody to the S-LPS complex of the *Brucella* cell, most of our information concerns the antibody mediated reactions.

After infection with virulent strains or vaccination with attenuated live *B. abortus* strain 19, antibody of the IgM class is the first to be detectable in serum, followed by antibody of the IgG class. Both IgG_1 and IgG_2 subclasses are detected. The type of serological test used influences the titer reported, as the immunoglobulin classes and subclasses differ in activity depending upon physical conditions. IgM antibody is active in agglutination tests at neutral pH, but with antigen buffered at pH 3.65 (Card or Rose Bengal Plate test) the agglutinating activity is reduced, and sera taken early after infection or vaccination may be negative. Treatment of serum with the acridine dye rivanol selectively precipitates out more IgM than IgG, and treatment of serum with the reducing agent 2 mercaptoethanol dissociates the IgM pentamer. Any of these treatments reduces agglutination due to IgM antibody without affecting activity by IgG subclasses (Report, 1971). IgM antibody in serum is relatively heat labile and its agglutinating and CF activity are reduced by heating serum at 56 to 58 °C for 30 to 60 min.

Agglutination by IgG_1 is enhanced at pH 3.65, or by use of antigens in 5% NaCl. Bovine IgG_1 is active in the CF test. IgG_2 is active in agglutination but does not fix complement (Diaz and Levieux, 1972; Alton, 1977).

The first appearance of antibody is related to the size and virulence of the inoculum and to the stage of pregnancy of the animal. On the average, antibody reaches diagnostic titers by 4 weeks after exposure during the fourth to sixth month of gestation, and at about 10 weeks after exposure in non pregnant animals or animals in the first trimester of gestation. However, significant numbers of newly infected animals will not have circulating antibody until 1 to 3 weeks after abortion or full term infected parturition (Plommet, 1977).

Infected cows secrete antibody in their milk, and there is a direct relationship between titer in the secretions from a quarter and presence or absence of infection in that quarter. Antibody can be titrated by agglutination tests using whey, or the milk ring test (MRT) using pooled negative milk as diluent. Preparturient animals concentrate serum immunoglobulin, especially IgG_1, in colostrum, and previously vaccinated cattle with low serum antibody titers have high colostral and early postparturient milk antibody titers.

Infected cattle produce antibody to protein antigens distinct from the S-LPS complex (Schurig *et al.*, 1978), and also produce antibody, predominantly

IgG_1, which is specific for a polysaccharide called poly B, extracted from a rough strain of *B. melitensis* (Diaz *et al.*, 1979).

The magnitude and duration of antibody response following vaccination with strain 19, is directly related to the age at vaccination and the number of organisms administered. In animals vaccinated at 3 to 8 months of age with ca. 5×10^{10} viable organisms, antibody is usually detectable within 2 weeks, and usually drops to very low levels 4 to 6 months later. Residual antibody is predominantly of the IgM class, and diagnostic tests which minimize its activity, such as the CF and rivanol tests, partially discriminate between infected and vaccinated non infected animals (Alton, 1977). Vaccinated non infected animals with antibody to the LPS complex are unlikely to have detectable antibody to protein antigens or to poly B (Schurig *et al.*, 1978; Diaz *et al.*, 1979). In infected environments, vaccinated animals will often develop transient increases in antibody titer. This is a reflection of antigenic stimulation in primed animals and its transient nature is a good indication that the animal has resisted the exposure.

Brucellosis is one of the bacterial infections in which effective antibacterial defense is considered to require participation of cell-mediated immune (CMI) reactions, which are presumed to operate through activation of tissue macrophages after recognition of specific antigens (McCullough, 1970).

Evidence for this concept is actually limited to the demonstration of intracellular killing of *Brucella in vitro,* and to the temporal relationship between correlates of CMI and infection, or of effective vaccinal protection in laboratory animals and cattle. These correlates include delayed type hypersentivity to brucella antigens *in vivo* (Jones and Berman, 1971; Fensterbank, 1977), and mitogenic responses of peripheral blood leukocytes (PBL) to *Brucella* antigens *in vitro* (Kaneene *et al.*, 1979).

The types of soluble *Brucella* antigens used to measure CMI reactions significantly affect the outcome of the tests. The most commonly used preparations are all complex mixtures of antigens extracted from *Brucella* cells in various ways. All contain a large number of different proteins, and either contain LPS or do not. The presence of LPS complicates the interpretation of delayed type hypersensitivity reactions both because of its toxicity and its participation in antibody-mediated cutaneous hypersensitivity (Jones and Berman, 1975). PBL from infected cattle have greater mitogenic responses to antigen mixtures containing *Brucella* LPS than to preparations which do not contain it, but the nature of this difference has not been adequately explored (Kaneene *et al.*, 1978).

Recently, some of the temporal relationships in the CMI response of cattle to infection with *Brucella* have been described. Relatively small numbers of animals have been investigated, so the following generalizations are incom-

plete, and individual variations are not considered, despite their importance in epidemiology.

Mitogenic responses of PBL stimulated in culture by antigen preparations containing LPS have been demonstrated as early as two weeks after infection with virulent brucellae, and the response was evident with some animals before antibody could be detected in their serum (Kaneene *et al.*, 1979). PBL from infected animals responded either weakly, or were unresponsive to LPS-free antigen preparations (Kaneene *et al.*, 1978). Cells from animals vaccinated 3 or more months previously with strain 19 were not stimulated in culture by either type of antigen preparation, even if they had residual antibody.

In skin tests for delayed hypersensitivity in infected cattle, LPS-free antigen, injected intradermally into the eyelid or caudal fold, produced induration, which reached maximum intensity at 72 hours. In one investigation in 27 infected herds (Fensterbank, 1977), approximately 55% of the animals were serologically positive, reacted to the skin test, or both. Of this group, approximately half gave positive reactions to both serological tests and skin test, 22% were positive by serology but were negative on skin test, and 27% were positive on skin test but negative by serology. Animals which had been vaccinated with strain 19 as calves were negative when skin tested at 20 or more months of age. In general, the skin test for delayed hypersensitivity detected infection on a herd basis, and in recently infected herds it detected more infected individuals than serological tests alone and detected some newly infected animals before the serological tests did.

Anti-infective Immunity

Effective protection against challenge by virulent brucellae is engendered either by self-limiting infection as is provided by attenuated strains, such as *B. abortus* strain 19, or by the use of killed organisms incorporated in water in oil adjuvants, such as *B. abortus* strain 45/20 vaccine.

The attenuation of virulence of strain 19 is stable, and although it can produce abortions when injected in large doses into pregnant heifers, there is no evidence of further spread. The protection induced is relative and not absolute. The factors which modify the extent of vaccinal resistance include degree of exposure, virulence of the infecting strain and stage of pregnancy of the individual animals at the time of exposure. In herds and areas, degree of exposure will be influenced by the proportions of vaccinated and unvaccinated animals at risk.

The standard recommended vaccination procedure is administration of not

less than 5×10^{10} viable organisms subcutaneously to female calves 4 to 8 months old. Males should not be vaccinated. In the absence of, or at low levels of exposure to virulent organisms, most such vaccinated animals lose their agglutinins and CF antibody 16 to 18 months later. Persistence of antibody increases directly with age at vaccination with the standard dose of vaccine, and in mature animals is usually permanent. Persistence of antibody in mature cattle vaccinated with reduced dosage by the subcutaneous route decreases with the dosage. Doses of approximately 10^9 cells are protective, at least during the next year, and antibody as detected by the CF or rivanol tests, generally drops below diagnostic levels after 6 to 8 months (Nicoletti *et al.*, 1978a).

Doses of approximately 5×10^9 organisms administered twice at 4 to 8 month intervals by the conjunctival route were protective both in controlled and field exposure trials. This route of administration had the advantage of not producing serologic reactions which interfered with diagnosis, even when used in adult cattle (Plommet and Fensterbank, 1976; Nicoletti *et al.*, 1978b).

To overcome the problems of persistent post vaccinal agglutinin reactions, a killed vaccine of the rough strain *B. abortus* 45/20 in adjuvant has been developed and has wide use in many countries. It can be administered to mature cattle. Two initial doses of the vaccine are required and booster injections every 12 to 18 months are advocated in high prevalence areas. Protection engendered is generally equivalent to that produced by strain 19. The vaccine is not entirely non agglutinogenic, with low titers of antibody, including transient rises on CF tests, being seen after the second and booster injections. Antibody is detected with the antibovine globulin agglutination test (Coombs test) at high titers and this persists indefinitely after the second injection (Cunningham, 1977). Persistent delayed type hypersensitivity to LPS-free skin test antigen is seen even in the absence of exposure to virulent strains (Fensterbank and Pardon, 1977). Localized granulomas are produced at the site of the second and subsequent vaccinations.

In animals sensitized by strain 19 vaccination or by latent infection with a virulent strain, a single injection of 45/20 vaccine produces persistent high Coombs test titers and variable CF test titers. This so called anamnestic test is used as a diagnostic procedure for identification of latent carriers (Cunningham, 1977a). The quality of the adjuvant and dissociation of the strain influence the ability of a batch of vaccine to immunize effectively. For these reasons, potency testing of each batch in animals is essential (Cunningham, 1977b).

Under experimental conditions with both vaccines, when vaccinated animals were challenged in mid-pregnancy about 25 to 30% of nonvaccinated

animals were resistant to challenge with a dose of virulent *B. abortus* which infected about 25 to 35% of vaccinated heifers or cows. With higher infective doses, up to 100% of non vaccinated and as few as 50% of vaccinated animals became infected. With exposure under field conditions, because the age of the animals, their stage of pregnancy, and dose of virulent *B. abortus* are all highly variable, vaccination with strain 19 and probably with 45/20 may provide as much as 90 to 95% protection for vaccinated animals. This would be true particularly if all animals in the herd or area were vaccinated.

Because vaccinal protection from infection is not absolute, it is important to achieve high levels of vaccination of individual animals within herds and of the total population of herds for optimal results. However, there is also a lower limit to the prevalence which will be achieved by a control program which depends upon vaccination alone, even when universal vaccination is practiced. Experience in the USA suggests that under such conditions, with no removal of animals except for normal culling, the prevalence of infection stabilizes at about 2% of cows. Conversely, while vaccination of less than 70% of cows within herds and less than 70% of herds will reduce individual animal infection rates and abortion rates within the vaccinated herds, it will reduce the overall animal infection rate within the total population only slightly.

Diagnostic Methods

Unequivocal diagnoses of brucellosis can be made by isolation and identification of the organisms by culture or guinea pig inoculation of materials from aborted fetuses, placenta, uterine exudate, milk or lymph nodes from the affected cow. Methods have been published (Alton *et al.*, 1975) and will not be detailed here.

The serological tests based on detection of antibody in serum and milk have been reviewed by Alton *et al.* (1975), and more recent information has been discussed above. The use of an International Standard Anti-*Brucella abortus* Serum (ISAbS) is well established for standardizing antigens for the serum agglutination tests and a unitage system is widely used for expressing the antibody levels of sera (Report, 1970). Antigens used in other tests, such as the MRT, buffered antigen rapid plate tests (card or Rose Bengal antigen tests) and CF tests are all derived from the standardized agglutination test antigen.

The MRT is the most practical and economical method for locating infected dairy herds and for surveillance of brucellosis-free herds. If performed on

pooled herd milk in cans or bulk 3 to 4 times a year on each herd, it will detect the majority of infected herds. It can also be used to survey for herd infection in nomadic or semi-nomadic herds. Herds with a positive MRT can then be examined by serological tests or individual animal milk agglutination tests, to identify the infected individuals.

Surveys can be made on serum samples taken from animals at slaughter, when assembled at markets, or for periodic veterinary attention such as dipping or vaccination programs. If individual animals are identified so that they can be traced to area or herd of origin, this type of surveillance can be used in identifying foci of infection. The epidemiological fact to be stressed is that brucellosis is a highly contagious disease. Diagnosis of infection in individual animals in a herd means that the entire herd should be considered as an infected unit. Animals with direct or indirect contact with known infected herds are also at high risk of exposure. This can occur on common or adjacent grazing, communal watering facilities, in markets, or on migration routes.

Prevention and Control

It is not possible to lay out precise recommendations for prevention and control of bovine brucellosis which would be appropriate for all conditions found in the tropics. The variety of systems of husbandry, range of climatic conditions and variability in prevalence of infection, as well as in the availability of financial resources all influence what can be expected in control efforts.

The principles which govern control in infected herds and areas are based upon minimizing transmission. Hygienic procedures such as segregation of preparturient animals and keeping them separate for a week or more after parturition, prompt disposal of aborted fetuses and placentas are important whether in intensive husbandry or with traditional systems. Identification and slaughter or segregation of carriers removes them as a source of infection and vaccination both protects the susceptibles and reduces spread by lowering the abortion rate.

It is possible for individual herds and groups of herds to bring the disease under control, and even to eliminate it by these methods, but such brucellosis-free herds in high or moderate prevalence areas are at great risk of reinfection from neighboring herds. Continued vaccination, attention to hygiene, and acquisition of replacement animals from brucellosis-free herds or areas are all essential to maintenance of brucellosis-free status.

The Joint FAO/WHO Expert Committee on brucellosis in its fifth report

recommended programs for control and eradication of brucellosis. While the principles and procedures in those recommendations are still valid, they placed greatest emphasis on the eradication and control plans which require high costs on short term intensive activity. Thus, they are most appropriate for application in developed countries with highly organized veterinary services and adequate financial resources.

For most of the cattle populations in the tropics, particularly under traditional systems of husbandry, vaccination, with or without serological surveys to furnish current information on prevalence, will be the control method of choice for the foreseeable future. Recent information on the responses of adult cattle to reduced doses of strain 19 vaccine, makes it possible to give greater emphasis to whole herd vaccination in order to produce the maximum increase in herd immunity in the shortest time. Vaccination of more than 70% of the animal population must be continued as long as prevalence has not been brought to very low levels.

Killed 45/20 vaccine can have a useful place in control in the tropics. A description of its use in a well designed scheme to eradicate brucellosis in beef cattle in a large range enterprise in northern Australia has been published recently (Slatter, 1977).

The greatest cost effectiveness for scarce economic resources in moderate to high prevalence areas will be obtained by maximum coverage with an effective vaccine until prevalence is reduced to the point where it becomes economically feasible to take steps towards eradication. During that time, information programs must receive substantial emphasis. The support of livestock owners can be assured only when they can see the benefits to be derived. It is obvious what the decision will be on allocation of funds between vaccination to prevent a fatal disease such as anthrax, and vaccination against brucellosis, which might cause abortions once or twice in the reproductive life of a cow. The economic costs exacted by brucellosis increase in relative importance as husbandry becomes more intensive, and control of the disease is then likely to command a greater share of economic resources. Information on the human health hazard can play a decisive role, but probably only in situations in which the other major human infectious diseases have been greatly reduced in prevalence.

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23. JOHNE'S DISEASE (PARATUBERCULOSIS)

W. I. M. McIntyre and I. E. Selman

Abstract. Johne's disease is characterized clinically by progressive weight-loss and profuse diarrhea. Established cases always die and the major pathological feature is a massively proliferative enteritis. It is caused by the acid-fast organism *Mycobacterium johnei*. Despite the fact that Johne's disease is a condition of adult cattle, infection is usually acquired during the first few months of life by ingestion or else by intra-uterine infection. The organism localizes in the wall of the small intestine and in many cases after a few months a spontaneous recovery takes place. Alternatively, a carrier state is established which, in a relatively small proportion of cases eventually develops into the clinical disease. Confirmation of diagnosis in the living clinical case rests upon serological and microbiological investigation and can sometimes prove difficult; post-mortem diagnosis is based upon the demonstration of typical lesions and microbiology. The detection of cattle in the "carrier" state is often impossible due to the deficiencies of all present testing procedures. Control rests upon hygienic procedures and, sometimes, vaccination. There is no known effective treatment.

Etiology

Mycobacterium johnei (*M. paratuberculosis*) is a small, rod-shaped organism (1.0–2.0×0.5 μm). It is strongly acid-fast. In stained fecal smears from clinically-affected cattle, it may occur singly or in tight clumps reminiscent (presumably only to the Occidental eye) of Chinese writing. Sometimes these clumps can be seen occupying discarded epithelial cells. *Mycobacterium johnei* is aerobic, non-spore-forming and non-motile. It has rather fastidious culture requirements and only grows in the presence of egg yolk and a source of "growth factor", such as killed *M. phloei* or its extracts or else in highly specific synthetic media. Even when grown under optimum conditions, *M. johnei* grows extremely slowly and successful culture may take several weeks or even months. For greater detail regarding the cultural characteristics of this organism, the reader is referred to Soltys (1963).

Several different strains of *M. johnei* have been described but their relative prevalence in cattle and their relationship with those strains which are known

to infect sheep and goats, is unknown. At least one strain of the organism produces a pigment which stains affected areas of gut bright orange; however, this strain would seem to be primarily a pathogen of Scottish sheep.

Pathogenesis

Mycobacterium johnei has a very definite predilection for the small intestine and even localises there when organisms are administered intravenously; however, the normal route of infection is by ingestion.

Following infection, the organism enters the intestinal mucosa and undergoes a phase of multiplication; thereafter, the sequence of events largely depends on the natural resistance of the host (Gilmour, 1976). In the majority of infected animals spontaneous recovery takes place after a few months while in the less fortunate minority there is progression to a chronic "carrier" state. Some of these latter animals eventually become clinical cases although this usually takes a considerable time (see below). The fundamental reasons why only some animals develop the clinical disease is not understood but several workers have suggested that predisposing factors are important and cite as an instance the well-recognized relationship between the onset of clinical signs and recent parturition.

In cattle which are destined to become clinical cases, small lesions in the terminal ileum gradually extend, finally to become confluent. This process may take several years during which time an affected animal is not obviously unwell although a degree of stunting or weight-loss may occur. In dairy cattle, it is sometimes possible to confirm from a retrospective examination of milk records that the previous lactation was poorer than anticipated for no other apparent reason (Gilmour, 1976). Eventually, the animal develops a malabsorption syndrome and diarrhea with an accompanying "leak" of plasma proteins into the intestinal lumen (i.e. a protein-losing enteropathy), all due to the effect of the widespread and massive cellular reaction within the wall of the intestine.

The local (i.e. intestinal wall) changes which arise and which are responsible for this series of events are highly reminiscent of those which occur in bovine tuberculosis. The portal of entry is the wall of the small intestine and a "primary complex" type of lesion is established here and in the associated (mesenteric) lymph nodes. Occasionally, similar lesions may be found elsewhere, for example in the tonsils and retropharyngeal lymph nodes. The cellular reaction resembles that seen in a tubercle although intestinal ulceration does not occur and other subsequent changes such as fibrosis, caseation and calcification do not take place, at least in cattle. The intestinal cellular

reaction involves a massive infiltration of macrophages containing acid-fast organisms, epitheloid cells, multinucleate giant cells, eosinophils, lymphocytes and plasma cells; occasional areas of necrosis may also arise. The reaction brings about a marked distortion of the normal architecture of the small intestine and, while its presence must inevitably give rise to a considerable degree of impaired function, the basic mechanism(s) involved in the pathogenesis of diarrhea in Johne's disease is still a matter of some debate.

"Post primary dissemination" is not a major feature of Johne's disease although lesions may arise occasionally in other parts of the body (Jubb and Kennedy, 1970). Intra-uterine infections of calves of both clinically-affected and "carrier" cows is now considered to be far more important in the epidemiology of the disease than was one thought (Kopecky *et al.*, 1967).

Distribution

Johne's disease has been recognized as a fairly common condition in both Europe and North America for many years. Its spread to many other parts of the world can very probably be blamed on the introduction of European-type (carrier) cattle. While accurate data are lacking, it would seem that, once introduced, it is a disease which persists and spreads far more successfully in the more humid tropical areas. However, this will also be affected by varying local managemental and other factors (see below).

Epidemiology

Mycobacterium johnei is an obligatory parasite which is highly resistant and is able to survive for at least a year in dung or damp soil.

Experimental studies using orally and parenterally-administered organisms and also natural (contact) exposure to a heavily-contaminated environment have emphasized that calves aged less than six months are highly susceptible to infection and, once infected, commonly go on to develop the clinical disease. In contrast, attempts to infect adult cattle experimentally have frequently failed or else only transient infections have been established. On the few occasions when such attempts have been successful and clinical disease is claimed to have resulted, it has usually been of a mild nature and spontaneous recovery has often been the outcome. Under field conditions it is usually assumed that moderate to heavy challenge (and the pathogenic dose may be as small as 1000 organisms in sheep) a large proportion of calves may become infected at an early age. There is also evidence to support the view

that infection is much more likely to occur in the offspring of either a fecally-excreting (carrier) animal or else a clinical case of Johne's disease. Clearly, fecal contamination of calves and teats is a major hazard under such circumstances and it is just possible that infection is made even more likely by the occasional excretion of *M. johnei* in the milk of infected cattle (Blood *et al.*, 1979). However, when the subject of early infection is discussed it must always be remembered that infections may arise *in utero* and, as yet, the relative importance of the two methods of infection have not been defined.

Although infection is most probably almost always acquired early in life, the insidious nature of Johne's disease is such that it is unusual to encounter clinical cases in cattle aged less than three years. Usually the peak age incidence is five or six years (i.e. in second or third calvers) and the disease is uncommon in cattle aged over eight years. On very infrequent occasions, Johne's disease may be found in yearling cattle; when such cases occur they are usually ranch-type (beef) cattle and it is assumed, rightly or wrongly, that either challenge was extremely high during the early neonatal period or else intra-uterine infection occurred.

Johne's disease occurs in bulls and sometimes the course of the disease is particularly short in such animals with cases dying within one week of the onset of clinical signs. In females, it is common for the disease to become clinically apparent shortly after parturition although (as stated above) the bodily condition or productivity of these animals may have given cause for concern prior to this.

Johne's disease may be a problem in both beef and dairy cattle although managemental factors may play a part in either exaggerating or suppressing the clinical aspects of the disorder. In beef cattle, the prolonged and intimate contact between dams and offsprings, the use of traditional calving areas, reliance upon natural (particularly stagnant) water sources and a reluctance to cull sick cows at other than traditional times (i.e. at weaning) all combine to make the disease quite often a regular clinical problem. In dairy cattle on the other hand, a disappointing early lactation may well result in an animal being culled purely on commercial grounds long before clinical signs develop. An additional problem, shared by beef and dairy cattle, is a growing reluctance on the part of many ranchers and farmers to seek veterinary advice for individual animals and also a widespread tendency not to investigate and record the reasons for culling in adult cattle.

The mortality rate *for established cases* of Johne's disease is 100%; the morbidity rate is usually relatively low, at around 1% per annum. This latter figure is presumably related among other things to the survivability of *M. johnei* under local conditions and the level of environmental reinfection. Since the organism is known to be highly resistant and also faecal excretion may

occur, albeit intermittently at first, during both the pre-clinical and the clinical phases of the disease (i.e. for up to several years in some individuals) it is not surprising that once permanent pasture is infected it probably remains so almost indefinitely. On certain farms and ranches, the morbidity rate may approach 10% per annum although the basic reasons for this are not known. Some workers have suggested that soil factors may increase the survivability of *M. johnei* (Blood *et al.*, 1979) but it is equally possible that on such farms only a slight increase in the morbidity rate in one year may susbstantially increase pasture reinfection rates and hence the challenge that calves are subjected to in subsequent years.

This question of reinfection rates and levels of challenge poses very relevant questions *vis-à-vis* certain modern intensive management systems although the answers to these questions are not, as yet, forthcoming. Many newer systems bring cattle of all ages into daily contact with liquid manure (slurry) often for several months of each year if not continuously; at other times of the year cattle graze fields on which vast quantities of slurry have been pumped or sprayed or else they are fed cut products from such fields. Under these conditions it is just possible that in the future *M. johnei* infection may occur and give rise to much more trouble than it has under more traditional systems. Clearly, much more needs to be learned about the survival rates of the organism in slurry stored in either tanks or "lagoons" and on slurry-dressed pasture.

Many different species of animal have been shown to be susceptible to *M. johnei,* apart that is from cattle. However, of the domestic species it is important to emphasise that infections have been described on several different occasions in buffaloes, camels, goats and sheep.

Clinical signs

The clinical signs of Johne's disease typically arise in a thin cow which has very probably calved its second or third calf within the last few weeks. Given sufficient opportunity (or interest) the attendant may have noticed the animal passing loose feces on one or two previous occasions. There may also be the suggestion that the animal's condition or productivity has been giving cause for some concern for several months.

On examination, the animal will be found to be bright, alert and interested in food, despite its poor condition. The only other abnormality which may be found in an uncomplicated case is profuse diarrhea. The feces will probably contain bubbles (although this is in no way pathognomonic for Johne's disease); dysentery is not a feature of the condition and the dung does not

smell unpleasant. In many cases, particularly if the animal has been housed on dry feed for a day or two or without adequate provision of water, the feces will be relatively normal in amount and consistency and the only sign to support the history of previous diarrhea will be fecal staining of the tail and hindquarters. Since considerable weight-loss may have occurred over a relatively short period, there may be folds of loose skin in the intermandibular space and on the dewlap and brisket. This is often mistaken for subcutaneous accumulations of edema. Certainly subcutaneous edema may occur but it is a rare clinical finding (limited to about 5% of cases) and usually it is only a transient feature of early cases, disappearing as the severity of diarrhea increases.

In later cases, an affected animal becomes much weaker and is finally unable to rise. However, even at this stage it may well be still interested in food and water. Eventually, though, its condition worsens to the point when its fluid output (in feces) exceeds its fluid intake. At this stage, the animal becomes increasingly depressed and anorexic and it usually dies within a few days. Terminally, there may be a marked bradycardia (ca. 40/min).

Pathology

The most striking feature on first examining the body of a cow which has died of Johne's disease or else been slaughtered in the terminal stages is the extreme degree of emaciation. Further investigation usually reveals a rather "wet" or edematous carcase which has not set properly. Varying accumulations of a gelatinous edema fluid are to be found in the subcutaneous tissues, between muscle bundles and in the mesentery and abomasal folds; there is usually a (minor) degree of ascites.

The small intestine is not usually markedly abnormal and in fact a histological examination has to be carried out in about 20% of severe cases in order to confirm the presence of a proliferative enteritis. However, in most cases a careful examination of the terminal part of the small intestine will reveal a thickened intestinal wall and on section the lining will be seen to be thrown up into corrugations. (This change has to be differentiated from the "normal" thickening which occurs when small intestine lays on a cold necropsy room floor; this can be done by stretching the opened bowel between the fingers when, in the latter instance, the corrugations disappear.) There is no ulceration of the intestine. Some workers (Blood *et al.*, 1979) claim that lesions always exist on the ileo-caecal valve, ranging from a degree of reddening to marked swelling and edema; however, this is not universally agreed upon. The mesenteric lymph nodes are often swollen and edematous.

Histological examination reveals the changes described in the section on pathogenesis, although it should be emphasized that in a relatively high proportion of cases, tissue has to be collected for examination from several different parts of the small intestine in order that lesions are not missed.

Immune response

The fact that adult cattle are much more difficult to infect experimentally than are calves suggests that some form of age resistance to *M. johnei* operates although it is by no means clear that this is of an immunological nature. Moreover, the insidious character of Johne's disease is such that it is always possible that many of the experimental adult cattle which were used had undergone exposure to the organism in earlier life and had thereby acquired resistance in that way.

Certainly, *M. johnei* is capable of stimulating several different immune responses in cattle. In the early stages of the disease (i.e. before the onset of clinical signs) antibodies are produced which are susceptible to the effects of 2, mercapto-ethanol (2ME) and which may be detected by the fluorescent antibody test (FAT). Later, other antibodies are formed, this time which are 2ME-resistant; these are detected by the complement fixation test (Yuai, 1974). It is also known that these latter antibodies may eventually disappear, some time during the course of the clinical phase of the illness. Skin sensitizing antibodies also arise during the early (i.e., preclinical) stages of infection and then often decline as the animal becomes clinically-affected. Finally, quite apart from these phenomena, it has to be remembered that the cellular events within the intestinal wall indicate that in all probability a cell-mediated immune reaction is operating at the site of infection.

Laboratory aids to diagnosis

It is convenient to subdivide this section into (i) post-mortem confirmation of diagnosis, (ii) confirmation of diagnosis in a clinically-affected animal, and (iii) identification of carrier cattle.

In general terms, the histopathological features of Johne's disease are sufficiently characteristic to enable a competent pathologist to make a definite diagnosis. If necessary, this may be complemented by an examination of an impression smear of the small intestinal mucosa, using the Ziehl-Neelsen staining technique for acid-fast micro-organisms. While culture of organisms may be carried out, the time taken (i.e., up to 12 weeks) means this is of little practical value except under special circumstances.

Confirmation of diagnosis in the living animal is sometimes more difficult although with local knowledge the disease does not usually present a major problem to the clinician. Techniques for confirmation are essentially microbiological (on feces) or serological. Again, culture of feces is usually of no practical merit but an examination of a fecal smear may yield positive results in about 60% of cases provided that proper preparation of the smear is carried out (Cunningham and Gilmour, 1959) and the Ziehl-Neelsen staining technique is used. However, it must be emphasized that a "positive" result can only be established if *clumps* of organisms are seen; single acid-fast organisms may be other mycobacteria and therefore should be viewed as "doubtful" and a retest requested in one week. The only useful serological tests are the fluorescent antibody test (FAT) and the complement fixation test (CFT). Both tests are of approximately equal merit (Gilmour, 1976) and both suffer the disadvantage that clinical cases may become seronegative as the course of the disease progresses. In addition, false positives may occur, particularly with the CFT. Approximately 90% of cattle with advanced disease are positive to the CFT (Gilmour, 1976). Certain veterinarians have also claimed that a "pinch biopsy" removed from the rectal mucosa *per rectum* and then stained for acid-fast organisms is also a useful laboratory aid in the living animal. This author has no personal experience of this technique but feels that many cases would yield repeated negative results since the disease would need to be widespread (and terminal) before clumps of acid-fast bacteria could be demonstrated by this technique. It is unlikely that other biopsy techniques (Blood *et al.*, 1979) would find practical and/or commercial support under present day farming conditions.

The most difficult problem to be faced in this area is the detection of carrier animals since currently there is no single test which enables this to be done (Gilmour, 1976). Again, attempts are basically either microbiological or immunological.

Examination of fecal smears is notoriously unreliable in carrier cattle and while fecal culture has been advocated under certain circumstances (Gilmour, 1976) the time taken is again a major practical disadvantage. The CFT is of little use because on the one hand it gives rise to false negatives and on the other to false positives, due to cross-reaction with antibodies to other bacteria, notably *Corynebacterium renale* (Gilmour, 1976). The FAT is more specific but is said to have the disadvantage of detecting antibodies which are produced only sporadically (Gilmour, 1976), hence a single test is of doubtful value. Skin testing, using "Johnin" (the *M. johnei* equivalent of tuberculin) is certainly useful in the early stages of the diseases but is subject to false positive reactions, largely due to cross-reactivity with *M. avium* (Blood *et al.*, 1979). Several other tests have been devised and used under field conditions

(Goodswaard *et al.*, 1976) but these have been found to be inferior to the CFT and the FAT.

Prevention and control

On farms where Johne's disease has been defined as an endemic problem worthy of action, attention should be directed towards prevention of environmental contamination and the avoidance of known or fancied danger areas. This is particularly important in ranch-type cattle since it is about the only approach that is practically feasible. All clinical cases and "suspicious-looking" cattle should be culled, together with their offsprings. In the absence of reliable immunological tests it has been suggested that fecal culture might have a role to play although the disadvantages of this have already been discussed. Since permanent pastures, once contaminated, can be viewed as being likely to be infected almost indefinitely, any known or suspected areas should be avoided at least as calving areas and as grazing for young stock. In a similar way, any stagnant water sources should be fenced off whenever this is at all possible. Ideally, water should be available from head-high troughs so that fecal contamination is much reduced or eliminated.

Vaccination is an approach which may also be used in conjunction with the above precautions. The vaccines available are adjuvanted products containing either live or dead strains of the causal organism. Unfortunately, there are several drawbacks associated with this approach in that (i) while it reduces the number of clinical cases, it does not eliminate infection but merely decreases the rate of fecal excretion, (ii) it also creates skin sensitivity to avian and mammalian tuberculin, thus creating possible problems in the interpretation of the tuberculin test, (iii) it has to be administered subcutaneously to calves under one month of age and it often induces the formation of unsightly fibrous nodules, a possible problem in pedigree herds, and (iv) since its successful use demands that calves must be reared away from contact with adult cattle, its use under ranch-type conditions is not possible and it is precisely this type of animal which in some countries is most affected.

As yet, there is no effective treatment for Johne's disease. Case management therefore rests upon early recognition of clinical cases and prompt slaughter in order to minimize environmental contamination and also realise the greatest salvage value possible.

The only other aspect worthy of further consideration is the problem of import and export certification. Clearly, countries or even farms which consider themselves free (or relatively so) of Johne's disease should take the best precautions possible to prevent introduction of that condition. However, the

fact that no laboratory test is capable of accurately determining whether or not an individual animal is a carrier means that strict control is not possible. Under these circumstances, perhaps the best precaution that an importing country should take is to insist (i) that all cattle should originate only from herds where the disease has not been recognized for a specified number of years (while at the same time recognizing the limitations of this sort of approach) and (ii) that all cattle should be shown to be negative to the intra-dermal (Johnin) test; this latter approach will inevitably produce many false positives but these are not a major problem to prospective importers except insofar as they reduce the number of cattle available for purchase.

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24. TUBERCULOSIS

I. E. Selman

Abstract. In many countries, tuberculosis is a relatively common disease of cattle; in others, its prevalence is naturally lower or it has been rendered thus by various control measures. It is almost always due to infection with *Mycobacterium bovis* although occasionally other mycobacteria may be responsible. The organism is usually acquired by inhalation of organisms excreted by other affected cattle but other modes of infection may sometimes occur, particularly through ingestion. The syndromes which arise depend on the nature, extent and site of the lesions. Cattle may show respiratory or abdominal signs, lymphadenopathy, mastitis, nervous signs or reproductive tract disease; there is usually marked weight and production loss, weakness and eventually death. The pathological picture is dominated by a granulomatous inflammatory reaction, with fibrosis, necrosis, caseation and calcification. Given adequate resources and facilities, it is possible to eradicate bovine tuberculosis from a country or region by the use of one or other of the tuberculin tests. Bovine tuberculosis is a very serious and often fatal disease hazard for man and the most common route of transmission is via contaminated milk.

Etiology

In cattle, tuberculosis is almost always caused by *Mycobacterium bovis* although occasionally the disease has arisen from infection with other species, notably *M. avium* and *M. tuberculosis* of bird and human origin, respectively.

Mycobacterium bovis is a slender, rod-shaped bacterium ($3.0-5.0\times0.4$ μm) which may occur singly or in clumps; in culture, it may exhibit more marked pleomorphism. It is aerobic, it does not form spores, and it is only capable of multiplication within the animal body. Usual staining techniques, such as the Gram method, are not readily applicable to tubercle bacilli, and their most characteristic staining property is their acid-fastness by the Ziehl-Neelson staining method. *Mycobacterium bovis,* like all other pathogenic mycobacteria, grows very slowly in culture. For a more detailed description of the organism and its behavior, the reader is referred to Soltys (1963).

Pathogenesis

It is generally accepted that the most common portal of entry in adult cattle is via the lungs following inhalation of the causal organism. A lesion is produced at the point of entry which rapidly assumes the appearance of a typical tubercle. The tubercle and the similar lesion in the associated lymph node(s) are termed the "primary complex". Such a lesion may also arise following infection by other routes although when cattle develop tuberculosis following ingestion of tubercle bacilli (e.g., when a young calf drinks contaminated milk) the lesion at the point of entry is often absent or overlooked. The most common lesions resulting from an oral infection are in the pharyngeal and/or mesenteric lymph nodes. This, and the high affinity displayed by *M. bovis* for pulmonary tissue, has prompted certain workers to suggest that inhalation infections in cattle are overrated and that infection by ingestion is probably more common in all ages of cattle than is generally accepted.

In many cases, infection eventually spreads within the body, a process termed "post-primary dissemination". The nature, extent, and rate of dissemination varies markedly between individual cattle although the disease is generally considered progressive in this species. There is an extension of lesions due to local spread, infiltration, and transport of infection across the body cavities and along blood and lymphatic vessels. If an animal survives long enough, a final stage is reached where rapid extension of the disease occurs presumably due to a total collapse of host resistance; this is termed the stage of "late generalization".

The syndrome which arises depends upon the degree of involvement of different organs and tissues with associated weight-loss, decreased productivity and weakness, leading finally to recumbency and death. The reasons for these profound systemic effects, however, are difficult to explain since tubercle bacilli are not toxin-producing in the accepted sense. The organisms are capable of provoking host resistance and a state of hypersensitivity. Far from being advantageous to the host, these factors are largely responsible for the major features of the disease, i.e., a highly prolific cellular and fibrous reaction on the one hand and tissue necrosis with "caseation" on the other. It has been suggested that the different pathogenicity of the various mycobacteria might be a function of varying abilities by the host to provoke these reactions. It has further been suggested that the lipid fraction of the organism is responsible for inducing the cellular reactions while the tuberculo-proteins induce hypersensitivity.

Distribution

Bovine tuberculosis is a worldwide problem although its veterinary and public health significance varies markedly between countries (Myers and Steele, 1969). It has been estimated that only one-third of the world's one billion cattle live in areas where the disease is under control; elsewhere the disease is either widespread but of undefined prevalence, or else common. In general, bovine tuberculosis exerts its greatest effects in underdeveloped and developing countries (Myers and Steele, 1969).

Epidemiology

Knowledge regarding the epidemiology of bovine tuberculosis has grown over many years as the result of clinical observations, pathological and microbiological examinations of sick cattle, microbiological examinations and routine monitoring of milk and other dairy products, abattoir surveys, experimental studies on artifically-infected animals, and the results of widespread tuberculin testing. The subject is reviewed in great detail by Myers and Steele (1969).

Cattle with tuberculosis commonly excrete organisms, but the route varies according to the form of the disease and the site of the lesions. Tubercle bacilli may be passed out in exhaled air, sputum, feces, urine, vaginal discharges, semen, lymph node, wound discharges or milk. The organism does not form spores but is capable of surviving for extended periods, particularly in damp, dark conditions. Feces may remain infective in fields for up to 6–8 weeks and may survive in slurry for up to five months. The major hazard for susceptible cattle is a nearby infected animal, usually bovine, which is excreting organisms. Infection may be acquired, however, from contaminated instruments and even semen.

Most authorities agree that in the majority of cases, cattle become infected following inhalation of the causal organism in airborne droplets. This view is supported by clinical and pathological findings. A few decades ago in Britain, for example, the disease spread far more rapidly in dairy cattle in the winter when they were housed (and usually neck-tied) than in the summer when they were in pasture and hence presumably having occasional access to herbage contaminated by infected feces. In general, the disease is more common in dairy cattle than in ranched beef cattle, probably for the same reasons. Similarly, the claim that Zebu-type cattle (*Bos indicus*) are relatively resistant to bovine tuberculosis may merely reflect the fact that Zebu cattle are commonly managed under extensive conditions and not under feedlot

conditions. Zero-grazing may also be associated with a high prevalence of bovine tuberculosis.

The most common situation involving infection by ingestion of mycobacteria is when young calves drink milk produced by cows with tuberculosis mastitis. In the same way, infected cow's milk may also give rise to tuberculosis (bovine strain) in other species including cats, pigs and humans (see below). Calves may develop tuberculosis congenitally when their dams have a tuberculous metritis, although it is generally considered to be a relatively rare event. Infection may also result when infected feces contaminate foodstuffs, pasture or drinking water. Tuberculosis may become a major problem on ranches when contamination of stagnant water sources occurs; running water sources pose far less of a problem.

The biggest hazard for susceptible cattle are infected cattle in the herd actively excreting mycobacteria. Nevertheless, the problems posed by other species must not be overlooked. Under farm conditions, cattle appear to be quite susceptible to infection with *M. avium* either from domestic poultry or wild birds. This is usually a self-limiting event for the cattle infected with the avian strain but it does not create difficulties in interpreting the tuberculin test (see below). Severe disease occasionally occurs which is clinically and pathologically indistinguishable from *M. bovis* infection, however. Cattle may develop tuberculosis, of either the bovine or another strain, as the result of infections derived from wildlife. This may occur occasionally under unusual conditions when, for example, cattle are kept in or near zoological gardens and are exposed to various forms of tuberculosis commonly found in captive animals. Alternatively, cattle may acquire infection when grazing a pasture which has been infected by tuberculous wild animals. Recently in certain localized areas of Britain, cattle acquired *M. bovis,* presumably via the oral route, from wild, clinically-affected badgers, *Meles meles meles.* It seems likely that this and similar situations may occur elsewhere in the world and create difficulties in eradicating tuberculosis from the cattle population.

Clinical signs

Bovine tuberculosis is usually a progressive disease accompanied by marked weight-loss, decreased milk production and weakness, finally leading to recumbency and death. The duration of the clinical course of the disease is usually measured in weeks or months although in certain cases, for example when acute miliary tuberculosis supervenes, deterioration occurs much more rapidly.

The syndrome which arises depends on the location and extent of the

lesions. The most common form is pulmonary tuberculosis in which signs are referable to a chronic progressive bronchopneumonia with or without pleurisy. In the early stages, respiratory signs are minimal or unnoticeable at rest although exercise may provoke an occasional soft cough and slight tachypnea. Later, the clinical picture worsens and the animal shows marked respiratory signs (i.e., tachypnea, hyperpnea or even dyspnea) at rest and more frequent coughing although this is rarely loud or paroxysmal. At this stage there may be an intermittent nasal discharge. There is little reliable published evidence regarding the findings on auscultation and percussion in tuberculous cattle and there is some doubt whether pleurisy gives rise to thoracic pain. Henning (1956), however, suggests that extension to the pericardium may result in signs of congestive cardiac failure.

Alimentary tract infections may sometimes produce ulceration but this is often not associated with diarrhea (Blood *et al.*, 1979). Such a lesion might extend through the wall of the intestine and result in dysentery or perhaps perforation and septic peritonitis, but detailed information on this point is lacking. Palpation of the right paralumbar fossa may occasionally reveal hepatomegaly. Extension of the lesions into the peritoneal cavity may produce multiple nodules and widespread lymphadenopathy which may be detected with rectal examination (Henning, 1956). A variable degree of ascites may be found in some cases. Other intra-abdominal lesions such as those affecting the female genitalia may also be palpated *per rectum*.

Lymphadenopathy is often marked and may occur with or without the above signs. Fistula formation and the development of discharging tracts is an inconsistent finding. When the palpable, subcutaneous lymph nodes are involved, enlargement is often unilateral or unequally bilateral. Retropharyngeal node enlargement results in loud snoring on both inspiration and expiration because the enlarged nodes bulge into the pharynx. Bronchial node enlargement may exert pressure on a major airway in the thorax, giving rise to loud wheezing which is mostly audible only on auscultation. Enlargement of the mediastinal lymph nodes exerts a downward pressure on the esophagus and interferes with eructation, gradually bringing about a stage of chronic ruminal tympany.

Other forms of bovine tuberculosis may occasionally arise (e.g., generalized tuberculosis and tuberculous meningitis) usually in very young calves and a whole variety of disorders of the male and female reproductive tract (Henning, 1956; Blood *et al.*, 1979).

Finally, but of enormous epidemiological significance for both cattle and other species (including man), there is tuberculous mastitis, a condition which may or may not be accompanied by signs referable to tuberculosis elsewhere in the body. On clinical examination, a typical case will show marked indu-

ration and enlargement of the affected quarter(s). Classically the disease predominantly involves the posterior glands and starts dorsally but spreads rapidly to involve the whole gland. It is painless and, in most cases, palpation will reveal marked unilateral or bilateral enlargement of the supramammary lymph nodes. On macroscopical examination, milk from even severely affected quarters may appear to be normal until the later stages of the disease (Soltys, 1963).

Pathology

The portal of entry within the bovine lung may be anywhere; however, it usually arises in a subpleural part of the dorso-caudal diaphragmatic lobe. The initial lesions, which may be single or multiple, start as a terminal bronchiolitis and later extend into the alveoli; consequently, lesions are lobular or multilobular. Extension of the original tubercle(s) may occur with the formation of satellite lesions. A similar process occurs in the associated lymph node(s), and together the lesions make up the "primary complex". The histopathological appearance of the lesions at this stage is a central zone of macrophages containing tubercle bacilli with epithelioid and multinucleate giant cells, surrounded by a zone of undifferentiated monocytes and lymphocytes. Later a fibrous capsule is formed, central necrosis occurs, followed by caseation and, later still, liquefaction or calcification. A variable degree of exudation also occurs.

Occasionally the disease may proceed no further than this and sometimes healing may occur. Bovine tuberculosis, however, is usually a progressive disorder and local extension of lesions and dissemination of infection is facilitated by the formation of small cavities within the affected tissue. Cavitation rarely becomes a major feature of tuberculosis in cattle. Eventually, large, single or multiple, bronchopneumonic foci are formed with marked fibrous capsule formation, caseation and calcification. Lymph node lesions may extend along lymphatics to other nodes; erosion of either small or major blood vessels may result in local and generalized spread. The main form of spread in lung tissue is via the bronchi, either by direct extension or by aspiration of infected material into other parts of the lung. Tuberculous ulcers may arise in the larger bronchi, the trachea or the larynx due to infected sputum coughed up and implanted in these areas. Occasionally, similar lesions may arise in the nares (Henning, 1956). Spread to the pleura is common, leading to a proliferative or caseous pleurisy with or without a pleural effusion, and a similar process may involve the pericardial sac. Finally, the disease may become widespread due to either "post-primary dissemination"

or "late generalization" and lesions may arise in almost all major organs, the peritoneal cavity, the meninges and the skeleton.

Infection following ingestion of *M. bovis* classically arises in the calf fed on tuberculous milk. A lesion at the portal of entry is only occasionally obvious at post-mortem examination but there is a marked tuberculous reaction often with gross enlargement in the pharyngeal, anterior cervical and/or mesenteric lymph nodes. Sometimes lesions may also be found in the tonsils and the lymphatic tissue in the posterior ileum. Post-primary dissemination in these cases usually gives rise to hepatic lesions.

According to Soltys (1963), tuberculous mastitis has been classified into four major types depending on the pathological changes involved. In all these forms, the detailed changes in the udder tissue are similar to those described in the pulmonary form of bovine tuberculosis. Three forms of tuberculous mastitis are the result of hematogenous spread but in one type (type 4), the lesions are restricted to the duct system and it is postulated that the condition arose as the result of infection via the teat duct. It should be emphasized, however, that the teat ducts are involved in all four types of the disease and it is likely each type results in the production of contaminated milk. Milk from affected quarters commonly appears to be normal on macroscopic examination until the later stages of the disease.

Immune response

In the natural course of tuberculosis, it has been shown that the host animal quickly develops both a resistance to tubercle bacilli and a hypersensitivity to tuberculoproteins. Unfortunately, at least in cattle, the resistance is not usually capable of totally overcoming the infection; at best, it may only temporarily halt the progress of the disease by stimulating the formation of a fibrous capsule around the tubercles. In certain species (e.g., man), this situation may give rise to prolonged protection from the infective contents although the capsule may break down with increasing age or as a result of intercurrent disease. The situation in cattle, however, appears to be different due to the nature of the lesions and, in particular, to the presence of small cavities. Thus the fibrous capsule in cattle is rarely a completely efficient shield against extension of the infection. The hypersensitivity reaction as a component of cellular immunity is also of doubtful protective value, however, the hypersensitivity reaction is the basis for diagnostic tests for detecting early (i.e., usually pre-clinical) infections.

Reasons for the variable rate of bovine tuberculosis progression are not properly defined. While it is possible that this is due to variations in the

immune response, it seems more likely that in many cases the rate of progress is determined more by other factors such as social and managemental conditions and nutritional status. Similarly, the reason for "late generalization" may be specifically immunological but it is likely to be more complex than this and possibly related to a total breakdown in the host defense mechanisms.

Laboratory aids to diagnosis

The frequent gross appearance and distribution of lesions makes bovine tuberculosis a striking and distinctive disease. This, coupled with the characteristic histological appearance, means that a fairly sound diagnosis can usually be made on pathological findings alone. Confirmation of diagnosis rests upon the demonstration of typical acid-fast organisms either on smears or in histological sections, using the Ziehl-Neelsen technique. Biological examinations, while necessary under some circumstances, have the disadvantage of taking several months to complete.

In comparison with the relative ease of establishing a diagnosis in dead cattle, the confirmation of a suspected case in a live animal sometimes presents problems. In excreting cases, an examination for acid-fast organisms can be made of sputum, feces, urine, discharges from nostrils, the reproductive tract, wounds and lymph nodes. Similarly, it is a relatively simple matter to carry out a biopsy of a suspicious lesion (e.g., an enlarged superficial lymph node) and to carry out histological and bacteriological examinations on the specimen obtained. The major problem lies in attempting to confirm diagnosis in the live animal which is excreting only small numbers of organisms or none at all. There is as yet no reliable (pre-mortem) skin or blood test for use in the clinical stages of the disease. It should be emphasized that while the results of a tuberculin test may be useful to define the tuberculosis status of the animal's herd of origin, the test is of no real value as a diagnostic test and in the later stages of the test may well yield negative results. In such cases, the best diagnostic procedure is prompt slaughter followed by a careful pathological examination. If this is impossible, the animal should be isolated and sampled twice weekly.

Prevention and control

This subject is dealt with in great detail by Myers and Steele (1969). In addition, the advantages and disadvantages of the tuberculin testing tech-

niques are discussed by Blood *et al.* (1979).

The first important point regarding the eradication of bovine tuberculosis is that, despite all of the obvious difficulties involved, several countries (e.g., Britain, Canada, Japan, the Netherlands, Scandinavia, Switzerland and the United States) have reduced the disease to almost negligible proportions. These successful countries have good communication systems and excellent medical and veterinary services; they also have well-developed organizations for the monitoring of milk and meat production and the distribution of these commodities and their by-products. Another distinct advantage is a well-educated and developed agricultural industry in which formal identification of individual cattle, stock movement controls and quarantine are all possible and where an adequate supply of replacement cattle is available if needed. Finally, the above countries are, or have been, relatively wealthy and have been able to employ the large number of veterinary personnel needed for tuberculosis eradication schemes and in some instances, to pay for compensation schemes for farmers whose stock have been slaughtered, and for bonus payments to beef and dairy farmers who have successfully eradicated the disease. Bovine tuberculosis is much more of a problem now in underdeveloped and developing countries. It is doubtful they have the resources for the type of eradication programs carried out in large parts of Europe and North America. Furthermore, as the cattle industry, and in particular dairy farming, develops in many of these less-fortunate countries, bovine tuberculosis may increase in prevalence and become a major problem even if it is not recognized as such at the moment.

Unfortunately there is no cheap or simple way to control or eradicate bovine tuberculosis. Results of vaccination trials using B.C.G. have shown that it has no place in control schemes. Similarly, long term chemotherapy and/or chemoprophylaxis using, for example, isoniazid, also has tremendous drawbacks and has been advised against, at least at the moment. The only proven effective eradication schemes are those which involve tuberculin testing and the prompt disposal of reactors. From a national viewpoint, slaughter is the best method of disposal. Voluntary schemes are rarely successful, but schemes with built-in compensation and/or bonuses are unacceptable to many governments. Other "cheap" alternative schemes, such as the detection and disposal of clinical cases (or merely cattle with tuberculosis mastitis), are also generally unsuccessful.

A country faced with this problem is probably best advised to define the prevalence and distribution of bovine tuberculosis by abattoir surveys and tuberculin testing in selected herds and areas. The test best suited to an initial investigation in a developing country is probably the single comparative intradermal test because (i) only two initial visits to a particular farm are

needed and (ii) it reduces interpretation problems due to nonspecific infections, i.e., *M. avium, M. johnei, Nocardia farcinicus* and skin tuberculosis.

The implementation of such an exercise might even serve to underline the financial losses which occur as a result of bovine tuberculosis. Quite apart from this, however, is the problem that the disease may be responsible for a huge morbidity and mortality rate in the human population.

Bovine tuberculosis as a zoonosis

The staggering statistics of this problem are spelled out by Myers and Steele (1969). Since the beginning of this century it has been accepted that humans may contract tuberculosis from cattle under a wide variety of different circumstances. Infection derived from meat and meat-products can occur although the striking nature of the lesions usually results in prompt condemnation at the time the carcase is examined by the meat inspectors. However, such a system is not always operant thus posing a certain risk for all, including veterinarians, public health officials and laboratory workers who are involved in the handling and preparation of carcases. Close contact (as in an intensive cattle unit) with actively excreting clinical cases, however, is a far greater risk to animal attendants. One worker (Henning, 1956) stated that working in infected cattle stables was more dangerous than working in a tuberculosis hospital.

The greatest risk for humans comes from drinking contaminated milk. From the milk production viewpoint, tuberculosis milk is a major problem because it may carry vast numbers of tubercle bacilli without appearing abnormal (i.e., "mastitic"). When used as a milk producer for a family, a single tuberculous cow may be responsible for several human cases, particularly in children. Even in large units where tuberculous milk may be bulked up with normal milk, widespread dissemination of infection may occur. Of course, pasteurization of milk virtually eliminates the risk to humans but unfortunately such a procedure is unlikely to protect rural populations from bovine tuberculosis, particularly in the less developed areas of the world.

Skin tuberculosis

This condition is a chronic and benign granulomatous disorder of cattle which affects the skin, usually around the neck, shoulders and upper forelegs. In some cases the lesions may be nodular and covered with normal skin, and in others they may protrude through the skin and resemble a small hard cauli-

flower. The process may occasionally involve the lymphatics but never spreads to the associated lymph nodes. Usually the lesions range from one to four centimeters in diameter. On histological examination, "tuberculoid" lesions often contain acid-fast bacteria although these unclassified bacteria are generally considered to be nonpathogenic.

The only significance of skin tuberculosis is that it is one cause of 'nonspecific reaction' to the tuberculin test.

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25. ANTHRAX

I.E. Selman

Abstract. Anthrax is a widely-distributed and usually fatal disease of cattle caused by the bacterium *Bacillus anthracis*. This organism readily produces highly resistant spores when it is exposed to air. In cattle, infection is usually by ingestion which may occur when contaminated feeding stuffs or drinking water are consumed or, under pastoral conditions, when cattle graze contaminated pastures. Infection usually gives rise to either rapid death (peracute anthrax) or, less commonly, a slightly more protracted syndrome characterized by extreme dullness, high fever, widespread hemorrhages, dysentery and usually death within 48 hours. Once soil is contaminated with spores it usually remains so for many years. Control methods should involve prompt diagnosis, prevention of contamination, preferably by the deep burial of unopened carcases and disinfection of the immediate environment. In countries where the disease is endemic or in non-endemic countries where anthrax is a persistent endemic problem, annual vaccination should be carried out. Anthrax is a zoonosis and the infection may be acquired by man from infected animals, animal products and contaminated laboratory equipment.

Etiology

Bacillus anthracis is a very large (6–8 μm × 1 μm), straight, rod-shaped organism with square ends. In smears made from a fatal case it is usually surrounded by a thick capsule and occurs singly, in groups or in short chains. It is Gram positive. Spores are readily produced when the organism is exposed to air. The spores of *B. anthracis* are very highly resistant and may persist in soil almost indefinitely. There are some suggestions that the organism is capable of an independent vegetative existence under optimal soil conditions. The organism is also able to withstand many industrial and manufacturing processes, the effects of a large number of disinfectants and also many standard bacteriological staining techniques. For information regarding other characteristics of this bacterium, the reader should consult the work of Soltys (1963).

Both intracellular and extracellular toxins are produced by *B. anthracis*.

Pathogenesis

In cattle, most cases arise as the result of ingesting the spores of *B. anthracis*, however, infections may result from inhalation and even wound contamination.

The organism passes through the mucous membrane, probably by way of abrasions in the mouth and elsewhere. Dissemination is by way of the lymphatics to the bloodstream where there is an overwhelming terminal bacteremia. The exotoxins of *B. anthracis* are considered more pathogenic than those which are produced intracellularly, and they give rise to widespread edema and tissue damage. The disease proves to be almost invariably fatal and death generally occurs within a matter of a few hours.

Distribution

Anthrax is a worldwide disease although its prevalence and public health significance tend to be much higher in less-developed tropical areas.

Epidemiology

In developed, temperate countries, anthrax is usually an infrequent, sporadic event involving only one, or at most a few, cattle in a herd. Frequently, the source may be attributed to the ingestion of concentrated feed which contains imported components contaminated with the spores of *B. anthracis*. Since early diagnosis, reduced sporulation rates, and legal control measures exist in developed countries with lower ambient temperatures, it seems likely anthrax would not persist if it were not for the occasional importation of infected feed. However, even under relatively mild, temperate conditions anthrax may occasionally be seen as a regular occurrence in a certain location. Frequently the area in question is a single field which has a history of occasional anthrax cases dating back over several generations. It is common for such an area to be adjacent to, or downstream from a tannery, knacker's yard, fertilizer processing plant or an abbattoir. Investigation may reveal that these locations were once used as a place for burying dead carcases or offal.

In tropical or sub-tropical environments the epidemiology of anthrax is somewhat different. Regular confirmation of diagnosis is less frequently sought and far more difficult to accomplish, particularly under extensive grazing conditions or when poor communications exist. In addition, there are major practical problems involved in burying or otherwise disposing of infected carcases. Even when attempts have been made to do so, there is the

added complication that various carrion feeders may either eat or carry off pieces of infected material and scatter them over a wide area. These factors, together with temperature and humidity conditions favorable for sporulation, make anthrax a much more significant disease threat in the tropics and subtropics.

When soil-borne infections occur they frequently exhibit a seasonal pattern. In some areas this may be due to a sudden change to favorable climatic conditions when, for example, heavy rain follows a prolonged drought. Alternatively, the disease may be seen as a dry season problem, presumably due to the greater chance of oral lesions arising when more fibrous material is eaten. Also, under such conditions cattle commonly ingest large amounts of soil along with herbage.

Anthrax may be seen in all ages of cattle but is common in animals over one year of age.

A respiratory form of anthrax may rarely occur in cattle and is analogous to "woolsorter's disease" in man. Also, the organism may occasionally contaminate wounds and perhaps may be spread to cattle by insect vectors (Blood *et al.*, 1979).

Two forms of the disease may be seen in cattle: peracute and acute anthrax. In the former, the course may be as short as 1 to 2 hours; in the latter it may be as long as 1 to 2 days. It has frequently been stated that the peracute disease is seen at the beginning of an outbreak. It may be well that this is merely a reflection of the greater vigilance which is stimulated as losses continue.

Clinical Signs

The incubation period under field conditions is unknown but experimentally it has been shown to vary from 1 to 10 days.

In most instances, cattle are observed as cases of sudden death. Only occasionally does an animal survive long enough for clinical signs to be observed. The animal may be extremely depressed, highly febrile and totally recumbent or nearly so. Extreme dyspnoea is often present with tachycardia and congested, sometimes petechiated, oral and conjunctival membranes. Dysentery occurs frequently and sometimes the scanty amount of milk which is available is bloodstained. There may also be localized areas of edematous swelling, particularly in the throat and along the floor of the belly. If a pregnant animal survives, it may abort.

Pathology

In cases of sudden death due to anthrax, the animal has usually died quietly; there is often dark, unclotted blood at the nostrils and anus. Bloating and putrefaction are quick to occur but rigor mortis is delayed. Given this situation it is advisable and, in some countries, a legal necessity to proceed no further until a blood smear has been examined (see below). If the smear is positive, the carcase should be left unopened in order to reduce chances of spore formation. The pathological features of the disease are: the blood is unclotted and often widespread ecchymotic hermorrhages are found in all areas of the body; there may be subcutaneous plaques containing blood-stained edematous fluid; there is an accumulation of sero-sanguineous fluid within the body cavities, the abomasum is highly congested, and the small intestine contains blood-stained material; often there is a dramatic, massive splenic enlargement and the organ contains a semi-solid, dark red material highly reminiscent of raspberry jam.

Immune Response

Recovered cases are assumed to have a solid immunity to the disease.

Laboratory Aids to Diagnosis

Diagnosis in a live animal may prove to be difficult since bacteremia tends to be a fairly terminal feature of the disease, consequently a blood smear is often misleading. However, when the disease is suspected, a blood smear should be attempted as well as a smear of fluid from edematous plaques. Extreme care should be taken to avoid spillage of blood or fluid. Whenever possible, the samples should be collected in a "vacutainer" bottle. In the dead animal, a blood smear should be collected from an ear vein and the small incision should be burned with a hot iron or knife. The ear then should be bound up either with elastoplast or a polythene bag. The smear should be dried and stained with either Giemsa or polychrome methylene blue. Extreme care should be taken; gloves should be worn and all materials and instruments should be carefully incinerated.

Unfortunately, infection may be difficult or impossible to detect once putrefaction has set in. In a case of anthrax in a hot climate, organisms may only be demonstrable for a few hours after death. Under such circumstances, it is advisable to submit swabs from the blood which has oozed from the anus or

nostrils for more detailed laboratory studies. Contaminated material or hide may also be submitted, but in all cases samples should be very carefully packaged and identified.

Organisms may still be found in mesenteric lymph nodes and spleen in an animal which has survived long enough to have received antibiotic therapy.

Prevention and Control

In countries where anthrax is sporadic and incidents can usually be attributed to ingestion of *B. anthracis* in imported feeding stuffs, there is little point in vaccination. However, where recurrent cases occur, the use of a vaccine should receive serious consideration (see below). In either event, it is essential that all suspicious incidents are properly investigated including at least an examination of a suitably-stained blood smear. Confirmed cases are preferably disposed of by deep (2 m) burial in quicklime or, if this is not possible, by controlled incineration. The animal's immediate surroundings should be thoroughly disinfected using either formalin (10%), caustic soda (10%) or peracetic acid (3%). Contaminated clothing, etc., should be buried or thoroughly disinfected with formalin. A careful watch for further cases should be carried out. In the event of other cattle becoming ill, they should be treated with extreme suspicion and receive appropriate treatment unless there are definite indications to the contrary (see below).

In countries where anthrax is common, there is a clear need for continued vaccination. Probably the most commonly-used preparation is a living, avirulent (uncapsulated) spore vaccine. This is usually administered to all cattle annually although in areas where challenge is particularly high, the dosage may be increased to twice yearly. Quite commonly, the anthrax vaccine is combined with other vaccines, e.g., blackleg.

When faced with an outbreak of anthrax, vaccination should be instituted immediately and all cattle movements in the area stopped. Further cases may occur over the following week or so. The use of antiserum is generally not advisable unless a killed vaccine is being used. Clinical cases should be isolated and treated with maximal doses of either penicillin, streptomycin or a combination of these drugs for at least five days. Antiserum is also considered to be beneficial if given daily over the same period in daily doses of 100-250 ml. This is an extremely expensive procedure, however, and not recommended for routine purposes (Blood *et al.*, 1979).

Anthrax as a Zoonosis

Although anthrax may occur in man as a fatal septicemic disease, most cases would appear to arise as either cutaneous abscessation (e.g., hideporter's disease), pneumonia (e.g., woolsorter's disease), or else as a severe gastro-intestinal disturbance with dysentery (e.g., when infected meat is inadvertently eaten). In each syndrome fever, depression, and sometimes death may occur although the clinical course of each of these syndromes depends on how quickly a correct diagnosis is made and effective treatment instituted. Laboratory technicians, animal attendants, abbatoir workers, knackers and veterinarians may occasionally contract the disease, usually in the cutaneous form. Extreme care should be exercised whenever a suspicious case is being examined. In the case of the veterinarian particularly, care should be taken when blood smears are obtained from suddenly-dead cattle. For similar reasons, the rectal examination of dull and febrile cows with dysentery is not recommended.

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26. TETANUS AND BOTULISM

I.E. Selman

A: TETANUS (LOCKJAW)

Abstract. Tetanus in cattle is usually of a sporadic nature and is frequently fatal. It arises as the result of a neurotoxin produced by the anaerobic bacterium, *Clostridium tetani*. The clinical signs are highly distinctive and the syndrome is characterized by hyperasthesia, generalized muscular stiffness and ruminal tympany. In severe cases the disease progresses to lateral recumbency with extreme extensor rigidity, opisthotonus and convulsions. Treatment of established cases is frequently unsuccessful but the disease may be prevented either by proper wound management together with the use of antitoxin or by routine vaccination.

Etiology

Clostridium tetani is a long, slender rod-shaped bacillus (2.0 μm×0.5 μm) with slightly rounded ends. It may occur singly or in short chains. It grows well on all ordinary media provided that cultural conditions are strictly anaerobic. The appearance and behavior of the organism varies according to age. In young cultures, it is Gram positive, motile and may form long filaments whereas in cultures older than 48 hours, it stains Gram negative and is inclined to sporulate. Different strains of *Cl. tetani* exhibit a varying tendency to sporulate but when present, spores are situated terminally and give the bacillus the appearance of a drumstick. Various other cultural properties of *Cl. tetani* have been listed by Soltys (1963). Ten strains of the organism have been identified, all of which produce the same antigenic type of toxin which can be neutralized by a single antitoxin.

Under suitable conditions, *Cl. tetani* produces a highly potent toxin which has been shown to contain at least two toxic factors: a hemolysin (tetanolysin) and a neurotoxin (tetanospasmin). The production of the latter factor is dependent upon the age of the culture, the culture medium and the strain of *Cl.tetani* (Soltys, 1963).

Pathogenesis

Contamination of a wound with the spores of *Cl. tetani* does not lead inevitably to clinical tetanus since local conditions have to be favorable for proliferation of the bacterium. The affected tissue, in particular, must have a low oxygen tension. Nevertheless, if local conditions are, or become, optimal (and the contaminating strain of organism is sufficiently toxigenic) there is a distinct risk that the clinical disease will ensue. Bacterial proliferation remains localized to the wound. The neurological effects occur as a result of neurotoxin absorption by the peripheral (motor) nerve endings which pass the toxin along the nerve trunks to the central nervous system. The role, if any, played by the hemolytic factor in these events is unknown although it has been postulated that it may play a supporting, albeit local, part in producing tissue necrosis (Soltys, 1963). The mechanism by which the neurotoxin exerts its effects is unknown and it is possible that it operates centrally in the spinal cord and brain stem, or peripherally via the motor nerves, or in both locations simultaneously (Brooks *et al.*, 1957). In any event, the result is a generalized or sustained contraction of locomotor and respiratory musculature. When death occurs, it is usually due to asphyxiation following respiratory paralysis; occasionally cattle will die from the effects of severe ruminal tympany or aspiration pneumonia.

Distribution

Clostridium tetani is widely distributed in the soil and in the feces of many different animal species, particularly the horse. There is, however, a marked tendency for local variations to exist regarding the prevalence of the clinical condition, and in some areas, tetanus is a commonly-encountered disease although the basic reasons for this are not clear.

Epidemiology

The highly resistant spores of *Cl. tetani* are present in large number in garden earth, street dust, hay and manure (Henning, 1956) and their numbers are continually being replenished by fecal contamination (Fildes, 1925). Therefore, circumstances favoring wound contamination are often present under normal farming conditions. If local conditions encourage proliferation of a toxigenic strain of the organism there is a distinct disease risk.

Susceptibility of domestic species appears to vary markedly to the effects of

the neurotoxin of *Cl. tetani*. Cattle are considered to be somewhat resistant in comparison to man and the horse. Most cattle and other domestic ruminants have detectable levels of circulating antitoxin. It has been suggested that this is the result of *Cl. tetani* proliferation in the forestomachs, absorption of toxin and consequent natural antigenic stimulation; thus the relative resistance of cattle to *Cl. tetani* neurotoxin may be acquired rather than natural. Nevertheless, it must be emphasized that the mortality rate in cattle affected with tetanus is very high (see below).

Tetanus may be seen in all ages of cattle under a variety of epidemiological conditions. While the classic situation is generally taken to be a deep puncture wound which becomes contaminated with bacterial spores, this does not seem to be the most common portal of entry in cattle. In the absence of reliable data, this author's impression is that the most frequent tetanus situations, in descending order of prevalence are as follows: (i) the postparturient cow (especially if there has been placental retention), (ii) the recently castrated steer (particularly if the operation was carried out using the rubber ring technique), (iii) the recently dehorned yearling and (iv) the neonate (presumably infected via the umbilicus). Many cases, however, are encountered where there has been no obvious or probable portal of entry although it is possible that such cases have had a prolonged incubation period and the original wound has healed. Finally, tetanus may occasionally be epizootic, usually occurring in immature (6–12 month) housed or grazing cattle. Problems of differential diagnosis arise but when all efforts have been made to exclude, for example, various mycotoxicoses and strychnine poisoning, it is generally assumed that the problem is due to absorption of *Cl.tetani* toxin via a breech in the epithelial lining of the alimentary tract.

Clinical Signs

The incubation period may vary from days to months but usually in cattle when infection can be related to a previous event, such as parturition, it is between 7 and 14 days. The course and severity of the disease also varies considerably. Mild cases commonly recover but often take up to two months to do so. Severe cases usually have a survival time ranging from 5 to 10 days after which death or humane destruction occurs. The overall tetanus mortality rate in cattle probably exceeds 90%.

The first clinical signs in adult cattle are an apprehensive appearance, an excessive reaction to mild, every-day stimuli, slight muscular tremors at rest and a slightly stiff stance and gait. At this stage the animal may continue to eat and drink a little, there may be a slight dribbling of saliva, ruminal

tympany and, if feces are passed, they are likely to be dry and dark in color.

As the condition develops, the animal will experience difficulty in eating, chewing and drinking although it may still wish to do so, at least for a time. Generalized but mild muscular stiffness gives way to severe extensor rigidity. The animal stands on the tips of its toes with its legs straight and extended in a pose reminiscent of a rocking horse. Walking is extremely difficult. The head is extended forward, the ears are stiffly pricked, the nostrils flaring and the facial muscles taut and hard. Trismus (lockjaw) may be present although it is usually possible to open the mouth a little. A prolapse of the third eyelid (membrana nictitans) may or may not be present. Salivation is more prominent and there is often marked sweating; there is tachypnoea, tachycardia, a tense abdomen (the whole body feels extremely hard), and the tail is held stiffly. Urination is difficult, particularly in females, mainly due to the difficulty in taking the appropriate posture. Sudden movement, noises and handling may precipitate a panic reaction and the animal may fall to its side in a tetanic convulsion. Eventually, the animal remains in lateral recumbency with extreme extensor rigidity and opisthotonus. At this stage the rectal temperature may rise to around 105 °F, presumably as the result of the protracted muscular contractions and convulsions and there will be marked sweating and severe distress. In addition, animals often inflict traumatic injuries upon themselves, particularly around the orbit, the coronets, hocks and elbows.

Pathology

There are no gross or histopathological, neuropathological or muscular lesions in tetanus although in many cases there is an obvious infection site.

Immune Response

The fact that in normal cattle it is common to detect low circulating levels of tetanus antitoxin has already been referred to. This would suggest that recovered cases are likely to develop even higher levels of antitoxin and be quite resistant to reinfection; however, detailed information on this point is lacking.

Laboratory Aids to Diagnosis

There is no satisfactory laboratory test to confirm *Cl. tetani* infection apart from culture of possible infection sites.

Prevention and Control

The first consideration in the prevention of tetanus in cattle is the management and treatment of potential infection sites. Good operative technique and clean instruments are of prime importance of routine tasks such as castrations and dehornings. Similarly, cattle with (or with a possibility of developing) metritis and/or placental retention should receive proper attention with antibacterial cover. In the case of accidental wounds, proper wound cleansing and disinfection with antibacterial cover is necessary.

In areas where the disease has not been recognized in cattle, there is little point in instituting anything other than the measures outlined above. In an area where tetanus is a definite and consistent hazard, in addition to the above measures, antitoxin should be administered subcutaneously ranging from 3000 to 7500 i.u. depending on the size of the animal. The effect of antitoxin is transient (i.e., persists for only about two weeks) and to obviate the need for haphazard and repeated antitoxin administration in high risk areas, it is advisable to vaccinate susceptible cattle with an alum-precipitated, formalin-treated toxoid. Protective immunity which develops two weeks after vaccination lasts for about one year. A second dose of the vaccine is likely to confer life-protection in cattle. The use of antitoxin or toxoid in tetanus areas depends on local considerations, and in particular the relative costs of the products.

In stark contrast to the efficacy of the above prophylactic measures, the treatment of cattle with clinical tetanus is frequently unsuccessful. Much depends on the stage and severity of the condition at the time the animal is first presented for attention. In mild cases (i.e., when the animal can still stand, walk and eat), the chances of recovery are fairly good although the course of the disease over the following few days is critical. Such an animal should be moved to a quiet, preferably darkened, area with comfortable bedding. Food and water should be offered at head height and every effort should be made to raise it quietly at least twice daily. If the animal has difficulty drinking, it should be stomach-tubed and water or normal saline given at the rate of at least 2 liters/50 kg body weight daily. Females with difficulty in urinating should be catheterized regularly. A search should be made for the infection site and, if found, should be thoroughly cleansed and

disinfected. Antibiotic (e.g., procaine penicillin) therapy should be instituted with the intravenous administration of antitoxin (i.e., up to 100 000 i.u. antitoxin). In some instances it is suggested that antitoxin is made effective if given by the epidural route and infiltrated around the wound is a better method. This seems unnecessary in view of the fact that many "mild" cases recover spontaneously or with limited individual care. The use of ataraxic drugs such as acetyl promazine (0.05 mg/kg body weight) twice daily for at least one week is also to be recommended. The prognosis must always be guarded over the first few days of treatment and even when an animal appears to be progressing satisfactorily. Any time within the first two or three weeks, a rapid deterioration of condition may take place.

The management and treatment of severe cases must be approached with extreme pessimism. If a case is at the stage of lateral recumbency and severe extensor rigidity when it is first presented for treatment, or progresses rapidly to this stage, it is probably best to carry out humane destruction. If attempts are made to save a severe case, however, the therapeutic and nursing regime is merely an extension of that already described. Fluid and electrolyte therapy have to be carried out by the intravenous route; antitoxin has to be administered either intrathecally or via the epidural space; the animal has to be kept on deep bedding and rolled several times daily; sedation is essential. Again, there is a tendency for even severe cases to improve transiently just prior to death and signs of improvement must never be taken as definite indications that the animal is making satisfactory progress. Finally, it should be emphasized that cattle with severe tetanus (unlike horses) should be managed and nursed on the ground and that slinging is contra-indicated.

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B: BOTULISM

Abstract. Botulism is an almost invariably fatal disease in cattle. It arises as the result of the ingestion of a pre-formed neurotoxin produced by the anaerobic bacterium, *Clostridium botulinum* and which is released following lysis of that organism. Cattle become intoxicated following the ingestion of decomposing animal and vegetable matter. The clinical signs are highly distinctive; the syndrome is sometimes characterized by sudden death but more frequently by flaccid paralysis of variable severity involving the muscles of locomotion, mastication and swallowing. Usually impairment starts posteriorly and progresses forward. Treatment of established cases is almost always unsuccessful but the disease may be prevented by vaccination. Identification and avoidance of the source of contaminated ingesta and control of predisposing factors also play a key role in prevention of botulism.

Etiology

Clostridium botulinum is a very large bacillus (4.0–8.0 μm×1.0–1.5 μm) which may occur singly, in pairs or in short chains. It grows well on most common media but is a strict anaerobe. In young cultures *Cl. botulinum* is motile and Gram positive although variation occurs in depth of staining between different bacilli. The organism is saprophytic and spore-forming. The spores, which are larger than the diameter of the bacterium and hence create a bulge, are usually situated subterminally. For other information regarding the cultural and biochemical properties of the organism, the reader is referred to the work of Soltys (1963). At least seven types of *Cl. botulinum* have been identified (A, V, Cα, Cβ, D, E, F), each elaborates a distinct toxin which exhibits marked variation in toxicity. The toxins are highly potent neurotoxins and are released from the bacterium when it undergoes lysis.

Pathogenesis

The toxins of *Cl. botulinum* vary greatly in their relative pathogenicities for different animal species but in general, cattle are highly susceptible. The ingested toxin is rapidly absorbed and becomes distributed throughout the body, finally to be absorbed by the peripheral nervous system. The incubation period is variable and in experimental cases may range from 18 hours to 16 days (Henning, 1956).

The effects of the neurotoxin appear to be localized to the peripheral nervous system (Payling Wright, 1955) inducing a wide range of clinical signs, almost all of which may be attributed to motor paralysis.

In the acute form of the disease, death is due to respiratory paralysis but in other instances cattle may die from aspiration pneumonia or be slaughtered as the result of prolonged recumbency.

Distribution

The different types of *Cl. botulinum* are widely distributed throughout the world. Those known to have caused disease incidents in cattle (i.e., C , D and, to a lesser extent, B) have been identified in North America, Australia, Europe and certain parts of South Africa.

Epidemiology

The spores of *Cl. botulinum* are extremely resistant and even the toxin is capable of prolonged survival when protected from leeching (Fourie, 1946). The organism is a common inhabitant of the alimentary tract of cattle and other herbivores from whence it may contaminate ranges and pastures. The organism proliferates only in decaying animal and plant matter. It is ingested toxin that is responsible for producing the clinical signs; the organism itself is generally assumed to be incapable of toxin production once inside the animal's body (Soltys, 1963).

In cattle, classic botulism was first recorded in South Africa in 1780 and from that time onwards much interest was focused on the condition until its etiology was finally defined in 1927 (Henning, 1956). The condition affected range cattle during the latter part of the dry season. It was established that the cattle were ingesting toxin due to their (seasonal) habit of eating bones from decomposing carcases in their grazing areas. This habit, pica, arose during the dry season as the result of severe phosphorus deficiency which was less severe after the onset of the rains. Such a situation has occurred and occasionally given rise to botulism in other dry areas such as parts of the United States and Australia. It has been emphasized, however, that in some areas the danger lies not in cattle eating the bones of other domestic farm animals but rather eating or chewing the remains of many small animals (Fourie, 1946). The bodies of dead tortoises are reported to be ideally suited for anaerobic growth and toxin formation, after which their shells become heavily contaminated; Fourie (1946) suggested that almost 100% of dead tortoises may be toxic in endemic areas and may be responsible for up to 80% of all cases of botulism in certain areas.

The disease has been confirmed in cattle drinking lake water infested by

the contaminated bodies of wild fowl, in cattle eating hay and silage contaminated by the bodies of dead rodents and in cattle grazing good pastures which have been fertilized with chicken manure containing the remains of dead birds (Blood *et al.*, 1979). In addition, it has also been claimed that the disease may arise occasionally as the result of cattle eating decaying pasture, decomposing hay and silage and heavily contaminated brewer's grains (Blood *et al.*, 1979).

Clinical Signs

The incubation period under experimental conditions may vary from 18 hours to 16 days but in most cases it ranges between two and six days (Henning, 1956). For obvious reasons, the incubation period under field conditions is usually impossible to assess. The syndrome has been divided into four distinct forms:

Peracute botulism

This is defined as the situation in which the period between the onset of clinical signs and death is less than 24 hours. Under field conditions it is characterized by sudden death; under experimental conditions it usually follows the administration of a large dose of toxin and a short incubation period. When clinical signs are seen (i.e., in closely observed experimental cattle) they are basically similar to those which occur in the acute form of the disease albeit contracted into a much shorter time scale.

Acute botulism

Acute cases are almost invariably fatal; the time between onset and death is one to two days and the evolution of clinical signs is much more striking. Initially cattle may be able to rise without assistance and move about in an incoordinated stumbling fashion. Soon, however, the animal is unable to rise although still able to maintain itself in sternal recumbency. At this stage, the head is often held around to the flank. The animal is afebrile and usually uninterested in food or water. Eventually, the muscles of mastication and swallowing become paralyzed, there is drooling of saliva and boluses of grass may accumulate in the back of the mouth.

Subacute botulism

This form of the disease lasts between three and seven days; it is the syndrome most commonly encountered under range conditions and almost invariably proves fatal.

The signs are similar to those described above but since they develop over a longer time period, they are more obvious. Affected cattle are again dull but afebrile. They may be able to rise with encouragement for several days but generally prefer to lie in sternal recumbency. When forced to walk, their gait is usually sluggish or incoordinated and knuckling of one or more fetlocks is quite common. At this stage, animals are inclined to collapse suddenly if harassed.

In this form of the disease the characteristic progression of the paralysis from the hind limbs forward is easily seen. Many animals will make reasonably coordinated attempts to rise on to their knees while totally unable to move their hind limbs. The paralysis is of a flaccid nature and sensation is usually retained until the later stages of the disease. Latterly, the animal is unable to raise its head and usually is unable to chew or swallow. This is not always the case, however, and some continue to eat for several days although chewing movements are slow, and difficulty in swallowing arises with a consequent drooling of saliva. Terminally, the tongue may be paralyzed and extended and the animal lapses into lateral recumbency and coma.

Chronic botulism

In this form, the animal lasts for more than seven days and a small number may even recover after three or four weeks of illness.

The clinical picture is generally one of dullness without fever and, at least initially, a reasonably good appetite; there is usually some difficulty in rising or walking and the animal prefers to lie for prolonged periods. In most cases the desire to eat remains regardless of impairment to chewing or swallowing. The condition rapidly worsens. Occasionally in chronic cases respiratory stertor (snoring) may arise, presumably due to pharyngeal paralysis. This sign may persist for several months in recovering animals.

Pathology

There are no characteristic gross or histopathological lesions in either renal or muscular tissue. Certain types of foreign body in the fore-stomachs, such as bones, pieces of tortoiseshell, sticks or stones may suggest that the animal has had a depraved appetite.

Extreme care should be exercised when carrying out post-mortem examination on suspected cases since the toxin has been known to be absorbed through minor wounds and abrasions.

Immune Response

Since most cases prove fatal in a very short time, little is known about the antibody response in natural infections. It is assumed that recovered cases are solidly immune thereafter.

Laboratory Aids to Diagnosis

In problem areas, laboratory diagnosis is seldom necessary since the disease is clinically distinctive.

In endemic areas, *Cl. botulinum* is often present in the feces of normal cattle, consequently, the identification of the organism in ruminal intestinal contents is not considered to be of confirmatory significance. The identification of toxin in intestinal contents is considered as conclusive evidence, however, it is absorbed so rapidly from the gut that it is likely to be found only in peracute and some acute cases and these are not so commonly encountered under field conditions. The identification of toxin in liver may be viewed as definite proof of botulism.

Prevention and Control

Little reliance should be placed on any treatment of botulism, although specific and polyvalent antitoxins are available. When cases occur, prompt vaccination using specific or polyvalent toxoid must be carried out. In most cases, a single dose is sufficient to confer protection for approximately two years and revaccination is recommended for cattle retained after this period.

It is essential that suspected outbreaks be investigated as carefully as possible to define the precise source of toxin, otherwise losses may continue almost indefinitely and there is the possibility of spread to the human population under certain circumstances. Once the source is established, evasive action can be planned, depending on the type of cattle involved and the way in which botulinum toxin is acquired.

In range cattle, all carcases should be disposed of when possible and

attempts made to correct phosphorus and other deficiency states. All attempts should be made to avoid using food, water or fertilizer which have been identified as hazardous.

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27. ANAPLASMOSIS

Miodrag Ristic

Abstract. Anaplasmosis is an infectious and transmissible disease manifested by progressive anemia and the appearance of other characteristic disease symptoms. It is a world-wide tick-borne disease of cattle and some wild ruminants caused by the rickettsia *Anaplasma marginale*. A short-term *in vitro* propagation of this agent was achieved recently. Serums from convalescing animals interact with *A. marginale* antigens in agglutination and complement fixation tests. Consequently, these are the most frequently used tests to detect anaplasma carrier cattle. Passive transfer of sera from immune to susceptible cattle, however, does not seem to confer protection against the infection and development of the disease. Studies which employed various tests for measuring cell-mediated immune (CMI) responses in association with simultaneously collected information on antibody activity, have shown that both humoral and cellular immune responses are needed for the development of protective immunity in anaplasmosis. It was further shown that an active replication of anaplasma is essential for induction of these two types of immune responses. Consequently, live virulent and attenuated immunogens fulfill requirements for induction of protective immunity. The use of a virulent agent for immunization (premunization), however, presents a risk, it is a laborious procedure and requires control of the initial infection by drugs.

Many drugs, including arsenicals, antimalarials, antimony derivatives, and dyes, have been employed in an effort to cure or prevent anaplasmosis. Dithiosemicarbazones have been described as having a specific chemotherapeutic effect on anaplasma. Because of their residual tissue toxicities, they are not approved for commercial sale. To date, however, only the tetracycline compounds (chlortetracycline, tetracycline, and oxytetracycline which are available commercially have been found to have a useful effect on the rate of multiplication of anaplasma.

Anaplasmosis is an infectious and transmissible disease of cattle, characterized by progressive anemia associated with the presence of intraerythrocytic inclusion bodies designated as anaplasma. At the beginning of the century, Theiler (1910) described a small punctiform body which occurred in the erythrocytes of African cattle suffering from an acute infectious anemia. On the basis of staining characteristics, the author concluded that the organism lacked cytoplasm and used the term "anaplasma" to indicate this property and the term "marginale" to describe the peripheral location of the organism

within erythrocytes. Beginning with the classic studies of babesiosis by Smith and Kilborne and for many years thereafter, *A. marginale* was considered to be a protozoan blood parasite. During the last 2 decades, considerable knowledge has been acquired on biologic and physical properties of the organism to justify its classification as a rickettsia.

Etiology

In the 8th edition of Bergey's Manual, *A. marginale* is the representative species of the genus *Anaplasma,* family Anaplasmataceae, order Rickettsiales (Ristic and Kreier, 1974). The organism (initial body) was shown to enter the erythrocyte by invagination of the cytoplasmic membrane with subsequent formation of a vacuole. Thereafter, the initial body multiplies by binary fission and forms an inclusion body which consists generally of four to eight initial bodies (Fig. 1). Inclusion bodies are numerous during the acute phase of infection, however, low-level infections persist for several years thereafter

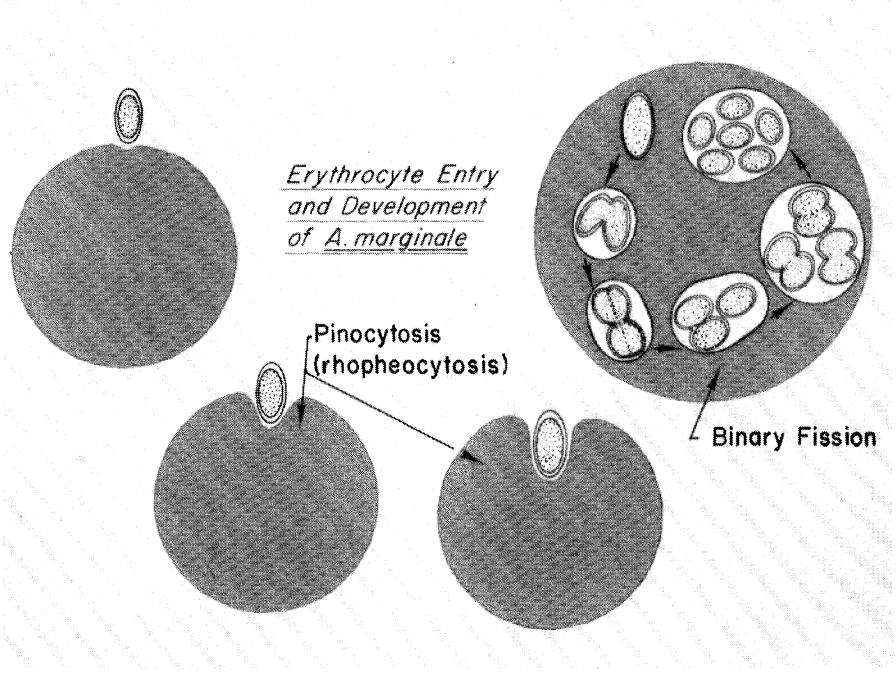

Fig. 1. Proposed erythrocytic cycle of development of *Anaplasma marginale.* The initial body enters erythrocyte by invagination of cytoplasmic membrane and in the process forms parasitic vacuole. In the vacuole, initial body multiplies by a binary fission-like process and forms an inclusion body.

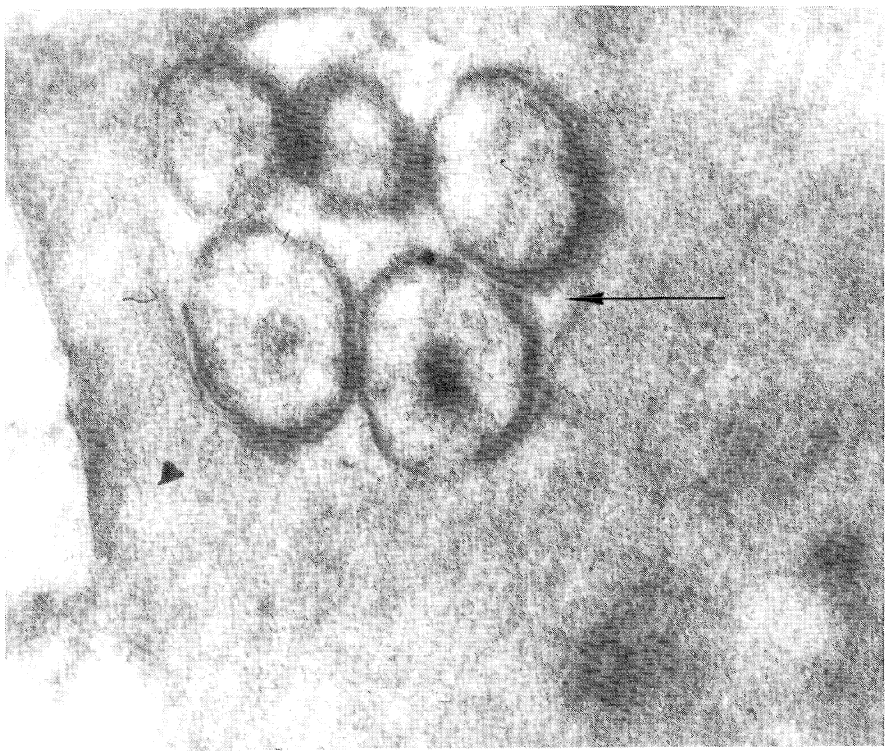

Fig. 2. An electron micrograph of an ultrathin section of marginal *Anaplasma* body in bovine erythrocyte. Note that the marginal body is composed of five initial bodies. ×50 000.

(Fig. 2). Of the three anaplasma species, *A. marginale* is the most pathogenic for cattle. *Anaplasma centrale* causes a relatively "mild" form of bovine anaplasmosis in Africa and *Anaplasma ovis* is the cause of ovine and caprine anaplasmosis.

Paranaplasma caudatum (genus *Paranaplasma*) was initially found in Oregon cattle in a mixed infection with *A. marginale*. Inclusion bodies of *P. caudatum* have appendages usually in the form of a tapering tail, a loop, or a ring, demonstrated only by use of special techniques. Studies in our laboratory showed that manifestation of *P. caudatum* appendages is a function of bovine host erythrocytes and does not occur in infected deer erythrocytes (Carson *et al.*, 1974).

The various anaplasma organisms have at least one species-specific antigen and are senstitive to the tetracycline group of antibiotics. Anaplasmas morphologically resemble other hemotropic rickettsiae, i.e., *Haemobartonella* and *Eperythrozoon* groups of organisms, and share common antigens with these agents. Ristic (1960, 1968, 1970) has compiled general reviews on anaplasmosis, the parasite itself, and the host response.

Host Range and Properties

Infections with *A. marginale* are wide-spread throughout tropical and subtropical regions of the world. In addition to cattle, the organism may infect zebu, water buffalo (*Babalus babalis*), bison (*Bison bison*), African antelopes, gnu (*Connachaetes gnou*), blesbuck (*Damaliscus pygargus albifrons*), duiker (*Sylvicapra grimmia*), American deer (southern black-tailed Rocky Mountain mule deer), Virginia white tailed deer (*Odocoileus* sp.), elk (*Alces alces*), camel (*Camelus bactrianus*) and wildebeest (*Connochaetes taurinus*). Sheep and goats may develop a transitory, subclinical infection. The African buffalo (*Syncerus caffer*) is refractory. Common laboratory animals, rabbits, guinea pigs, rats, mice, dogs, ferrets, cats and chickens, all are refractory to infection with *A. marginale*.

Infectivity of *A. marginale* can be destroyed by heating the organism at 60 °C for at least 50 min, by exposure to sonic oscillation at 35 °C for at least 90 min, or by X-ray irradiation at 100,000 R or higher. Histochemical analysis of *A. marginale* reveals DNA, RNA, protein and organic iron. Deoxyribonucleic acid from isolated marginal bodies and calf erythrocytes infected with *A. marginale* was found to be double stranded and to contain 51 moles percent guanine plus cytosine.

Anaplasma marginale can be preserved for at least 4 years by low temperature freezing of parasitized blood to which glycerin and/or dimethylsulfoxide (DMSO) has been added.

Some indications on metabolism of *A. marginale* are derived from *in vitro* studies with labeled amino acids. Relatively high levels of uptake of radioactive isoleucine, glycine, and methionine were detected in trichloracetic fractions of partially purified anaplasma bodies, indicating that the parasite is capable of protein synthesis outside its erythrocytic environment. Total adenosine triphosphatase (ATPase) activity associated with erythrocyte membrane was significantly increased during anaplasmosis. The increase in ATPase activity was partially attributed to *A. marginale*.

Because the host cell of anaplasma, the erythrocyte, is deprived of nucleus, mitochondria, or endoplasmic reticulum, it is postulated that the organism possesses numerous metabolic capabilities. Smith *et al.* (1972) studied biochemical environment of the organism by measuring enzymatic activity of erythrocytes in cattle infected with *A. marginale*. The enzymes included eight in the glycolytic pathway, two in the hexose monophosphate pathway, and two in other pathways. Activity did not change significantly for all enzymes during the incubation period, but all enzymes except pyruvate kinase were elevated significantly during reticulocytosis.

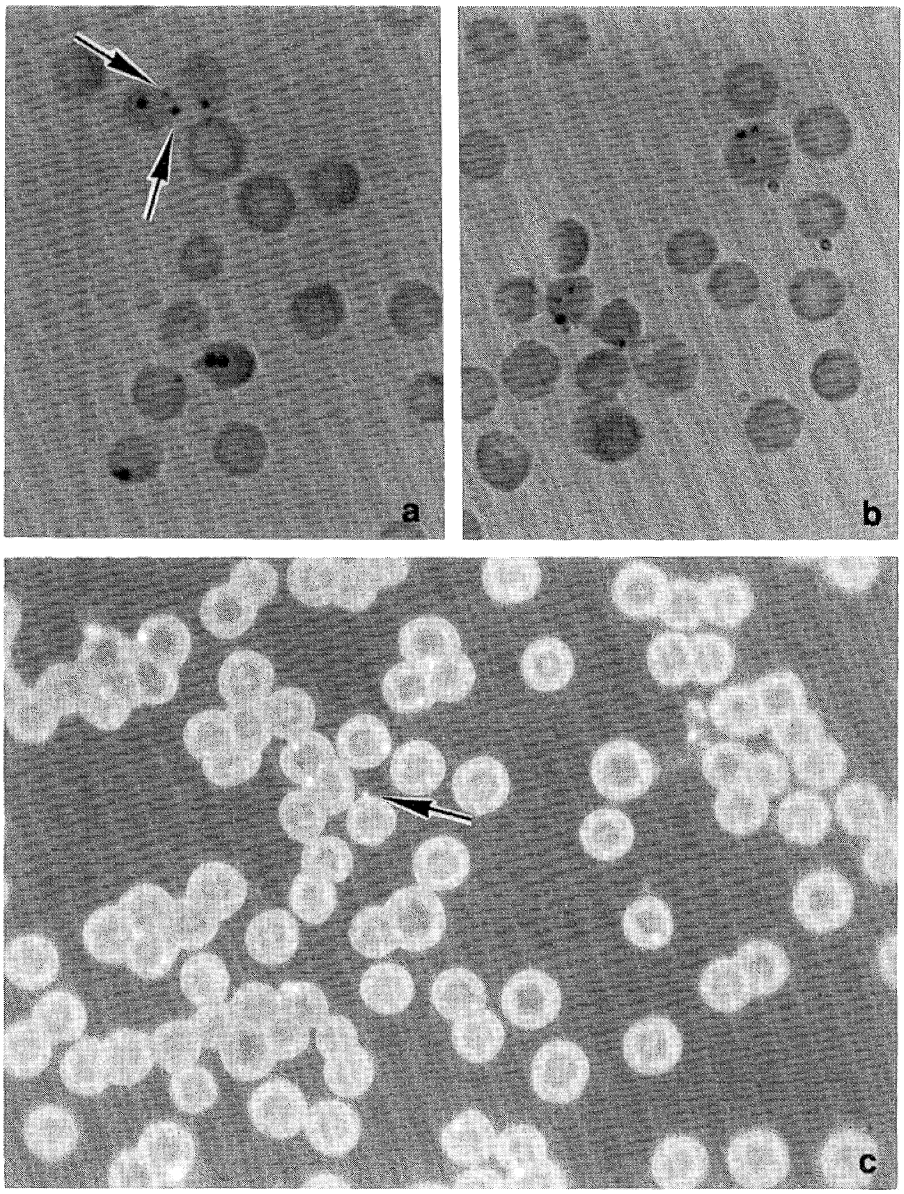

Fig. 3. Samples of *Anaplasma marginale* propagated in cell cultures. Note multiple erythrocytic infections with inclusion bodies varying in size and position with reference to the periphery of the erythrocyte. Also present are a few extraerythrocytic organisms. Giemsa (a+b) and fluorescent antibody staining method (c). ×1000.

In Vitro Cultivation

Over the years, various efforts were made to maintain and/or cultivate *in vitro* *A. marginale*. Cell culture systems of mammalian origin included bovine bone marrow, rabbit bone marrow, and bovine lymph nodes. Although some suc-

cesses were reported, studies were not continued and there is no information on results being reproduced by other investigators. Cultures derived from the mosquito *Aedes albopictus* were also used for cultivation of *A. marginale*. When erythrocytes from a calf in the acute phase of anaplasmosis were introduced in the above system, cultured mosquito cells phagocytized both infected and uninfected erythrocytes. The organism remained viable in these cells, as determined by calf inoculation, for 21 days.

None of the above systems produced evidence of active replication of *A. marginale*. Recently Kessler *et al.* (1979), and Kessler and Ristic (1979) used a modification of a method for propagation of *Plasmodium falciparum* to culture *A. marginale*. In various trials, which employed different procedures for diluting the original inoculum by serial passages, the organism was maintained *in vitro* between 28 and 60 days. A maximum intraerythrocytic parasitemia increase of more than 1000% was produced. Multiple erythrocyte infections with inclusion bodies varying in size, arrangement and position were abundant. Organisms on or in proximity to erythrocytic membrane were also present. Actual invasion of new erythrocytes by the organism was demonstrated by using the attenuated *A. marginale* which transgressed from ovine into bovine erythrocytes and developed in the latter cells. Slides of samples stained by Giemsa and fluorescent antibody methods were used to reveal the organisms (Fig. 3). Spectrophotometric and electron microscopic examination of culture medium revealed a considerable quantity of extraerythrocytic anaplasma bodies being produced by the system. Thus the true quantum of parasitic mass produced by this cell culture system is greater than that revealed by enumeration of intraerythrocytic forms. The organisms of 30- to 60-day-old cell culture consistently induced infections in susceptible cattle. In all instances, incubation periods and the virulence of the organism were similar to those produced by infections with whole blood derived from an animal in the acute phase of disease. Adaptation of the organism for *in vitro* growth was increased by alternate passages between the host and the cell culture.

Arthropod Vectors and Epizootiology

Experimentally, at least 20 tick species have been shown to transmit anaplasmosis, although field evidence indicating the tick as the principal disease vector is lacking. Histochemical staining, fluorescent antibody methods, and electron microscopy have been used for identification of the organism in the tissues of vector ticks. The organism was demonstrated in the gut contents and in the Malpighian tubules of engorged *Dermacentor andersoni* ticks;

however, very little is known regarding its developmental life cycle (Ristic, 1968). Experimental and epizootiological evidence incriminates horseflies (*Tabanus* sp.) as the most significant insect vector of anaplasmosis. Transmission by flies is affected by the direct transfer of blooed from infected to susceptible cattle and must take place within a few minutes after feeding on an infected animal. Experimental evidence of transmission was also produced with stable flies (*Stomoxys*), deerflies (*Chrysops*), horseflies (*Siphona*), and mosquitoes of the genus *Psorophora*. In more recent studies, transmitting abilities of mosquitoes (*Anopheles quadremaculatus*), the tick (*Dermacentor andersoni*) and horse flies (*Tabanus* sp.) have been comparatively examined. The tick transmitted the infection more readily by far than the two hematophagous insects (Peterson *et al.*, 1977).

In addition to carrier cattle, wild deer have been shown to play an important role in the epizootiology of anaplasmosis. Extensive studies in California indicate that anaplasma may survive in nature in the absence of cattle through deer-to-deer transfer via appropriate vectors. Transmission of infection from deer to cattle has also been demonstrated by several investigators (Ristic, 1968).

Pathogenesis

Pathogenesis of anaplasmosis in adult cattle can usually be divided in discrete intervals. The incubation or prepatent period seems to be indirectly related to the quantum of parasitic mass used to infect the animals. Under laboratory conditions using needle-induced infections with carrier blood, this period lasts 3 to 5 weeks. The patent period which follows is usually more intense and longer lasting in adult cattle than in calves.

During the patent period, infected erythrocytes are rapidly removed from the circulation by phagocytes of the reticuloendothelial system, especially of the spleen. Maximal anemia occurs 1 to 6 days after the peak of parasitemia, at which time an increasing number of apparently intact anaplasma-free erythrocytes are phagocytized by macrophages of the spleen and bone marrow. This new clearance mechanism, which apparently contributes greatly to the reduction of hematocrit values, arises in part from an autoimmune process which may develop during the course of the infection. Increasing anemia may persist for 4 to 15 days; during this period, animals may lose up to 70% of their circulating erythrocytes (Ristic, 1980a).

In animals which survived the patent phase of the disease, convalescence began with accelerated hematopoiesis characterized by reticulocytosis, macrocytemia, and granulopoesis. Recent studies in cell-mediated immunity (CMI)

showed that mature cows which demonstrate an early response in the CMI, occurring prior to or concurrently with the beginning of the patent period, survived infection with virulent *A. marginale*. On the other hand, similar cows which had little or no evidence of an early CMI response died of the disease (Ristic and Carson, 1977). Thus, it appears that the competence of the reticuloendothelial system and immunologic responsiveness of individual animals are the factors governing the outcome of the patent stage of anaplasmosis in an adult cow. The patent period is followed by a gradual convalescence. Convalescence usually lasts 1 to 2 months, but in aged animals it might be prolonged to 3 or more months due to parasitic recrudescence.

It is generally agreed that both parasitemia and anemia is mild in young calves following experimental infection with virulent *A. marginale*. Various evidence, in most cases hypothetical, can be advanced to explain this young age resistance to anaplasmosis. Among possible factors are promptness of immune response being greater in young cattle; competence of thymus function, particularly as this relates to cell-mediated immunity; vigorous erythropoietic bone marrow activity, and the protective role of fetal hemoglobin.

In the field, however, infections and clinical disease have been observed in young calves and in certain regions of South America these are known to cause actual economic losses. Successful intrauterine infection was accomplished by inoculating a 100-day-old bovine fetus *in utero* with blood containing 70% erythrocytes infected with *A. marginale*. At 41 days after inoculation, the fetus was removed from the cow by Caesarean section. The fetal serum reacted in the amplasmosis complement fixation (CF) test, its erythrocytes contained anaplasma bodies, and the whole fetal blood was infectious for a susceptible calf. The possibility that inoculation of the fetus may have resulted in damage of the uterine wall permitting passage of maternal antibodies into fetal circulation was ruled out on the basis of a considerable discrepancy of CF titer between the dam and the fetus. As a sequel to this finding is a report of naturally acquired clinical anaplasmosis in a 4-day-old calf. The infection has apparently been contracted ante-natally. This calf showed weakness in both front and hind legs, 30 to 40% of its erythrocytes contained *A. marginale* bodies, and its blood serum was positive in the anaplasmosis agglutination test. Infections via ocular route have been described and this may be a factor during terminal pregnancy and delivery (Ristic, 1980a).

Role of the spleen

The spleen appears to be the principal organ involved in limiting the uncontrolled spread of the anaplasma during the acute phase of infection. This role

is best illustrated by the fact that if splenectomized and nonsplenectomized calves are each injected with comparable numbers of the organism, the splenectomized calves undergo a more severe and frequently fatal attack, while the nonsplenectomized animals show less severe signs of the disease and usually survive. Occasionally splenectomy has been used in the field to demonstrate latent anaplasma infections. The second role of the spleen is to maintain continuous control of parasitic crises during the convalescent and carrier phases of anaplasmal infections. This function possibly contributes to the production and maintenance of the extrasplenic cellular and humoral elements necessary to control the infection efficiently at the latent level.

Clinical Forms

There are mild, chronic, acute, and peracute forms of anaplasmosis which are grouped according to variations in the severity and duration of the disease. The malady is generally mild in calves up to 1 year of age; acute but rarely fatal in cattle up to 2 years of age; acute and occasionally fatal in cattle up to 3 years of age, and often peracute and frequently fatal in cattle over 3 years of age.

The signs of acute anaplasmosis usually consist of anemia, weakness, febrile reaction, constipation, icterus, inappetence, depression, dehydration, labored respiration, and abortion. The acute crises often occur unexpectedly, with no prior evidence of illness. In bulls, temporary infertility has been observed.

Peracute anaplasmosis constitutes the most severe and usually fatal form of the disease. It occurs frequently in purebred animals or in high-producing milk cows, which succumb within a few hours after the onset of infection. In addition to anemia, milk flow is suspended, extensive salivation and very rapid respiration are noted, and animals so affected often exhibit irrational behavior and signs of nervousness.

Necropsy Findings

Gross pathological changes are typical of an acute anemia in which erythrocytes are removed by the reticuloendothelial system. The prominent changes are icteric mucous membranes, enlarged spleen, and obstructed gall bladder. Petechial hemorrhages may be observed on the epicardium and pericardium, and the heart is usually pale and flabby.

Diagnosis

During the acute stage of anaplasmosis, the diagnosis is made on the basis of clinical symptoms, hematocrit test, and microscopic examination of stained peripheral blood films for intraerythrocytic *Anaplasma* inclusion bodies. Giemsa staining is the oldest and most frequently used method (Fig. 4). Other staining methods include toluidine blue, and acridine orange. The latter method requires an ultraviolet microscope for visualizing the marginal anaplasma bodies.

In contrast to the ease with which acute forms of anaplasmosis are recognized, identification of the carrier stage is difficult. Although carrier cattle serve as reservoirs of infection, they cannot be clinically differentiated from unifected cattle and anaplasma usually cannot be demonstrated in blood films from the carriers. Various soluble and corpuscular antigens extracted from the blood of infected animals have been used for serologic diagnosis of anaplasmosis. Currently used tests are complement fixation (CF), capillary tube agglutination (CA) and card agglutination (CT) (Ristic, 1968, 1980a).

These tests are relatively accurate means of detecting subclinical carriers of *A. marginale*. The CF test is used in the laboratories with trained personnel while CA and card tests are useful diagnostic tools in the field. The CA test

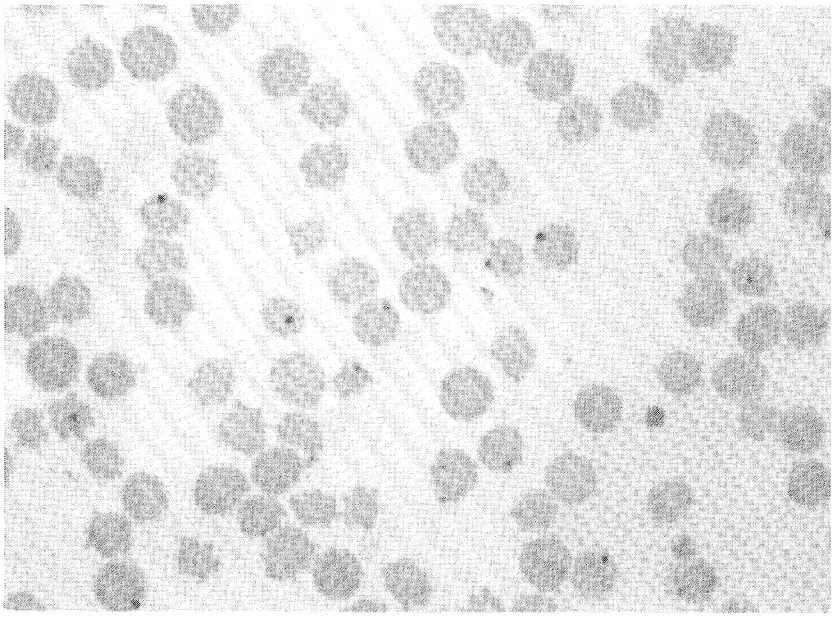

Fig. 4. Blood film of a cow with acute anaplasmosis stained by Giemsa method. Note *A. marginale* usually situated in close proximity to the erythrocyte membrane. ×1100.

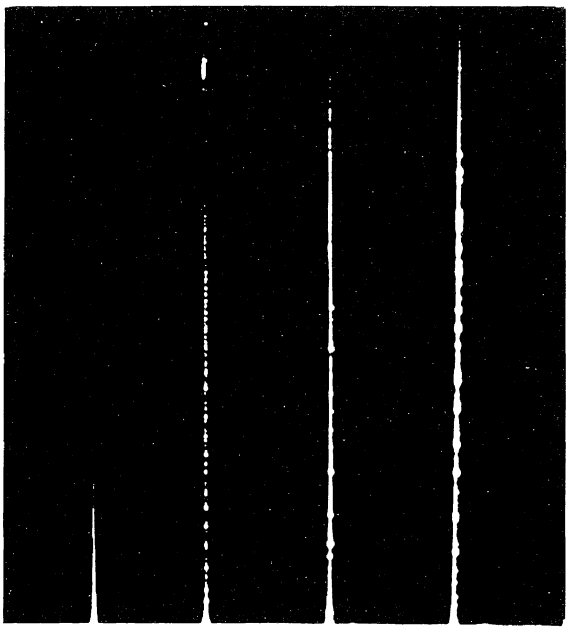

Fig. 5. Anaplasmosis capillary tube agglutination (CA) reactions. For routine field diagnosis, undiluted serum samples are used. Three degrees of positive reactions and a negative sample are shown.

was also effectively used to detect anti-anaplasma antibodies in the colostrum and milk of carrier dams. Serologic results suggest a colostral transfer of noninfectious (sterile) immunity from the dams to their calves (Fig. 5).

Immunity

In most cases, immunization with killed vaccines against the facultative intracellular parasites neither stimulates cell-mediated immunity (CMI) nor gives marked protection against challenge infection. Live vaccines are highly active in both respects. It is also significant that immune serum confers little, if any, protection against any of these agents. Serums from animals convalescing from anaplasmosis are active in precipitation, agglutination, complement fixation, and fluorescent antibody tests. However, transfer of sera from

immune to susceptible animals does not seem to confer protection against the infection and development of the disease. It may appear, therefore, that antibodies which coat naked parasites or cells harboring parasites have no protective capacity. Observations based on passive protection obviously are incomplete and to a degree misleading. One logically expects that antibodies interact *in vivo* with the macrophage system in bringing about anti-parasitic action.

The classical concept of protection in anaplasmosis has been that maintenance of protective immunity depended upon maintenance of the carrier state "infection immunity." Recently several laboratories reported that anaplasma carriers which were freed from virulent organisms by chemotherapy demonstrated considerable protection to challenge. In a similar study conducted in our laboratory, two previous carrier cows were challenged with virulent *A. marginale*. A prompt response was noted in the leukocyte migration inhibition (LMI) test after the challenge and the animals were clinically protected. These and other experiments indicate that CMI response in anaplasmosis seems to parallel anti-bacterial cellular immunity in the requirement of living cells to produce a continuous stimulus with concomitant and residual sensitivity of circulating leukocytes subject to an anamnestic secondary response. Lasting protection is seemingly afforded only by the presence of a carrier state following active infection with virulent *A. marginale* or the attenuated vaccinal agent.

Based upon the above observations, one could propose possible immunologic sequences in the development of protective immunity in anaplasmosis. An initial exposure of cattle to replicating *A. marginale* (virulent or attenuated organism) presents the host reticuloendothelial system with sufficient initial antigenic stimulus to induce a CMI response which may serve as the protective mechanism in concert with the simultaneously induced humoral response. Continuous antigenic stimulation results from the perpetual low-level infection. Correlation of protection and CMI, as measured by the LMI test, implies the presence of an *in vivo* mechanism which is directed toward neutralization of the invading organism. Humoral antibody may serve as opsonins in promoting the phagocystosis of *A. marginale*-infected erythrocytes by macrophages which have been "stimulated" and are attracted to that particular area by lymphokines released from sensitized lymphocytes. Reexposure to *A. marginale* stimulates memory T lymphocytes to multiply and afford a magnified protective response due to the increased numbers of sensitized lymphocytes capable of specific response.

Immunologic Aspects of Anemia

It has been known for many years that the severity of anemia in infected animals was not always proportional to intensity of parasitemia. Fixed and free-serum autohemagglutinins have been demonstrated in anaplasma-infected cattle and proposed that anemia might be, in part, of immunologic etiology. It was then shown that autohemagglutinins apparently contributed to the onset of erythrophagocytosis in the spleen and bone marrow of infected animals. Opsonins were found in the serum of infected cattle and by an *in vitro* test demonstrated that these induce phagocytosis of normal bovine autologous and homologous erythrocytes. Further studies showed that the opsonins could be eluted from erythrocytes of infected animals before they were detected in the serum. The detection of opsonins in the erythrocytic eluate and serum preceded the appearance of parasites in the peripheral blood and the onset of anemia. Maximal opsonic titers coincided with the anemic crisis (Morris *et al.*, 1971). These findings of an apparent role of immunologic elements in the pathogenesis of anemia in anaplasmosis are further strengthened by more recent data in which cell-mediated cytotoxicity to ^{51}Cr labeled autologous erythrocytes was demonstrated (Ristic, 1980b).

An analysis of all immunologic data in anaplasmosis indicates that in the course of the disease, immune responses contributing to the development of anemia appear first to be followed by protective immune elements which become most pronounced after recovery of the acute phase of the disease. With the attenuated *A. marginale* vaccine, the latter protective immune responses are equally pronounced as with the virulent agent but the mild nature of the agent seems to instigate development of little, if any, anemia associated immune factors. Only transient and low levels of anti-erythrocyte cytotoxicity mediated by lymphocytes and humoral elements takes place following administration of the attenuated *A. marginale* (Ristic, 1980b).

Immunization

Prophylactic immunization against anaplasmosis used naturally occurring *Anaplasma* species (premunization), laboratory-attenuated *A. marginale* of ovine origin, and inactivated *A. marginale* of bovine and ovine origin.

Naturally occurring Anaplasma marginale *and* A. centrale

Early use of field isolants of anaplasma for premunization consisted of injecting blood from known carriers into susceptible cattle. This procedure pre-

sented a certain risk, since it was often necessary to control the initial infection with drugs so subsequent recovery and development of the carrier state could occur. A method for premunization using a small inocula to induce protection has also been reported but later results indicated that severe disease and mortality can result from even very small doses of *A. marginale*.

Prior infection with *A. centrale* did not prevent infection with *A. marginale* but reduced the severity of the superimposed disease. On this basis, Theiler (1910) developed the method of *A. centrale* vaccination which is still used in several countries. Variable resistance to *A. marginale* resulting from vaccination with *A. centrale* was later described. Furthermore, it was observed in Australia that cattle inoculated with *A. centrale* (South African strain) developed severe symptoms of the disease. *Anaplasma marginale* and *A. centrale* have more recently been compared on a clinical, hematological, and serological basis. Heterologous challenge in all cases produced a definite hematological reaction but *A. centrale* usually reduced the severity of a subsequent *A. marginale* challenge.

Laboratory attenuated Anaplasma marginale

Detailed description of the development of the attenuated *A. marginale* from the virulent Florida anaplasma isolant is reported elsewhere (Ristic, 1968). The basic methodology used for attenuation of the Florida isolant has been (1) induction of an apparently accelerated rate of mutuation of the organism by exposure to irradiation; and (2) selection of an avirulent *A. marginale* strain by serial passage of irradiated organisms in splenectomized deer (two passages) and sheep (138 passages). Lots of sheep blood-derived seed material of established immunogenicity, growth pattern, and safety were preserved by storage in liquid nitrogen. For vaccine production, the seed material is reactivated in splenectomized sheep, using four to five passages at 7- to 9-day intervals. The vaccine is dispensed in liquid nitrogen and inoculated intramuscularly in 1- to 2-ml doses. The prepatent period in inoculated animals varies between 4 and 6 weeks after which the organism may be detected in approximately 0.5 to 8% of the peripheral blood erythrocytes. Vaccinated animals mount strong humoral and cell-mediated immune response (Fig. 6). A slight hematocrit decrease usually not exceeding 5 to 10% of the preinoculation value, generally occurs. These manifestations are transitory and are in evidence for 1 to 2 weeks. On challenge with the virulent agent, vaccinated animals are not clinically affected.

A series of laboratory and field experiments conducted in the United States, Peru, Venezuela, Colombia, Mexico (Ristic and Carson, 1977), and Brazil

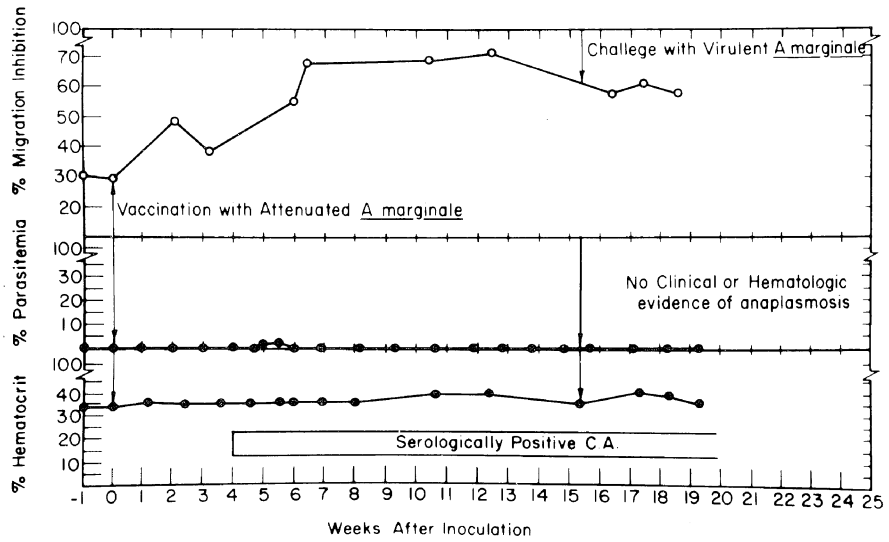

Fig. 6. Hematocrit, percentage of parasitemia, percentage of leukocyte migration inhibition (LMI), and results of capillary tube agglutination tests for an adult cow following vaccination with the attenuated *A. marginale* and challenged with virulent *A. marginale*. Note a prompt cell-mediated immune response following vaccination as measured by the LMI test and clinical protection following challenge.

(Novas, 1978) have shown that the immune response induced by this vaccine protects adult susceptible cattle against challenge with virulent endemic strains.

Inactivated Anaplasma marginale *of bovine and ovine origin*

Use of a vaccine containing killed anaplasma organisms has been described in Africa (McHurdy and Simpson, 1973) and the United States (Brock *et al.*, 1964). The United States vaccine presently used commercially, Anaplaz,* was prepared from blood of infected animals collected at the peak of parasitemia. Blood cells were washed, lysed, and lyophilized. The desiccated material was reconstituted with an oil adjuvant. Two doses of the vaccine were given subcutaneously at 4- to 19-week intervals. A degree of protection from the clinical signs of anaplasmosis has been reported to be afforded 2 weeks after the second injection. Vaccinated animals became carriers after field challenge. An annual booster injection is also recommended. Studies of the vaccine in our laboratory showed that on challenge, vaccinated animals developed lower

* Ft. Dodge Laboratories, Ft. Dodge, IA.

parasitemia, however , the anemia was equally pronounced as that in non-vaccinated controls. Aside from its low protective effect, the greatest drawback of this vaccine is induction of isoimmunization and erythrolysis due to the bovine blood group components which it contains (Dennis *et al.*, 1970).

A preparation similar to Anaplaz was developed in our laboratory using ovine erythrocytes infected with the attenuated *A. marginale*. The Anaplaz adjuvant has been used to reconstitute the lyophilized material and the aforementioned vaccination regimen followed. A degree of resistance to development of parasitemia has similarly been detected after challenge with virulent *A. marginale*. Cattle inoculated with this vaccine, however, did not develop isoimmunity to bovine erythrocytes.

Very recently, a new method of vaccination against anaplasmosis using soluble organism-free cell culture derived *A. marginale* antigen was developed in our laboratory. The immunogen appears to be a surface coat antigen of the initial *Anaplasma* bodies. The antigen is apparently essential for attachment and entry of the organism into erythrocytes. Isoimmunization and other side effects caused by the Anaplaz are eliminated by this new procedure.

Treatment

Prior to the development of tetracyclines, many chemotherapeutic compounds, i.e., arsenicals, antimalarials, antimony derivatives and dyes have been employed in an effort to treat acute froms of anaplasmosis. Supportive therapy, including hematinics, electrolytes, and whole blood transfusions were similarly used as additives to chemotherapy.

Two new compounds, Gloxazone † (alpha-ethoxyethylglyoxal dithiosemicarbazone) and Imidocarb †-3,3′-bis (2-imidazolin-2-yl) carbanilide dihydrochloride or dipropionate), have been described as having a specific chemotherapeutic effect on anaplasma. These drugs, however, are not approved for commercial sale and consequently are available for experimental studies only.

The tetracyclines, oxytetracycline, chlortetracycline, and tetracycline are the only effective drugs for treatment of bovine anaplasmosis. The tretracyclines can be used parenterally and orally to treat both acute and latent infections. The application of these compounds on animals during the acute phase of the disease will retard and inhibit the cycle of development of the parasite and usually alleviate occurrence of severe parasitemic and anemic crisis, assuming the drugs were administered early in the course of the disease. In latent

† Burroughs Wellcome Co., Research Triangle Park, NC.

Table 1. Successful treatment regimens, utilizing tetracycline drugs for elimination of *Anaplasma marginale* carrier status.

Drug	*Route*	*Number of daily treatments*	*Dosage, mg/kg/day*	*Total drug (g) to eliminate infection in a 400 kg bovine animal*
Chlortetracycline	Oral	41	2.2	36.1
Chlortetracycline	Oral	120	1.1	52.8
Chlortetracycline	Oral	60	3.3	79.2
Chlortetracycline	Oral	45	5.5	99.0
Chlortetracycline	Oral	60	5.5	132.0
Chlortetracycline	Oral	30–60	11.0	132.0–264.0
Chlortetracycline	IV	16	33.0	211.2
Tetracycline	IV or IM	10	11.0	44.0
Oxytetracycline	IV or IM	12–14	11.0	52.8–61.6
Oxytetracycline	IV	5	22.0	44.0

Modified from Magonigle *et al.* (1975).

infections, the dose of drugs and the number of treatments can be calculated to eliminate the organism completely (Magonigle *et al.*, 1975).

Consistent elimination of latent infections by oral application of tetracyclines on a herd basis is frequently difficult to attain because of differences in consumption of medicated feed by individual animals. It appears important that the drug be mixed with highly palatable feed supplement so all cattle obtain the needed medication by free-choice feeding. The feeding of high levels of chlortetracycline (11 mg/kg/day) is an effective method for reducing the incidence and transmission of bovine anaplasmosis (Table 1). Disappearance of specific antibodies from the serum of treated animals, as revealed by serologic tests, can be used as a means of determining that anaplasma has been eliminated from such an animal. Agglutinating antibodies in card agglutination (CT) and capillary tube agglutination (CA) tests subside faster than complement fixing (CF) antibodies following destruction of the organism by chemotherapy.

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28. HEARTWATER DISEASE

G. Uilenberg

Abstract. Heartwater, or cowdriosis, is one of the main causes of death in imported and improved cattle in Africa south of the Sahara. It is an acute febrile disease of ruminants, associated with nervous, intestinal and pulmonary disorders, caused by the rickettsia *Cowdria ruminantium,* and transmitted by several species of *Ablyomma* ticks.

Local cattle in endemic regions have acquired considerable resistance through long natural selection. Endemic resistance without clinical disease is not attained in susceptible breeds. Only very young calves possess a considerable degree of age-resistance to the disease. Solid or partial protective immunity, lasting for several years, follows recovery. The nature of protective immunity is unknown.

The course of the disease, the post-mortem lesions and the pathogenesis of heartwater are reviewed. Unequivocal diagnosis of heartwater is possible only by the microscopical demonstration of rickettsial colonies in endothelial cells of blood vessels, especially in the capillaries in smears of brain cortex.

Acaricidal control of the vectors must be frequent and efficient if heartwater transmission is to be prevented. Immunization of cattle by infection and treatment is possible, but the method is rather unsatisfactory, unsuitable for application on a large scale, and far from optimally safe and effective. Attempts at attenuation of strains have failed. Tetracyclines are the best available drugs for treatment, but their action is slow and chemotherapy commonly fails if delayed until typical nervous systems have developed.

Disease

Heartwater is an acute rickettsial disease of ruminants in Africa south of the Sahara. It is caused by *Cowdria ruminantium* and transmitted by at least five species of *Amblyomma* ticks. The name is derived from the frequent occurrence of hydropericardium. Other names are cowdriosis and, less specifically, malignant rickettsiosis of ruminants. It is the most important tick-borne disease of cattle in many regions and is relegated to second place only in areas where pathogenic theilerias occur. Since Cowdry (1925) first discovered the causal agent, several authors have reviewed the literature (Alexander,

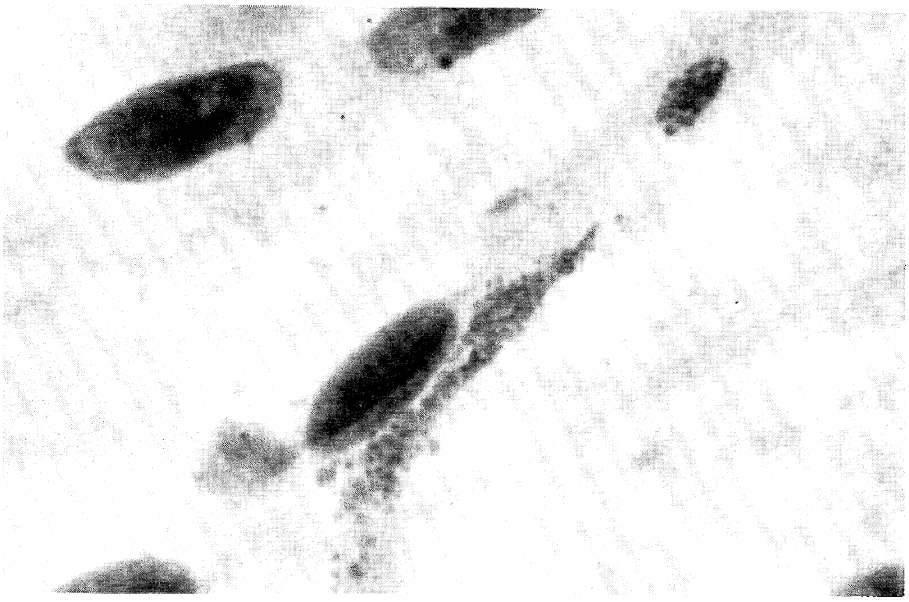

Fig. 1. Cowdria ruminantum. Groups of organism of intermediate size in brain capillary. Giemsa staining method. ×2200. (Original.)

1931; Curasson 1943; Haig 1955; Henning 1956; Neitz 1968; Andreasen 1974; Ilemobade 1976; Uilenberg 1971, 1977; and Ramisse and Uilenberg 1971).

Etiology

Heartwater is caused by the rickettsia *Cowdria ruminantium* (Cowdry, 1925). It occurs in close-packed colonies in the endothelial cells of blood vessels in various organs (Fig. 1). The number of granules in one colony varies from less than 10 to several hundreds. The rickettsias in one group are usually of similar size, but the individual diameter varies from group to group from approximately 0.2 μm to over 1.5 μm. Viewed with the light microscope, the smaller granules are coccoid, larger ones also occurring as rings, horseshoes, rods and as irregular masses. The differences in size and shape suggest the possibility of a growth cycle. Multiplication appears to be mainly by binary fission. There are suggestions that *Cowdria* may initially develop mainly in reticuloendothelial cells of the lymph nodes, before invading endothelial cells of the vessels, and the organism has been reported to occur in various other cell types, including macrophages, monocytes and cells of the renal tubules. Although animal inoculation shows that the organism also occurs in the blood, it cannot be detected microscopically in blood smears. Most workers

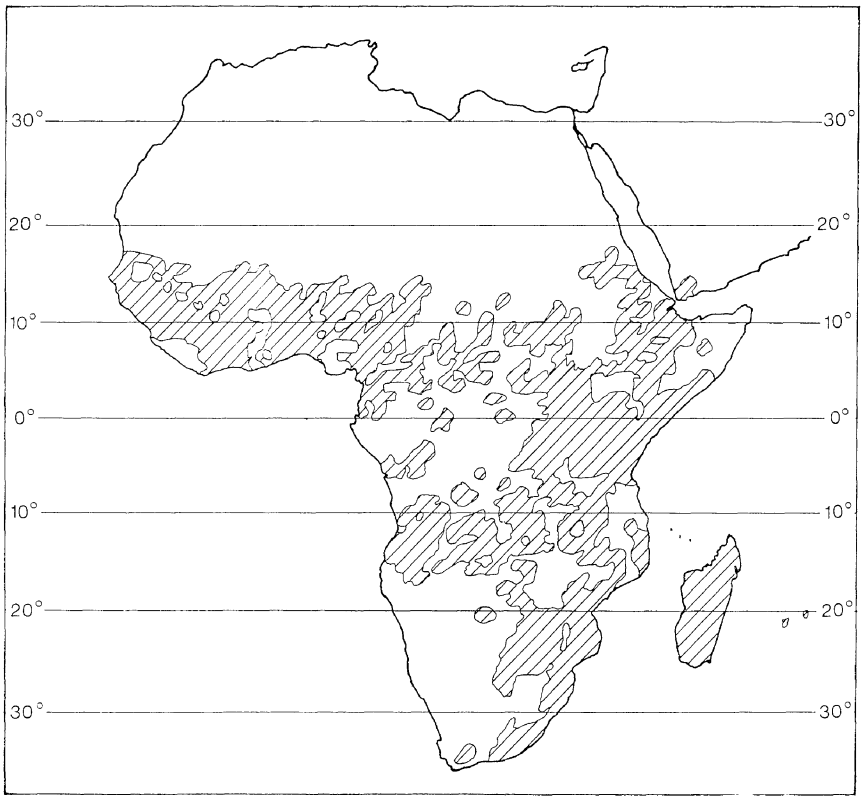

Fig. 2. Approximate distribution of the five known vectors of heartwater disease in Africa as recorded by Morel (1969). (Adapted.)

now seem to agree that it is associated with blood leukocytes, but there are conflicting experimental results; it has also been demonstrated to occur, although not consistently, in the plasma.

Little work has been carried out on the distribution and cycle of *C. ruminantium* in the tick vector. Its presence has been demonstrated in the tick intestine. Eearly suggestions that it is inoculated by regurgitation from the alimentary canal are supported by recent work in South Africa, where saliva was found to be rarely infected and then only slightly so (Bezuidenhout, personal communication, 1979).

Distribution

Heartwater has been reported from many countries in Africa south of the Sahara, but in most areas little is known about the incidence of infection and

disease because of deficient diagnosis and because its existence often is recognized only after the introduction of particularly susceptible foreign breeds of ruminants. Large numbers of these were first introduced into South Africa, and for this reason the disease has been most extensively studied in this country. The potential, and possibily the actual distribution of heartwater, covers at least that of the tick vectors *Amblyomma hebraeum, A. variegatum, A. pomposum, A. gemma,* and *A. lepidum*; because of their various ecological requirements, these ticks occupy most of Africa South of the Sahara as well as Madagascar and other off-lying islands (Fig. 2). *Amblyomma variegatum* has been introduced into the Caribbean region (e.g., Guadalupe, Antigua, Puerto Rico) and cowdriosis has recently been demonstrated to occur on Guadalupe. Reports on its occurrence in southeastern Europe have not been confirmed. The assumption that it might be endemic in the Near East, as blackheaded "Persian" sheep proved to possess considerable resistance to heartwater when they were first introduced into South Africa (Alexander, 1931), may be based on a misunderstanding of the origin of this breed, which appears to be unknown in Iran (A. Rafyi, personal communication, 1977), but is common in the endemic regions of the Horn of Africa and East Africa.

Susceptibility

Apart from cattle, other domestic ruminants such as sheep, goats and Asian buffalo, are susceptible to infection and the disease is in fact as important for sheep and goats as it is for cattle. The susceptibility of the camel has been suspected, but not proved. Some species of African antelopes (as well as European fallow deer) have been shown to be susceptible to experimental infection; although the antelopes usually did not show clinical symptoms, natural fatal cases of the disease have been seen in springbuck and eland antelope.

Animals other than ruminants are refractory or difficult to infect. The rickettsias may survive in laboratory rodents for as long as 3 months, but infection is latent and can be demonstrated only by subinoculating ruminants. Serial passage in rodents of such latent infections has always failed, except for one instance in Madagascar where a large number of serial passages was achieved in cortisone-treated mice; this was possible only when intravenous subinoculation with blood was adopted (J.J. Ramisse, unpublished reports, 1970-1973). Even then, the mice did not show any clinical sign of disease and the micro-organism could not be detected microscopically in their tissues. There are some reports of *C. ruminantium* clinically affecting various laboratory animals, with microscopic detection of the organism in affected animals.

Evidence of the infection being that of *C. ruminantium* was not given, or was not quite convincing (Du Plessis and Kumm, 1971). Andreasen (1974) claims that the Malagasy strain, used by Ramisse, was pathogenic to congenitally athymic "nude" mice, and that he actually found the organism in such mice; so far it has not been possible to duplicate this work in our laboratory with a Nigerian and a South African strain.

Epidemiology

As far as is known, heartwater disease occurs in Africa only; thus the natural reservoir of the disease is undoubtedly to be found in wild African ruminants. Nevertheless, domestic ruminants alone suffice to maintain the disease at a high level of incidence, as is the case in Madagascar where wild ruminants do not occur.

Experimentally, five species of *Amblyomma* were shown capable of transmitting *C. ruminantium.* The elephant tick, *A. tholloni,* has recently been shown in Zimbabwe to be an experimental vector. A few other African *Amblyomma* spp. occurring on wild ruminants have adapted themselves locally to domestic cattle and might prove to be vectors, extending even more the potential distribution of the disease; this is, for example, the case with *A. astrion* on the islands of Sao Thome and Principe, and *A. cohaerens* in parts of Ethiopia. Heartwater has recently also been transmitted in our laboratory by an American tick, *Amblyomma maculatum* (unpublished).

Amblyomma spp. are three-host ticks. It is generally accepted that transmission is transstadial only, so that unfed larvae are always free of infection. However, recent work at Onderstepoort has shown transovarial transmission in one conclusive experiment (J.D. Bezuidenhout, personal communication, 1979). Transovarian transmission is probably infrequent. A larva feeding on an infective ruminant is able to transmit the disease not only in the next instar, the nymph, but is still infective in the adult stage, even if the nymphal feeding took place on a non-susceptible species of animal. On the other hand, as larvae commonly feed on non-susceptible animals, it is often the nymph that first acquires infection and in that case, only the adult transmits the disease. As is known in some other tick-borne diseases, infected ticks do not start to transmit the disease immediately upon attachment. They usually feed for a variable period of time before transmission occurs. This has practical implications for the frequency of acaricidal treatment necessary to stop disease transmission in the case of babesiosis and theileriosis, where this pretransmission feeding period is commonly a matter of days, but this does not appear to be so in heartwater where the period is said to be less than 24 hours (Neitz, 1968).

Artificial transmission is possible by means of blood and tissues of sick animals. All authors agree that intravenous injection is necessary in order to achieve reliable transmission. The subcutaneous route of infection was successfully used only with brain tissue material derived from an infected animal (Ilemobade, 1976). Intravenous inoculation of brain tissue emulsion usually kills the animal by shock.

Local cattle in endemic regions usually suffer little mortality, a considerable degree of innate resistance presumably having been acquired through long natural selection. Only a few reliable figures are available to that effect, however. Although Neitz and Alexander (1945) found calves of the local Afrikander breed susceptible to heartwater, Bonsma (1944) reports that at one particular breeding station only 5% of Afrikanders were lost to the disease before the age of $2\frac{1}{2}$ years, against 60% of European beef cattle. Henning (1956) and Uilenberg (1971) confirm the resistance to heartwater disease of cattle raised in endemic areas. While most indigenous cattle are zebu, the resistance is not linked to any particular breed. Foreign zebu, such as Brahman and Sahiwal, and even African zebu breeds from heartwater-free areas, are highly susceptible; on the other hand, African *Bos taurus* cattle, such as the Ndama and similar breeds, maintain themselves successfully in heartwater regions without tick control, contrary to European breeds.

Calves, even of highly susceptible stock, are relatively resistant to the disease for a 2- to 3-week period immediately after birth. This resistance is independent of the immune status of the dam. Most calves infected during this period recover, but as the majority are not infected until later after the age resistance is lost, a state of endemic stability without apparent disease (such as commonly occurs in babesiosis) does not exist. The infection rate in tick populations in the field is apparently so low that individual cattle commonly escape infection for several months (Alexander, 1931; Bonsma, 1944) or even years if tick numbers are not excessive (Neitz and Alexander, 1945; Uilenberg, 1971). The reason for the low infection rate in the field may partly be the fact that the blood is infective for only a limited period, approximately from the time the fever reaction starts until one week to two months, most often two to three weeks after recovery. These results have been obtained from studies in small ruminants; no such information is available for cattle. Ilemobade (1976) found the blood of one calf positive on subinoculation 19 days after spontaneous recovery, but negative on the 34th day; another calf was positive up to 40 days after treatment but negative at 55 and 72 days. Although reinfections may cause the blood to become temporarily infective again, at least in sheep (Neitz *et al.*, 1947), there is no permanent carrier state, so that ticks have a limited opportunity of becoming infected. Another factor for the low infection rate undoubtedly is the fact that a large proportion of the

immature stages of *Amblyomma* feed on non-susceptible animals such as small mammals, birds, and even reptiles.

Where the vector population is low, either by acaricidal treatment or by unfavorable ecological conditions, sporadic cases of heartwater may occur with long intervals of seeming absence of the disease. The fact that infection may be passed on from the larva through the nymphal stage, to the adult, combined with the longevity of *Amblyomma* ticks, explains that such long interepizootic intervals are possible. Ilemobade (1976) found that the infection is maintained in fasting adults for over 15 months. The disease may also be carried over long distances and reintroduced into pastures by nymphs on birds, if infection was acquired in the larval stage.

Heartwater occurs throughout the year, but its incidence may decrease during the dry season in countries with pronounced seasonal climatic changes, which influence the seasonal activity of the different stages of the vector.

Clinical signs

Few figures are available for the length of the incubation period in cattle following natural tick transmission. According to Neitz (1968) fever starts on the average 18 days after tick infestation. The incubation period to fever after artificial intravenous infection may vary from 1 to 3 weeks. The average incubation period may vary according to the tick strain, at least in small ruminants (Neitz *et al.*, 1947, and other authors), and possibly also in cattle (Uilenberg, 1971). After the onset of fever, a period of 1 to 9 days may elapse before definite clinical symptoms are seen; occasionally typical clinical signs are not preceded by fever.

The course of infection varies from subclinical to peracute. Subclinical and mild forms are common in local indigenous cattle of all ages, in partially immune animals, in very young calves of all breeds, and occur in older animals of susceptible breeds. If symptoms occur, they are slight and seldom noticed under field conditions although the animal is febrile. On close examination, the respiration rate may be increased and the animal may be listless to some extent, but recovers within a few days.

At the other extreme, peracute cases are fairly common in cattle of exotic breeds. An animal which appears to be normal, although its temperature may already be high, may suddenly collapse in convulsion and die. Auscultation before death would in most cases have revealed the existence of lung oedema and the presence of exudate in the bronchi.

In most cases in susceptible cattle, heartwater is an acute febrile disease,

accompanied by nervous disorders and often intestinal and pulmonary disorders. It starts suddenly with a high fever (often over 41 °C), which is not usually noticed as feeding remains normal at first. After a variable number of days, nervous symptoms appear, ranging from a staggering, drunken gait, circling movements, abnormal postures and, more rarely, aggressiveness, to merely frequent twitching of the eyelids, frequent sticking out of the tongue, a haggard facial expression, or tremors of individual muscles, which can often be provoked by a sudden noise or by touching the animal. Pregnant cows may abort. Finally the animal may collapse in convulsions with pedalling movements of the limbs, and frequently nystagmus, opisthotonus and chewing movements of the mouth. Death follows, either during the first crisis of convulsions or in the course of a subsequent attack, of which there may be several. Profuse, fetid diarrhea is frequent, and is sometimes the only clinical sign; the feces may be haemorrhagic. A moist cough is often noticeable and on auscultation bronchial rales are frequently heard. When marked nervous and/or intestinal symptoms have developed, recovery is rare and the animal usually dies within a few days.

In subacute cases, symptoms are similar but less marked and the disease may last for over a week, then, either collapse and death ensue, or the animal gradually recovers.

The mortality rate varies with the virulence of the strain and the susceptibility of the cattle. It is usually over 50% in exotic breeds, often far more, while it may be only some 5%, sometimes less, in local breeds in endemic areas. It is low, less than 10% in calves up to the age of 2 to 3 weeks irrespective of the breed.

The differential diagnosis varies according to the symptoms. Nervous disorders may simulate diseases such as rabies, cerebral babesiosis, hypomagnesaemic tetany, tetanus or strychnine poisoning. Haemorrhagic diarrhea is also found in coccidiosis and, in combination with the high fever and sudden death in peracute cases, may make blood-smear examination for anthrax necessary. High fever is, of course, found in many contagious and vector-borne diseases such as foot-and-mouth disease, rinderpest, theileriosis, babesiosis, anaplasmosis, trypanosomiasis, etc., but clinical and parasitological examination will usually reveal the specific lesions or the causal blood parasites associated with these diseases. The history of the herd and knowledge of the local disease situation is important.

Pathology

Lesions at autopsy are quite variable. None of them are pathognomonic, but the association of frequently occurring post-mortem changes may justify a

tentative diagnosis of heartwater, especially if suspect symptoms were observed prior to death. The predominant lesions reportedly vary with the strain of *Cowdria* (Alexander, 1931).

In peracute cases the most striking lesion usually is a very marked oedema of the lungs and the presence of froth in the trachea and the bronchi. These lesions are often so pronounced that they appear sufficient to explain death by asphyxia.

Hydropericardium, hydrothorax and ascites are frequent lesions in acute heartwater, either occurring together or singly, but they are far from being always present or marked. The lungs are frequently oedematous and froth in the respiratory passages is common. The mediastinal and the perirenal tissues may be oedematous. The lymph nodes are commonly swollen. Petechiae and haemorrhages occur in and on various organs, such as the heart, lungs and the gastrointestinal tract. The mucous membrane of the abomasum often shows local hyperaemia and scattered petechiae; marked enteritis with petechiae and often larger intestinal haemorrhages is common; haemorrhages may be present even in the rectum, in which case the feces appear haemorrhagic. The liver is usually engorged and the gall bladder distended. The heart muscle and kidneys are degenerated. The brain does not usually show any striking macroscopical changes, apart from frequent congestion of the meningeal vessels; petechiae and larger haemorrhages may be seen on the cut surface. Alexander (1931) and Henning (1956) state that splenomegaly is usually present, but in our experience it is rarely seen (Uilenberg, 1971); Andreasen (1974) never observed enlargement of the spleen, and Ilemobade (1976) found it in a minority of cases only. (Many of these observations refer to small ruminants.)

The pathogenesis of heartwater is poorly understood. As stated above, marked lung oedema may in some cases be directly responsible for death by asphyxia. The histopathological changes in the brain do not appear to be constant and severe enough to consistently explain the nervous symptoms associated with the disease. An important terminal decrease of the arterial blood pressure may be one of the main factors causing death, and is associated with an important terminal fall in plasma volume. The latter is presumably due to an increase in capillary permeability, leading to the transudates and oedema so often seen at autopsy; these accumulations of liquid frequently coagulate, indicating that molecules as large as fibrinogen are able to pass through the capillary wall. These findings, by South African workers, have been obtained in small ruminants. The occurrence of toxins has been offered as a possible explanation for some of the lesions in heartwater, but their presence has so far not been demonstrated.

Immune response

Most of the knowledge on immunity has been acquired in artificially infected small ruminants. After recovery from infection induced by inoculation of blood, sheep acquire immunity to artificial or natural reinfection, irrespective of whether the clinical reaction was mild or severe. In some animals the immunity wanes after 6 months, but even then the reaction to challenge is usually subclinical and limited to fever; partial or solid immunity may last for as long as 5 years.

Alexander (1931) states that in partially immune sheep a solid immunity is established after two or three reinfections. A temporary rickettsaemia may follow reinfection, even in sheep which show no febrile reaction (Neitz *et al.*, 1947).

In cattle recovered from artificial infection, cases of clinical and fatal disease following reinfection appear to be more common (Neitz and Alexander, 1945; Haig, 1955).

As has been stated above (Epidemiology), the immunity appears to be sterile. After a limited period during which the blood is still infective following recovery, the organism cannot be demonstrated any more by subinoculation of blood, or even by scrapings of the intima of blood vessels. Nevertheless, the rickettsias have been found in the brain of goats dying of heartwater up to 9 weeks after tetracycline treatment although their blood was not infective (Ilemobade, 1976).

There is no definite evidence of the occurrence of immunological differences between strains (unless the mouse-adapted organism of du Plessis and Kumm (1971) is really *Cowdria*), and strains from Nigerian and South Africa have given complete cross-protection in recent experiments in goats (A.J. van Winkelhoff and G. Uilenberg, unpublished). Great differences in virulence do exist between strains (Neitz *et al.*, 1947).

Nothing is known concerning the nature of the immune response. Serum or large amounts of concentrated gammaglobulins from hyperimmunized animals, administered before, after, or simultaneously with infective blood have no influence on the course of the disease in sheep (Alexander, 1931; Du Plessis, 1970). This, together with negative results obtained with the indirect immunofluorescent antibody test, led the latter author to the conclusion that humoral antibodies do not play a role in immunity to heartwater. It may be significant in this connection that Ilemobade (1976) found a decreased level of gammaglobulins in serum of affected animals suggesting an impairment of the humoral response. Splenectomy has no influence in heartwater.

It has not been possible to demonstrate the occurrence of passive immunity in calves, and the innate age resistance of very young calves is not associated with the immune status of the dam.

Laboratory aids to diagnosis

There is no practical and specific method for the unequivocal diagnosis of heartwater disease in a living animal. Two procedures used in diagnosis include microscopic detection of the organism in the capillaries of the brain cortex obtained by biopsy, or subinoculating blood of affected animals intravenously into a susceptible ruminant. Both methods obviously have no application in the field. Moreover, as reinfection of an immune animal may result in its blood being temporarily infective, a positive result of subinoculation does not necessarily prove that the donor suffered from the disease. A tentative diagnosis is often possible on the clinical signs and the history of the herd. The finding by several authors that the number of eosinophilic granulocytes decreases considerably during the disease (at least in small ruminants), may be of some help.

There are no serological tests for demonstrating present or past infection except for a recently developed capillary flocculation test using brain extract as antigen (Ilemobade, 1976). The period during which this test is positive is limited to a few weeks following recovery from the disease or after reinfection.

The organism can be demonstrated after death in brain smears. When handling brain material of an animal which has exhibited nervous symptoms, the possibility of rabies should always be kept in mind and due precautions taken. After opening the skull, a small fragment of grey matter (of the size of a match head), is snipped off the surface of the brain with curved scissors, placed on a slide, crushed with another slide, and while pressure is maintained the slides are drawn over each other lengthwise, which results in two smears. After methanol fixation and Giemsa staining, areas containing many capillaries are located with a low magnification and individual capillaries are then searched with the oil immersion lens. An oil immersion lens with a magnification of 40 to 60 times is useful, as it allows fast scanning; the 100 times lens is used in case of doubt. After some experience, it is easy to recognize the colonies of rickettsias (Fig. 1). Their color, after Giemsa staining, varies from reddish purple to blue. They may be extremely scanty, especially in peracute cases, and the search may have to be long. Nevertheless, if sufficient numbers of capillaries are examined, the result is regularly positive in experimentally infected animals (Uilenberg, 1971). Some strains consistently give more colonies in brain capillaries than others.

Sampling the cerebral cortex necessitates opening the skull, often a tedious exercise, especially if proper instruments are not at hand in the field. Schreuder (1980) has developed a method whereby samples of the cortex of the cerebellum can be taken through the foramen magnum, after severing the

head from the neck through the occipital articulation. The cerebellum can be seen by lifting the dorsal meninges from the medulla oblongata with forceps, and superficial cerebellar tissue collected with a curette, or even an ordinary teaspoon, inserted between the medulla and the meninges. Smears are made in the same way as described for cerebral cortex. Schreuder found the cerebellum to be positive in all cases where organisms were found in the cerebrum. There are conflicting reports in the literature, some authors finding less rickettsiae in the cerebellum, while others find more or as many as in the cerebrum.

Cowdry (1925) stated that unless samples are taken soon after death, the organism loses its staining properties. This myth unfortunately persists and is one of the main causes of insufficient sampling in the field. It is now known that the organisms maintain their stainability in brain kept in the refrigerator for up to 3 weeks, in fixed or unfixed smears kept at room temperature for at least a month, and in brain material kept in a freezer for several months at least (Uilenberg, 1971).

Prevention and control

Disease transmission can be prevented by control of the vector. *Amblyomma* species are three-host ticks and each stage remains on the host for a relatively short period. Ticks of this genus are moreover less susceptible to most acaricides than, for instance, *Boophilus*. Immature stages are indiscriminate feeders and may be carried over long distances on birds and wild mammals; adults also parasitize wild and non-target domestic mammals. Frequent acaricidal treatment is therefore necessary to prevent all disease transmission; as transmission may start within a day after attachment of the tick (see Epidemiology), dipping or spraying would have to be carried out at least every 3 days if the acaricide used has, for instance, a reliable residual activity of 2 days. Nevertheless, effective weekly acaricidal treatment will often bring down the disease incidence to an acceptable level. Eradication of *Amblyomma* ticks is bound to fail because of alternative hosts, and management of tick control is substandard in many countries. The future of vector control has been made more gloomy by the recent discovery of arsenic, organochlorine, and organophosphate resistance in African *Amblyomma* vectors.

As an aid to acaricidal control of the vector, a practical and effective method of immunization would be of the greatest importance. Many attempts have been made at adapting the organism to laboratory animals or to *in vitro* culture, in the hope of attenuating it. All have failed so far. Even after 100 serial passages in cortisone-treated mice (which succeeded only after the intravenous route of infection was adopted), no attenuation was achieved and

the virulence of the organism was also unchanged after more than a hundred serial passages in local Malagasy sheep (J.J. Ramisse, unpublished reports, 1970-1974). All attempts at serial *in vitro* culturing have failed. So far the only method of immunization is infection and treatment: infective blood is inoculated intravenously and chemotherapeutic treatment is administered to abort or treat the reaction.

As the degree of immunity does not depend on the severity of the reaction, and as immunological differences between strains have not been convincingly demonstrated, mild sheep-derived strains can be used as immunogen. Merino sheep, which usually have predictable and regular reactions, are most often used as donors. The risk of transmitting cattle diseases is also less with blood from sheep than from cattle. The donor should be free of infections, such as blue-tongue. The blood of the donor is collected into an anticoagulant on the 3rd or 4th day of a definite temperature reaction, or earlier if nervous symptoms develop. Animals to be immunized are inoculated intravenously with 5 to 10 ml of this blood. Apart from very young calves, as outlined below, it is absolutely necessary to take the rectal temperature daily (early in the morning before it is affected by the heat of the day), and to apply treatment as soon as the fever reaction begins. Pregnant cows should not be immunized. Although sheep may be immunized safely by treating a set number of days after the infection, the procedure is more difficult in cattle, where the incubation period is more variable, and where treatment applied during the incubation period merely prolongs it (Uilenberg, 1971). Temperatures should be taken from the 7th to the 30th day after inoculation. As soon as a definite temperature rise occurs, specific tetracycline treatment must be applied (see below); treatment should be repeated the next day, and if the hyperthermia continues, should be given daily until the temperature has returned to normal. Because relapses after treatment are frequent, the temperature should be monitored for at least a further 2 weeks after the last treatment. Blood parasites such as *Babesia, Anaplasma* and *Eperythrozoon* may complicate matters, and if at all possible, blood smears should be examined at the first rise of temperature, and also if hyperthermia continues in spite of treatment; specific treatment against blood parasites is then applied as required.

The mortality in artificially infected young calves (less than 3 weeks) is within the range of only 5%. Neitz and Alexander (1945) used this knowledge to immunize such calves without monitoring the temperature and treating them. The infection and treatment method, as indicated for older cattle, however, should be used for highly susceptible valuable pure-bred calves.

The immunizing strain can be maintained at low temperatures (in dry ice, in freezer at $-70\,^{\circ}C$ or below, or in liquid nitrogen). Infective blood, to which

an anticoagulant and a cryoprotectant have been added, is frozen rapidly; glycerol is unsuitable as cryoprotectant but dimethyl sulphoxide (DMSO) has been used with success at a concentration of 10%; there are conflicting reports on the necessity of adding a cryoprotectant. The strain may also be maintained in deepfrozen brain emulsion (Ilemobade and Blotkamp, 1978); using DMSO as a cryoprotectant, such material is fairly regularly infective when injected by the subcutaneous route (personal observations, 1976-1980). The most reliable material appears to be supernatant of homogenized engorged infective ticks, frozen with 10% DMSO, which is consistently and highly infective when inoculated intravenously (J.D. Bezuidenhout, personal communication, 1979, confirmed by observations in our laboratory). In the absence of deepfreezing facilities, strains may be preserved in *Amblyomma* ticks where they remain viable for long periods; alternating passages between the tick and ruminants are far cheaper than frequent serial passages in ruminants only.

The donor blood should be transported on ice to the field, where it must be used as quickly as possible, preferably less than 12 and not more than 24 hours after bleeding the donor. Alternatively, the reacting donor may be taken alive to the site of immunization and bled on the spot. Infective blood may also be deep-frozen and issued to the field in dry ice or liquid nitrogen whenever required, but as the minimum volume used for immunization purposes is 5 ml, spacious deep-freezing facilities are necessary.

Losses due to the immunizing infection are not negligible. In older cattle immunized by the infection and treatment method, peracute cases, animals dying without having shown a temperature reaction, cases with exceptionally long incubation periods, and failure of treatment are all responsible for losses of some 5%, in spite of close supervision (Fick and Schuss, 1952). In short, it is a rather unsatisfactory method, unsuitable for application on a large scale, reasonably safe only under close supervision, and far from optimally effective. It is the only one existing so far, however, and susceptible cattle cannot be raised in endemic areas without it, if tick control is not excellent.

Antibiotics of the tetracycline group are active in curing heartwater in an early stage. Oxytetracycline, chlortetracycline and n-pyrrolidinomethyl tetracycline have all been found effective. Dosage should not be less than 5 to 10 mg/kg. Two treatments, on consecutive days, give better results than only one (Ilemobade, 1976). Suspension formulations, administered intramuscularly, have given better results in small ruminants than soluble ones, presumably because their action may be more durable, but as the course of the disease is usually rapid, it may be advisible to start with an intravenous injection of a soluble preparation, especially if clinical symptoms already exist. The action of tetracyclines on heartwater is slow in any case and treatment is often ineffec-

tive when nervous or intestinal symptoms are apparent; as most cases in the field are not discovered until this stage, the results of chemotherapy are disappointing. The fact that the action of tetracyclines on *Cowdria* is not impressive, is illustrated by the finding that oral administration of oxytetracycline during 3 weeks at a dosage rate of approximately 10 mg/kg daily does not reliably eliminate the organisms in young calves, if administration is started 7 days after infection (Uilenberg, 1971).

Sulphonamides also possess some chemotherapeutic activity against heartwater, but even less than the tetracyclines, so that they have now been abandoned. An experimental drug, a dithiosemicarbazone, (Gloxazone®), has been shown to be active, possibly more so than the tetracyclines, but because of toxicity problems may not be released on the market. It is possible that long-acting preparations of tetracyclines, which have been used successfully in immunization against theileriosis and the treatment of anaplasmosis, may prove useful in the treatment of heartwater.

Because of the present unsatisfactory state of immunization and treatment, introduction of susceptible exotic breeds into heartwater areas must be strongly advised against unless strict, efficient acaricidal control can be guaranteed.

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29. BOVINE PETECHIAL FEVER

D.R. Snodgrass

Abstract. Bovine petechial fever, caused by *Ehrlichia ondiri*, occurs sporadically in highland areas of Kenya, particularly affecting exotic breeds of cattle. There is a marked spatial restriction on disease occurrence, often to areas as small as part of a paddock, and usually associated with forest edge or scrub. *Ehrlichia ondiri* can be harbored by bushbuck and possibly other wild ruminants, and may be transmitted by an arthropod vector.

The disease is clinically marked by fever and widespread petechiation of mucous membranes. At necropsy, edema and lymphoid hyperplasia are evident in addition to widespread hemorrhages. Diagnosis depends on demonstration of *E. ondiri*, either by examination of Giemsa-stained blood smears, or by inoculation of tissues to susceptible sheep.

It may be possible to protect valuable animals by artificial infection followed by treatment with a dithiosemicarbazone. Generally, control depends on keeping cattle away from known infected areas, and clearing undergrowth and scrub.

Synonyms: ondiriitis, ondiri disease, BPF.

Etiology

The causative agent is a rickettsia-like organism *Ehrlichia* (*Cytoecetes*) *ondiri* (Haig and Danskin, 1962). The organism initially multiplies in the spleen, and subsequently, during clinical reaction, it parasitizes the circulating granulocytes and monocytes (Snodgrass, 1975). *Ehrlichia ondiri* can be observed in blood smears as small bodies (0.4 μm), larger bodies (1-2 μm), groups of small and large bodies, and groups or morulae of small bodies, all staining blue with Giemsa (Krauss *et al.*, 1972). They occur in cytoplasmic vacuoles, and are most commonly seen in neutrophils (Fig. 1).

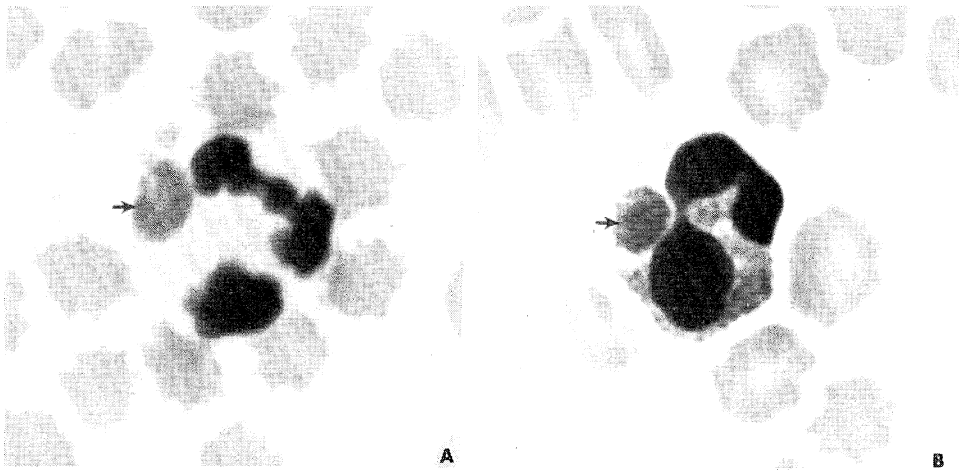

Fig. 1. Ehrlichia ondiri (arrowed) in cytoplasma of a) neutrophil and b) eosinophil, in Giemsa-stained blood smear from steer infected with bovine petechial fever.

Distribution

Bovine petechial fever (BPF) has been confirmed only from Kenya in areas of more than 1500 m altitude, which includes a large part of the most productive land in Kenya. In an epidemiological survey of sites where BPF has been confirmed, considerable variations in ecological type from humid to semi-arid were found (Walker *et al.*, 1974). The vegetation common to all sites was thick bush or understory cover giving heavy shade. Wild ruminants, particularly bushbuck and duiker, probably occurred in all sites, even in the Nairobi peri-urban area.

Bovine petechial fever has also been reported but not confirmed from areas of northern Tanzania with ecological similarities to the BPF zone of Kenya.

Epidemiology

Bovine petechial fever occurs usually in imported breeds of adult cattle; the Sahiwal may be most susceptible. The most striking epidemiological characteristic is the strictly localized occurrence of BPF, often in areas as small as part of one paddock, and usually associated with forest edge or scrub. The disease occurs sporadically and cattle can contract BPF within 1 to 2 weeks of being introduced to pasture unstocked for 2 years. Heavy losses may occur when farmers are compelled by drought to move animals from clean to infected areas. The spatial restriction and sporadic nature of the disease have led to the suggestion that an arthropod/wild mammal cycle exists.

It has recently been shown that bushbuck (*Tragelaphus scriptus*) can harbor *E. ondiri* in an endemic area, and that the organism can multiply after experimental infection in cattle, sheep, goats, bushbuck, impala (*Aepyceros melampus*), Thomson's gazelle (*Gazella thomsonii*), and wildebeest (*Connochaetes taurinus*) (Snodgrass *et al.*, 1975). Thus, bushbuck in an enzootic area constitute a reservoir of *E. ondiri*, and other species of wild ruminants are potential reservoirs.

Most cattle and sheep have been shown to develop latent infections with *E. ondiri* after a primary reaction (Snodgrass, 1975). This would facilitate transmission by an arthopod vector, but extensive efforts over many years have failed to incriminate ticks of the genera *Rhipicephalus*, *Amblyomma*, *Boophilus* and *Haemaphysalis*, biting insects of the genera *Stomoxys*, *Culex*, *Anopheles* and *Simulium*, and trombiculid mites (Kenya, 1937; Kenya, 1957; Walker *et al.*, 1974).

Clinical Signs

The incubation period following experimental infection ranges from 4 to 14 days, and in the field, cases have been observed within 10 days of moving

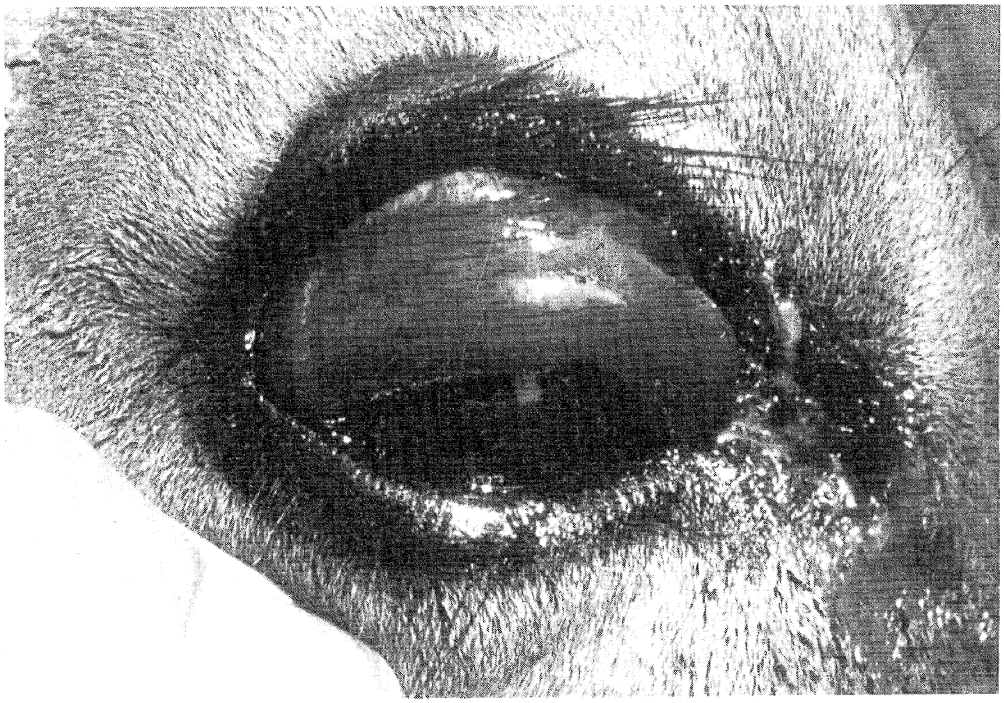

Fig. 2. 'Poached egg eye' from acutely-ill and subsequently fatal case of bovine petechial fever. There is marked conjunctival edema and hemorrhage, with excessive lachrymation.

cattle onto infected areas (Haig, 1966). The severity ranges from inapparent to fatal, and it is probable that many field cases are undetected or show merely as a fall in milk yield. The onset of symptoms is marked by fever and a drop in milk yield, which usually coincides with the appearance of patent parasitemia. This is followed on the second or third day by weakness, apathy, and a staring coat. Hemorrhages on mucous membranes also became evident at this stage, and can be observed on vulvo-vagina, conjunctiva, labial surface of the gums and ventral surface of the tongue. They first appear as petechiae, which enlarge over several days and then regress. On occasion, mucosal membranes are also congested, particular in the fatal cases. A characteristic lesion of BPF sometimes seen is 'poached egg eye', consisting of edema and hemorrhage of the conjunctiva (Fig. 2). The mortality varies from 10 to 20% and usually occurs in the first few days of illness.

Pathology

The appearance of BPF cases at necropsy is characterized by hemorrhage, edema and lymphoid hyperplasia (Piercy, 1953; Danskin and Burdin, 1963; Plowright, 1962). The hemorrhages are often large and are present extensively throughout the body. There is usually subcutaneous and intramuscular hemorrhage. The heart shows subepicardial fluid. Mucosal hemorrhages in the respiratory tract, urinary bladder and gall bladder, and mucosal and serosal hemorrhages in the alimentary tract give rise to free blood and tarry feces.

Edema is also widely present, often in subcutaneous and intramuscular tissues. Lung edema is particularly marked and may be the immediate cause of death.

Lymph nodes are often enlarged, with cortical and subcapsular petechiae, edema and hyperplasia. The spleen and liver may be enlarged and show petechiae.

Histologically, little significance other than petechiation has been observed, with the exception of the hyperplasia of the larger lymphoid cells. *Ehrlichia ondiri* may be seen in spleen cells and liver Kupffer cells.

Hematological changes are characteristic and consist of an absence of eosinophils, marked fall in lymphocyte counts followed by an equally pronounced drop in neutrophil levels.

Diagnosis

Ehrlichia ondiri has not been isolated *in vitro*, so diagnosis of BPF cannot be made by isolation of the organisms or by demonstration of a serological

response to it. The clinical and necropsy findings, although dramatic, are not pathognomonic. Bovine petechial fever may be confused with bracken and arsenic poisoning, hemorrhagic septicemia, heartwater, acute trypanosomiasis and acute theileriasis. In an area where BPF is known to be enzootic, a history of movement to rough grazing, coupled with clinical and necropsy examinations, may be sufficient to lead to a presumptive diagnosis of BPF. In areas where BPF has not previously occurred, however, diagnosis rests on the demonstration of *E. ondiri.*

This may be done directly by the observation of *E.ondiri* in Giemsa-stained blood or spleen smears, where it may be seen in granulocytes and monocytes or in spleen cells. However, severe illness and petechiae do not develop until the second or third day of febrile and parasitemic reaction, by which time visible parasitemia may be low or absent. Direct observation of *E. ondiri* may not succeed. As an alternative diagnostic method, tissue suspensions from the suspected case may be inoculated intravenously into susceptible cattle or sheep. The tissues with the highest titers of *E. ondiri* are spleen and lung although from a non-fatal case, blood is satisfactory. Giemsa-stained blood smears from the recipient animal should be examined daily for 10 days to determine the presence of *E. ondiri* in the granulocytes and monocytes. Because some isolates of *E. ondiri* do not cause febrile reactions on first passage in sheep or cattle, it is not sufficient to rely on blood-sampling only in the event of a febrile reaction.

Prevention and Control

Recovered animals are resistant to clinical reinfection for several years, but probably carry latent infections of *E. ondiri.* Similarly, wild ruminants may also act as reservoir hosts. Preventing spreading of infection to susceptible cattle depends on avoiding contact with the unknown vector, which in turn necessitates keeping cattle away from known infected areas. Where possible, clearing of the undergrowth and scrub should be carried out.

Tetracyclines have been shown to abort clinical reactions if given during the incubation period, or on the first day of clinical reaction. However, dithiosemicarbazone (Gloxazone, Wellcome), as a single intravenous injection at 5 mg/kg (Snodgrass, 1976), was found to be more effective than tetracycline. After experimental infection and subsequent treatment with the dithiosemicarbazone, cattle were shown to be immune. This would appear to be a feasible method of introducing valuable cattle to areas where BPF is endemic.

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30. DERMATOPHILOSIS

Allan C. Pier

Abstract. Dermatophilosis is an infectious, contagious disease of the skin of cattle and other animals caused by the aerobic actinomycete, *Dermatophilus congolensis*. Dermatophilosis is typified by an exudative dermatitis with suppuration, superficial epidermal necrosis, acanthosis and the build-up of scabs along entrapped hairs over the infected skin surface. The causative organism, an obligate parasite, has been cultured only from lesion materials; it has a multimorphic life cycle including a motile (flagellate) zoospore form, a branching filamentous form and a multiplanar septate form. The infection is transmitted by direct contact or by insect, arachnid and inanimate vectors. Moisture on the skin enhances transmissibility and causes exacerbation of existing lesions. The disease is most prominent in temperate to tropical climates during the rainy season. Diagnosis depends on microscopic demonstration of typical multiplanar septate forms in Giemsa-stained smears of exudate or cultural isolation by special techniques described. Serologic and fluorescent antibody applications are also described. The infection may be treated successfully by a number of bacteriocidal solutions including copper sulfate and by antibiotic injections including dihydrostreptomycin. Control of flies and other vectors and keeping animals dry are important considerations in controlling dermatophilosis in cattle. Some evidence exists indicating that an actively acquired immunity may be induced through experimental vaccination. The disease is economically important through reduced productivity of affected cattle and residual hide damage. Dermatophilosis is transmissible to man and is considered to be a zoonotic disease.

Etiology

The causative agent is an aerobic (facultative) actinomycete of the family Dermatophilaceae (Buchanan and Gibbons, 1976). There is but a single species in the genus, *Dermatophilus congolensis* (Gordon, 1964). The organism is strongly Gram positive, nonacid-fast, and has a multi-phasic life cycle which is depicted in Fig. 1. Presumably, the infectious form of the organism is the flagellate, motile zoospore (Richard *et al.*, 1976) (Fig. 1a) that is released from mature multiplanar septate forms (Fig. 1f and g) found in infectious crusts and exudates. This zoospore germinates (Fig. 1b), produces a branched fila-

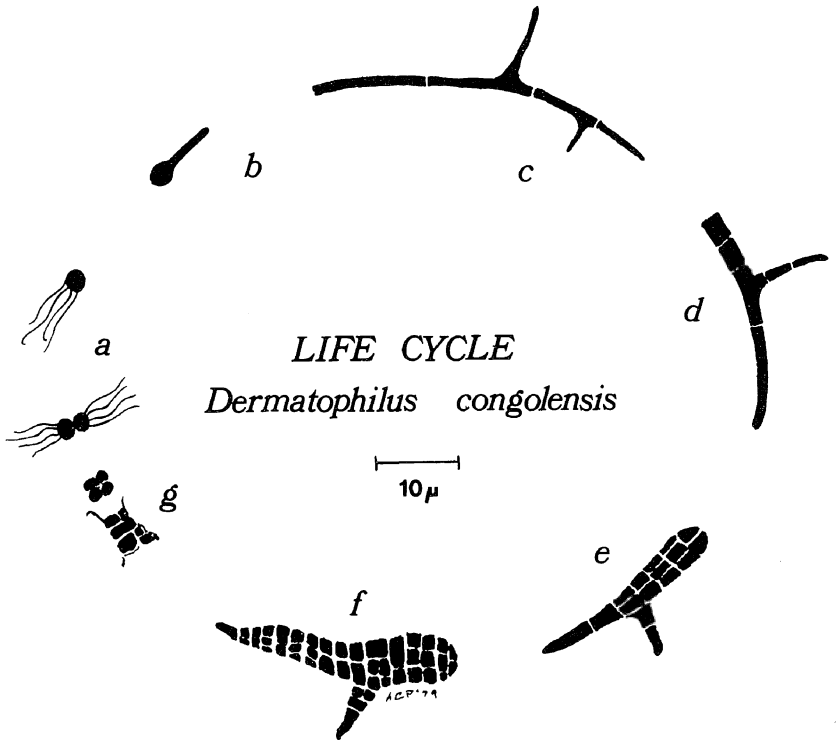

Fig. 1. Life cycle of *Dermatophils congolensis.*

mentous structure (Fig. 1c) which invades hair follicles, sweat glands and other epidermal structures, then enlarges and develops multiplanar septations in infectious exudates (Fig. 1d-f). *Dermatophilus congolensis* is highly proteolytic and regularly produces urease, hydrolyses casein, liquefies gelatin and coagulated serum (Cottral, 1978). These proteolytic abilities of the organism are considered important in the pathogenesis of dermatophilosis. *Dermatophilus congolensis* appears to be an obligate parasite; to date it has been isolated only from lesion materials and has not been found living freely in the environment of infected animals.

Distribution

Dermatophilosis occurs throughout the tropical to temperate regions of the world. Major economic losses associated with infections of cattle, sheep, goats, and horses have been reported from Africa, Australia, and New Zealand, Europe, The Middle East, North, Central and South America (Lloyd and

Sellers, 1976). Numerous species of animals have been reported to harbor natural infection including most domestic and wild ruminants, horses and other Equidae, dogs, fox, rabbits, raccoons, zoo-housed polar bears, seals, and certain species of monkeys (Richard and Shotts, 1976). While infections of both pigs and a cat have been reported, they were not conclusively confirmed by culture and apparently such infections are infrequent. Man is readily infected and dermatophilosis is considered a zoonotic disease (Dean *et al.*, 1961; Pier, 1979). Perpetuation of infection in endemic areas during dry and winter seasons by wild animal populations is considered likely.

Epidemiology

Infection results from transfer of infectious material by direct or mechanical transmission from contaminated brush, fence posts, halters, clippers, etc. and by arthropod vectors including flies and ticks (Richard and Pier, 1966). Skin abrasions may enhance transmission but are not a necessary precursor to infection. Extensive outbreaks have followed clipping excess hair from the udders of dairy cows or in bands of sheep after shearing with clippers that were contaminated from passing through infectious crusts entrapped in the

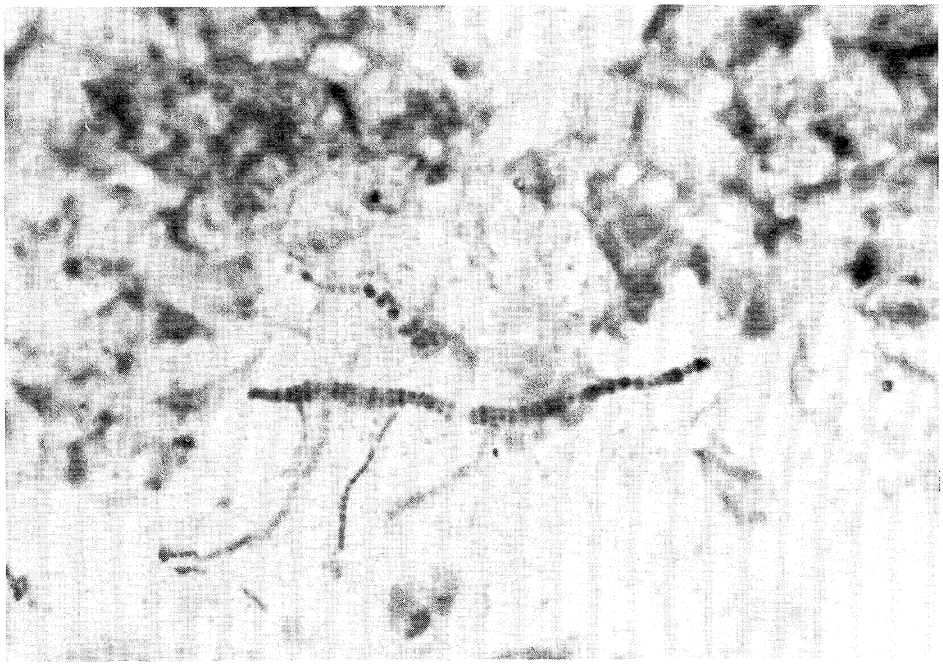

Fig. 2. Dermatophilus congolensis in stained smear of emulsified scab. Note multiplanar septations of mature form and filamentous branches. Giemsa stain, ×900.

hair or wool. Concurrent infections of sheep with *D. congolensis* and the virus of contagious ecthyma has been observed as has infection in cattle with herpes mammillitis virus and sea lions with pox virus. It is not known which agent infected first or whether infection was simultaneous but the several viral agents as well as *D. congolensis* are capable of primary pathogenicity.

Moisture enhances transmission of infection and major outbreaks in individual herds or in a geographic area are usually relatable to rainfall or some other exposure to water. Motile zoospores of *D. congolensis* are released from the mature, multiplanar septate forms in exudative crusts (Figs 1f and 2) when the crusts become wet. The zoospores swarm from existing lesions and establish new foci of infection or enlarge existing lesions in concentric waves. Direct contact with infected animals, mechanical transfer by flies feeding on moist exudates and transferring to fresh moist sites or other mechanical transfer readily transmits infection to other animals. Lesions often appear first on body areas where water exposure and retention are greatest (e.g. back, axillae, folds on flexor surfaces of joints, etc.). Young animals (i.e. birth to yearlings) appear to be most susceptible; presumably some immunity to routine exposure is acquired by older animals in endemic areas. As the rainy season wanes and flies abate, the rate of new cases generally recedes. Existing lesions become quiescent due to failure of new zoospore release at the skin surface, the exudative crusts are pushed out on the entrapped, growing hairs and eventually are removed or drop off. If rainfall occurs after a period of quiescence, new zoospore release causes exacerbation of the lesion, additional exudation and the accrual of thick, laminated exudative crusts.

The source of new infection in a herd of domestic animals presumably is from direct or indirect contact with infectious lesion material. Whether this exposure results from direct or indirect contact (e.g. via ticks or flies) with infected members of the herd, with infected wild animals (e.g. deer, rabbit, fox, raccoon, etc.) or from surviving elements in fallen exudative crusts is not known. How long *D. congolensis* remains viable in exudative crusts on the ground is not known, but it is known that *D. congolensis* unprotected by exudative material does not survive in soil (Roberts, 1970).

Clinical Signs

The initial lesion of dermatophilosis is a discrete focus of exudative dermatitis with erosion, suppuration and the formation of an exudative crust. The crust forms at the base of the hair and entraps it. Often the disease is first discovered by running a hand over the infected area and feeling bumps beneath the hair coat. In acute dermatophilosis, during periods of excessive

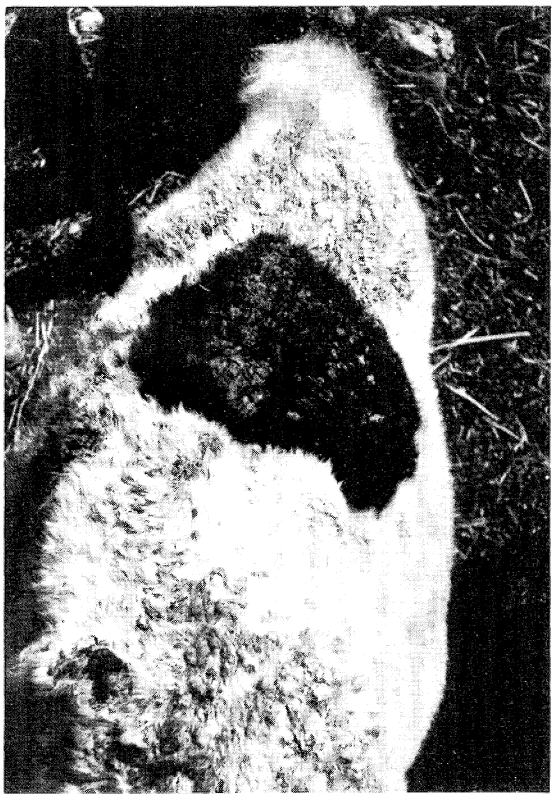

Fig. 3. Bovine dermatophilosis. Note widespread scattered lesions over the surface of the back and confluent lesions about the tail head.

rainfall, the exudative crust is usually thin, approximating 2-4 mm; individual foci of infection are scattered over extensive areas of the body (Fig. 3) or may coalesce to form large confluent lesions. When climatic conditions are favorable, this form of dermatophilosis may transmit readily to other animals, until nearly all young animals in the herd are clinically involved (Pier *et al.*, 1963). There is a purulent exudate below the crusts; the superficial cornified layers of the epidermis are eroded and removal of the crust reveals a moist, inflamed area of granulosa cells. Systemic signs of illness are usually absent or limited to a febrile response in moderate cases; milk production may drop in affected dairy cattle. With extensive acute involvement, secondary bacterial invaders may cause a fatal termination, presumably from endotoxemia (Kelley *et al.*, 1964).

A more chronic form of the disease occurs when moisture is less abundant. In this form, deep encrustations one to several centimeters thick, build up

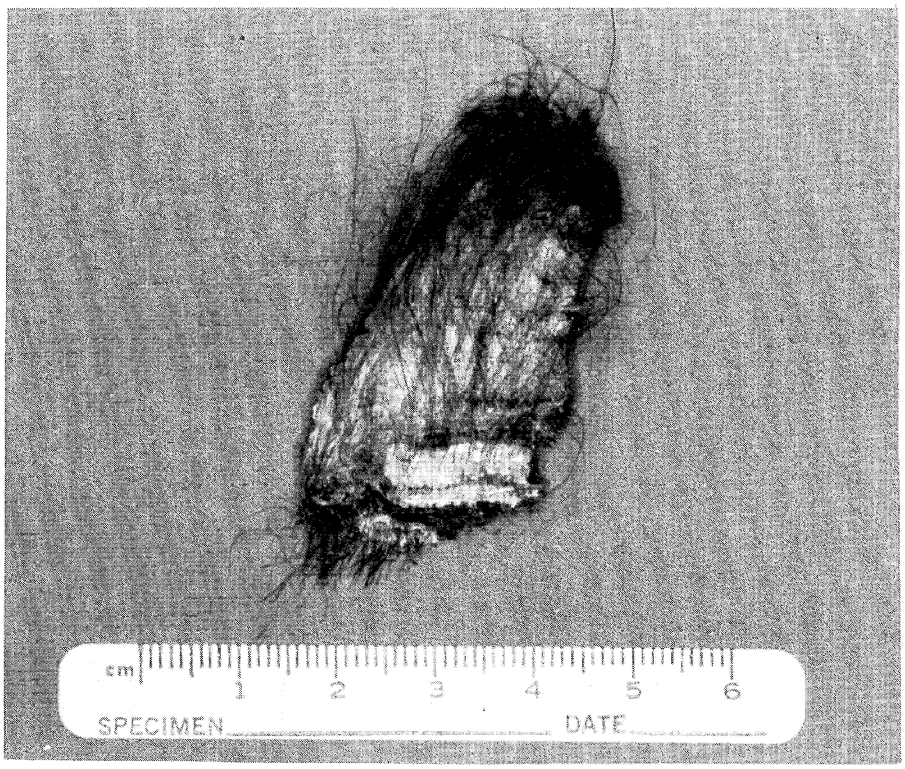

Fig. 4. Exudative crust and entrapped hair from chronic lesion of bovine dermatophilosis. Note laminations.

along tufts of entrapped hair. The crusts "grow" as successive moisturizing releases a new mass of zoospores which results in a new exacerbation of the lesion; as the lesion dries, inflammation wanes and the crust is pushed away from the skin by new hair growth. The crusts on such chronic lesions are often laminated as a result of the intermittent cycle of lesion activity (Fig. 4). Such activity is often seen in temperate zones where snow fall provides periodic moisturizing of the lesions. Examination of cattle 2 or more weeks in the wake of a herd epizootic often reveals only a few areas of encrustation on the distal ends of the hairs that resemble caked mud. These remnant crusts are often found in protected areas such as the axilla or inguinal region.

Pathology

The pathologic changes associated with dermatophilosis are largely confined to the epidermis with some reactive cellular infiltration of underlying dermal areas (Oduye, 1976). The organism, or those forms that are recognizable in

histologic sections such as filaments and mature multiplanar septate forms, is seen chiefly in the upper levels of the epidermis and hair follicles. Filaments are seen in cornified epithelium, between laminae of exudative crusts and occasionally in the lower strata of the epidermis but only rarely do they penetrate below the epidermis and then only through breaks in the stratum germinativum. Affected epidermis remains largely intact, becomes acanthotic and is covered with laminated crusts composed of cornified epithelial cells, leukocytic debris, filaments and mature packets of *D. congolensis*. There is a marked neutrophilic infiltration of affected epidermis and development of microabcesses in the epidermis near hair follicles. Cellular infiltration of underlying dermal areas is largely lymphocytic and histocytic. The neutrophilic infiltration of the epidermis begins very soon after experimental infection and by 24 hours has caused separation of the epidermis from the underlying dermis. New epidermis regenerates by 36 hours and laminar layers of old epidermis, neutrophilic leukocytes, cellular debris and new epidermis are evident by 48 hours. Barring exacerbation, scabs of primary lesions begin sloughing 3 days after experimental infection; the degree of acanthosis increases through 7 days then recedes.

Immune Response

Infection by *D. congolensis* elicits a variable serologic response depending apparently on the severity and duration of infection. Detectable antibody has been shown in serums of experimentally and naturally infected animals by gel diffusion precipitin, passive hemagglutination and agglutination tests (Pulliam *et al.*, 1967; Richard *et al.*, 1976). These reactions appear to be highly specific for *D. congolensis* but detectable titers may not result from a mild primary infection. In a limited survey, precipitins were detected in 39% of serums from 64 cattle in eight Iowa herds (Richard *et al.*, 1976) and positive serologic responses have been shown to persist in cattle for 8 through 18 months (longest period tested) following recovery from natural infection (Pulliam *et al.*, 1967). Whether this serologic reactivity imparts enhanced resistance to re-infection, however, is problematic. Laboratory animals, particularly rabbits, can be readily reinfected despite the presence of appreciable levels of circulating antibody. In nature, reinfection undoubtedly occurs although younger animals appear more susceptible, perhaps due to some level of acquired immunity in animals 2 years and older. While acquired antibody levels may not withstand experimental reinoculations, they may be helpful in clearing the few zoospores introduced by ticks or flies. It is also possible that an acquired immunity is independent of antibody levels but associated with cell

mediated immune reactions. Limited experimental vaccination trials in Zebu cattle indicate some level of vaccine protection is obtained (Provost *et al.*, 1976).

Laboratory Aids to Diagnosis

The most universally used diagnostic technique for dermatophilosis is microscopic examination of Giemsa stained smears of exudative crusts. Specimens removed from infected animals are emulsified in sterile saline solution on a microscope slide, air dried, fixed in absolute methanol and stained with Giemsa's stain. The smear is then examined diligently for mature, multiplanar septate bodies as shown in Figs 1f and 2. In very old lesions, typical definitive forms may not be present but aggregations of coccoid elements suggest the possible involvement of *D. congolensis*. This possibility can be confirmed in the diagnostic laboratory by staining the scab emulsion with specific fluorescent antibody (Pier *et al.*, 1964). Because of their distinctive morphology, demonstration of typical forms in a stained smear constitutes a strong presumptive diagnosis; there are, however, other organisms that may sometimes resemble *D. congolensis* in tissue. For this reason positive confirmation by fluorescent antibody or cultural isolation is recommended where the infection is suspected in new geographic locales or in new animal hosts.

Cultural isolation can be accomplished occasionally with ease but sometimes requires the use of special techniques to separate *D. congolensis* from more robust contaminants. A highly effective technique utilizes membrane filtration of scab emulsions (Pier *et al.*, 1964). Clinical specimens of exudative material are emulsified in sterile saline solution in a TenBroeck tissue grinder and the suspension allowed to stand undisturbed in the grinder tube at room temperature for 1 to 2 hours. Zoospores are liberated and congregate in the upper levels of the suspension and the more heavily contaminated materials settle to the bottom. One to two milliliters of the upper supernate is aspirated into a syringe which is then passed through a syringe-adapted 1.2 μm pore size membrane filter. A loopful or drop of the filtrate which contains zoospores is streaked for isolation on an enriched medium containing whole blood (e.g. BHI-blood agar) and incubated aerobically for 48 to 120 hours at 37 °C. Colonies of *D. congolensis* appear as small, smooth to granular, opaque, buff colored adherent colonies that are sunken into the surface of the medium. A narrow zone of hemolysis is usually present. Isolation may also be enhanced by applying emulsified clinical materials to shaved and moistened areas of rabbit skin; if exudative crusts appear these can be examined microscopically or culturally as above.

Prevention and Control

Present information indicates that *D. congolensis* is an obligate parasite of animals and man; free living forms in nature have not been found. Thus, a large part of prevention and control of dermatophilosis is associated with curtailing exposure to infectious sources. Flies and ticks are known to spread infection from animal to animal; control of these arthropod vectors is helpful, especially during warm wet weather when the moistened exudate materials are most infectious and the moist skin of recipient animals is most receptive (Richard and Pier, 1966). Similarly, removal of crusts shed from infected cattle, disinfecting stanchions, stables, corral fences, clippers, etc., with phenolic or quaternary ammonia bacteriocides or copper sulfate solution diminishes exposure of new cattle to infection. The course of infection on individual animals is shortened and the possibilities of dissemination are diminished when infected animals are protected from rain or other external water applications. Isolation and stabling such animals may be most helpful from the standpoints of keeping them dry, away from uninfected animals, and establishing fly control.

The subject of immunoprophylaxis remains to be definitively explored. Currently, preliminary information indicates that bacterins, prepared from killed whole cells are at least partially effective in curtailing epizootics (Provost *et al.*, 1976).

Dermatophilus congolensis is susceptible to a number of antibiotics. The problem is in delivering the antibiotic in effective concentrations to the site of active infection, i.e. the skin surface below exudative crusts. Intramuscular dihydrostreptomycin at the rate of 10 mg/kg as a single dose or repeated at 3 to 5 day intervals is highly effective when animals are kept dry. Topical applications of astringent antibacterial solutions such as copper sulfate, gentian violet, etc., may also be helpful in hastening recovery of early acute processes and diminish the effect of secondary bacterial invaders (Blancou, 1976). Human attendants of infected animals should remember that *D. congolensis* is a zoonotic agent that causes a severe pustular dermatitis of man; appropriate care and decontamination of arms, hands, and clothing should be observed after handling affected livestock.

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31. COCCIDIOSIS

John V. Ernst and Gerald W. Benz

Abstract. Coccidiosis in cattle is caused by protozoan parasites of the genus *Eimeria*. Numerous species of coccidia have been described from cattle, but only *Eimeria bovis* and *Eimeria zuernii* produce the severe clinical disease. Coccidiosis is found wherever cattle are present. The disease occurs most often in calves less than a year old, but may also occur in older cattle. Cattle become infected by oral ingestion of sporulated oocysts contained in fecal-contaminated feed or water. The severity of the disease varies from mild when only a slight diarrhea is present, to acute when the calf may die. Typical signs of coccidiosis include bloody diarrhea, straining during defecation (tenesmus), dehydration, rough hair coat, lack of appetite (anorexia), weakness, listlessness, and emaciation; sometimes convulsive seizures occur. Lesions of coccidiosis are caused by the sexual stages and include destruction of the epithelial mucosa in the large intestine. Diagnosis of coccidiosis is made on the basis of clinical signs, presence of oocysts of the pathogenic species in the feces, and history of the individual calf and herd. Several available drugs are effective for prophylaxis, but are not efficacious once signs of infection are present. The disease can often be prevented by proper sanitation and management of the calves.

Disease

Coccidiosis as a distinct clinical disease of cattle was first described by F. A. Zürn in 1878 (Becker, 1934). Zürn observed coccidian intracellular stages in sections of intestine from a calf that had died of enteritis. The tissue had been sent to him by a Swiss veterinarian named Pröger, whose letter describing the signs and lesions in the stricken animal appeared in Zürn's account. The disease encountered was given the name *rote Ruhr* ("red dysentery"). Unfortunately Zürn did not give a detailed description of the parasitic stages he saw and hence present-day investigators cannot distinguish that species from the other species which are now recognized from cattle. *Eimeria zuernii* is apparently the most common cause of *rote Ruhr* in Switzerland and may have been the species that Zürn saw.

Bovine coccidiosis has been given several common or colloquial names in addition to *rote Ruhr;* these include scours, bloody scours, hemorrhagic enteritis, dysentery, and bloody diarrhea. The most precise name for this disease is bovine eimeriosis, since all coccidial organisms of cattle belong to the genus *Eimeria.* However, bovine coccidiosis is the most commonly used name and is preferred by most investigators.

The terms coccidia and coccidiosis are used in this chapter in the most restricted meaning. Coccidia are protozoan parasites that belong to the family Eimeriidae. Coccidiosis is the disease caused by the coccidia. Much recent work has shown that the organisms *Toxoplasma, Sarcocystis,* and *Besnoitia* are closely related to the coccidia, but the diseases they cause are not discussed here. Futhermore, a taxonomic classification for the coccidia is beyond the scope of this chapter.

The coccidia that belong to the genus *Eimeria* usually infect only host animals that belong to a single genus. There are, however, exceptions to this rigid host specificity, and most investigators recognize that the same *Eimeria* species infect the bovine genera *Bos* and *Bubalis,* although some species are little known and have only been described once from one or the other of these genera. The term "cattle" as used throughout this chapter is meant to include the ox (*Bos taurus*), the zebu (*Bos indicus*), and the water buffalo or carabaos (*Bubalus bubalis*). Little or no information is available about the coccidia of other species of cattle found throughout the world. Most of the experimental work on bovine coccidiosis has been done on the ox, *Bos taurus.*

Etiology

The number of species of coccidia that occur in cattle is open to question, but most reviewers accept 19 to 22 (Davies *et al.*, 1963; Levine and Ivens, 1970; Pellérdy, 1974). Most coccidian species of cattle are known only from the oocyst stage, and several have been described only once from a single location. Some of the descriptions are so poor that the validity of the species is questionable. No attempt will be made here to discuss each species of coccidia that has been described from cattle because this chapter deals mainly with the disease bovine coccidiosis, and only two species, *Eimeria bovis* and *Eimeria zuernii,* have been implicated in clinical cases of the disease, although *Eimeria alabamensis, Eimeria auburnensis,* and *Eimeria ellipsoidalis* have been reported to cause diarrhea when large numbers of sporulated oocysts are given to experimental calves. Descriptions of the species of coccidia of cattle, along

with drawings of the oocysts, and discussions about validity of the species can be found in the reviews by Levine and Ivens (1970) and Pellerdy (1974). An additional spieces, *Eimeria kosti,* was described as an abomasal coccidium from a cow from Sudan after these reviews were published (Elibihari and Hussein, 1974).

A diagrammatic representation of a generalized life cycle of an *Eimeria* species is shown in Fig. 1. The infective stage of the coccidia is the sporulated oocyst. When the sporulated oocysts (1) are ingested by the host, the sporozoites (2) escape from the sporocysts and then the oocysts and enter host

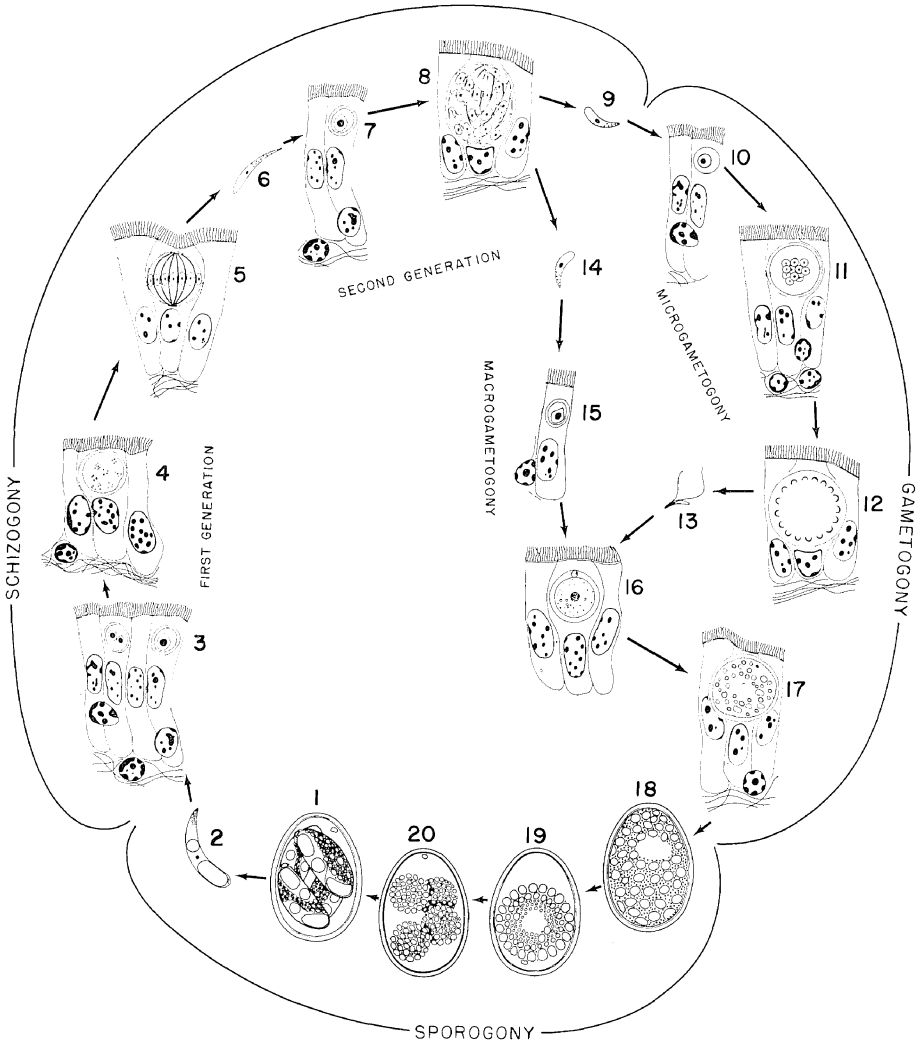

Fig. 1. Diagrammatic drawing of a typical coccidian life cycle. Numbered stages are described in the text. (Adapted from Vetterling, 1966.)

cells, usually intestinal epithelial cells, to form trophozoites (3). The trophozoite nucleus then begins to divide and a schizont is formed (4). Growth of the schizont is accompanied by further nuclear division, and elongate merozoites, each containing a nucleus, develop within the schizont (5). The process of schizont growth and merozoite formation is termed schizogony. When mature, the merozoites escape from the schizont (6), penetrate another host epithelial cell, and begin another generation of schizogony (7, 8). Schizogony continues for a specific number of generations, depending on the coccidial species. Eventually merozoites initiate the sexual cycle, termed gametogony. A merozoite of the last schizogonous generation (9, 14) penetrates a new host epithelial cell and becomes a female cell, a macrogamont (15), or a male cell, a microgamont (10); these processes are termed macrogametogony and microgametogony, respectively. During microgametogony, the nucleus divides many times (11). Each nucleus then elongates, cytoplasm forms around each nucleus, and two flagella develop. This flagellated, motile organism is a mature male cell, a microgamete. The mature microgamont containing the microgametes is called a microgametocyte (12). Unlike the nucleus of the microgamont, the nucleus of the macrogamont does not divide. The uninucleate macrogamont grows and when mature is called a macrogamete (16). When mature, the microgametocyte ruptures and releases the motile microgametes into the intestinal lumen (13). A microgamete finds and enters the host cell containing the macrogamete and fertilizes it, resulting in the formation of a zygote (17). A resistant wall develops around the zygote to form an oocyst. The host cell containing the oocyst ruptures, and the oocyst (18) is passed from the host in the feces into the external environment. At the proper temperature and humidity, the cytoplasm (the sporont) of the oocyst contracts into a ball (19) and then dvides (a process termed sporulation) into four sporoblasts (20). Each sporoblast elongates and becomes a sporocyst, and two sporozoites develop within each sporocyst (1). This process of sporozoite formation is known as sporogony. The stages occurring within the host are known collectively as the endogenous stages, and the stages outside of the host are known collectively as the exogenous stages.

The life cycle of *E. bovis* has been studied more intensively than that of any other species of bovine coccidia (Hammond, 1973). The mature first-generation schizonts are located in the central lacteals of the villi in the lower part of the small intestine. They are about 300 μm in diameter, and each contains about 120,000 merozoites at maturity. These first-generation schizonts require about 15 days to mature. The second-generation schizonts are found in epithelial cells of the crypts of the cecum and upper colon. They are about 10 um in diameter and contain about 30 merozoites. The second-generation schizonts require only 1.5 to 2 days to reach maturity. Macrogamonts and

microgamonts are found in the same location and at the same time as the second-generation schizonts. The sexual stages, especially the macrogamonts, are much more numerous than the second-generation schizonts in the infected tissues. Oocysts of *E. bovis* are first found in the feces on the 17th day after sporulated oocysts are ingested by the calf. The pathologic changes and signs seen in *E. bovis* infections are associated with the development and maturation of the sexual stages of the life cycle.

Coccidiosis due to *E. zuernii* is difficult to reproduce experimentally in cattle, and the complete life cycle of this species has only recently been reported (Stockdale, 1976). The endogenous life cycle of *E. zuernii* is similar to that of *E. bovis* in many respects. Mature first-generation schizonts of *E. zuernii* are also large, up to 234 μm, and contain numerous merozoites. They are in connective tissue cells of the small intestine. These schizonts are mature by the 16th day after inoculation of sporulated oocysts. Second-generation schizonts are located mainly in the surface epithelium of the cecum and colon, also on the 16th day after inoculation, and are about 19 by 15 μm. Macrogamonts and microgamonts are present in the same area as the second-generation schizonts, although not in great numbers, on the 16th day after inoculation. Stockdale (1976) reported that the second-generation schizonts and the sexual stages of *E. zuernii* occurred only in the cecum and proximal colon of experimentally infected calves. Davis and Bowman (1957) reported that the sexual stages of *E. zuernii* occurred in the lower small intestine, cecum, colon, and rectum in their experimentally infected calves. Most case reports of *E. zuernii* coccidiosis state that the sexual stages and oocysts are found in the cecum, throughout the colon, and in the rectum of the infected animals. Further experimental work is needed on the life cycle of *E. zuernii* to clearly define the location of the endogenous stages.

Descriptions of the known life-cycle stages of the other species have been compiled by Levine and Ivens (1970). Little has been added to the literature on life-cycle stages of the bovine coccidia since the publication of their monograph, probably because experimental infections in calves with many of the species are difficult to produce.

Distribution

Bovine coccidiosis has been reported from many parts of the world. The disease occurs in tropical, subtropical, and temperate regions, and it cannot be unequivocally stated to be more important in one geographical region than in another. Some investigators believe that the disease is more important in the cooler temperate region, but this is probably because the disease has been

more intensively studied in this region. In the tropical countries, other diseases of cattle have received more attention and study. Coccidiosis is probably a problem wherever cattle are raised.

Many surveys for coccidia of cattle have been done throughout the world. Reviews by Becker (1934), Orlov (1956), Davies, Joyner, and Kendall (1963), Levine and Ivens (1970), and Pellérdy (1974), among others, list the countries in which each species has been found.

Bovine coccidiosis is not a reportable disease in most countries, so information about the incidence of the disease and the losses it causes is difficult to secure. Coccidiosis has been listed as the third most important parasitic disease in cattle in the United States (Swales *et al.*, 1948), and Foster (1949) estimated that bovine coccidiosis in the United States was responsible for the loss of $10 million annually. Bovine coccidiosis is reported as a "minor disease" by the Canada Department of Agriculture, and Niilo (1970) estimated that it causes a loss of $3.8 million annually in that country. Fitzgerald (1972) stated that bovine coccidiosis accounted for an annual loss of at least $1/calf less than 1 year old. He estimated that this loss amounted to $47 million in the United States and $472 million worldwide.

Epidemiology

Cattle become infected with coccidia by ingesting oocysts along with their feed or water or by licking contaminated material, often the bodies of other cattle. Only sporulated oocysts are infective. Since all *Eimeria* oocysts are passed from cattle in the unsporulated stage, proper climatic conditions must be present for sporulation to occur. However, little is known about the factors necessary for proper sporulation of cattle coccidian oocysts except that moisture and oxygen are necessary. The occysts apparently sporulate in a range of temperatures from 15 to 30 °C. Temperatures above 30 °C kill the oocysts in a relatively short time. Temperatures below freezing also kill the oocysts, but not as quickly as high temperatures. Cold temperatures above freezing prevent sporulation but do not kill the oocysts unless exposure lasts many months.

Bovine coccidiosis is usually considered to be a clinical problem primarily in calves less than 1 year of age, although older cattle can be affected also. Animals with subclinical infections, regardless of age, may be the source of infection. Disease outbreaks are most likely to occur wherever there is an aggregation of young stock under conditions permitting the accumulation and sporulation of large numbers of oocysts. Because of the tremendous reproductive potential of the parasite, continued successive transmission of the para-

sites from one animal to another results in the dissemination of large numbers of oocysts to contaminate the environment. Hammond (1973) estimated that ingestion of a single sporulated oocyst of *E. bovis* by a calf could result in the passage of 12 million unsporulated oocysts. Over a period of time, enough oocysts become present in the environment so that cattle ingesting them develop clinical coccidiosis. The feeding of 100,000 sporulated oocysts to a 3-week-old calf frequently results in a fatal infection.

Older cattle can also be severely stricken with coccidiosis, usually when they are stressed by factors such as crowding, changes of feed, severe weather, castration, and dehorning. An outbreak of coccidiosis may occur 18 to 30 days after cattle have been shipped to a feedlot.

Clinical Signs

The severity of coccidiosis in cattle is directly related to the severity of damage to the intestinal epithelium in the host, which in turn depends on the number of sporulated oocysts of pathogenic species ingested by the animal. The ingestion of a few oocysts produces few or no apparent signs, except that

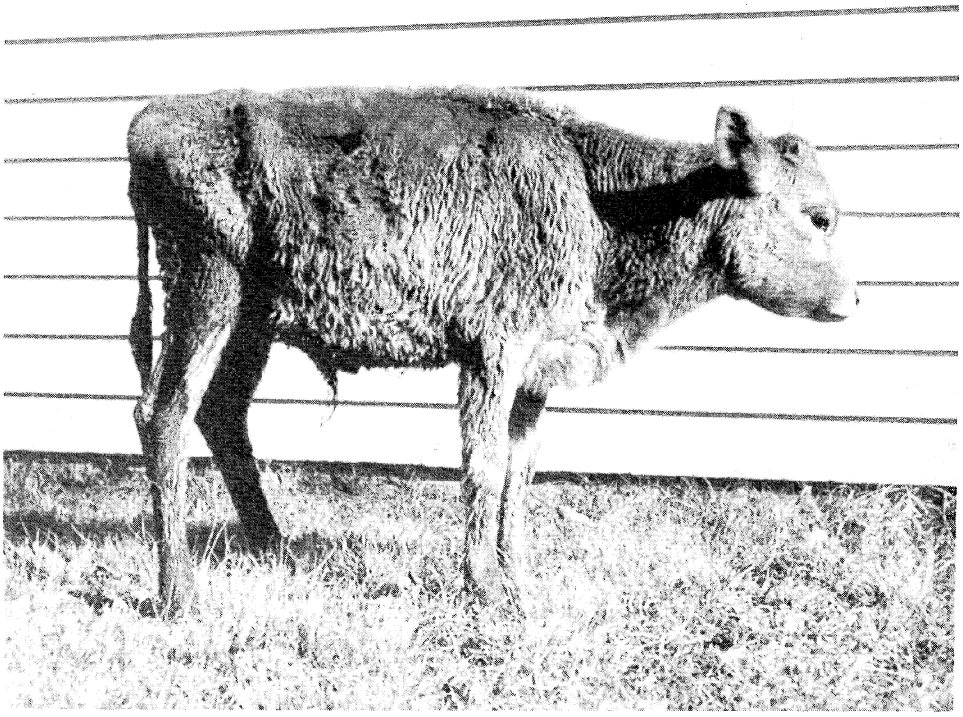

Fig. 2. Calf with clinical coccidiosis. (USDA Regional Parasite Research Laboratory file photograph.)

the feces may be less formed than normal. In mild cases, the calves have signs that include diarrhea, weakness, and loss of appetite (anorexia). Streaks of blood may be present in the feces. In acute cases, the feces are very fluid and are bloody. The feces may also contain strands of mucus and pieces of intestinal mucosa. During this acute stage of the disease, the calves may become emaciated, dehydrated, weak, and listless. They may have rough coats, drooping ears, and sunken eyes (Fig. 2). They often have severe straining while trying to defecate (tenesmus). The tail, hindquarters, and lower part of the body may be soiled with feces, and these areas often become denuded of hair. The fluid, bloody feces usually have an extremely foul odor. In this generally weakened condition, a calf may defecate without attempting to stand up.

The acute phase of the disease with severe diarrhea lasts for 3 to 5 days. Calves that succumb to the disease usually die 4 to 10 days after the acute phase has begun. Secondary infections, especially pneumonia, are common during the acute phase of the disease. Dehydration is another common sequelae. Calves that do not succumb recover slowly. The feces return to normal within a week after the acute phase has passed, but calves often require about 2 weeks to regain their strength and several weeks to regain their lost weight.

Some calves exhibit a central nervous system (CNS) disorder during coccidial infections. The CNS disturbance may be mild, with only minor muscular incoordination and twitching plus occasional circling and loss of balance. Often the CNS signs are more severe; the animals may have convulsive seizures or periods of body rigidity alternating with periods of relaxation. Although most reported cases have involved *E. zuernii,* the pathogenesis of this CNS disorder associated with coccidiosis is not well documented. One theory suggests that this syndrome is related to an electrolyte imbalance caused by severe losses of fluids following intestinal mucosa destruction, which results in cerebral edema and the subsequent signs. Another theory suggests that the CNS signs are caused by a toxemia resulting from the presence of the coccidia.

Pathology

Mature first-generation schizonts of *E. bovis* are visible grossly in the ileum at the tips of the villi, whereas those of *E. zuernii* occur in the ileum near the muscularis mucosa and are not visible grossly. Schizonts cause few if any gross lesions except perhaps for a slight hyperemia of the mucosa. Microscopically, the cytoplasm of the host cell containing a mature schizont of *E. bovis*

or *E. zuernii* is stretched into a thin covering layer. The host-cell nucleus is enlarged and the nucleolus is prominent. Schizonts of *E. bovis* are often surrounded by a thin multicellular envelope outside the host cell, and *E. zuernii* schizonts are often surrounded by one or more layers of lymphocytes. Large numbers of eosinophils are sometimes present in the lamina propria in association with *E. bovis*. Adjacent mesenteric lymph nodes are often enlarged but rarely contain coccidial stages.

Second-generation schizonts of *E. bovis* and *E. zuernii* in the cecum and colon apparently have little or no pathologic effect until they mature. The infected host cell then enlarges and the host-cell nucleus becomes indented because of the mass of the schizont. Little inflammatory reaction occurs, except that the lamina propria may have increased numbers of neutrophils, lymphocytes and macrophages.

The sexual stages of *E. bovis* and *E. zuernii* cause extensive lesions. Whereas *E. bovis* affects primarily the mucosa of the cecum and proximal large intestine, *E. zuernii* affects the cecum as well as the entire large intestine, including the rectum. Affected epithelial host cells lose their columnar shape, and destruction is in the surface epithelium as well as in the crypts. Some crypts become distended with oocysts and inflammatory debris. Hemorrhage is often extensive. In the lumen, numerous oocysts are usually present along with erythrocytes, leucocytes, strands of fibrin, and necrotic debris. The lamina propria contains numerous plasma cells, lymphocytes, neutrophils and eosinophils. Capillaries of the lamina propria may be directly exposed to the lumen in areas where the epithelial layer has sloughed.

The prominent gross lesions in the mucosa of the cecum and large intestine of severely affected calves include extensive hemorrhage and sloughing of epithelium. Feces are semiliquid and bloody in the early stages of such infections, but later they become formed masses of sloughed mucosa, blood cells, fibrin, and oocysts. These coagulated masses may resemble a pseudomembrane. Large areas of mucosa are destroyed in severe infections. The serosal surface of the cecum and large intestine is often reddened opposite the affected mucosal areas but otherwise appears anemic. Adjacent lymph nodes are often edematous and enlarged. Serous atrophy of the mesenteric fat frequently occurs. The other abdominal organs have few if any specific gross changes.

Immune Response

Little is known about the immune response to most species of cattle coccidia. Immunity to *E. bovis* has been most extensively studied, mainly because

experimental infections of *E. bovis* can be produced consistently in calves. A single oral dose of about 100 000 sporulated *E. bovis* oocysts stimulates a fairly strong resistance to reinfection in calves. Resistance is not complete as evidenced by the production of some oocysts from a challenge inoculation, but the number of oocysts produced is very much reduced and clinical signs are absent. Resistance of older cattle to experimental infections of *E. bovis* and *E. zuernii* is well established and is probably attributable to immunity developed from prior infections with the coccidia; keeping calves free of coccidia is almost impossible.

Controversy exists as to whether the host's immune response to coccidia is related to humoral or cellular factors, or both. Although humoral antibodies to *E. bovis* have been demonstrated in calves (Andersen *et al.*, 1965), inoculation of susceptible calves with blood cells or serum from immune calves did not produce any noticeable protection against infection, as measured by oocyst production and clinical signs (Senger *et al.*, 1959; Fitzgerald, 1964).

Cellular factors may also be involved in coccidial immunity in calves. An antigen prepared from unsporulated occysts of *E. bovis* was used to demonstrate delyaed hypersensitivity (a type of cell-mediated immunity) in calves infected with *E. bovis*. Delayed hypersensitivity to the oocyst antigen was transferred to previously nonreactive calves by inoculation of either lymphocytes or transfer factor (a product made from lymphocytes). Partial immunity to *E. bovis* infections, as measured by reductions in the number of oocysts passed and the deaths due to coccidiosis, was demonstrated in calves treated with transfer factor (Klesius *et al.*, 1975; Klesius and Kristensen, 1977).

Laboratory Aids to Diagnosis

With a certain amount of practice, coccidial oocysts of cattle can be identified to species. The sporulated oocyst is especially useful in differential diagnosis because it has many more morphological characters than the unsporulated oocyst. A routine laboratory procedure for the sporulation of oocysts is to mix feces containing oocysts in about two volumes of 2.5% potassium dichromate solution and place the mixture in a thin layer in a petri dish with the top left ajar. The mixture is maintained at about 20 °C and gently stirred daily; after several days, with the exact time depending on the species, the oocysts will be sporulated. This process is time-consuming, however, and most investigators identify the species on the basis of the unsporulated oocyst. A calibrated ocular micrometer is needed in either case to measure the oocysts. The measurements for the sporulated oocysts given in the reviews of Levine and

Ivens (1970) and Pellérdy (1974) can also be applied to the unsporulated oocysts, because there are no significant differences in size.

In addition to size, other features useful in identification of unsporulated oocysts include the shape, the color and texture of the oocyst wall, and the presence or absence of a micropyle. Photomicrographs and drawings of unsporulated and sporulated oocysts from cattle can be found in the reports of Christensen (1941), Lee and Armour (1959), Joyner *et al.* (1966), and Levine and Ivens (1970). Photomicrographs of unsporulated oocyts of *E. bovis* and *E. zuernii* are presented in Fig. 3.

The simplest and fastest method of examining feces for oocysts is a direct fecal smear. In this procedure a small amount of feces is placed on a glass slide and mixed with a drop of water. A coverslip is placed on the mixture and the fecal suspension is examined microscopically. Although this technique is fast and requires little equipment, oocysts may not be found because of the small amount of feces examined.

Most investigators use one of a variety of flotation techniques that concentrate the oocysts in the feces. Many solutions can be used as the flotation medium, including zinc sulfate, magnesium sulfate, sodium nitrate, sodium chloride, and sucrose. Sheather's sugar solution has the advantage that the solution evaporates rather slowly and does not distort the oocysts as some salt

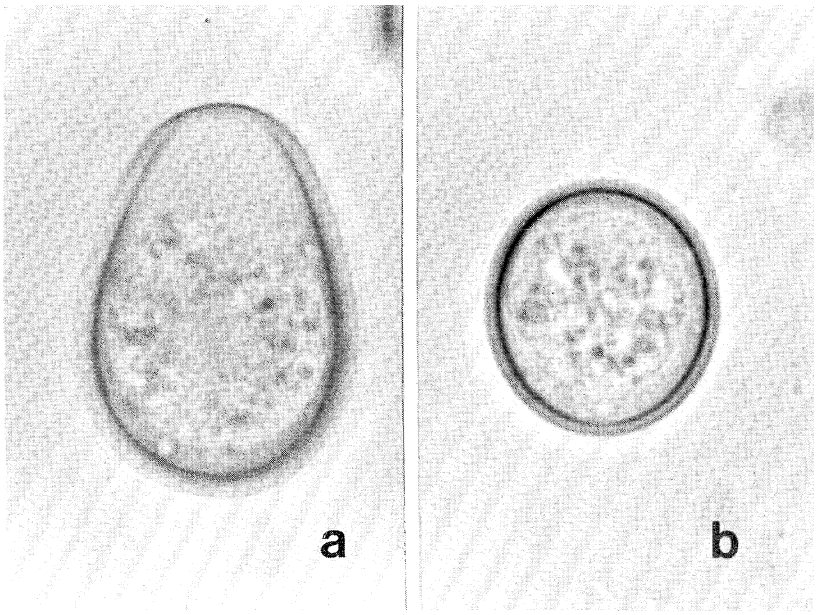

Fig. 3. Photomicrographs of unsporulated oocysts. (a) *Eimeria bovis* (b) *Eimeria zuernii*. 1800×. (Original.)

solutions do. It is prepared by adding 500 g of sucrose to 320 ml of water. This mixture is boiled gently, with frequent stirring, until the solution is clear. The solution is allowed to cool and 6.5 g of melted phenol is added as a preservative.

A simple technique to concentrate oocysts by flotation is as follows:

1. Mix about 2 to 3 g of feces in about 1/3 test tube of water.
2. Place a piece of cheesecloth or gauze over a 15-ml centrifuge tube and pour the fecal mixture through the cheesecloth into the centrifuge tube.
3. Fill the centrifuge tube to the top with Sheather's sugar solution.
4. Place a coverslip on the top of the centrifuge tube so that the liquid touches the coverslip.
5. Wait 5 minutes.
6. Centrifuge at about 400 *g* for 5 minutes.
7. Remove the coverslip from the tube, place it on a microscope slide, and examine the sample for oocysts with a microscope at 100×.

There are many modifications of this technique. One that does not require centrifugation of the sample is to add 3 g of feces to about 30 ml of flotation solution in a beaker or paper cup, mix well, strain through a single layer of gauze into a vial, place a coverslip on the vial so that it contacts the liquid, wait about 10 minutes, remove the coverslip and place it on a slide, and examine the sample as above.

Oocyst counts are important in some areas of research on coccidia, such as studies on the effects of drugs on the parasites, but are not extensively used in clinical diagnosis. Oocyst counts are also used in field surveys to determine the intensity of coccidial infections in cattle. A technique often used for counting oocysts is a modification of the well-known McMaster technique and is as follows:

1. Place 15 g of feces in 135 ml of water and mix well, preferably with an electric mixer.
2. Add 1 ml of the above solution to 2 ml of Sheather's sugar solution in a small vial and mix well.
3. Fill a McMaster chamber with the feces-sugar solution mixture. (McMaster slides are available commercially, and instructions for their construction are given by Whitlock [1948].)
4. Allow the preparation to stand for at least 5 minutes to allow the oocysts to rise to the top of the chamber.
5. Count the oocysts in the ruled area of the chamber and multiply the number of oocysts by 200 to give the number of oocysts/g of feces. (Note: Commercial McMaster slides have two chambers on each slide, so if both chambers are used for the same sample, the total number of oocysts is multiplied by 100.)

Care must be taken in assessing the significance of the number of oocysts of a pathogenic species present in the feces and their relationship to disease. A count of 5000 oocysts/g of feces is considered high enough by some investigators to warrant the judgment that the animal has the disease. Other investigators have routinely found 10 000 *E. bovis* oocysts/g of feces in pastured calves without visible signs of coccidiosis. In some acute *E. zuernii* infections, clinical signs are present before the infection becomes patent (before oocysts may be found in the feces). A diagnosis of coccidiosis must be based on the presence of signs, the presence of oocysts of *E. bovis* or *E. zuernii* in the feces, and the appropriateness of the clinical history of the individual calf and herd. A diagnosis of clinical coccidiosis should not be based merely on the presence of large numbers of oocysts, especially if they are of the other, apparently nonpathogenic species that are frequently encountered in feces of young calves.

Microscopic examination of scrapings and stained sections of intestinal tissues are also used in the diagnosis of coccidiosis at necropsy. Either living or fixed life-cycle stages can be identified in infected tissue. The pathogenic endogenous stages of both *E. bovis* and *E. zuernii* occur in the cecum and large intestine, in which areas that have gross coccidial lesions (see Pathology) should be examined microscopically. *Eimeria zuernii* usually produces lesions in the entire large intestine from the cecum to the rectum, whereas *E. bovis* lesions are usually confined to the cecum and upper part of the colon. In scrapings of infected tissue, oocysts of the two species can be easily differentiated, since those of *E. zuernii* are usually subspherical and those of *E. bovis* are usually ovoid. Also, many *E. zuernii* schizonts are present in the mucosa and are easily observable along with the sexual stages and oocysts, whereas with *E. bovis* sexual stages and oocysts are apparent but schizonts are few and not readily apparent because of their small size.

Large schizonts found in scrapings and tissue sections from the small intestine may be those of *E. bovis, E. zuernii,* or *E. auburnensis.* In addition, the mature microgametocyte of *E. auburnensis,* which occurs in the same location, is large and may be misidentifified as a schizont. These stages are easily confused and are not readily identifiable to species except by an expert. First-generation schizonts of *E. bovis* protrude from the tips of the villi into the lumen and may be seen grossly at necropsy as white, pinpoint dots on the surface of the epithelium; crushing one of these in saline releases many motile merozoites, visible on microscopic examination. The mature first-generation schizont of *E. zuernii,* and the mature first-generation schizont and the mature microgametocyte of *E. auburnensis,* are located deeper in the tissue and are not visible grossly.

Prevention and Control

Because coccidia are transmitted by fecal contamination, and animals become infected by ingesting sporulated occysts in contaminated feed or water or by licking other contaminated animals or material, sanitation and isolation are important in preventing coccidiosis.

Dairy calves should be isolated soon after birth and kept separately in individual stalls or pens. Before use, stalls or pens should be thoroughly cleaned, preferably with high-pressure steam, and allowed to dry completely. Any water left standing in the stall or pen becomes a good environment for sporulation of oocysts. Then pens should be cleaned daily and old bedding should be replaced at regular intervals, before it becomes heavily soiled with feces. Buckets and other utensils used to feed and water calves should be marked and used for individual calves. After use, they should be washed with hot water and detergent and rinsed thoroughly with hot water. Feed and water containers should be placed high enough in the stalls to prevent fecal contamination.

Calves that cannot be isolated in individual stalls or pens should be segregated by age in large holding pens; younger calves should never be penned with older calves. Pens should be kept clean and dry and care should be exercised so that feces are not carried from one pen to another.

Fig. 4. Feedlot cattle with signs of coccidiosis, including tenesmus and feces-stained hindquarters. (Photograph courtesy of Dr. Robert Pierson, Colorado State University, Fort Collins, Colorado.)

Pastures or ranges for birth and rearing of beef calves should be clean and well drained. Overstocking and crowding should be avoided. Wet areas around feeding places should be filled in or drained. Feeding sites should be changed regularly.

Caution should be exercised in moving cattle to feedlots. The stress of moving and feedlot entry procedures frequently triggers outbreaks of clinical coccidiosis. The feedlots should be well drained and cleaned as often as possible. In feedlots where coccidiosis is a persistent problem because of heavy contamination with coccidial oocysts, the surface layer of soil should be removed and replaced with small gravel. Figure 4 shows feedlot cattle with typical signs of coccidiosis, including tenesmus and feces-stained hindquarters.

Calves with clinical signs of coccidiosis should be isolated to prevent contamination of other calves. The remaining apparently healthy calves in the pen should also be treated for coccidiosis because some of them are probably in the early stages, or prepatent period, of the disease.

The difficulty with treatment of clinical coccidiosis is that the signs of the disease do not become evident until the disease is far advanced, the parasite has already passed through the part of the development cycle in which the available drugs are effective, and the digestive tract is already severely damaged. In some cases, medical treatment is to no avail when signs are present, and the infected calf either dies or recovers on its own. This is the reason that so many home remedies have been reported to be successful in the treatment of bovine coccidiosis. Treatment of the infected animal at the first sign of clinical coccidiosis with an anticoccidial drug at a therapeutic level will stop development of the coccidia that have not completed their cycle. In addition to using the anticoccidial drug, using electrolytes to control dehydration and antibiotics to control secondary infections helps in severe cases of coccidiosis.

Several drugs have been shown to be effective in the treatment of bovine coccidiosis. The sulfonamides probably have been used most widely in the past and are still extensively used today in many parts of the world. Among the sulfonamides that have been used effectively against bovine coccidiosis are sulfaguanidine, sulfamethazine, sulfabromomethazine, sulfamerazine, and sulfaquinoxaline. Recently, other drugs have been developed that are highly effective against bovine coccidiosis; these include amprolium, decoquinate, and monensin. These drugs are used for both therapeutic and prophylactic treatments. All drugs used for treatment of bovine coccidiosis should be used only in accordance with the manufacturer's direction or as prescribed by a veterinarian.

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32. EAST COAST FEVER *

A.D. Irvin and M.P. Cunningham

Abstract. East Coast fever (ECF) is a tick-borne disease of cattle occurring in large areas of East and Central Africa. It is currently the major constraint to development of the livestock industries in the countries affected. The disease is caused by *Theileria parva* which is transmitted by the ixodid tick *Rhipicephalus appendiculatus*. The parasite undergoes a complex life cycle in both the vector and the bovine host, and the pathogenic stage, the macroschizont, occurs in bovine lymphoid cells. In susceptible cattle there is high mortality as a result of lymphoid cell destruction. Animals which recover from infection have a long-lasting immunity to homologous challenge, but may be susceptible to challenge with other strains, particularly those of buffalo origin.

Control of the disease is essentially by acaricidal control of vector ticks, but immunization and chemotherapy, both of which are still in the experimental stages, show encouraging developments and should soon make an important impact in limiting or even eradicating the disease.

Introduction

East Coast fever (ECF) is a tick-borne protozoal disease of cattle caused by *Theileria parva*. Apart from cattle the only species known to be susceptible are the closely-related buffaloes *Syncerus caffer* and *Bubalus bubalis*. The disease occurs across a large area of east and central Africa, particularly in Uganda, Kenya, Tanzania, Rwanda, Burundi, Zambia, Zaire and Malawi. It has been estimated that one animal dies approximately every minute from ECF; this represents an annual loss of around fifty million dollars. This figure does not take account of indirect losses from reduced productivity nor of the vast sums of money expended on disease prevention, particularly tick control. The overall economic loss may therefore be ten or even more times higher than the above figure; this represents an enormous and unacceptable drain on the resources of developing countries. The current chapter emphasises the biolog-

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ical and practical aspects of ECF as it occurs in the field. Further details of the disease can be obtained from recent reviews by Barnett (1977), and Purnell (1977).

Classification

Theilerial parasites are placed in the sub-phylum Sporozoa (Apicomplexa). Many taxonomists then split the sub-phylum into parasites with a proven sexual cycle (malaria, coccidia etc.) and those without (piroplasms). Since, however, it has recently been shown that a sexual cycle almost certainly does occur in piroplasms, the separating of them from the main body of the Sporozoa seems no longer tenable. A more satisfactory classification for *T. parva* in the light of recent knowledge is as follows:

Phylum	Protozoa
Sub-phylum	Sporozoa (Apicomplexa)
Class	Teleospora
Sub-class	Coccidia
Order	Eucoccida
Sub-order	Piroplasmorina
Family	Theileriidae
Genus	*Theileria*
Species	*parva*

Other genera of the Theileriidae have been described including *Gonderia, Haematoxenus* and *Cytauxzoon,* but the current tendency is to drop these names as more details of life cycles become revealed and, although *Cytauxzoon* may survive, theilerial parasites may eventually all be classified under the genus *Theileria.*

Life cycle

In the bovine host

The life cycle of *T. parva* is shown in Fig. 1. Some of the stages still remain to be elucidated, but the recent evaluation of the sexual cycle by Schein and his colleagues (Schein *et al.*, 1977) has resolved the largest area of confusion.

Sporozoites from infected ticks are injected into cattle during the course of normal tick feeding. If ticks feed on a susceptible animal there is an occult phase of about 5 days before parasites can be detected in the local drainage lymph node adjacent to the site of tick bite. It is not known what happens

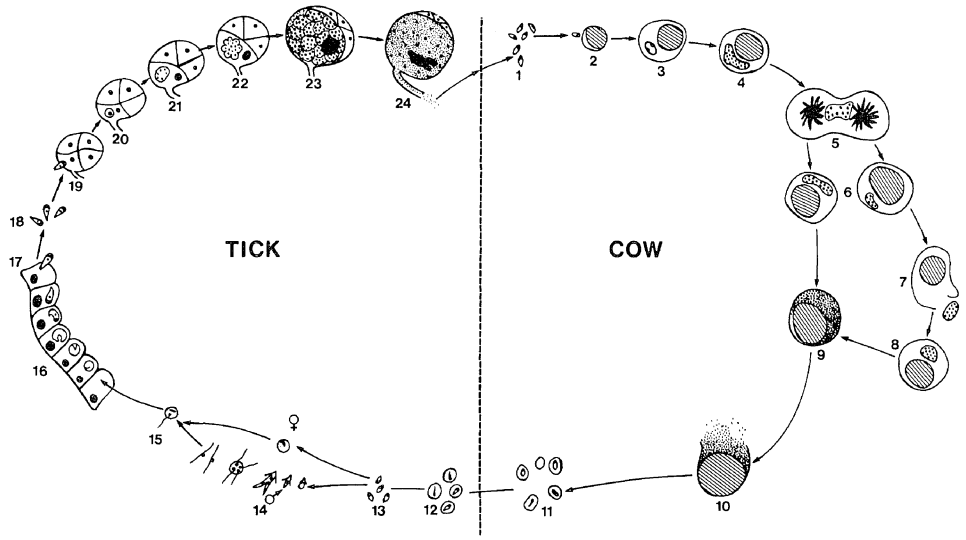

Fig. 1. Life cycle of *Theileria parva*.

SCHIZOGONY

1. *Sporozoites* injected with saliva into bovine host when infected tick feeds.
2. Sporozoite invades lymphocyte.
3. Transformation of lymphocyte to lymphoblast is induced by the parasite which differentiates into a *macroschizont* in the cell cytoplasm.
4. Macroschizont grows as disease progresses (Average size 4.8 μm).
5. Macroschizont infected cell divides, macroschizont nuclear particles become aligned along the spindle.
6. Two infected daughter cells are formed.
7. Macroschizont-infected cell degenerates releasing free macroschizont.
8. Free macroschizont may be able to invade an uninfected lymphoid cell.
9. Macroschizont differentiates to a *microschizont*.
10. Microschizont-infected cell ruptures releasing *micromerozoites* (1–1.5 μm).
11. Micromerozoites invade erythrocytes to form *piroplasms* (3–5 μm).

GAMETOGONY

12. Piroplasm-infected blood taken into tick gut as tick feeds.
13. Erythrocytes lyse; *micro-* and *macrogamonts* develop from piroplasm ring forms.
14. Formation of male *micro-* and female *macrogametes*.
15. Anisogamy and *zygote* formation.
16. Zygote invades gut cell and undergoes differentiation to a *kinete* (19 μm).
17. Motile kinete breaks out from gut cell.
18. Free kinetes in tick hemolymph.

SPOROGONY

19. Kinete invades salivary gland acinar cell (usually type III acinus).
20. Kinete rounds up.
21. Parasite nuclear division and formation of *sporont* (primary fission body).
22. Invagination of sporont and formation of buds. Futher development is delayed until tick starts to feed in its next instar.
23. *Primary sporoblasts* develop from sporont buds to form *cytomeres* (secondary fission stage) Hypertrophy of host cell and cell nucleus.
24. Division of primary sporoblasts to *secondary sporoblasts* (tertiary fission stage) and formation of *sporozoites* (1–1.5 μm) which are released into salivary duct. Host cell and nucleus degenerate, parasite residual bodies remain.

during these 5 days but it is likely that sporozoites very rapidly enter target lymphoid cells, and thus escape the phagocytic, lytic and immunological defences of the host. Infected (and possibly uninfected) lymphocytes transform to lymphoblasts, with larger and less dense nuclei and increased cytoplasm. There is rapid proliferation of these cells and growth of macroschizonts. Expansion of this infected lymphoblast population is mainly by division of infected cells. This division appears to be stimulated by the presence of the parasite, which itself divides in synchrony with the host cell to produce two infected daughter cells. Invasion of uninfected lymphoid cells by free macroschizonts probably also occurs. The mechanism of the last mode of infection is not clear but may be through membrane fusion of cells in close apposition.

A proportion of macroschizonts differentiate to microschizonts from about day 14 after tick attachment. The mechanism of differentiation is not known. Microschizont-infected cells rupture to release micromerozoites which invade erythrocytes and differentiate to piroplasms. Piroplasm-infected erythrocytes are then available to infect ticks feeding on the blood of the infected animal. Piroplasms of *T. parva* only rarely appear to divide within erythrocytes, in contrast to *T. mutans* and *Babesia* spp.

In the tick

The normal vector of ECF is the ixodid tick *Rhipicephalus appendiculatus;* this is a 3-host tick: the three instars (larva, nymph and adult) all feeding on separate hosts. Transmission of ECF is transstadial: a larva which becomes infected by feeding on a parasitemic cow can transmit infection as a nymph, and a nymph which becomes infected can transmit infection as an adult. There appears to be no trans-ovarial transmission (as occurs in *Babesia* infections), nor can ticks infected as larvae transmit infection as adults, since there is no carry over of infection from the nymph.

Nymphs feeding on an infected cow take piroplasm-infected erythrocytes into their gut. Erythrocytes lyse releasing the piroplasms; many of these are digested but a proportion of the ring forms differentiate into macro- and microgametes. Fusion by a process of anisogamy is assumed to occur to produce a zygote about 6 days after nymphal repletion; this invades a cell in the gut wall and during the course of the tick moult develops to form a motile kinete which is released into the tick hemolymph. Kinetes invade the tick salivary glands and undergo differentiation to sporonts. Further development is delayed until the now-adult tick begins feeding on a new host. Rapid parasite development then occurs, as shown in Fig. 1, and after about 3 days of feeding, mature sporozoites develop which are released in the saliva. Peak

sporozoite production is reached by day 5 but sporozoite release may persist throughout the feeding period of the ticks (normally about 10 days for females; males may feed intermittently over a much longer period).

Epidemiology

Effect of climate

East Coast fever is confined to areas where the vector tick *R. appendiculatus* occurs, and the incidence and occurrence of infection is closely linked with the size and activity of the tick population. *Rhipicephalus appendiculatus* occurs from sea level to altitudes over 7000 ft provided that there is adequate vegetation and a rainfall in excess of 20 inches a year. In many ECF endemic areas rainfall is markedly seasonal; tick activity follows the onset of rain and this in turn results in outbreaks of ECF. During the dry seasons tick activity is minimal and the number of ECF cases falls accordingly. In other areas where there is more constant rainfall, for example around Lake Victoria, in highland areas, or along parts of the coast, tick activity may be continuous and ECF can occur at any time.

In areas where there is constant challenge, cattle are continually exposed to infection from birth. If challenge is severe there can be high mortality in young animals. Surviving cattle, however, become immunized and are fully resistant to subsequent challenge and are able to thrive in such areas. On the other hand, in areas where challenge is seasonal or where survival of *R. appendiculatus* is marginal, exposure of cattle to infection may be very irregular and, in immunized animals, immunity may wane. This means that when the challenge rises, at the onset of the rains for example, cattle losses can be enormous because of the presence of young animals not previously exposed and of a proportion of older animals with reduced immunity. The same type of increased challenge can occur when stock owners are forced, in times of drought, to move out of marginal areas to graze their stock in wetter endemic areas. Similarly, in times of prolonged and heavy rain, areas that are normally marginal for *R. appendiculatus* may become ecologically suitable for the tick with disastrous effects on cattle.

Cattle

Cattle in many endemic areas show a high degree of resistance to ECF; much of this is acquired as a result of challenge, but there is good evidence that genetic selection of resistant stock can occur and there are instances where

progeny of a particular sire or dam have shown high innate resistance to ECF. Unfortunately this finding has not been exploited nor have the genetic factors responsible for resistance been determined, although hemoglobin type may be one such factor. One genetic feature which seems clear however is that cattle of *Bos indicus* type (Zebu) are naturally more resistant than *Bos taurus* type (European breeds). Much of the evidence for this strain difference is circumstantial and experimental proof is scanty. *Bos indicus* populations as a whole may have an innate resistance to ECF or African populations may have been naturally selected for resistance to the disease. At this stage the mechanism is not known. Similarly circumstantial evidence suggests that young animals may be naturally more resistant than older animals, but again properly-designed experiments have to be conducted to confirm or refute this.

From a practical point of view, the introduction of susceptible cattle into an ECF-endemic area will result in a high death rate irrespective of age or breed. In *Bos taurus* cattle morbidity and mortality rates can approach 100%. In *Bos indicus* these figures may be lower, but losses will nonetheless be severe.

Parasite species and strains

The causative agent of ECF is classically *T. parva,* but in the field the presence of other theilerial parasites can complicate the picture. The two commonest species isolated are *T. lawrencei* and *T. mutans. Theileria lawrencei* occurs naturally in buffalo (*Syncerus caffer*). There is a high morbidity rate but mortality rate is not known; however, surviving animals frequently become carriers of the parasite and experimental work has shown that they can continually infect ticks for at least 3 years. The vector is *R. appendiculatus* and consequently in areas where cattle and buffalo populations overlap there is every opportunity for cattle to become infected. In cattle, *T. lawrencei* causes high mortality, but macroschizont-infected cells are few and piroplasm production scanty. This means that infection cannot readily be transmitted to ticks from cattle, but only maintained by buffalo in which piroplasms are constantly circulating. If however *T. lawrencei* is passaged through cattle, as has been done experimentally, it quickly reverts to a parasite indistinguishable from *T. parva,* which causes a syndrome indistinguishable from ECF. The obvious inference from this is that *T. parva* is a cattle-adapted variant of *T. lawrencei* which causes high mortality in an abnormal host but only low mortality in the natural host. In view of this apparent conspecificity it would seem rational to adopt the recently suggested nomenclature of *T. parva parva* for the cattle parasite and *T. parva lawrencei* for the buffalo one.

When African buffalo are infected experimentally with *T. parva,* either of cattle origin or transformed from buffalo, they undergo mild or inapparent

infections. This is in contrast to the Asiatic or water buffalo (*Bubalus bubalis*) which usually dies – a factor which has prevented the exploitation of these animals in Africa.

Theileria mutans occurs widely in both cattle and African buffalo. The reported vectors are *Amblyomma variegatum, A. cohaerens* and *A. gemma.* The parasite is normally apathogenic, but pathogenic strains have been encountered in a number of countries. The parasite divides in the erythrocytes and can cause an anemic syndrome which may be fatal. An asymptomatic *T. mutans* carrier state is common in both cattle and buffalo.

Theileria parva parva, T. parva lawrencei and *T. mutans* frequently occur together in the field, making a complex clinical syndrome which would be diagnosed as ECF, but which could not be controlled effectively until the complexities were unravelled. An example of such a situation has been described at Aitong in Kenya (Irvin *et al.*, 1972).

In addition to the buffalo (*Syncerus caffer*) a number of other wild animal species have been implicated in the epidemiology of ECF; they include eland (*Taurotragus oryx*), bushbuck (*Tragelaphus scriptus*), kongoni (*Alcelaphus buselaphus*), wildebeeste (*Connochaetes taurinus*), waterbuck (*Kobus ellipsiprymnus*) and others. Much of the evidence is speculative or circumstantial but a number of facts have been established: a parasite, *T. taurotragi* isolated from eland, can be transmitted to cattle and cause a mild theilerial syndrome; most African antelopes and other Bovidae harbour piroplasms in their blood indistinguishable from *T. parva,* but attempts to transmit these experimentally to cattle have largely failed; tick populations, including those of *R. appendiculatus,* can be maintained by wild Bovidae in the absence of cattle.

The occurrence, in the same area, of different theilerial species can result in a complex field syndrome, but perhaps even more important from the practical view point is that different immunogenic strains of *T. parva* exist in different areas. A strain from one area may not therefore cross protect with a strain from another area. This makes the question of control by vaccination much more difficult, since a multiplicity of strains may be required to protect cattle which are moved from one area to another.

Vectors

The normal vector of *T. parva* is *R. appendiculatus* and it seems likely that the disease cannot be maintained in areas from which this tick is absent. However, other species including *R. evertsi, R. pulchellus, R. carnivoralis, R. compositus, R. jeanneli, R. pravus, R. simus, Hyalomma truncatum* and *H. dromedarii*, have been shown capable of transmitting infection experimentally but, with the possible exception of *R. evertsi,* they probably play no significant

role in the epidemiology of the disease since, in most cases, the nature of their life cycles precludes their acting as vectors of ECF.

The survival of *R. appendiculatus* in the pasture is determined very much by climatic and other ecological factors, but it has been shown that infected ticks have survived in suitable areas left fallow for nearly two years. At the end of this time they were still able to infect cattle. Under less favorable conditions, survival of infected ticks is of the order of a few months.

The infection rate in ticks in endemic areas is usually low; there is a high percentage of immune animals which will cleanse infected ticks, and at any one time only a small number of animals will have piroplasms circulating in their blood. Infection rates in ticks are then of the order of 1 to 2%. This means that for a susceptible animal to become infected with ECF it must be subjected to a high tick challenge.

However in areas where there are many susceptible cattle, infection rates in ticks can become much higher once ECF becomes established, since there will then be a high proportion of parasitemic animals and a much greater opportunity for ticks to acquire infection. This in turn means that susceptible animals can become infected with ECF after infestation with only a few ticks.

Clinical signs

The clinical signs vary according to the level of challenge and range from inapparent or mild to severe and fatal infections. The prepatent period and severity of reaction are directly proportional to the quantum of sporozoites injected by the ticks. However, there is a minimum prepatent period of about 5 days which is not quantum dependent, and even in the face of massive experimental challenge this time cannot be reduced. Further factors that modify the course of the disease are the susceptibility of the host, the duration of time over which ticks emit sporozoites, and the different strains and species of theilerial parasites encountered by the host.

In uncomplicated *T. parva* infection in susceptible animals the first clinical signs usually occur about 2 days after the appearance of macroschizonts in the lymph nodes draining the site of tick attachment (i.e. between days 7 and 10). The parotid lymph nodes are usually the first ones affected since they are adjacent to the ears which are the preferred sites of feeding for *R. appendiculatus*. Swelling of the nodes occurs and the animal becomes pyrexic with a temperature in excess of 39.5 °C. Pyrexia continues throughout the course of infection and may exceed 42 °C. Lymph node swelling becomes pronounced and generalized, and peripheral nodes, particularly the parotids, prescapulars

and precrurals, become prominently enlarged. Anorexia develops and the animal rapidly loses condition; lacrimation and nasal discharge may occur. Terminal diarrhea and dyspnea are common symptoms and the animal normally becomes recumbent. Just before death there is usually a sharp fall in temperature and severe dyspnea with massive pulmonary exudate which pours from the nostrils. The animal normally dies as a result of asphyxiation resulting from edema of the lungs. Death can occur within 14 days of initial infection, but more commonly occurs after 18 to 24 days. The mortality rate in fully susceptible animals approaches 100%. Animals which survive beyond 4 weeks normally recover, but some field strains can produce chronic debilitating infections which may ultimately be fatal.

Occasionally nervous symptoms are seen, usually in partially immune animals subjected to massive challenge. The condition known as "Turning Sickness" is characterized in the severe form by rapid turning, dizziness and collapse. In the less severe form, animals circle slowly and frequently press their heads against walls or trees. Both forms are fatal. The etiology of the condition is uncertain but there is a good correlation between the presence of macroschizonts in cerebral tissues and clinical and histopathological findings.

In partially immune animals or those subjected to continuous low level challenge, the disease takes a more chronic course, and animals frequently recover. Lymph node enlargement is the most prominent feature, but there is usually intermittent pyrexia, anorexia and loss of condition. This syndrome is commonly seen in calves in endemic areas. The effects of constant ECF challenge, frequently coupled with chronic malnutrition, helminthiasis and other conditions, result in severely retarded animals which only rarely achieve their production potential if they recover. The mortality rate of calves in such areas is related to the severity of challenge but may, in extreme cases, reach 50%.

Pathology

Severity of pathological lesions varies according to the course of the disease, but the most constant lesion is depletion of lymphoid tissues, due to destruction of lymphoid cells. Initially there is hypertrophy and hyperplasia of lymph nodes, but terminally there is usually lymph node edema, some hemorrhage and cellular necrosis. Macroschizonts are first seen about 5 days after infection in lymph nodes draining the site of tick attachment; 2 to 3 days later there is generalised lymphoid cell infection, macroschizonts being detectable in most lymphoid organs as well as peripheral blood lymphocytes. This infection increases for about 5 to 7 days, then there is also an increase in

extracellular macroschizonts but this is usually followed terminally by a marked fall in detectable macroschizonts. Microschizonts are usually seen from about day 11 onwards and piroplasms appear shortly afterwards. Piroplasm parasitemia can be very variable but normally reaches about 30% to 40%; however it may even exceed 70% or fail to reach 5%. Parasitemia levels vary with different strains of *T. parva*.

In circulating blood there is a progressive fall in total leukocytes, often to less than $1000/mm^3$. Granulocytes and polymorphs fall as well as lymphoid cells. Erythrocyte counts, packed cell volume and hemoglobin levels are frequently unchanged, but terminal anemia may occur with some strains.

The most striking lesion seen at post mortem examination is massive pulmonary edema, hyperemia and emphysema. The alveoli, bronchi and trachea become filled with frothy pulmonary exudate. There may also be some pleural and pericardial exudate. Hemorrhages are commonly seen on the serosal and mucosal surfaces of many organs including the pleura, viscera, spleen, heart, kidneys, bladder and under the tongue. Hemorrhages on the

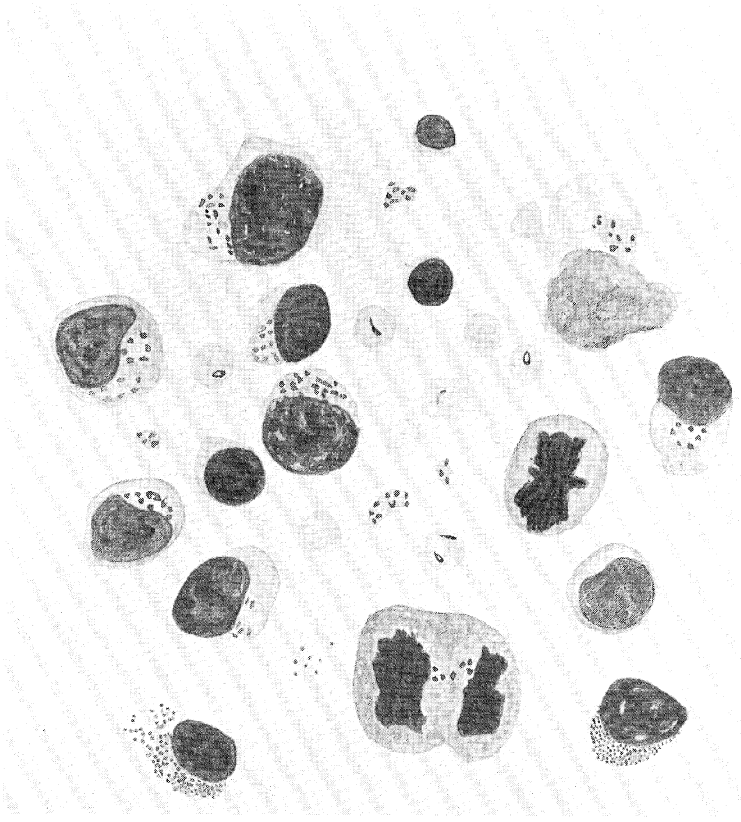

Fig. 2. T. parva-infected lymph node, composite picture show intra- and extra-cellular macroschizonts, microschizonts and piroplasms.

mucosal surface of the abomasum frequently ulcerate. In protracted cases ulcers are also seen in areas where Peyer's patches have been depleted of lymphoid cells. The cortex of the kidneys is frequently congested and pseudo infarcts of lymphoid tissue may be seen as raised white areas on the kidney surface. Changes in the spleen can be very variable; in texture it is usually friable or mushy; in appearance it can be shriveled but, in common with the liver, is often enlarged. Post mortem degeneration is very rapid.

The post mortem features can vary according to the course of the disease and also with the strain of parasite with which the animal was infected.

Diagnosis should be based on the detection of macroschizonts in lymph node biopsy or impression smears (Fig. 2). In the absence of facilities for such examinations, the clinical and post mortem findings coupled with the presence of the vector tick should be sufficient to make an accurate diagnosis. Diagnostic serology can be valuable where appropriate facilities are available, the indirect fluorescent antibody test being the most reliable.

Immunity

Cattle which have recovered from ECF are strongly immune to homologous challenge, but may be partially or totally susceptible to challenge with a different strain. Recovered cattle show a sterile immunity which lasts for more than 3 years, even in the absence of re-infection. This immunity does not appear to be reduced by stress but there is some waning of immunity with time since, when animals of long-standing immunity are subjected to homologous challenge, a number will show mild febrile and parasitemic responses. Such responses are rare in animals recently immunized. The duration of immunity under field conditions is difficult to study because of the frequent exposure to challenge and hence constant boosting of immunity.

The nature of the immunity has not yet been fully determined but the main mechanism seems to be cell mediated. An autologous mixed lymphocyte reaction is generated *in vitro* and *in vivo* by the host against macroschizont-infected cells. Stimulation, *in vitro* of lymphocytes from immune animals by autologous infected cell lines, results in generation of cytotoxic lymphocytes. The specificity of this latter reaction and the mechanisms of other cell-mediated immune responses have yet to be elucidated.

Following infection, animals show a high titre of antibodies to both macroschizont and piroplasm antigens. Titres reach a peak 4 to 6 weeks after infection and may persist for 6 months or more. Titres to piroplasm antigen usually wane first. There may be no correlation between the level of antibodies and the degree of resistance to ECF, since animals which have become

serologically negative may be totally resistant to infection. There is no evidence that passively transferred antibodies, either from colostrum or from the sera of recovered animals, confer any protection against challenge.

Serology plays a useful role in identifying animals which have been recently exposed to infection, and it can be helpful in species differentiation. The indirect fluorescent antibody (IFA) test is the most widely used. The complement fixation (CF), capillary tube agglutination (CA) and indirect hemagglutination (IHA) tests have also been used but appear to be less reliable. Recently, promising results have been obtained using the enzyme-linked immunosorbent assay (ELISA) test, if these are confirmed, the test could supersede the IFA test since it is more specific and more easily quantified.

Another advance of great potential is the raising of monoclonal antibodies against individual theilerial antigens using "Hybridoma" techniques. Immunoglobulin-secreting myeloma cells are fused with mouse spleen cells primed with theilerial antigens, the resultant hybrid cells are cloned; clones of cells secreting appropriate monospecific antibodies are isolated. These clones can then be grown up in bulk, either in mice or in culture, with large quantities of antibody being obtained. Using such antibodies it is possible to determine, with great precision, the different antigenic components of different parasite strains and ultimately it may be possible to identify and isolate the specific antigens required to induce protective immunity.

Cross immunity between different strains of *T. parva parva* and *T. parva lawrencei* is very variable and may be totally absent; animals resistant to one strain may, for example, be fully susceptible to heterologous challenge. This is particularly true of animals immunized against *T. parva parva;* these may frequently be susceptible if challenged with strains of *T. parva lawrencei,* although the reverse does not usually apply. Under field conditions, exposure to *T. parva lawrencei* normally occurs when cattle are grazed in areas frequented by buffalo. Under these conditions breakdowns of immunity can readily occur, partly because of lack of cross protection between *T. parva parva* and *T. parva lawrencei* and partly because the antigenic nature of *T. parva lawrencei* harbored by buffalo seems to change with time. Cattle immunized against a strain of *T. parva lawrencei* isolated from a buffalo may be fully susceptible to challenge with a strain isolated from the same buffalo several months later, even though fully resistant to challenge with the original strain. In other words buffalo can produce antigenically different organisms at different times, and have even been shown to produce antigenically distinct organisms at the same time. The nature of the antigenic change or drift is not known.

This situation obviously complicates any potential vaccine program in areas where buffalo and cattle overlap. Fortunately a similar situation does not

appear to prevail where only strains of *T. parva parva* are concerned since, in contrast to buffalo, cattle recovered from infection appear to have a sterile immunity and do not continually circulate piroplasms in their blood. The carrier state in buffalo appears to be maintained by continuous slow division of macroschizonts and possibly also of piroplasms. Piroplasms may however, circulate in cattle which are exposed to continual or repeated challenge, particularly if they are only partially immune. This situation may be common in calves in an endemic area.

Laboratory studies

Many of the laboratory studies conducted with *T. parva* are not of direct relevance to the theme of this chapter, but there are a number which should be described since they represent significant advances in the study of the disease and have a direct bearing on practical issues.

Macroschizont-infected lymphoblastoid cells taken from infected cattle can be grown and maintained in continuous suspension culture. This achievement was first reported by Malmquist *et al.* (1970) and recent developments have been summarized by Brown (1979). The most important of these has been the *in vitro* transformation of bovine lymphocytes by sporozoites of *T. parva* and the isolation of macroschizont-infected cell lines (Brown *et al.*, 1973). With such a system it is possible to study very effectively the immunological relationship between syngeneic infected and uninfected lymphoid cells, having on one hand an infected cell line and on the other the uninfected syngeneic donor animal. Macroschizont-infected cell lines are an important source of theilerial antigen for serology, they have provided a valuable tool for drug screening, and they may have an application in vaccination against ECF. This last potential has yet to be realised since problems have been encountered with the large number of cells needed, the variable response induced and conflict between histocompatibility antigens on cells of the donor and recipient animals. No effective method has yet been developed to obtain free viable macroschizonts for potential vaccine material free of the complications described.

Another important advance has been the development of a means to produce stabilates of theilerial sporozoites which can be cryopreserved, which when injected, will reproducibly infect cattle over long periods of time and which can be titrated down to fractions of a tick-equivalent dose. This development enabled workers to confirm that ECF reactions in cattle were sporozoite quantum dependent rather than strain dependent. This work has

been reviewed in detail by Purnell (1977). At present the only reliable way to quantitate infective sporozoites is by titration of stabilate material in cattle. The *in vitro* transformation of lymphoid cells is beginning to be used as a means of titrating sporozoite material, but this has yet to be correlated with infectivity for cattle. Another approach to this problem has been to try and develop a laboratory model for ECF, and although some success has been achieved in establishing macroschizont-infected cells in athymic nude mice (for review see Irvin, 1977), sporozoites of *Theileria* will not infect any of the wide range of laboratory animals so far tested.

Control

There are four main ways that ECF can be controlled: by vector control, by cattle control, by immunization and by chemotherapy. All have a part to play and, irrespective of the efficacy of one or other method, effective control will only be achieved by efficient integration of all four methods.

Vector control

In theory the most effective way to control ECF is by elimination of ticks. It may be possible to achieve this aim under exceptional circumstances of geographical isolation, but in most areas tick eradication is impractical. The best that can be achieved is to prevent ticks feeding on cattle by constantly treating the cattle with acaricides. The most common ones in current use are organochloride and organophosphorus compounds. The method of application varies according to the facilities available and the resources of the farmer. The system most applicable to the small farmer is the use of a hand pump and spray to treat all animals individually, paying particular attention to predeliction sites of tick attachment. This method is inexpensive and can be very effective, provided the farmer has some knowledge of tick behavior. Where small numbers of animals are involved, hand dressing predeliction sites of tick attachment (particularly the ears) with acaricidal grease can be a valuable adjunct to spraying.

Where larger numbers of cattle are involved the use of a spray race or a dipping tank is essential. The former is expensive to install and maintain but running costs are relatively low. The latter is also expensive to construct, maintenance costs are lower but constant monitoring is essential to ensure the dip is maintained at correct strength. The choice between spray races and dips is governed by many factors including the availability of materials and

labour, maintenance facilities, availability of water, availability of power to run a spray race, the number of animals to be dipped and the finances available. Once a system has been chosen, the effectiveness of an acaricidal programme will depend on the vigilance and conscientiousness of the operator. The commonest fault is use of under-strength acaricide; this may be because of a misguided attempt by the farmer to economize or, particularly in the case of dips, because of dilution by rain, replenishment of water level without replenishment of acaricide and degradation of acaricide with time and organic debris. Constant monitoring of acaricide dips and washes is essential to ensure that the correct strength is maintained. If the concentration of acaricide falls not only will tick control be less effective, but there is a very real danger that ticks will develop acaricide resistance and populations of ticks will emerge that are resistant to acaricide even when it is used at normal strength. More details of measures to control ticks with acaricides are given elsewhere (Wellcome Research Organisation, 1976).

In an ECF-endemic area acaricide treatment of cattle may need to be carried out twice a week. This obviously involves considerable cost and furthermore populations of fully susceptible cattle are thus established. This means that should acaricidal control break down for any reason losses from ECF can be enormous. Acaricide treatment should also include control of ticks on other livestock and exclusion by fencing of wild animal populations which can act as tick reservoirs.

Cattle constantly exposed to ticks acquire resistance to infestation. Some breeds or individuals have a greater potential for developing resistance than others. Resistant animals can play an important role in reducing tick populations on pastures and, if the animals are also ECF-resistant they can cleanse ticks of infection. Attempts are being made to induce tick immunity artificially in cattle and to exploit this potentially important means of control.

Cattle control

In most countries, where ECF is endemic, legislation has been drawn up which incorporates policies to control ticks, restrict animal movement, implement quarantine restrictions and impose slaughtering of infected cattle. The legislation, however, is not always implemented or enforced and may therefore be ineffective. In Rhodesia and South Africa legislation was enforced and ECF eradicated, but the cost and effort were enormous. It is doubtful if similar means could be applied in other countries where the disease is endemic, partly because of cost and partly because the epidemiological situation may be more complex.

Even in the absence of legislation, the farmer can do much to control ECF

by sensible management and cattle control, coupled with acaricidal control of ticks. Proper fencing will prevent access of nomadic cattle and game to his farm and will isolate areas particularly suited to ticks. Grass burning, rotational grazing of pasture and alternately using land for pastures and crops, will help to avoid build up of ticks and infection. Grazing of pastures with sheep, goats or immune cattle can reduce or even eliminate infected ticks. Newly-acquired cattle should be quarantined. If an outbreak occurs it may be possible to contain it by slaughtering infected cattle; prohibiting movement of animals, people, hay and grass from infected areas; and establishing a buffer zone, free of animals, between infected and clean areas.

Immunization

Many methods of immunization against ECF have been tried. Until recent years these were either ineffective or else too severe in that many animals died. Immunization procedures are still in the experimental stage but the advent of *T. parva* stabilates, which meant that single controlled infections could be administered to cattle, provided the means for a series of new approaches to immunization. These have been summarized by Cunningham (1977). The most successful method has been termed "infection and treatment" (Radley *et al.*, 1975). Animals are infected with a normally-lethal dose of stabilate on day 0 and the ensuing infection is then controlled with oxytetracycline drugs for 5 days. The animals thus treated undergo mild or inapparent infection and are subsequently fully resistant to homologous challenge. In practice it has been found that a cocktail of stabilates (two *T. parva parva* strains and one *T. parva lawrencei*) given to cattle on day 0 followed by a single injection of a long-acting oxytetracycline formulation confer a high degree of protection against a wide range of *T. parva parva* strains encountered in the field (Uilenberg *et al.*, 1977). Protection against different strains of *T. parva lawrencei* is harder to achieve and, at present, it is difficult to envisage effective immunological control of this infection in areas where cattle are exposed to contact with buffalo.

For an animal to mount an effective immunity against ECF it seems to be necessary for the parasite to become established within the host and to be presented on a syngeneic background. This poses problems of controlling the ensuing infection. The infection and treatment method goes a long way to achieving this, but other methods of immunization have also been sought. The problems of developing a cell culture vaccine have already been discussed. The use of killed parasite preparations has been disappointing and the possibilities of developing an attenuated sporozoite vaccine are just now being explored.

Chemotherapy

Oxytetracycline is effective in controlling ECF if given at the same time as infection, but is only slightly effective against clinical disease and then only when used in massive doses. Certain anti-malarial compounds, notably chloroquine, can be effective against the non-pathogenic piroplasm stages, but only recently have compounds been found which are effective against the pathogenic macroschizonts. Studies *in vitro* showed that the anti-folates, aminopterin and methotrexate, and the hydroxy napthoquinone, menoctone, selectively killed macroschizonts. Studies with menoctone and derivatives have now been extended to the *in vivo* situation (McHardy *et al.*, 1976), and the compounds have been shown to be highly effective against clinical ECF. Much work remains to be done before a suitable agent is commercially available, but there is now every hope that an ECF-specific therapy may soon be developed.

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33. *THEILERIA* INFECTIONS OTHER THAN EAST COAST FEVER

G. Uilenberg

Abstract. Cattle are infected by at least five species of *Theileria* other than *T. parva,* of which *T. annulata,* the cause of Mediterranean or tropical theileriosis, is by far the most important as a disease problem. Other species are of low pathogenicity (*T. mutans, T. taurotragi*) or avirulent (*T. velifera*) and cause benign theileriosis while other yet unidentified, mostly non pathogenic theilerias occur on all continents and are possibly related to the *T. sergenti/T. orientalis* group of the far eastern USSR, the agents of East Asian theileriosis. *Hyalomma* ticks are the vectors of *T. annulata while mainly Amblyomma, Rhipicephalus* and *Haemaphysalis* spp. are involved in the transmission of other species of the organism.

Imported European breeds are far more susceptible to Mediterranean theileriosis than local cattle in endemic regions, which may live with the disease in a state of endemic stability.

Mediterranean theileriosis is an acute febrile disease in susceptible cattle, with a mortality rate which may reach 70%. Involvement of lymphoid tissue is caused by invasion by the schizontic stage, anemia is associated with the erythrocytic piroplasm stage. The course, the post-mortem lesions and the pathogenesis of the various theilerial infections are reviewed.

Recovery from infection by all species concerned is followed by the piroplasm carrier state, but persistence of the parasite is not necessary for immunity, which may last several years. Humoral antibodies are unimportant in protection and immunity is likely to be cell-mediated.

Presumptive diagnosis on clinical signs and post mortem lesions require parasitological confirmation based on demonstrating schizonts and/or a high parasitemia. In addition to morphological characteristics, serological techniques are necessary for species differentiation.

Intensive short-interval acaricidal treatment prevents transmission of theileriosis but there are numerous problems associated with tick control, and effective methods of immunization and treatment are required. Immunization against *T. annulata* is carried out in some countries, using attenuated schizonts grown in cell culture. Sporozoite vaccine promises to be even more effective. The occurrence of immunologically different strains is a complicating factor in immunization. Certain antimalarial drugs affect the erythrocytic stage of *Theileria,* while the only schizonticidal drug on the market is the recent coccidiostat halofuginone.

Disease

Theileriosis in cattle is caused by several species of the tick-borne protozoan genus *Theileria*. Two of these are responsible for some of the most important bovine tick-borne diseases in the tropics. *Theileria annulata* causes Mediterranean or tropical theileriosis. Neither of these names is appropriate as the disease is not limited to the Mediterranean basin or to the tropics. The second name is more confusing as more kinds of theileriosis occur in the tropics than in the Mediterranean area. The second species, *T. parva* with biological subspecies causing East Coast fever, January disease, and corridor disease in Africa, is dealt with in another chapter. Mention should also be made of East Asian theileriosis, caused by *T. sergenti,* an important pathogen on the littoral of the eastern USSR according to Russian workers. Clinical theileriosis reported in imported cattle in tropical and subtropical countries of eastern Asia might be due to this or to a related species. Infections due to other species of *Theileria* in the tropics have often been grouped under the name of benign bovine theileriosis, which in some cases is somewhat of a misnomer. Some progress has recently been made in unravelling the confused taxonomy of bovine theilerias, and for clarity's sake this will have to be dealt with at some length.

The accent in this chapter will be on *T. annulata* which is by far the most important cause of *Theileria* infections other than those due to *T. parva*. As the field is so large, it is impossible in the present context to quote all relevant literature references. The most recent general reviews of theileriosis are those by Barnett (1968, 1977), while the review by Neitz (1957) is also still of value in many respects, and that by Sergent *et al.* (1945) still gives a useful account of Mediterranean theileriosis. Proceedings of a 1976 conference at Edinburgh (edited by Wilde, 1978) also contain much recent information.

Etiology

Theilerias are tick-transmitted Sporozoa, with extraerythrocytic schizonts occurring in the mammalian host, most commonly in lymphocytes, in addition to erythrocytic stages (piroplasms) which do not produce pigment. Sporozoites are injected into the host with tick saliva. The infection induces a transformation of lymphocytes into lymphoblasts in the lymphatic glands and stimulates mitosis of these cells. The first stage discovered so far in the mammal is the macroschizont in the cytoplasm of lymphoblasts (Fig. 1). During the mitotic division of infected lymphoblasts, the schizont situates itself in the dividing plane and is torn into two halves, resulting in two

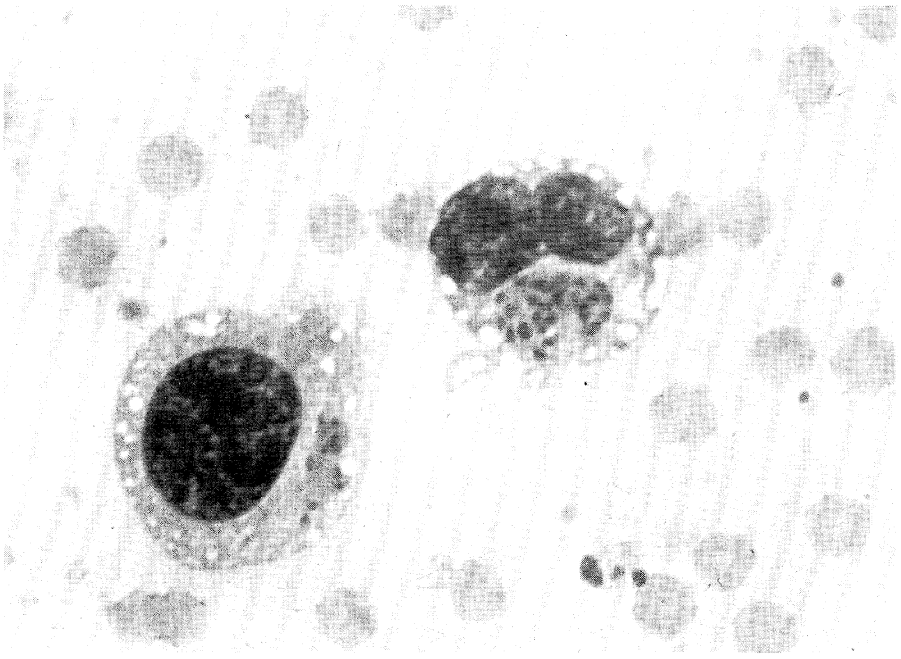

Fig. 1. Macroschizonts of *T. annulata* in lymph gland smear. Giemsa staining method. ×1650.

infected lymphoblasts; an infected lymphocytic cell line is thus established. In addition to this method of multiplication, some transfer of schizont material to uninfected lymphocytes undoubtedly also occurs and this appears to be more frequent in *T. annulata* than in *T. parva* infection. The next stage is the microschizont which develops directly from the macroschizont by nuclear multiplication; it contains numerous small, dense nuclei which on disintegration of the schizont become merozoites and enter red cells where they become piroplasms (Fig. 2). These multiply again by division into four, with the formation of a Maltese cross. In some species schizonts are few, and multiplication is effected mainly by division of the piroplasms (for instance, *T. mutans*), in others both schizogony and division of piroplasms contribute materially to piroplasm numbers (*T. annulata*), while in *T. parva* most piroplasms originate directly from the numerous schizonts and do not themselves divide to a great extent.

Transmission is transstadial. The cycle in the tick appears to include sexual development in the gut, with formation of macro- and microgametes and zygotes; the latter change into kinetes which move through the hemolymph to the salivary glands where cytomeres and sporoblasts are formed. These remain dormant until, after moulting, the next tick stage starts to feed; intense multiplication results in the formation of large numbers of infective

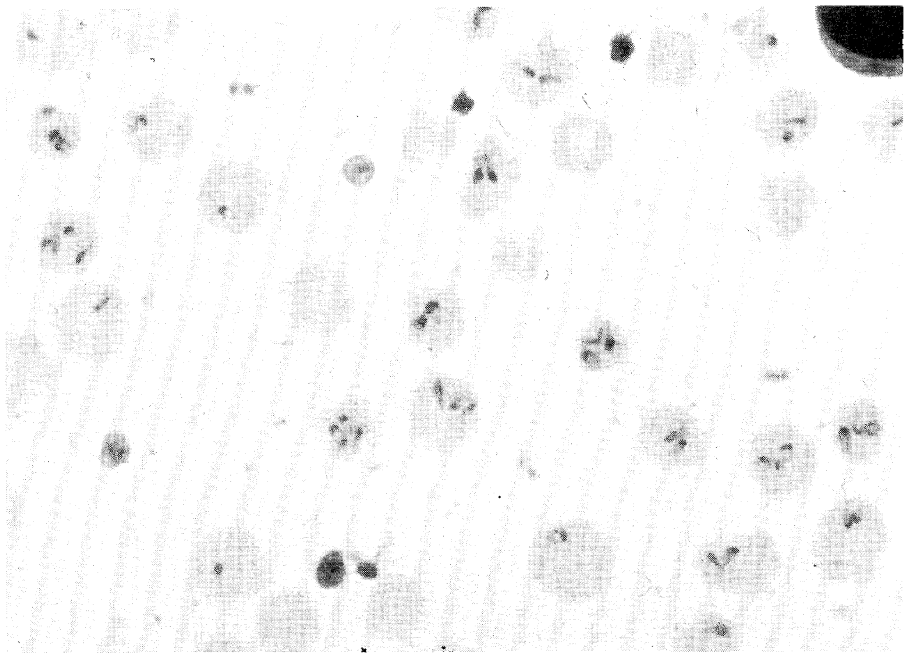

Fig. 2. Piroplasms of *T. parva* in blood smear. Giemsa staining method. ×1400.

sporozoites in the acinus cells of the salivary glands. Recent work on the cycle in the vector has mainly been carried out by Schein and co-workers in Berlin.

The various species of *Theileria* are distinguished by differences in the morphology of both piroplasms and schizonts, serological differences (the indirect fluorescent antibody (IFA) test being the main one used), differences in vector species and differences in pathogenicity; knowledge of biochemical differences is still virtually nonexistent. The shape of the piroplasms varies from round and oval to elongate, rod and comma shaped, but the percentages of these different forms are to some extent variable and cannot be considered as a reliable criterion for the distinction of species, although in combination with other characters they may be of some help. In some species a bar-like structure and/or an oval or rectangular body consisting of crystalloid hemoglobin-derived material occurs in the cytoplasm of the infected red cells (Fig. 3); for further details of these structures, the reader is referred to Van Vorstenbosch *et al.* (1978) and Young *et al.* (1978a). The schizonts of a few species have not yet been discovered, while *T. mutans* is distinguished by its large macroschizonts, containing relatively few large nuclei.

Until recently only three or four species were generally accepted, but it has become apparent that the taxonomy of bovine *Theileriae* is far more complicated than was recognized, and it should be realized that bovine theilerias in

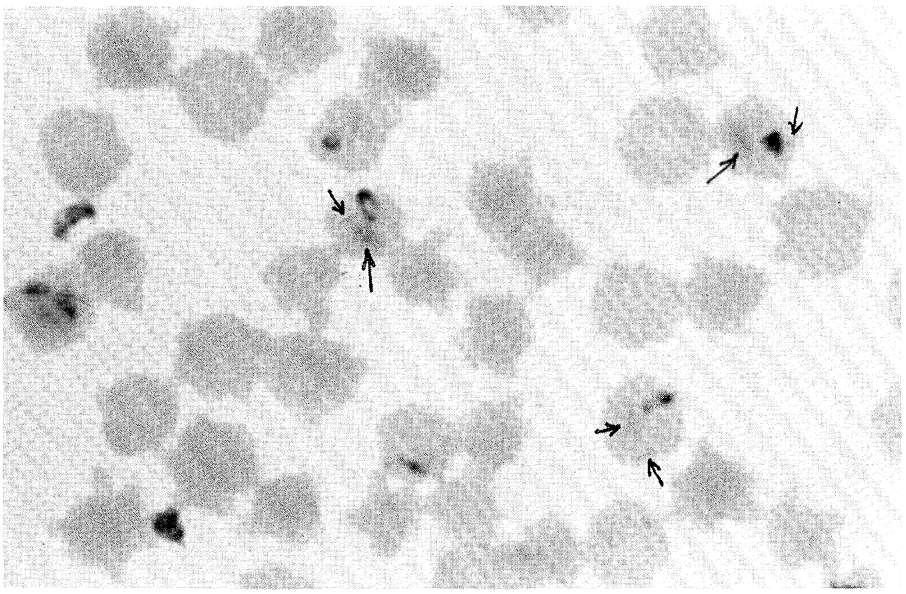

Fig. 3. Theileria sp. (England) in blood smear. Note associated bar as thin line and crystalloid body as dark oblong patch (arrows). Giemsa staining method. ×1600.

many parts of the world have not yet been properly examined as to their relationship with characterized species and strains.

Apart from *T. parva* reviewed in another chapter, we recognize the following species and groups of strains in cattle:

Theileria annulata. Pathogenic, causing Mediterranean or tropical theileriosis. Both schizonts and piroplasms are numerous in clinical cases. The schizonts are indistinguishable from those of *T. parva* while piroplasms are predominantly round and oval in shape (as opposed to *T. parva,* with numerous comma- and rod-shaped forms). Piroplasms are not associated with intraerythrocytic bar structures or crystalloid bodies. *Hyalomma* spp. are vectors. *Theileria annulata* occurs in Africa, Asia and Europe.

Theileria mutans. Although infection usually is subclinical or benign, clinical and even fatal disease has been reported. Macroschizonts are scanty, large with large nuclei (Young *et al.*, 1978b). Piroplasms are numerous in clinical cases; oval and round forms are common, but elongated and rod-shaped organisms with a long nucleus also occur in significant proportions. They are not associated with bars or crystalloid bodies in the erythrocyte cytoplasm. *Amblyomma* spp. are vectors. *Theileria mutans* occurs in Africa and is not a cosmopolitan parasite as was believed until recently.

Theileria ?taurotragi. Infection is usually subclinical or mild, however, a low mortality rate may occur. Both macroschizonts and piroplasms are normally scanty and are not numerous even in clinical cases. Macroschizonts are quite

similar to those of *T. parva,* but piroplasms are predominantly round and oval. There are no associated intra-erythrocytic bars or crystalloid bodies in cattle. This African species, the name of which is still tentative, confused in the past with *T. mutans* on one hand and with mild *T. parva* on the other, is probably derived from a *Theileria* of African eland antelope (*Taurotragus oryx*), infective to cattle (Grootenhuis *et al.*, in press). Furthermore, Grootenhuis (1979) who also summarizes the literature on the subject, makes a good case for the identity of this eland *Theileria* with the eland parasite described as *Cytauxzoon taurotragi.* *

Theileria velifera. Non-pathogenic. Schizonts have not yet been described. Piroplasms are usually scanty, but even in the rare cases with a high parasitemia, infection is subclinical. Piroplasms are predominantly rod-shaped and, except for the smallest forms, are associated with a distinct rectangular crystalloid body, appearing to arise laterally from the parasite; there is no bar structure. *Theileria velifera* is transmitted by *Amblyomma* spp. and occurs in Africa.

Theileria sergenti. Reportedly pathogenic, although experimental infections were less severe than field cases (Markov, 1962). The cause of East Asian theileriosis. In clinical cases schizonts are reportedly numerous although they do not appear to have been properly described, and piroplasm parasitemia is high. Piroplasms are predominantly rod-shaped and often very long. It is as yet unknown whether they are associated with bars or crystalloid bodies. *Theileria sergenti* has been assigned by most western workers to either *T. annulata* or to *T. mutans* but there are clear-cut morphological, serological and vector differences with *T. annulata* (Markov, 1962), while *T. mutans* has now been shown to be strictly an African species. *Theileria sergenti* is transmitted by *Haemaphysalis* spp. It occurs on the littoral of eastern USSR and probably elsewhere, but the identity of species associated with clinical disease in European breeds of cattle in other Asian countries remains to be settled. Japanese bovine theilerias are now commonly called *T. sergenti* but they do not appear to be very pathogenic by themselves (Ishihara, 1968) and, so far, the occurrence of schizonts has not been reported.

Finally, there is a group of normally non-pathogenic parasites in Australia, Asia, Europe, northern Africa and America, all being called *T. mutans.* Their status as yet is uncertain, as is that of inadequately described parasites in the eastern USSR, called *T. orientalis*, of which the validity is in doubt. It was originally described as non-pathogenic, but according to Gamaleev (1973), it may cause chronic disease with progressive anemia. Schizonts have not been

* The genus *Cytauxzoon* has been separated from *Theileria* by some workers on the basis of the type of host-cell parasitized by schizonts and the morphology of the latter.

reliably observed in infections caused by any of these parasites. Piroplasms are usually scanty (except in splenectomized animals).

Distribution

Theileria annulata, associated with its *Hyalomma* vectors, is known to occur in Portugal, Spain, Italy, the Balkan countries, Bulgaria and Greece in particular, Turkey, southern Russia and central Asia, the Near and Middle East, Pakistan and India, while in Africa its distribution is limited to the northern littoral, Morocco, Algeria, Tunisia and Libya, except in north-eastern Africa where it reaches down through Egypt to at least as far south as the 13th parallel in the Sudan and is reported from Erythrea.

Theileria mutans and *T. velifera* are present in most of Africa south of the Sahara, wherever their *Amblyomma* vectors occur, and *T. mutans* is suspected of occurring on some Caribbean islands where African *A. variegatum* has been introduced.

Theileria ?taurotragi is probably spread throughout the area of distribution of the vector *Rhipicephalus appendiculatus,* eastern, central and southern Africa.

Theileria sergenti and, if valid, *T. orientalis,* occur on the East Asian littoral, but may well have a wider distribution (see above), while as yet nameless theilerias, usually non-pathogenic, are known from all continents, Australia, Asia, northern Africa, Europa and, more rarely, North and South America. Clinical theileriosis is unknown in the Americas. Apart from American strains, all these parasites appear to be associated mainly with *Haemaphysalis* spp.

Epidemiology

Transmission of theileriosis in the field is only by means of ticks. Survival of *Theileria* species in the tick is transstadial but not transovarial. The infection does not pass through the egg to the next generation, thus unfed larvae are always free of the agent. In two-host ticks, in which the larva and nymph feed on one individual animal, only the adult can be infective. In three-host species, the larva can acquire the agent and transmit it as nymph, but the nymph frees itself of infection while feeding, consequently, the subsequent adult is not infective. However, a nymph acquiring the parasite from a positive animal can transmit it in the adult stage. Transmission does not normally being immediately when the infective tick attaches for feeding; parasites in the salivary glands mature to the infective sporozoite stage only after the tick has started to feed. This usually results in a safe pretransmission feeding period, which may last for 2 to 4 days and has obvious potential

implications for the frequency of acaricidal treatment aimed at preventing disease transmission. However, high temperatures to which ticks are exposed while on the host appear to be a major stimulus for sporozoite maturation (Samish, 1977), thus the pretransmission feeding period may be very short or nonexistent during very hot weather.

Theileria annulata is transmitted by ticks of the genus *Hyalomma*. Taxonomy in this genus was for a long time extremely confused, but most authors now follow the concepts of Hoogstraal, Kaiser, and co-workers. The major vector role appears to be played by the three-host *H. anatolicum anatolicum* (formerly *H. excavatum*) and the two-host *H. detritum*. The former is widely distributed in Eurasian and African areas where *T. annulata* occurs. *Hyalomma detritum* is limited to warm regions from China to northwest Africa. *Hyalomma asiaticum* is also a vector in southern Asia. *Hyalomma lusitanicum* has been suspected to play a role in western Mediterranean countries. Transmission has been achieved experimentally with several other species but these are unlikely to be important vectors in nature since their immature forms normally feed on small mammals and birds. Among the latter species are *H. dromedarii, H. anatolicum excavatum* (formerly *H. anatolicum*), and subspecies of *H. marginatum*. *Hyalomma scupense*, a one-host species, can also transmit *Theileria* experimentally, through the transfer of unfed adults, fed as nymphs on parasitemic animals, to susceptible hosts.

The epidemiology of Mediterranean theileriosis varies according to the climate and the vector species. In subtropical regions the disease has a pronounced seasonal character, the great majority of cases occurring in summer when adult ticks are most active. Seasonality may be less pronounced in some tropical countries where ticks are active to some extent the year around.

Recovered cattle remain healthy carriers of the parasite, and cattle alone are sufficient as a reservoir for the infection of ticks. Nevertheless, Asian water buffalo can be infected and generally have mild infections, so that in areas where they are common they may well be important additional reservoirs for the disease in cattle. Sheep are refractory, while fatal infection has been reported in American bison in a zoological garden, and the yak has also been reported as susceptible. The original host of *T. annulata* is unknown, but should be looked for among Asian ruminants such as the water buffalo or wild species of cattle.

Local cattle in areas where the disease is endemic are on the whole far less susceptible than imported stock. It is not quite clear whether animals of the same local breed but from theileriosis-free areas are also genetically more resistant, or whether the lower susceptibility of populations in endemic areas is solely due to natural genetic selection by local disease pressure. Breeds from

western Europe are highly susceptible, especially dairy cattle, while zebus in general may be less so and, on the whole, Mediterranean theileriosis becomes a major disease problem when European breeds are imported to replace or upgrade the local resistant zebu.

Calves appear to be more resistant than adult cattle, but whether this is solely or even partly due to maternal antibodies which have been demonstrated in the colostrum of immune dams is uncertain.

Differences in the virulence of individual strains of *T. annulata* and immunological responses of the host may influence the clinical picture of the disease.

Theileria mutans is transmitted by at least four species of African *Amblyomma*: *A. variegatum, A. cohaerens, A. hebraeum* and *A. gemma,* all three-host ticks. The epidemiology has hardly been studied. Calves in endemic regions are all infected early in life and remain life-long carriers. Fatal cases in East Africa have been seen in older animals which had not been exposed to infection, and it appears that some strains are more pathogenic than others. The African buffalo (*Syncerus caffer*) is almost certainly the original host of *T. mutans* as it is of *T. parva.*

Theileria ?taurotragi is transmitted by *Rhipicephalus appendiculatus,* a three-host tick which is also the vector of *T. parva.* Eland strains of this theileria have also been experimentally transmitted by *R. pulchellus,* a dry area tick in eastern Africa, so the potential distribution of the parasite extends outside *R. appendiculatus* areas. The eland antelope is probably its original host.

Theileria velifera is transmitted by at least three species of African *Amblyoma: A. variegatum, A. lepidum* and *A. hebraeum.* Infected animals remain carriers, probably for life. The African buffalo is almost certainly the original host. As it is not a disease problem, there is no need for further consideration of this species.

Theileri sergenti is transmitted by *H. longicornis,* while *H. japonica* and *H. concinna* are the vectors of *T. orientalis* in the east of the USSR, according to Gamaleev (1973). Nonpathogenic theilerias in western Europe are transmitted by *H. punctata.*

Experimental mechanical transmission with blood is easy with most species of *Theileria* except for bovine strains of *T. parva* where large numbers of schizonts have to be present in the inoculum to ensure infection. Mechanical transmission of theileriosis does not play a role in the field. Intra-uterine transmission has been observed, but it is an exceptional occurrence.

Clinical Signs

The incubation period (between tick attachment and onset of fever) of Mediterranean theileriosis is on the average 2 weeks with extremes of 8 and 30

days, according to Sergent *et al.* (1945), while shorter periods were reported by Gill *et al.* (1977). Incubation periods of some 10 to 15 days are common for most *Theileria* species, but longer periods may be encountered and Zolotarev (1956) reports 35 days as the average for *T. sergenti*.

Mechanically transmitted *T. annulata* may on occasion become clinically apparent only after several months but on the average the incubation period is only slightly longer than after tick-transmitted infection. Intravenous infection with blood is, as a general rule, followed by a shorter prepatent period than subcutaneous infection.

The severity of Mediterranean theileriosis depends on the susceptibility of the animal and the virulence of the strain. It is also dose dependent, the more sporozoites injected by ticks, the more severe is the disease on the average, and this is probably true for *Theileria* infections in general.

In typical acute *T. annulata* infection, as is commonly seen in European dairy cattle, the disease starts with high fever and swelling of the superficial lymph glands. This is preceded by swelling of the regional lymph gland draining the area where the infective ticks are feeding but this is overlooked in the field. General symptoms associated with febrile infectious diseases soon follow: listlessness, accelerated pulse and respiration rate, while the eyelids are often swollen and lachrymation is commonly seen. Milk production drops suddenly, rumen movements stop, and constipation is common but may be followed by diarrhea. Abortion may occur. The appetite may remain normal in the early stages. Anemia dominates the picture at the last and icterus may also occur; cutaneous nodules are sometimes present. In fatal infections the animal dies in an emaciated state after having remained recumbent for a variable number of days, often in hypothermia. Death usually occurs one to two weeks after the onset of clinical signs but in hyperacute cases the animal may die as early as 3 days after the first obvious symptoms are noticed.

In cattle that will recover, symptoms are less marked and gradually wane from one to several weeks after their first appearance but it may take a long time before a full recovery takes place. Cases are on record where the disease has suddenly flared up again after several weeks or even months, with rapid death. In local cattle, especially young calves, symptoms are often so slight that the infection passes unnoticed. The mortality rate by *T. annulata* in susceptible adult European dairy cattle is usually at least 70% of the infected animals.

Depending on the history of the herd and the disease situation in the region, a probable diagnosis of Mediterranean theileriosis is often possible on the basis of clinical signs. Differential diagnosis should take into account other diseases associated with general febrile symptoms and anemia.

Theileria mutans infection also starts with fever and swelling of the super-

ficial lymph glands, often limited to the local draining one. Fever, usually moderate, lasts for a short time, often only one day. Anemia becomes apparent in some cases a few weeks after the onset of fever, but the slight symptoms are usually inapparent under field conditions, even in European breeds of cattle. Some strains appear to be more pathogenic and may cause fatal infection associated with severe anemia and icterus but it is unlikely that even such strains cause serious problems in endemic areas where calves are exposed to infection early in life. Clinical diagnosis of *T. mutans* infection is virtually impossible; laboratory examination of blood smears is necessary to differentiate the condition from other causes of anemia (babesiosis, anaplasmosis, trypanosomiasis, etc.), while serological techniques are needed to distinguish between this and other species of *Theileria*. If characteristic schizonts are found in lymph or blood smears, specific diagnosis is virtually assured.

Little is known concerning the symptomatology of *T. ?taurotragi* infections. Kenyan and Tanzanian strains have consistently caused subclinical infections, mild fever and pronounced swelling of the local draining lymph glands being the only symptoms. Rhodesian strains, however, have sometimes caused alarming clinical signs in experimental animals in our laboratory although all have recovered; animals showed listlessness, swollen eyelids, lachrymation and accelerated respiration. Here again, clinical diagnosis is very difficult, and differentiation from *T. parva* (with which it shares the morphology of the macroschizont) and from *T. mutans* (which it resembles in its usually low pathogenicity) is possible only in the laboratory.

According to Markov (1962), typical *T. sergenti* infection in the field is an acute disease with symptoms which are comparable to those reported for *T. annulata* but the experimental disease was less severe. The mortality rate may be high, according to Zolotarev (1956). *Theileria orientalis,* usually considered to be non-pathogenic, is reported by Gamaleev (1973) as causing chronic debilitating infection.

A cerebral form of theileriosis has been described as "turning sickness", associated with *T. parva, T. annulata* and *T. mutans*, or more likely *T. ?taurotragi*. Various lesions have been reported in the brain, hemorrhages, perivascular encephalitis, necrosis, and the condition may be associated with the presence of schizont-infected lymphoblasts in the brain capillaries. This form sometimes occurs in immune animals, possibly associated with stress.

Pathology

Not all aspects of the pathogenesis of theileriosis are well understood. It is related on one hand to the schizontic stage and its effect on the reticuloen-

dothelial system and on the other hand to the piroplasm stage affecting the erythrocytes. The existence of toxins has been postulated in an effort to explain some of the phenomena.

The infection has an unexplained mitogenic effect on the lymphocytes, starting with hyperplasia of the local lymph gland draining the area where the infecting ticks feed. Transformation of lymphocytes into lymphoblasts and intensive mitosis begins even before schizonts are detected. The hyperplasia, followed by the presence of schizonts, spreads to other lymph nodes and lymphoid organs, mainly in *T. annulata* infections, less so in other species. An unexplained fact is that while leukopenia is characteristic of *T. parva* infection, the number of circulating leukocytes increases in Mediterranean theileriosis. Local hyperplasia due to disseminated infected lymphoblasts gives rise to lesions in the kidneys, liver, and occasionally in the skin.

A reduction in erythrocyte number occurs during parasitemic crisis. This appears to be due to removal of the infected erythrocytes by phagocytosis rather than desctruction by the parasite. It has also been suggested that an autoimmune mechanism might contribute to anemia. Bilirubinemia and bilirubinuria associated with icterus are also observed.

In Mediterranean theileriosis, both the schizontic and the piroplasm stages contribute to pathogenesis, while in *T. mutans* pathogenesis is almost solely due to the erythrocytic stage. Curiously, in east coast fever where parasitemia

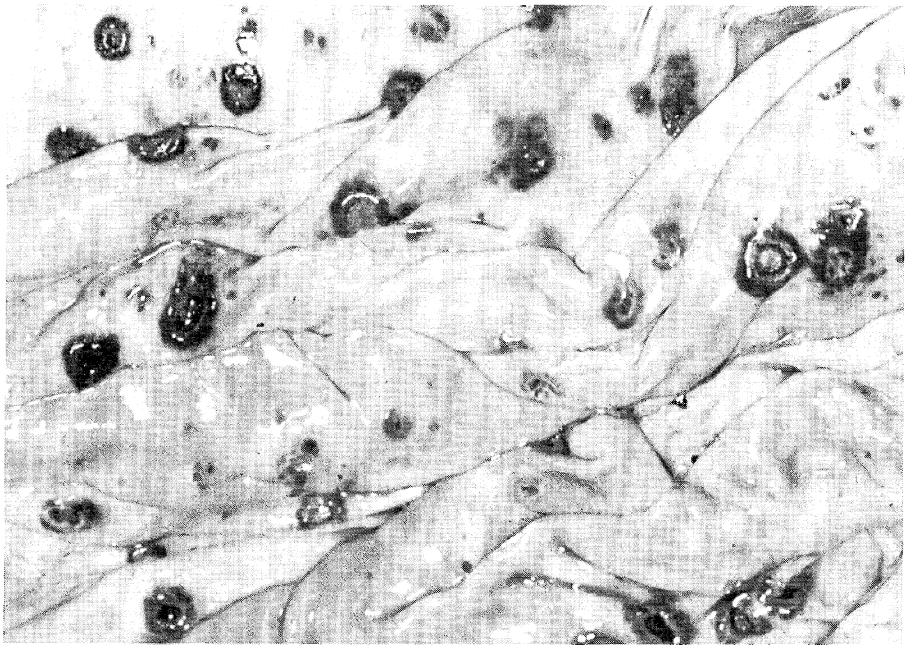

Fig. 4. Abomasal ulcers in a fatal *T. annulata* infection.

is commonly extremely high, anemia is not usually an important feature of the pathogenesis.

Lesions at autopsy in Mediterranean theileriosis vary, depending to a large extent on the duration of the disease. The carcass is usually emaciated, anemic and often icteric, except in hyperacute cases. Classical lesions are pronounced hypertrophy of the spleen and lymph nodes, hemorrhages on and in the heart, in the skin, the pleura, the peritoneum, various mucosae and elsewhere, walled ulcers with a necrotic center in the abomasum (Fig. 3), and whitish or red foci at the surface of the renal cortex and sometimes the liver. Ulcers may also occur in the intestine and there may be hemorrhagic gastroenteritis. The liver is enlarged and the gall bladder distended. Lung oedema is frequent and froth may be present in the respiratory passages. All these lesions are not always present, especially not in hyperacute cases.

Animals which have died of *T. mutans* infection show a severe anemia, often icterus and brown urine, while the spleen and liver may be enlarged.

Symptoms in *T. sergenti* infection as reported by Markov (1962) are emaciation, anemia, icterus, splenomegaly, hypertrophy of the lymph glands, hemorrhages in serous and mucous surfaces, and ulcers in the abomasum.

Little work has been carried out on the microscopical pathology of theileriosis. Interested readers are referred to Gill *et al.* (1977).

Immune Response

Recovery from natural infection is followed by solid immunity to the homologous strain. The duration of immunity is not well known and it may start to wane gradually after 2 or more years. The nature of the immunity has not yet been satisfactorily elucidated. Although high titers of antibodies, detectable by various serological techniques, are often present shortly after recovery, these antibodies appear to play little if any role in protection, and immunity persists even after they have become undetectable. Immunity is therefore presumed to be cell mediated, and some indications of this have been reported.

Except for classical cattle-adapted strains of *T. parva,* recovery from *Theileria* infection is followed by the carrier state, which is often life-long. Piroplasms are present in the blood although there may be so few that they are difficult to detect microscopically; a parasitemic relapse of most species can be induced by splenectomy. Whether schizonts also persist is uncertain.

Laboratory Aids to Diagnosis

Although the case history, clinical signs and lesions are often indicative of theileriosis, a definite diagnosis is possible by laboratory methods only.

Thin smears of blood and of samples obtained by needle biopsy of enlarged superficial lymph nodes are stained by the Giemsa method and examined microscopically. Interpretation of the results is not always easy, and it is important to know which species of *Theileria* may be encountered in the area. If schizonts are found in the lymph node material and in blood smears, the diagnosis of theileriosis is confirmed. If a low piroplasm parasitemia is found in the absence of schizonts, however, a diagnosis of theileriosis is not justified as the animal is likely to be a healthy recovered carrier. On post mortem examination, schizonts may be found in most organs including the spleen and the liver.

Aside from direct recognition of the organism in stained smears, serologic diagnosis may be used as an auxiliary method. Most frequently used is the indirect fluorescent antibody (IFA) test. The antigen for this test is derived from the blood of acutely infected animals or schizont-infected lymphocyte cultures. A peak antibody titer in the IFA test is manifested shortly before death or at early recovery from the acute stage of infection. Other serologic tests for theileriosis are the complement fixation test, a capillary agglutination test and the indirect hemagglutination test, however, their reliability is not always proven. Antigens are species-specific, although some cross-reactivity sometimes occurs. The high antibody titers in recently recovered animals decline and often disappear after a variable number of months, thus random serological surveys are not a good indicator of the number of immune animals in a population.

Prevention and Control

Under natural conditions, theileriosis is transmitted by ticks only. There is no hope of eradicating the vectors over large regions because they are two- and three-host tick species which remain on the host for relatively short periods. Various wild and domestic animals constitute the vector's reservoir hosts with immature stages often feeding on small wild animals. Emerging acaricide resistance is a further complicating factor, consequently, effective short-interval dipping of cattle is recommended for prevention of disease transmission. In situations where tick life is maintained by wild and untreated domestic animals or by uncontrolled stray cattle, the required safe frequency of treatment with most acaricides is twice a week. On well-managed fenced farms without significant wild life, weekly dipping will give good results once the tick population has been practically eliminated.

It should be remembered that in situations where local cattle live in a state of endemic stability with *Theileria* spp., intensive tick control is unnecessary,

uneconomical and even harmful as it will disturb the stable situation, not only as far as theileriosis is concerned, but also for other tick-borne infections as babesiosis and anaplasmosis. Control should be limited to preventing harmful direct effects of excessive tick numbers.

In some countries imported cattle are kept in stables under conditions of zero-grazing to avoid all contact with ticks. This method of control is laborious, expensive and of little help to the small farmer. Moreover, ticks are sometimes brought in with fodder. In some subtropical regions *Hyalomma* vectors of Mediterranean theileriosis behave as barn ticks by engorging and hibernating in cracks of walls. Improvement of masonry and spraying acaricides on the walls should be advised in this case.

Strains of *Rhipicephalus* and *Amblyomma* spp. resistant to arsenicals, organochlorine and organophosphate have been found in countries where acaricidal control has been carried out intensively (usually because of the presence of *T. parva*).

Drug resistance in *Hyalomma* and *Haemaphysalis* is still rare. Should acaricide resistance problems become unmanageable, alternative tick control methods such as those used against *Boophilus* ticks promise little success for the control of multi-host tick vectors.

Immunization methods against *T. annulata* are thoroughly reviewed by Pipano (1974, 1977a, 1977b). Formerly, blood vaccines containing schizonts of a mild mechanically transmitted strain of *T. annulata* which had lost the capability of producing erythrocytic forms, were used with some success against Mediterranean theileriosis (Sergent *et al.*, 1945). This method is now replaced by the use of live schizonts grown in lymphocyte cell culture, attenuated by prolonged *in vitro* passage. Attenuated schizonts do not produce erythrocytic forms so that immunization does not produce reservoirs of infection for ticks. There have been no indications of reversal to virulence. Calves and beef cattle immunized with attenuated schizonts withstand tick-borne challenge, but highly susceptible dairy cattle may react clinically although rarely fatally, and pregnant animals may abort. Immunity induced by schizonts protects against schizonts of the homologous strain but only partially against sporozoites. Experiments on the infection and treatment method of immunization developed for *T. parva* using sporozoites obtained from prefed ticks and simultaneous tetracycline treatment (see chapter on East Coast fever), have also been undertaken for *T. annulata* with promising results (Gill *et al.*, 1978).

Immunization with attenuated schizonts is at present carried out on a significant scale only in a few countries. In Israel the vaccine is used in the form of small pellets frozen in liquid nitrogen. The vaccine organism will remain alive for 3 days at 4 °C. Because of a possible immunologic difference

between various isolants of *T. annulata,* it is advisable to use local strains for production of vaccine.

If *T. mutans* is a problem, it is possible to immunize animals with blood from a carrier of a mild strain.

Up to very recently, there was no specific drug on the market for the treatment of Mediterranean theileriosis. The future has now become considerably brighter since it was found that a recent coccidiostat, halofuginone, cures clinical cases of Mediterranean theileriosis, East Coast Fever, corridor disease as well as *T. mutans* infection (Schein and Voigt, 1979; G. Uilenberg *et al.*, unpublished). The drug appears to be mainly schizonticidal and is curative in doses of 1 to 2 mg/kg, administered orally, as a commercially available premix (Stenorol®), containing 0.6% of the active ingredient. It is active on schizonts in cell culture in concentrations as low as 0.02 ppm. The toxic safety margin appears to be unfortunately rather narrow. Another drug, a hydroxy napthoquinone (Menoctone), is also curative in East Coast Fever and Mediterranean theileriosis, but has been found to be unsuitable for marketing and the search for related compounds is on.

The use of tetracyclines is limited to the infection and treatment method of immunization, where they effectively suppress the development of clinical disease if administered simultaneously with and for a short further period following the administration of a controlled dose of sporozoites.

Some antimalarial aminoquinoline compounds, such as primaquine and pamaquine, are active against the erythrocytic forms of various theilerial species (see Neitz, 1957).

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34. BESNOITIOSIS AND GLOBIDIOSIS

R.D. Bigalke

A: BESNOITIOSIS

Abstract. Bovine besnoitiosis, previously known as globidiosis, is a disease which is fairly widespread in Africa but not limited to that continent in its distribution. It is caused by the protozoan parasite *Besnoitia besnoiti* of which cattle are the intermediate and felids the final hosts. Clinically affected animals pass through an acute, febrile, anasarca stage and a chronic scleroderma stage of the disease. The mortality rate is low but affected animals suffer a severe loss of productivity and bulls are frequently rendered permanently infertile. Clinically inapparent cases are common. A significant aspect of the pathogenesis of the disease is the destruction of endothelial cells by the proliferating obligate intracellular endozoites. The large, thick-walled cysts are pathognomonic. Recovered animals are immune to reinfection. In some countries cattle are immunized by inoculation with a live vaccine consisting of a blue wildebeest (antelope) strain of *B. besnoiti* grown in cell culture.

Disease

Bovine besnoitiosis of cattle was previously known as bovine globidiosis, globidiose cutanée du boeuf and sarcosporidiose cutanée chez bovins (Pols, 1960). Colloquial descriptive names such as olifantsvelsiekte (Afrikaans) or elephant skin disease, and anasarque du bœuf or elephantiasis du bœuf (Pols, 1960) have been coined in countries where the disease is or was well known.

Bovine besnoitiosis is either a severe but usually non-fatal disease of cattle or a mild disease caused by the protozoan parasite *Besnoitia besnoiti*. The severe form of the disease is characterized by fever, anasarca, inappetence, hyperemia of the skin and muzzle and orchitis in the early acute stage, which is associated with proliferation of parasites in endothelial cells of blood vessels in superficially situated tissues. This is followed by a chronic stage, characterized by scleroderma, hyperkeratosis, alopecia, loss of necrotic epidermis and atrophy and induration of the testes of bulls. This stage is associated with the development of cysts in the same tissues where the initial proliferation occurred.

Description and Life Cycle

Besnoitia besnoiti, synonyms *Sarcocystis besnoiti* and *Globidium besnoiti* (Marotel, 1912), is both morphologically and in its developmental cycle similar to *Toxoplasma gondii.* Both protozoan parasites are coccidian in nature and have been classified in either the family Toxoplasmatidae (Frenkel, 1974) or Eimeriidae (Levine, 1977) of the class Sporozoa.

Based on observations made on other *Besnoitia* spp. (reviewed by Dubey, 1977, and Rommel, 1978) and a single, as yet unconfirmed, investigation on *B. besnoiti* (Peteshev, 1974), it is reasonable to assume that cattle contract besnoitiosis by ingestion of mature isosporan-type oocysts shed in the feces of members of the cat family. Peteshev showed that both domestic cats and a wild cat, *Felis lybica,* shed such oocysts after ingestion of cyst-containing tissues.

The sporozoites which emerge from the oocysts enter the circulation and multiply by endodyogeny in endothelial cells of blood vessels, particularly in the dermis, subcutis, fascia and upper respiratory tract (Basson *et al.*, 1970; Rommel, 1978). Merozoite-like endozoites (= tachyzoites) (Fig. 1; Table 1) emerge from damaged cells to re-invade adjacent or more distant cells to produce further endozoites. This cycle of development is superseded by cyst formation.

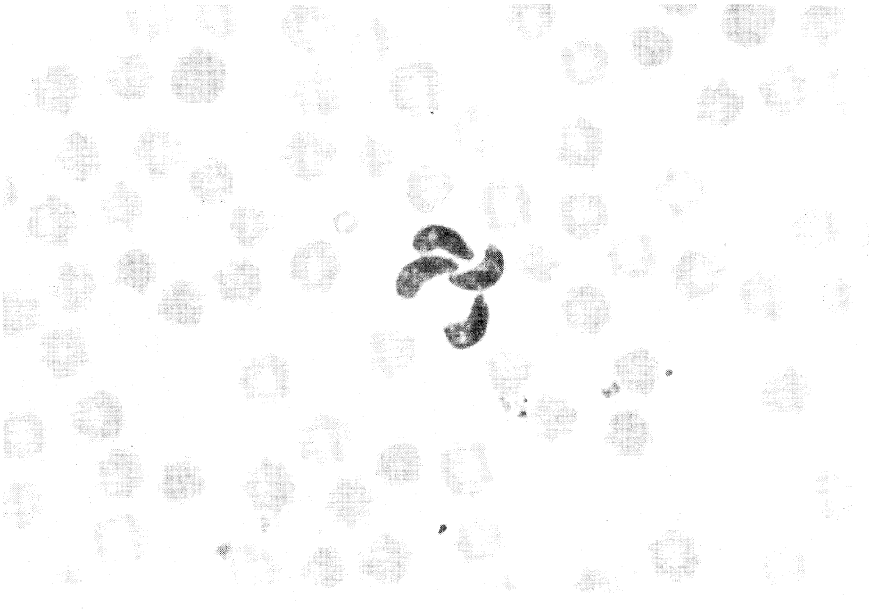

Fig. 1. Endozoites (= tachyzoites) of *Besnoitia besnoiti* in a blood smear prepared from an experimentally infected steer on the 5th day of the febrile response (8th day post-infection). ×1200.

Table 1. Important features in the life cycle of *B. besnoiti.*

	Bovine (intermediate) host			*Felid (final) host*		
	Endozoites (= Tachyzoites)	*Cystozoites (= Bradyzoites)*	*Cysts*	*Schizonts*	*Gametocytes*	*Oocysts*
Size (μm)	5.9 × 2.3 (4.5–7.5 × 1.5–3.8)	8.4 × 1.9 (6.7–10.4 × 1.5–3.7)	Up to 394	Not known	Not known	15.1 × 12.9 (14.2–16.0 × 11.6–14.2)
Shape	Crescentic to pyriform	Crescentic to pyriform	Spherical to subspherical	Not known	Not known	Ovoid
Host cells	Endothelial	Activated histiocyte	Activated histiocyte	Not known	Not known	—
Multiplication	Endodyogeny	Endodyogeny	—	Schizogony	Gametogony	Sporogony (outside host)
Sites	Dermis subcutis fascia ant. resp. tract	—	Dermis subcutis fascia ant. resp. tract	Gut wall	Gut wall	Feces

Cyst formation commences approximately 1 week after the initial cycle of proliferation (Basson *et al.*, 1970) when activated, hypertrophic cystozoite-containing histiocytic cells, in or in close association with blood vessel walls, become detectable in the same sites where the endozoites were formed (Table 1). The merozoite-like cystozoites (= bradyzoites) multiply by endodyogeny (Rommel, 1978) in vacuoles of the markedly enlarging host cells with hyperplastic and hypertrophic nuclei. The characteristic thick-walled cysts (Fig. 2) reach a size of up to about 400 μm and contain approximately 200 000 cystozoites each, which are similar to endozoites in appearance (Table 1). Cyst formation is remarkably synchronous and there is no evidence that cystozoites from disintegrating cysts give rise to further cysts or endozoites in the same animal (Pols, 1960; Bigalke, 1968).

The developmental cycle of *B. besnoiti* in the carnivorous (final) host has not been studied in detail but it is probably similar to that of other *Besnoitia* spp. (reviewed by Dubey, 1977, and Rommel, 1978). Following the ingestion of cyst-containing tissues, the liberated organisms presumably enter the gut wall in which a typical coccidian life cycle of schizogony followed by gametogony occurs with the production of unsporulated oocysts (Peteshev, 1974) which are passed into the environment with the feces (Table 1). Sporogony follows on the ground with the development of infective isosporan-type oocysts awaiting ingestion by the intermediate (bovine) host.

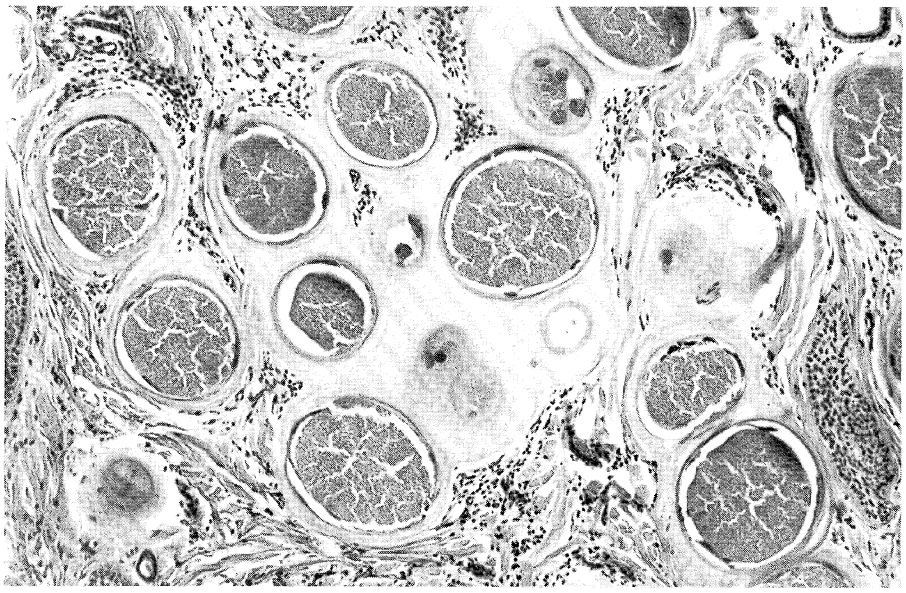

Fig. 2. Mature cysts of *Besnoitia besnoiti* in the stratum papillare of the dermis of the skin of a naturally infected bull. ×75

Table 2. The distribution of *B. besnoiti.*

Continent	*Country*	*Natural hosts*	*Level of endemicity*
Africa	South Africa	Cattle	Enzootic – regional
		Blue wildebeest (viscerotropic strain)	Enzootic – regional
		Impala (viscerotropic strain)	Enzootic – regional
		Kudu (unidentified *Besnoitia* sp.)	Enzootic – regional
	Swaziland	Cattle	Not known
	Botswana	Cattle	Not known
	South-West Africa/Namibia	Cattle	Sporadic
	Zimbabwe	Cattle	Sporadic
	Angola	Cattle	Not known
	Zaire	Cattle	Not known
	Kenya	Cattle	Not known
		Goats (unidentified *Besnoita* sp.)	Not known
	Tanzania	Cattle	Not known
	Uganda	Cattle	Not known
	Sudan	Cattle	Not known
	Cameroon	Cattle	Not known
	Nigeria	Cattle	Not known
Europe	France	Cattle	Not known
	Portugal	Cattle	Not known
Asia	Israel	Cattle	Enzootic
	Iran	Goats (unidentified *Besnoita* sp.)	Not known
		Wild goats (unidentified *Besnoitia* sp.)	Not known
	USSR	Cattle	Enzootic – regional
	South Korea	Cattle	Not known
South America	Venezuela	Cattle	Not known

Distribution

The current distribution of bovine besnoitiosis is summarized in Table 2. Although the disease was originally recorded in southern France, it now seems to be more important in Africa where it is widespread, and notably so in the Republic of South Africa where it presents a definite economic problem. Distribution of the disease suggests preference for a less temperate, even subtropical, climate. In South Africa the majority of fresh cases occur during the warmer, moister months of the year. Considerable research has also been devoted to the disease in southern Russia, Israel and South Korea, where it is also of economic importance.

Epidemiology

The epidemiology of bovine besnoitiosis awaits further elucidation, particularly as regards the role played by the carnivorous host.

Susceptibility of Cattle and Incidence

All breeds of cattle seem to be susceptible to besnoitiosis and in South Africa most cases occur in the Africander, a *Bos indicus* breed, which is the commonest breed in the enzootic region. For unknown reasons infections are rarely encountered in calves under 6 months of age. The highest incidence of infection was detected in the 18-month to 6-year-old animals on a farm where the disease was prevalent. Comparatively few animals, though, develop the typical clinical signs of the disease. Out of a total of 5018 head surveyed by scrutiny of the scleral conjunctiva for cysts, 427 (8.5%) were infected, of which 74 (17.3% of infected animals and 1.5% of the total number examined) showed typical clinical signs of chronic infection (Bigalke, 1968).

Carriers

There is good experimental and circumstantial field evidence that chronically infected cattle harboring large numbers of cysts serve as carriers of the disease (Bigalke, 1968). Although a variety of laboratory rodents such as rabbits, gerbils, hamsters and mice, and sheep, goats and black wildebeest have been found to be susceptible to artificial infection with bovine strains of *B. besnoiti* (Pols, 1960; Bigalke, 1968, and unpublished observations; Dubey, 1977), no naturally infected hosts other than cattle have been found. Strains of *Besnoitia*

isolated from two species of antelope (blue wildebeest and impala), though antigenically closely related to bovine strains of *B. besnoiti,* were viscerotropic rather than dermatotropic in bovine and rabbit hosts, and relatively apathogenic (Bigalke *et al.*, 1967). Consequently, it seems most unlikely that antelopes infected with the above-mentioned strains would serve as carriers for the typical bovine disease. The relationship of the *Besnoitia* sp. found in goats (Bwangamoi, 1967; Cheema and Toofanian, 1979) and *B. tarandi* of reindeer (Dubey, 1977) to *B. besnoiti* has not been determined.

Mechanical transmission of bovine besnoitiosis from chronically infected cattle to susceptible ones by hematophagous insects, although probably involved in the epidemiology of the disease, does not solve the transmission riddle since only clinically inapparent cases were thus produced (Bigalke, 1968).

It is clear that a carnivorous host must also exist for *B. besnoiti. Felis lybica,* shown by Peteshev (1974) to produce isosporan-type oocysts is, for instance, fairly common in the enzootic region in South Africa. Domestic cats, however, are absent on many infected farms. Moreover, it is difficult to understand how a small wild cat such as *F. lybica* can become infected from cattle on such farms. It is therefore probable that a hitherto unknown cycle of transmission involving a carnivorous host occurs in nature.

Clinical signs

Typical clinical signs of bovine besnoitiosis appear in two distinct sequential stages, namely, the acute anasarca stage, which is mainly associated with proliferation of endozoites, and the chronic scleroderma stage which is mainly associated with cyst formation (Table 3).

Anasarca Stage

The first clinical sign to appear is a pronounced febrile reaction. An incubation period of 4 days has been recorded after infection with sporulated oocysts of feline origin (Peteshev, 1974), whereas a mean interval of 13 days has been observed after mechanical transmission of the disease by Bigalke (1968) on whose observations much of the following description is based. The temperature reaches 40 to 41 °C or even higher, and may remain at that level for 7 days or more.

Pyrexia is accompanied by progressive inappetence, leading to complete anorexia in severe cases, and loss of weight. The respiratory rate increases while animals seek the shade, are listless and prefer the recumbent position,

walking with a slow gait if forced to move. Hyperemia of the muzzle, periorbital skin and scrotum is usually more clearly noticeable in light skinned animals and appears a few days after the rise in temperature. Anasarca also usually appears at about this time, often presenting itself as nothing more than a slight filling of the face, or thickening of the skin folds of the neck, back or chest region. In more severe cases, the skin of virtually the entire body may be edematous. The testes of bulls are swollen and very sensitive to palpation. In many cases, however, early anasarca goes unnoticed and its presence is revealed only when edema fluid begins to accumulate along the lower reaches of the body such as the inter-mandibular, brisket, sternal, abdominal, preputial, tail and limb regions. Occasionally the anasarca stage is not detected at all.

There may either be a distinct break between the anasarca and scleroderma stages, with disappearance of edema by the end of the third week of the reaction, subsidence of temperature and some improvement of appetite, or a merging of the two stages (Table 3).

Scleroderma Stage

The most characteristic symptom of this stage is scleroderma manifested by progressive thickening, hardening, decreasing elasticity and consequential prominent folding, and puckering of the skin (Fig. 3), a feature which usually

Table 3. Sequence of appearance of clinical signs in a typical (experimental) case of bovine besnoitiosis.

	Sequence of appearance of clinical signs	*Days post-infection*
	Pyrexia	3
	Inappetence	3
	Polypnea	3
	Hyperanemic muzzle	4
Anasarca	Endozoites appear (blood smears)	4
stage	Weakness in hindquarter	6
	Anasarca	7
	Endozoites disappear (blood smears)	12
	Cysts (skin sections)	13
	Temperature normal	14
	Necrosis of skin	21
	Anasarca disappears	23
	Alopecia	25
Scleroderma	Scleroderma	25–35 (various sites)
stage	Lymph nodes enlarge	29
	Cysts in scleral conjunctiva	36
	Cysts mature (skin sections)	71

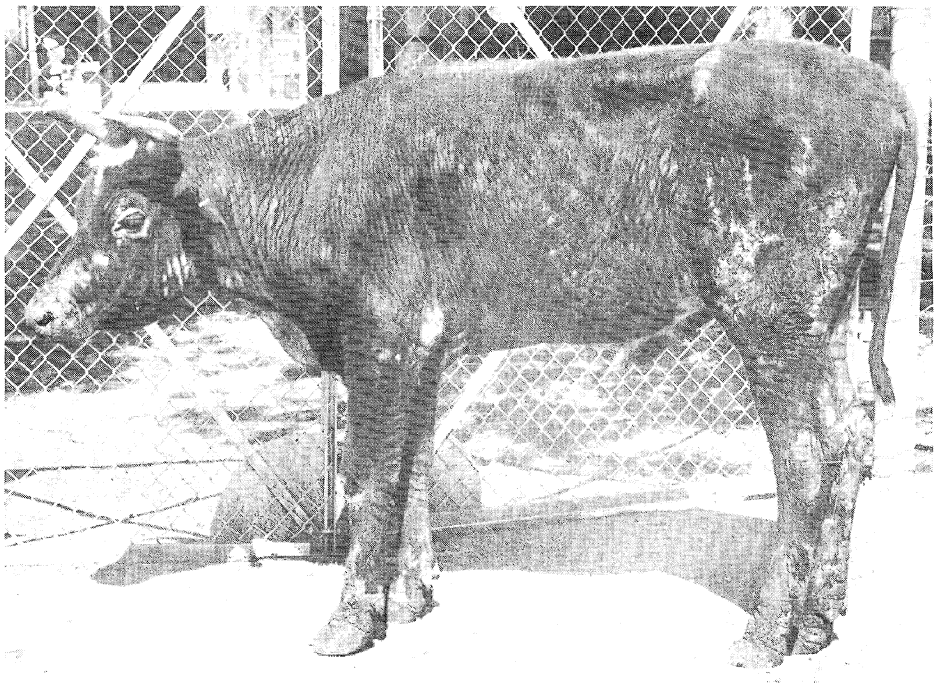

Fig. 3. Naturally infected ox in the scleroderma stage of the disease. Note thickened skin folds, alopecia, seborrhea, nasal crusts and poor condition.

appears from 3 to 4 weeks after the rise in temperature. This is accompanied by progressive loss of hair, which is more pronounced over the severely affected parts and may be quite extensive in severe cases. The epidermis may even be shed in patches, leaving grey seborrheic areas, and sitfasts are commonly seen over bony prominences as well as over the ventral portion of the scrotum of bulls. Deep, raw, maggot-infested fissures sometimes develop between folds of skin in sites such as the breech and flexor surfaces of the limbs. Seborrhea is a characteristic, chronic feature in all cases. Nodule formation accompanied by loss of hair sometimes occurs around the eyes and mouth in milder cases. The superficial lymph nodes are invariably swollen.

A mucopurulent nasal discharge, which forms crusts and clogs the nostrils (Fig. 3), and stridor are not uncommon in severe cases. An undulant, low level febrile reaction may persist during the first few weeks of this stage, the animal is listless, its appetite poor and emaciation is invariably present to a greater or lesser degree. One or both testes may become permanently atrophic and indurated. Bulls invariably develop an aspermatogenesis, which is usually permanent on account of the severe testicular lesions which usually develop 2 weeks after the initial clinical reaction (Pols, 1960). Cysts become visible

about 6 to 7 weeks after the rise in temperature in a site such as the scleral conjunctiva (Bigalke, 1968).

Death may occur in either the anasarca or scleroderma stages, and although most animals survive, convalescence is slow. In severe cases the characteristic scleroderma and alopecia are life-long features despite a gradual improvement in the appearance of the animals.

Clinically inapparent infections which go unnoticed by farmers but possibly manifest themselves by mild febrile reactions and decreased appetite, represent the majority of cases in the field.

Pathogenesis

As obligate intracellular parasites, endozoites proliferating in cells of blood vessel walls cause degenerative and necrotic vascular lesions, vasculitis and thrombosis in mainly the medium and smaller veins and capillaries of the dermis of the skin, subcutis, nasal mucosa, larynx, trachea and testes (Basson *et al.*, 1970). These lesions, together with a postulated toxic effect, apparently cause an increased permeability of blood vessels, with anasarca and subcutaneous edema as a consequence, ischemic infarcts and areas of necrosis in the above-mentioned sites.

The radical circulatory disturbances also account for the alopecia and necrosis of the skin and mucous membranes of the upper respiratory tract seen in the scleroderma stage of the disease. Millions of rapidly growing, thick-walled cysts (Fig. 2), especially in the dermal papillae and other parts of the stratum papillare, and the granulomatous reaction and accompanying fibrosis, hyperkeratosis and acanthosis account for the typical scleroderma (Basson *et al.*, 1970).

Lesions, which were described in the previous section, are largely confined to the visible or palpatable portions of the body.

Immune response

There is good circumstantial field evidence that cattle that have contracted either clinical (Hofmeyr, 1945, cited by Pols, 1960) or subclinical besnoitiosis (Bigalke, unpublished observations) are immune for life.

Naturally infected cattle and experimentally infected cattle and rabbits that were challenged by inoculation with homologous or heterologous bovine strains showed no evidence of a reaction; in other words, there was good protection to challenge (Pols, 1960; Bigalke, 1967, 1968).

In an extensive field vaccination trial, Bigalke *et al.* (1974), found that 100% of the cattle inoculated against besnoitiosis with a blue wildebeest strain of *B. besnoiti* on farms where the disease was severe were protected from the clinical form of the disease over an observation period of 1 to 4 years. Percentage protection to subclinical infection varied from 75% to 100%. This means that the blue wildebeest strain does not induce complete cross-immunity to natural challenge with bovine strains of *B. besnoiti.* These observations were confirmed by laboratory studies in cattle (Bigalke *et al.*, 1974).

A serological survey conducted by means of an indirect fluorescent antibody test in Israel revealed the presence of specific antibodies to *B. besnoiti* in a high percentage of animals (Neuman, 1972). The exact nature of the immune response is, however, unknown but cellular immunity is probably an important component, as appears to be the case with *B. jellisoni* (Lindberg and Frenkel, 1977).

Laboratory aids to diagnosis

The following methods may be used in diagnosis of the disease:

1. Free (Fig. 1) or intracellular endozoites of *B. besnoiti* may be demonstrated for several days (Table 3) in Giemsa-stained blood smears of the peripheral blood at the height of the febrile stage of the disease, but they are scarce and often difficult to find. Lymph node smears are less reliable. Blood smears made from chronic cases often contain cystozoites originating from punctered cysts. Both endozoites and cystozoites are merozoite-like in appearance and are therefore not pathognomonic.

2. An experienced pathologist will recognize endozoites by histo-pathological examination of skin biopsies, but a differential diagnosis would be even more difficult than is the case with blood smears.

3. Large numbers of cysts are present in the dermis of the skin of typical scleroderma cases (Fig. 2) and their demonstration by histo-pathological examination of biopsy material is the most reliable method to confirm the diagnosis.

4. Cysts can best be seen in the living animal in the scleral conjunctiva from about 6 to 7 weeks after the start of the reaction. They are plentiful in this site in typical clinical cases but rare in clinically inapparent ones (Bigalke and Naudé, 1962; Bigalke, 1968). Their identity may be confirmed if they are removed under surface anesthesia and if smears are prepared with the aid of a dissection microscope.

5. Cysts can be seen in large numbers in carcasses of typical scleroderma cases at abattoirs or at post-mortem examination in the subcutis, the connective tissue, on and between muscles and tendons, the nasal mucous membrane, scleral conjunctiva, and the superfical veins such as the facial vein and those of the lower limbs (McCully *et al.*, 1966).

6. Serological diagnosis with immunofluorescence for example, is possible but more detailed studies are required on the correlation between infection and the presence of serum antibodies.

Prevention and control

A live vaccine against bovine besnoitiosis has been developed in South Africa (Bigalke *et al.*, 1974), and been in use there since 1974. It is administered subcutaneously to weaners or older animals and each dose contains approximately 1×10^6 organisms of a blue wildebeest strain of *B. besnoiti* grown in cell culture. A single dose was shown to protect cattle from the clinical form of the disease for observation periods of up to 4 years, but did not entirely prevent subclinical infection from taking place. Annual re-vaccination for 2 to 3 years is recommended.

Until the epidemiology of the disease is better understood, it will not be possible to determine whether preventive measures based on the elimination of intermediate or final hosts will be feasible in practice.

Hitherto, no effective drug has been found. Sulphonamides and pyrimethamine, whether used alone (Pols, 1960) or in combination (Bigalke, unpublished observations), have no noticeable effect on the course of the infection in cattle and rabbits.

Supportive treatment in the form of antibiotics, anti-inflammatory agents, wound dressings, myasis control, and nutritious food and water *ad lib* is of some value.

B: GLOBIDIOSIS

Bovine besnoitiosis, among other designations, was previously known as bovine globidiosis, a name derived from the generic name *Globidium* which was used for protozoan parasites with large cyst-like structures in the gastrointestinal tract. Nöller, who revised the nomenclature of parasites such as *Besnoitia* and *Globidium* in 1920 (see Pols, 1960, for a review), regarded the cutaneous cysts of *B. besnoiti* as being morphologically indistinguishable from

Globidium cysts. Since the latter was the older name it enjoyed preference and Nöller named the cutaneous parasite *Globidium besnoiti* (Marotel, 1912).

It is now generally accepted that the cyst-like structures found in the abomasum and intestines of herbivores are giant schizonts of *Eimeria* spp. (coccidia), some of which have not been identified to species level (see Levine, 1973).

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35. BABESIOSIS

Miodrag Ristic

Abstract. Babesiosis is a tick-transmitted disease of cattle manifested by anemia, occasional hemoglobinuria, and the appearance of infecting protozoa in the host erythrocytes. Various species of babesia are known to infect cattle, however, the most economically important are those caused by *Babesia bovis* (*Syn. argentina*) and *B. bigemina*. Babesiosis is presently considered as one of the most important constraints in production of cattle in most regions with tropical and semitropical climates. The course of infection depends on the species of babesia involved. Animals infected with virulent babesias usually need chemotherapy before acquired immunity develops. Babesia infection commonly persists for long periods of time. Maintenance of immunity is not dependent on the presence of the parasite. Antibody titers detected by serologic tests do not always relate to levels of protective immunity. Humoral-antibody protection, however, has been demonstrated by passive and active immunization. The role of cell mediated immunity (CMI) in protection has not been clearly documented. Active immunization against babesiosis has been achieved with virulent strains, irradiated parasites and inactivated parasites in adjuvant.

A scientific breakthrough in the study of babesiosis was recently achieved by development of cell cultural systems for continuous propagation of *B. bovis*. Antigens derived from these cultures have been proven effective and safe immunogens. Using this new technique, vaccines for prevention of bovine babesiosis should be commercially available in the near future.

Babes in 1888 was the first to describe the babesia parasite in blood of African cattle showing hemoglobinuria (Babes, 1888). The classic work of Smith and Kilborne (1893) which was under way in the United States at approximately the same time, signified the beginning of the scientific history of babesiosis. It is through this study that the tick was recognized for the first time as an active and later, biologic vector of *Babesia bigemina,* one of several *Babesia* spp. known to cause bovine babesiosis. For several decades thereafter, the disease known as Texas tick fever was causing serious losses among the U.S. cattle population. While the disease was gradually eliminated from the U.S. by costly eradication of the tick vector, *Boophilus annulatus,* it remained as one of the economically most important infectious diseases of cattle in all

tropical and semitropical regions of the world. It is estimated that today half a billion cattle throughout the world may be endangered by the disease caused by one or more *Babesia* spp.

A century long investigation of bovine babesiosis and its causative agents was limited to use of its natural host, the cow. Very recently a major breakthrough in a century-long struggle against the disease was achieved by development of cultural methods for continuous *in vitro* propagation of *Babesia bovis*, one of the most important agents of the disease. This achievement has opened a vast horizon of possibilities for easier and more accurate studies of biologic properties of the organism and for development of modern and more effective immunoprophylactic and serodiagnostic methods for control of the disease. These and other pertinent aspects of bovine babesiosis are being discussed here.

Disease and causative agents

Babesiosis is a tick-transmitted disease of animals which is manifested by anemia, occasional hemoglobinuria, and the appearance of infecting protozoa in the host's erythrocytes (Ristic, 1970). Currently, 17 distinct *Babesia* spp. are known to infect various vertebrate hosts. An earlier suggestion that a sexual phase in the development of *Babesia* spp. may exist in the tick was documented by recent light and electronmicroscopic studies of *B. bigemina* in heavily infected *Boophilus microplus* ticks.

Babesia is a member of the suborder Piroplasmidea. The suborder includes certain erythrocytic parasites of mammals belonging to the families Babesidae and Theileridae which do not produce the pigment hemozoin in erythrocytes following digestion of hemoglobin. *Babesia* is the most important genus in the family Babesidae. The presence of a complex organelle on the anterior extremity of *Babesia, Plasmodium, Toxoplasma, Sarcocystis, Besnoitia* and the coccidia is the basis of a new subphylum, Apicomplexa. This organelle is visible only with the electron microscope and for this reason its significance was formerly unknown. The morphologic characteristics and dimensions of this organelle vary among different genera.

Dominant tick-borne bovine Babesia species include *Babesia bigemina, B. bovis* (synonymous *B. argentina*), *B. divergens* and *B. major*. *Babesia bigemina* is a large piroplasm measuring approximately 4 to 5 μm long by 2 to 3 μm wide. *Babesia bovis* is a small piroplasm measuring approximately 2.4 μm long and 1.5 μm wide (Fig. 1). Both agents occur in Europe, Africa, Australia, Asia and Central and South America. The disease caused by *B. bovis* is more severe and more difficult to control than that caused by *B. bigemina*. *Babesia*

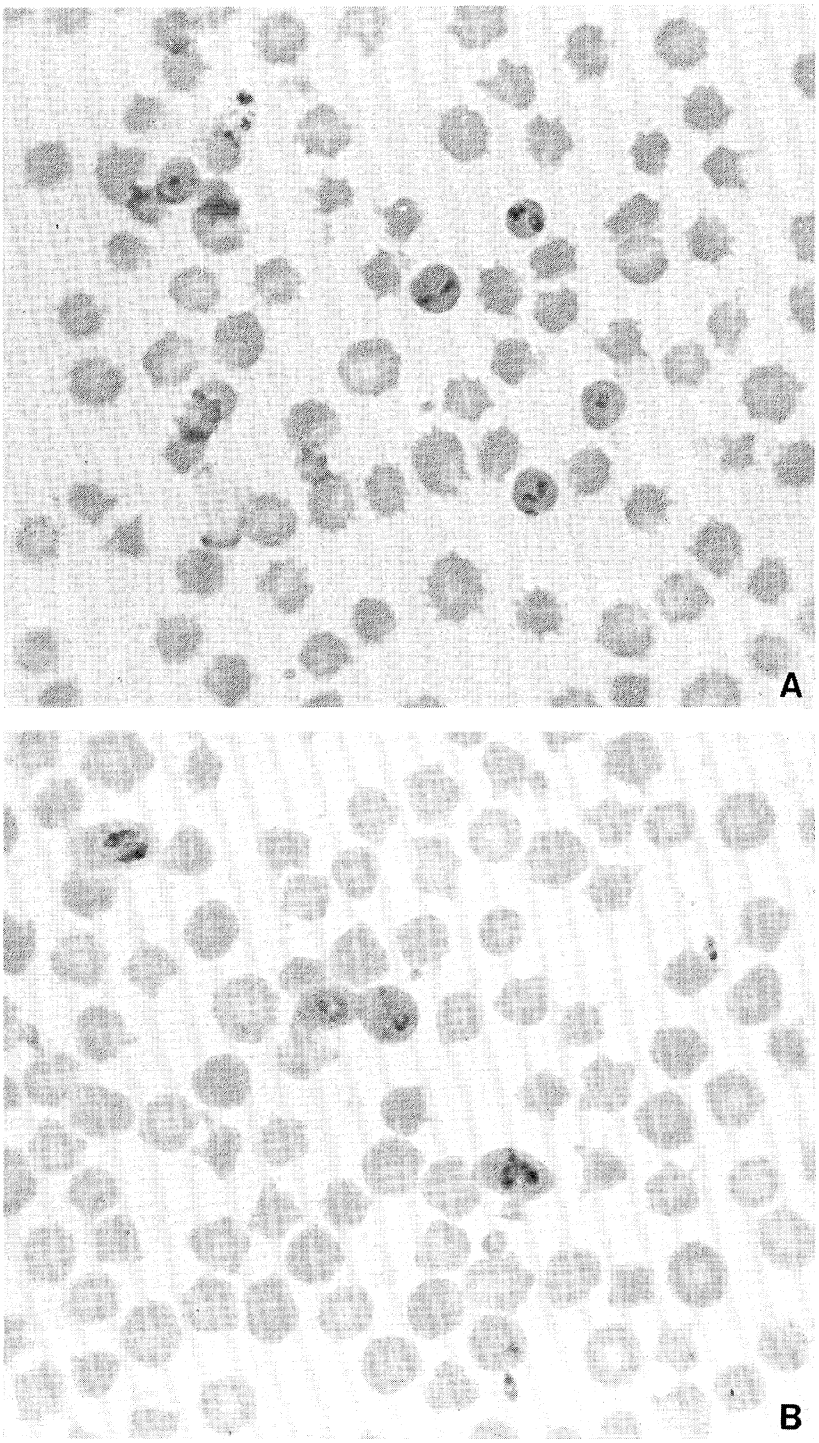

Fig. 1. Microscopic appearance of *Babesia bovis* (A) and *Babesia bigemina* (B) in Giemsa-stained blood films from cattle in the acute phase of babesiosis. ×940. Courtesy of Dr. Ronald D. Smith, University of Illinois, Urbana, Ill.

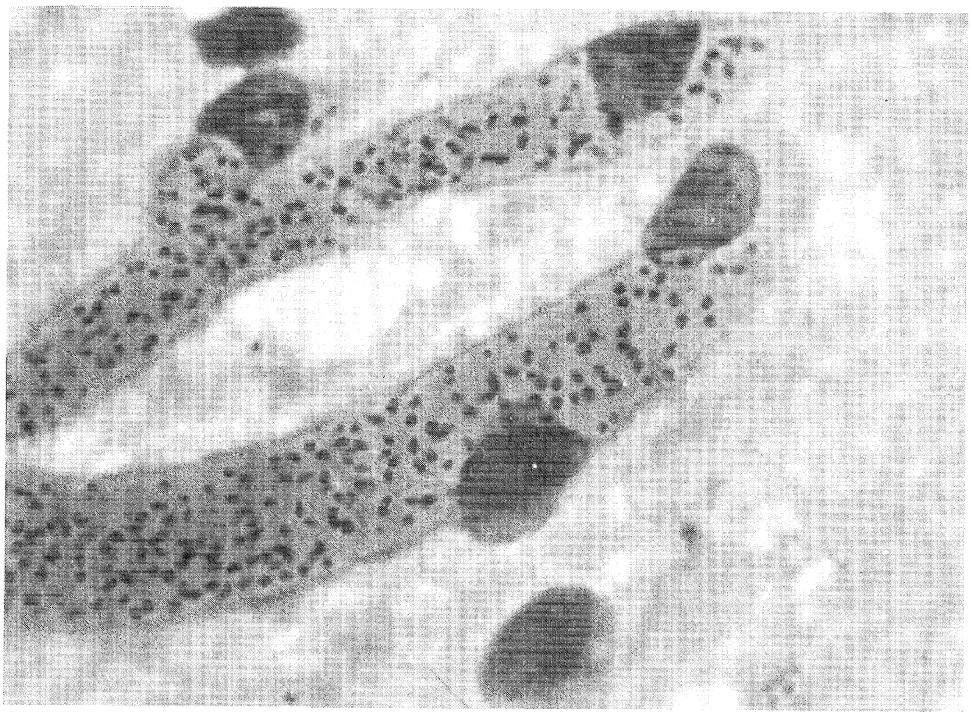

Fig. 2. Intravascular agglutination in the cerebral cortex of a cow with the acute form of *Babesia bovis* infection. Giemsa-stained specimen. ×1400. Courtesy of Dr. Ronald D. Smith, University of Illinois, Urbana, Ill.

bovis frequently causes a cerebral form of the infection characterized by formation of thrombi and emboli in the brain capillaries (Fig. 2). Such animals may develop clinical signs of rabies and usually die before any appreciable parasitemia is manifested in the peripheral blood. In general, and from the economic point of view, infections caused by *B. bovis* are considered the most important of all bovine babesial infections. *Babesia divergens* occurs more frequently in northern Europe and the United Kingdom; it is often confused with *B. bovis,* being a pyriform, paired, or club-shaped organism about 1.5 μm long and 0.4 μm wide. *Babesia major,* found in many parts of the world, is similar to but smaller than *B. bigemina.*

Dimethylsulfoxide (DMSO) and glycerol have been used to preserve infectivity of *Babesia* spp. by low temperature storage of infected blood. The addition of DMSO reduced the percentage of hemolysis of the infected blood following frozen storage from 82.8% to as low as 6.7%. Blood frozen with glycerol and given subcutaneously after thawing was highly infective, whereas blood frozen without glycerol was noninfective by this route.

Pathologic mechanisms of babesiosis

Two states of infection have been described as babesiosis and babesiasis. Babesiosis refers to the period when there is rapid growth and multiplication of the parasite and there are clinical signs of disease. The most common signs of acute babesiosis include fever, hemoglobinuria, anemia, and associated parasitemia. Babesiasis refers to the subclinial infections observed in animals which have recovered from the clinical disease and in passively immunized young animals.

Many of the pathologic effects of babesiosis are due to the indirect effects of babesial multiplication in the host. Immune complex disease and associated glomerulonephritis, anemia and thrombocytopenia have been described in rodent babesiosis caused by *B. rodhaini*. Circulating babesial antigens also appear to attach to parasitized and nonparasitized erythrocytes, The formation of immune complexes on the erythrocyte surface may increase the permeability and fragility of circulating erythrocytes. Formation of such complexes in the plasma may serve as a means of evasion of parasite destruction by immune antibodies.

During the exit of *B. bovis* and *B. bigemina* parasites from infected bovine erythrocytes, two or more parasite-associated proteolytic enzymes are released into the plasma. These enzymes and/or similar parasite metabolic products are believed to interact with blood components and are ultimately responsible for several of the pathologic signs and symptoms associated with bovine babesiosis, including increased erythrocyte fragility, hypotensive shock, and disseminated intravascular coagulation. Anemia is caused by intravascular destruction of erythrocytes by escaping babesias (Callow and Pepper, 1974). The reduction of hematocrits in acutely infected animals may also be associated with activation of kallikrein.

A peculiarity of babesiosis caused by certain species of babesia, including *B. bovis* and *B. canis,* is the clogging of small capillaries of the skin and brain with both parasitized and unparasitized erythrocytes. It is believed that the coating of erythrocytes by parasite antigens neutralizes the normal surface charge of these cells, thus favoring erythrocyte autoagglutination. This effect is particularly striking in *B. bovis* infections. Despite peripheral blood parasitemias being below 1%, parasite rates in brain capillaries usually exceed 90%. This relationship persists following recovery and affords a method of diagnosing persisting babesial infections in clinically normal cattle through the use of brain biopsies.

The fever, anemia, and parasitemia were considered to be valid parameters of the intensity of host-parasite interactions in cattle infected with *B. bovis*. An estimate of the fever, however, was considered the best on physiological

grounds because of the ease of measurement and because it appeared to be less susceptible to extraneous effects, error and subjective judgement than the other two parameters.

Studies of the pathogenesis of *B. bovis* and *B. bigemina* infections in splenectomized calves showed a decrease in serum potassium levels in some animals while urine potassium levels were increased in all cases. Tubulonephrosis and degeneration of the convoluted and collecting tubules were present in severe infections. Vascular congestion was frequently observed, but vascular stasis only occurred in severe *B. bovis* cases. Glomular shrinkage was usual in moderate and severe cases. Severe hepatic centrilobular and midzonal degeneration and necrosis was common in all severe cases.

Erythrocytes parasitized with *B. bovis* were more resistant to lysis than either normal erythrocytes from control animals or unparasitized erythrocytes from infected animals. On the other hand, erythrocytes parasitized with *B. bigemina* were larger and osmotically more fragile than uninfected erythrocytes from the same animal, which were in turn more fragile than normal erythrocytes from control animals. There is an indication that *B. bigemina* preferentially invades young erythrocytes.

Babesiosis in man

Until 1957, babesia infections had not been reported in man. The first case of human babesiosis was reported in a Yugoslav farmer in 1957 (Ristic and Healy, 1981; Healy, 1979). This person, as with a number of other cases, was splenectomized prior to acquiring the infection. However, not all patients who have developed the acute form of babesiosis were splenectomized. The latter is particularly true for individuals who contracted infections with *Babesia microti*. Other *Babesia* spp. which caused human infections are of bovine and, in one case, apparently equine origin.

In most acute cases of babesiosis, the initial diagnosis was malaria. Usually after anti-malaria chemotherapy (chloroquine) was instituted, differential diagnosis of babesiosis was established. Certain clinical signs observed in patients with acute babesiosis were high fever, chills, headache, and pain in the back. Other symptoms included hemoglobinuria and icterus. Of the total of 23 cases thus far reported, there have been three fatalities.

The diagnosis of the disease was made by serologic means, isolation of the organism in hamsters and monkeys, or microscopic observation of babesia in stained blood films (Ristic *et al.*, 1971). In hamsters infected with a human isolant of *B. microti,* the patent phase of the disease was characterized by severe anemia and marked parasitemia which occurred between the 6th and

41st day following infection. The patent phase was followed by development of a carrier state; this was demonstrated by relapse following splenectomy 113 days after infection. An indirect fluorescent antibody (IFA) test was used to survey a human population residing in an endemic area of babesiosis in Mexico. Using the results of the test, efforts were made to isolate babesia from selected cases of serologically positive patients. The organism was isolated from three of the patients. These individuals apparently never exhibited any clinical signs of babesiosis (Osorno *et al.*, 1976).

Based upon a limited number of reported cases of human babesiosis, one would conclude that the public health importance of the disease is limited, however, only a few places in the world are free of babesiosis. Farmers and other agricultural workers who spend a great deal of time in the field are likely to come in contact with babesia-infected ticks. The risk of contracting babesiosis is increased if these individuals are splenectomized. Factors which govern human susceptibility to babesiosis are not known at this time. For individuals with intact spleen conditions such as concomitant infections, metabolic and endocrinologic disorders may be among some of the possible disease precipitating factors. Virulence of a specific babesia strain and the nature of a vector tick must also be considered as elements governing the initial infection and the pathogenesis of the disease. In all probability, there are many cases of human babesiosis that have escaped proper diagnosis. The following factors may be attesting to that effect: there is morphologic similarity between trophozoites of *Plasmodium falciparum* and many *Babesia* spp. which may complicate differential diagnosis; symptoms of acute malaria and babesiosis are similar, thus, further complicating clinical diagnosis; finally, some of the common anti-malaria drugs may also be effective against babesiosis (antimalarial drugs are not effective against rodent *B. rodhaini*). These and other factors may suggest that at least some cases of human babesiosis could have been misdiagnosed as malaria. In addition, experience in Mexico indicates that there are subclinical babesial infections which obviously remain undetected in the absence of a special diagnostic effort as made in that country (Osorno *et al.*, 1976). The significance and long-term effects of subclinical infections at the present are unknown. Babesia infections, however, could be disseminated by blood transfusion from individuals with subclinical infections to susceptible subjects.

In most of the above human babesiosis cases, ticks have been incriminated as the vectors of various *Babesia* spp. It is well-established that differences exist among various tick species with respect to their ability to feed on man. Certain ticks, such as the *Boophilus* spp. which attack cattle, are extremely host-specific and rarely, if ever, attack man. Others such as the *Dermacentor* spp. are known to readily attack man and a wide range of other animals. On

Nantucket Island off the east coast of the United States where more than a dozen acute cases of human babesiosis were diagnosed between 1969 and 1979, the vector was *Ixodes dammini* (deer tick) similar to *I. scapularis*. *Ixodes ricinus* has been identified as an important vector of bovine babesiosis.

Epizootiology and tick control

Enzootic zones typically have a rather stable population of ticks with numbers sufficient to ensure calf exposure to *Babesia* spp. prior to 9 months of age. Colostral antibodies and/or age resistance protect exposed calves from developing severe reactions and a state of premunition, or infection-immunity, ensues. Tick transmission studies and serologic data indicate that infections between cattle, and possibly between cattle and wild ruminants, are common in those areas. Mixed infections with *Anaplasma marginale* are frequently observed in enzootic zones.

Marginal zones, in contrast, experience significant variations in vector tick numbers over seasons or years with the result that a number of animals escape exposure to tick-borne babesiae until after 9 months, and sometimes until 2 or more years of age. Infection at this time is more severe and death may occur. The severity of reactions on a herd basis is directly related to the proportion of susceptible animals, i.e., those which were not exposed to infected ticks as calves. It is important to note that stable, enzootic regions may be converted into unstable, marginal areas through the use of acaricides. If the tick population is artificially reduced for a number of months or years and then allowed to increase to earlier levels, a real probability of epidemic babesiosis exists (Curnow, 1973).

An important part of any tick-borne disease control program is vector control. This has traditionally involved the use of acaricides applied as dusts, sprays, dips or through hand application. Unfortunately, ticks have developed resistance to arsenicals, DDT, BHC, toxaphene, dieldrin, organophosphates and carbamates. Although acaracide resistance in Australia has received considerable attention, similar resistance problems to arsenicals, DDT, BHC and toxaphene have appeared in Argentina, Brazil, Venezuela and Colombia. Organophosphate resistance is also reported to occur in the Americas.

Mechanisms of resistance are variable reflecting the genetic makeup of individuals in any tick species. *Cross-resistance* describes the situation where a single property provides cross-protection to various toxicants, in *multiple resistance* different defense mechanisms exist in the same tick strain, and in *duplicate resistance* at least two mechanisms exist in the same resistant strain to protect individuals from one compound. Some investigators believe that

genetic selection for insecticide resistance is more intense when (1) lethal concentrations of insecticide, as opposed to sub-lethal, are used and (2) the insecticide has residual effect thus providing continuous selection pressure. Other techniques that have been tried with limited success to control ticks include the burning of infested grasslands, pasture treatment with acaricides, the use of tick parasites and predators, and pasture spraying.

Development of babesia in invertebrate and vertebrate hosts

Little is known about the early stages of parasitic development in the tick, but various bodies have been detected and designated sexual stages. Although union of these stages has not been seen, the various forms seen in tick gut content could be gametes which unite to form a zygote. There is enough evidence available to suggest that such fusion of gametes may occur (Rudzinska, 1979). Such sexual union is followed by sporogonic multiplication, giving rise to numerous vermicules in epithelial cells of the gut. Large numbers of vermicules penetrate all tissues of the tick and repeat the

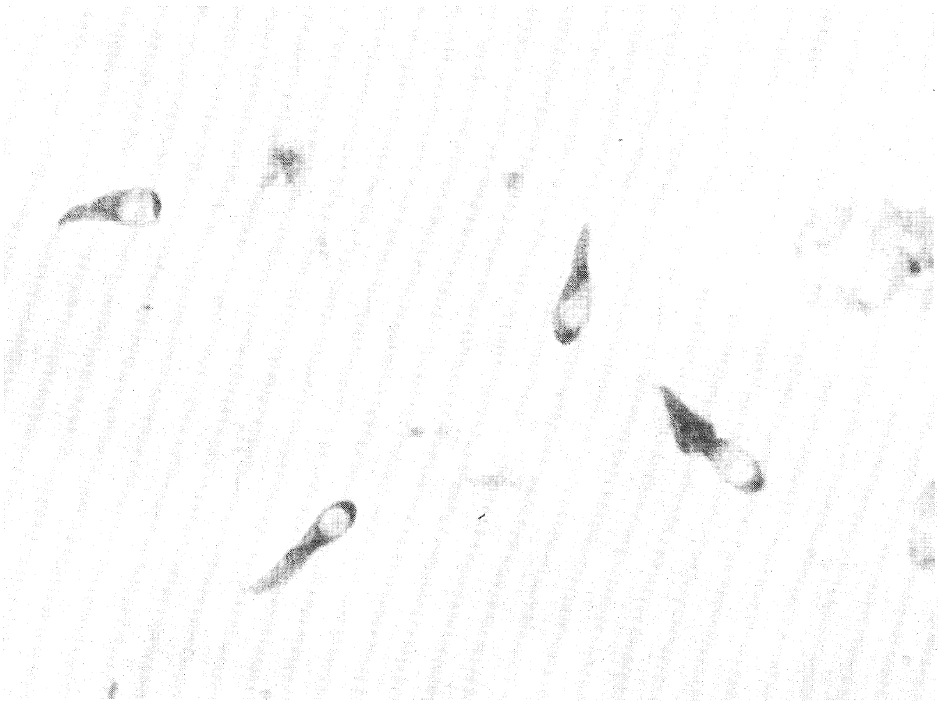

Fig. 3. Mature vermicules of *Babesia bovis* with vacuolated cytoplasm in the hemolymph of an adult *Boophilus microplus* tick. ×1650.

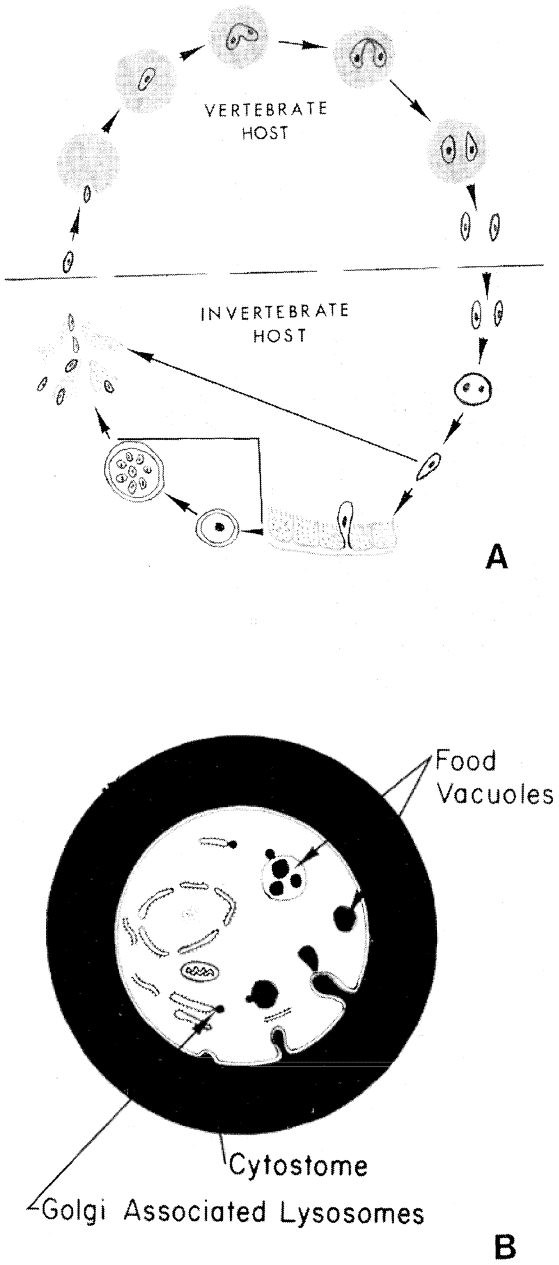

Fig. 4. Postulated life cycle of *Babesia bigemina* in the cow and the tick (A). Feeding mechanism of babesia during erythrocytic phase of development. An engulfment of droplets of erythrocytic cytoplasm (phagotrophy), with hydrolysis of subsequently formed intracytoplasmic food vacuoles, are biochemical pathways leading to formation of parasitic proteins (B).

multiplication cycle (Fig. 3). Some vermicules invade the eggs and divide in the gut epithelium of the developing larva. After the larva feeds, vermicules enter cells of the salivary gland and grow into large bodies which generate the small round and pear-shaped forms, infective for the vertebrate host.

After infection by the tick, "sporozoites" enter the blood circulation and invade erythrocytes. Intraerythrocytic larger parasite forms designated tropozoites divide by binary fission or a budding-like process. Each dividing trophozoite produces two to four merozoites which are capable of invading new erythrocytes. At the time of their exit from the erythrocytes, merozoites synthesize a surface coat which seems to be essential for attachment to and penetration of erythrocytes. Merozoites invade erythrocytes by formation of a parasitophorous vacuole consisting of erythrocytic membrane. This vacuole temporarily surrounds the newly-arrived parasite and then gradually disappears by fusing with the plasma membrane of the parasite. The parasite again enlarges into trophozoites and the cycle of multiplication and invasion of erythrocytes continues. The final step in the erythrocytic growth cycle is marked by differentiation of the organism into micro- and macrogametes which enter into the sexual cycle of development in the tick. Developmental cycles of babesia in vertebrate and vector hosts are projected (Fig. 4A).

The feeding mechanism of *Babesia* during the erythrocytic growth phase is similar to that of *Plasmodium*. The parasite engulfs large droplets of erythrocytic cytoplasm by pinocytosis or phagotrophy, leading to the formation of food vacuoles. These vacuoles are then hydrolyzed and resulting amino acids utilized for building parasitic proteins (Fig. 4B). Unlike plasmodium, there is no residual pigment (hemozoin) detectable in the cytoplasm of babesia following digestion of the food vacuole. A diffusion through the erythrocytic membrane is another apparent means for the parasite to derive needed nutrients from the host blood plasma.

Methods for *in vitro* propagation of *Babesia*

Until recently, the only method for producing babesia antigens for serologic and vaccine purposes was the use of the organism and its products collected from acutely infected cattle. The first *in vitro* method for short-term cultivation of *B. bovis,* called the spinner flask method, was recently achieved (Erp *et al.*, 1978). Subsequently the technique was adapted as a continuous cell culture for *B. bovis* (Erp *et al.*, 1980). The method was later adapted to the cultivation of *B. canis*. Levy and Ristic (1980) radically modified Erp's spinner flask method into a microaerophilous stationary phase (MASP) culture system. The latter method fulfilled the apparent maximum requirements of *B.*

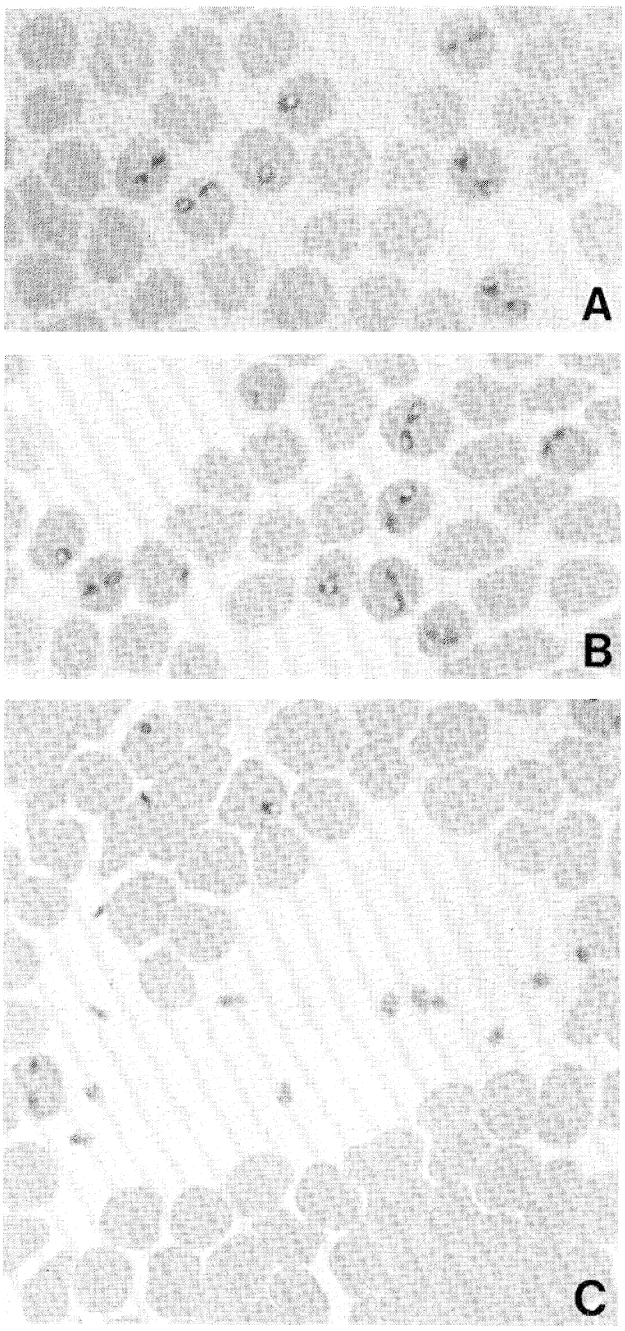

Fig. 5. Babesia bovis in microaerophilous stationary phase (MASP) culture. Various intraerythrocytic growth forms (A) and (B), and extraerythrocytic merozoites (C) are shown. Giemsa staining method. ×1280.

bovis for low oxygen tension. Lowered oxygen tension within the layer of host erythrocytes results in a darkening of infected cultures and provides a rapid means of evaluating parasite growth. The MASP method is a continuous culture system capable of generating massive quantities of soluble and merozoite antigens for use as immunogens and serodiagnostic reagents (Fig. 5C). In addition, the method is beneficial for evaluation of chemical assays of antibabesial drugs.

Deprivation of CO_2 in the MASP cultures causes the merozoites to accumulate in the medium rather than invading new erythrocytes (Fig. 5). This phenomenon provides a tool for collection of cell-free merozoites and for study of various biologic parameters of host cell-parasite interaction. This finding led to the development of the first *in vitro* merozoite neutralization test (MN) for a hemotropic parasite. In the MN test measured quantities of immune babesia serum are used to inhibit invasion of erythrocytes by the extracellular merozoites. Consequently, the MN test provides a means for measurement of protective antibodies in babesiosis.

Babesia antigens from cell cultures

Most of the data on structural, biochemical and antigenic properties of *Babesia* spp. obtained from cell cultures used the organism isolated by differential centrifugation and Ficol density gradient. Upon separation of erythrocytes by low speed centrifugation, the supernatant was subjected to high speed centrifugation producing soluble antigen in the supernatant and merozoites in the pellet (Gravely *et al.*, 1979). Upon extraction of merozoites in cold (4 °C) phosphate buffered saline, soluble antigens were eluted from the surface of these organisms. When *B. bovis* and *B. canis* merozoites were subjected to examination by electron-microscopy, a surface coat analogous to that observed in *Plasmodium* spp. was demonstrated (Fig. 6). Based on preliminary immunologic studies, it appears that the merozoite surface coat antigens are identical with the soluble antigens found free in the supernatant of the spinner flask and MASP cultures. The surface coat of babesia appears to carry the immuno-dominant characteristics. An *in vitro* effect of immune bovine serum on the merozoite surface coat antigens is shown in Fig. 7. It has been demonstrated that soluble babesia antigens and merozoite surface antigens are immunogenic and confer a good protection against homologous challenge (Ristic and Levy, 1979).

Various immunochemical methods have been used to further purify soluble babesia antigens and consequently examine their biologic properties. With *B. canis* and *B. bovis* it was shown that 40 to 50% ammonium sulfate precipita-

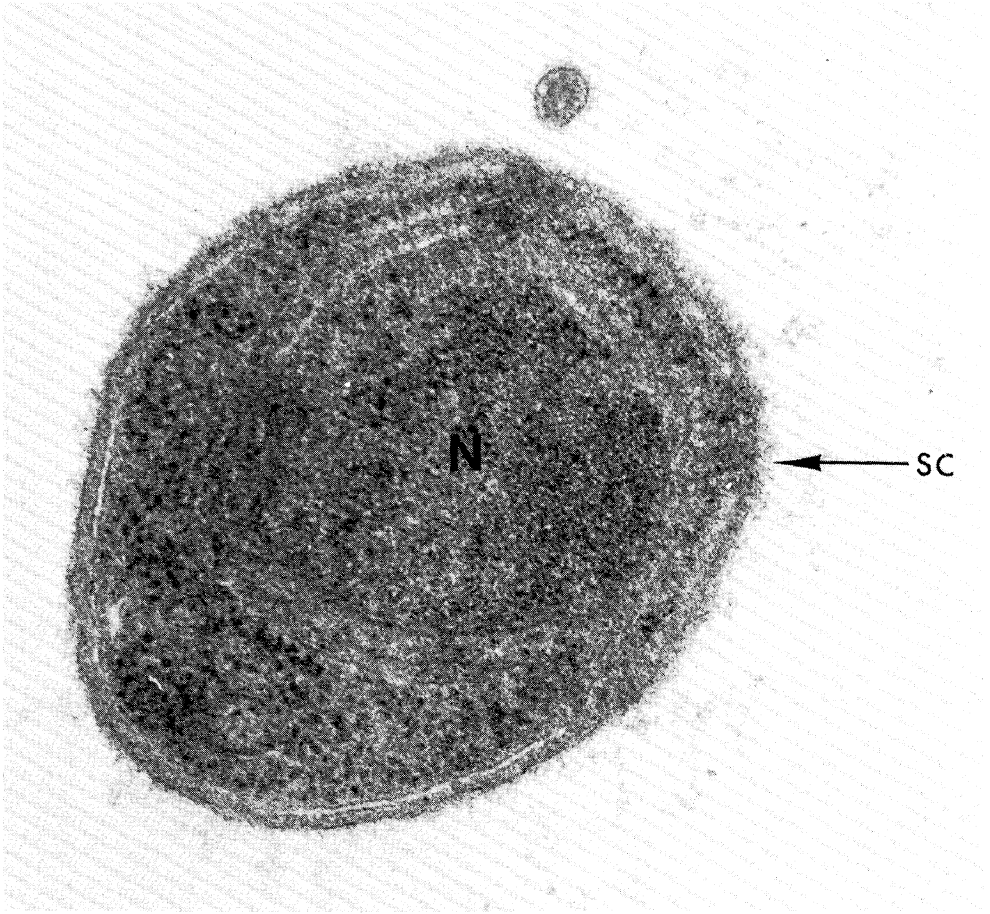

Fig. 6. An electron micrograph of a cell-free *Babesia canis* merozoite derived from *in vitro* cell culture. Note the prominent nucleus (N) and the very distinct surface coat (SC). ×40 500.

tion removes most of the antigens from the culture supernatant. With *B. bovis,* relatively pure soluble antigens were obtained by the immunoadsorbent procedure adapted from Avrameas and Ternynek (1969). In this method, specific gammaglobulins are insolubilized by treatment with 2.5% glutaraldehyde and used as an immunoadsorbent substrate. Gel filtration methods showed that soluble antigens eluted in the void volume coincident with IgM. Small quantities of parasite specific antigens were also isolated by use of isoelectric focusing technique. Very recently, *B. bovis* was adapted to growth in a rabbit erythrocyte-serum system using the MASP method. This achievement provided an excellent means for application of immunologic systems to isolation and purification of soluble babesia antigens from a corresponding *in vitro* bovine system.

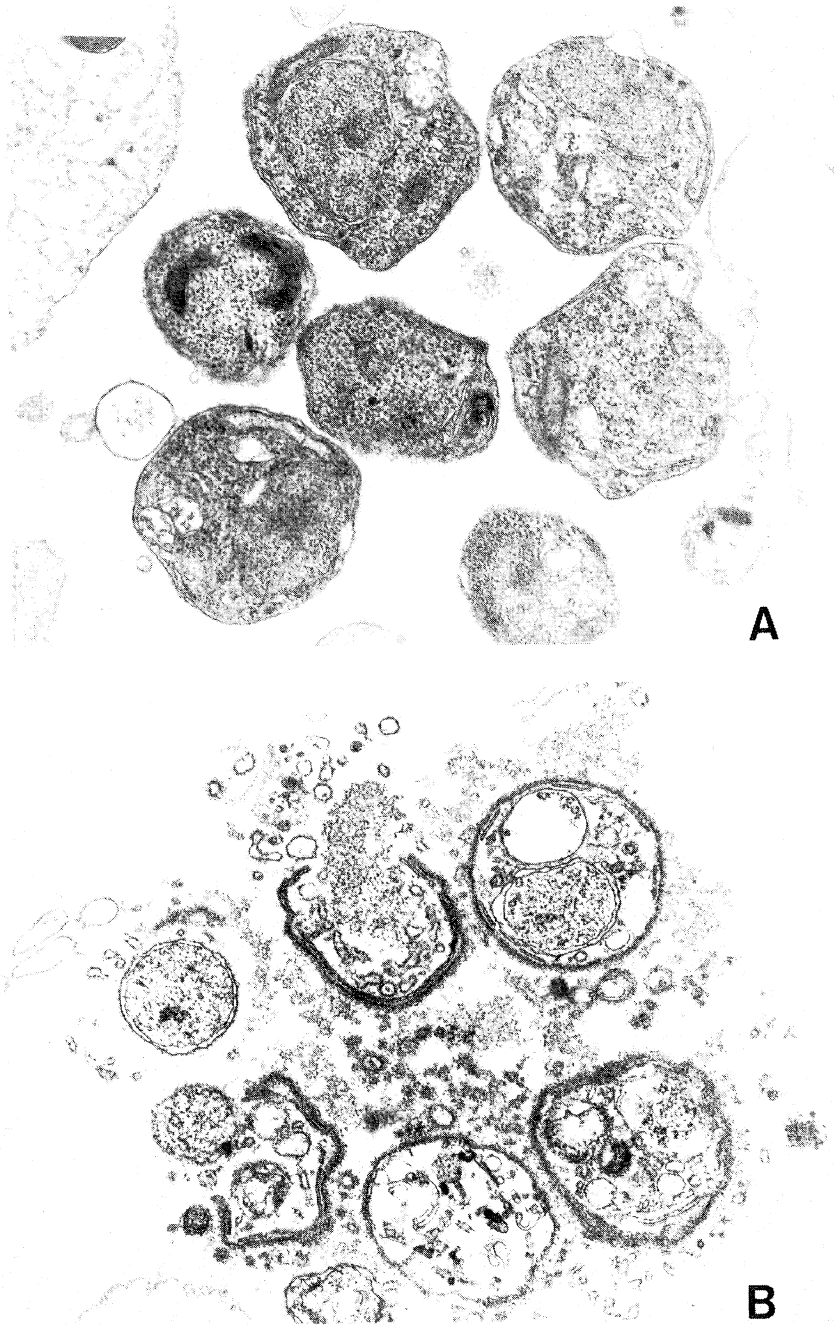

Fig. 7. Electron micrograph of cell culture-derived merozoites of *Babesia bovis* incubated for 30 min at 37 °C in a normal (A) and *B. bovis* immune serum (B). Note strong agglutinating and lytic effects in merozoites incubated in immune bovine serum. ×10 500.

Based upon their reaction with convalescent sera, cell culture-derived soluble *B. canis* antigens were found generally thermolabile following treatment at 100 °C for 10 min. However, these still reacted with hyperimmune canine sera indicating the presence of a residual carbohydrate antigen moiety. This finding is further substantiated by reduction of antigenic activity manifested following treatment of soluble antigens with amylase. The antigens were also degradable by treatment with 2-mercaptoethanol and to a variable degree with trypsin. The overall results indicate that the surface coat antigen is a rich protein moiety with polysaccharide residues (glycoprotein).

Resistance

Innate factors are possessed by the host as natural protective elements unrelated to a particular pathogen. This narrow host-specific resistance to babesia infection, however, must be reevaluated in view of recognized occurrence of babesiosis in human beings cuased by various babesia strains. Different breeds of cattle are known to differ in their susceptibility to infection and manifestation of clinical signs of babesiosis (Aragon, 1976). *Bos indicus* cattle are more resistant than *Bos taurus* to *B. bovis*. Age resistance was demonstrated with *B. bovis* and *B. bigemina* in 4- to 7-month old calves from nonimmune mothers. Other workers failed to demonstrate this nonspecific resistance and showed that protection was passively transferred from cow to calf. Passive protection lasted 1 to 2 months after birth. Animals concurrently infected with other hemotropic parasites may show resistance to babesiosis. This resistance may be related to nonspecific stimulation of reticuloendothelial systems and possibly alteration of the erythrocytes with a concurrent agent making them less suitable for babesia.

Nonspecific stimulation of resistance with diethylstilbesterol, which increases phagocytic activity, and by inoculation with the endotoxins and BCG was thus far demonstrated in murine babesiosis only. From evidence of studies in malaria, there seems to be a considerable difference in the mechanism of resistance and possible protective immunity between rodent and human malaria. Similar phenomenon may be operational between rodent and cattle babesiosis. Consequently, not all immunological data derived from studies of infections in mice caused by *B. rodhaini* and *B. microti* may be applicable to infection of cattle with *B. bovis* and *B. bigemina*.

Immunity

According to the well-established concept of "premunition", persistence of solid immunity to clinical babesiosis depends upon continuous maintenance of the causative agent in the blood. The presence of the organism in the tissue in a metabolically active state apparently supplies the necessary antigenic stimulus for continuous maintenance of humoral and cellular defense. There are numerous reports, however, to show that maintenance of acquired immunity to babesia parasites in cattle is not dependent on a continued presence of the parasite (Mahoney *et al.*, 1979). A sterile protective immunity following elimination of *B. bigemina* and *B. bovis* by chemotherapy, by vaccination with nonliving antigens derived from infected animals, and more recently from cell cultures, has been clearly demonstrated. The earliest indication of the possible role of serum antibodies in protection is indicated by the following observations: (1) the concentration of specific antibodies usually falls below detectable levels before immunity wanes, (2) passive transfer of immunity to *B. bovis* from mother to offspring does take place, presumably *via* colostral antibody, (3) a delay in the onset of parasitemia occurs if the recipient animal is given the antiserum at the time of infection.

Passive transfer of serum from cattle immune to *B. bovis* to splenectomized calves infected with the same agent indicated that an effector mechanism was mediated by antibodies which reacted with parasitized erythrocytes. It was concluded that the protection manifested in calves was due to opsonization of infected erythrocytes and consequently removal by phagocytic elements.

Limitations are obvious in studying the kinetics of protective anti-babesia antibodies using a living host. The newly-developed MASP culture system described above provides a simpler and more accurate means for such studies. From preliminary studies thus far completed, it is indicated that the major antigenic determinants associated with induction of protective immunity to babesia are localized in the surface coat of the merozoites. It was further shown that antibodies to these antigens are fully responsible for preventing penetration of erythrocytes by extracellular babesiae. Based upon electron microscopic evidence, the interaction between these antibodies and the parasite results in disruption and/or lysis of the organism (Fig. 7). The protective role of the merozoite surface coat antigens is further reflected by their apparent interaction with specific receptor sites on the surface of erythrocytes. Merozoites deprived of the surface coat do not seem capable of attachment and penetration of erythrocytes.

Of the cellular elements involved in protection of a living animal, phagocytic cells obviously play a significant role. There is an indication that their activity is being prompted by opsonins and/or cytophilic antibodies. Nothing

is known of any cell-mediated immune processes which may be operating although indirect evidence points to their involvement. Thymus-derived lymphocytes (T cells) may be involved in a helper capacity for antibody synthesis and in the activation of macrophages leading to increased parasite destruction.

Role of the spleen

Splenectomy of cattle with latent *B. bovis* infections rarely precipitates a patent relapse parasitemia, whereas in latent *B. bigemina* infection splenectomy is regularly followed by nonfatal patent parasitemias. Presumably in the former situation, extra splenic sites more readily take over the immune function of the spleen.

Three functions have so far been ascribed to the spleen in immunity to babesia: (1) it may remove parasites from infected red cells by the process of "pitting" as described for malaria infections and thereby reduce parasite numbers even before a specific immune response is under way; (2) the spleen is a major site of phagocytosis and (3) it is a site of antibabesial antibody production.

Antigenic variations

Babesia infections are frequently of long duration with the parasites surviving in the semi-immune host. Survival of the parasite may reflect its ability to evade the host's immune response by undergoing antigenic variations. Antigenic differences between field isolates of the same species have also been reported. Vaccination studies, however, have shown that one strain conferred active protection against the other. It is suggested that the mechanism of cross-immunity is based on priming of the host's immune system by protective antigens of the vaccine strain so that a secondary response against the heterologous strain occurred soon after challenge (Mahoney *et al.*, 1979).

Diagnosis

During the acute phase of the disease, the organism can be microscopically detected by examination of stained blood films. *Babesia bovis* can also be detected in stained brain smears. In the procedure, a sample of grey matter of the cerebral cortex is placed on a slide near one end, the tissue is crushed

using another slide and then spread toward the other end of the first slide, fixed and stained.

Soluble and corpuscular antigens derived from the blood of infected animals have been used in various serologic tests for diagnosis of babesiosis (Todorovic, 1975). These tests include complement fixation (CF), indirect hemagglutination (IHA), capillary and slide agglutination (CA), indirect fluorescent antibody (IFA), precipitation in gel (PG), and the enzyme-linked immunospecificity assay (ELISA) tests.

The length of the period during which antibodies are detectable depends on the test employed. For example, in bovine babesiosis the titer of CF antibodies rises to a peak 2 to 3 weeks after infection and then gradually declines. The CF test could be used to identify *B. bigemina* and *B. bovis* infected animals for 4 and 7 months after infection, respectively.

The IFA test is species specific, that is, it differentiates *B. bovis* from *B. bigemina* infections. Detectable levels of antibody using this technique persist for a relatively long time. Antibodies to *B. bovis* and *B. bigemina* have been detected for nearly 1 and 2 years, respectively. A comparison of the CF, IFA, and the ELISA tests in bovines showed that although CF titers were detected earlier, they were consistently lower than those determined with the other two tests and declined to undetectable levels at a time when IFA and ELISA tests were clearly positive.

Based upon the results of field and laboratory studies, the IFA test seems to be the most widely used serologic test. An evaluation of the accuracy of the IFA test for detecting antibodies to *B. bovis* showed that the test is a reliable indicator of current infection with the organism. The probability of the test not detecting animals infected with *B. bovis* (false negative) was 0.003 (95% confidence limits 0.001 to 0.01). The probability of the test giving a false positive result was 0.028 (95% confidence limits 0.02 to 0.05). Dried paper-absorbed blood samples were also used as a source of antibodies for the IFA test. Consequently, the IFA test appears useful for epizootiological studies of bovine babesiosis.

The IHA and the CA tests have demonstrated persistent antibodies after prolonged periods of time. Accordingly, these tests appear useful for detection of long-standing carriers.

Pathology

Gross pathologic changes of an animal which dies during the acute phase of babesiosis are characteristic and have been described by many investigators. Principal changes include icterus of all tissues, excessive serous fluid in body

cavities, subcutaneous and pulmonary edema, enlarged spleen, congested liver and swollen and frequently hemorrhagic kidneys.

Microscopic changes caused by an infection of cattle with *B. bovis* include the packing of erythrocytes, the majority of which are parasitized in the capillaries of the grey matter of the brain and in the kidney. Variable degrees of centrilobular necrosis of the liver and renal nephrosis with cast formation are common findings. Biliary retention with distension of canaliculi is another prominent abnormality. Excess hemosiderin may be present in macrophages of the liver, lymph nodes, lung and, to a lesser extent, in the spleen and kidney. Active phagocytosis of erythrocytes and cell debris may be evident. Mobilization of lymphocyte reserves and hyperplasia of the reticuloendothelial system is marked. Excessive numbers of plasma cells are present in the spleen, liver and kidney of the animal in which the course of disease is prolonged.

Immunization

Various vaccination methods have been developed and studied under laboratory and field conditions as immunoprophylactic means against bovine babesiosis. In all instances, the vaccine antigens were derived from animals in the acute phase of infection, usually splenectomized, and were introduced into recipient animals in live or inactivated forms. The recent development of cell cultures for continuous propagation of *B. bovis* has provided the necessary means for producing the first vaccine against an intraerythrocytic parasite in a standard manner commonly used in preparation of vaccines against viral and bacterial diseases.

1. Live Vaccines

The oldest form of immunization against babesiosis consisted of inoculating susceptible animals with the whole blood of carrier cattle. This procedure resulted in disease and occasional losses in inoculated animals. The method could be modified by infection and treatment of inoculated animals, the procedure being known as "premunition".

A limited degree of protection was given to cattle injected with irradiated *B. bovis, B. bigemina* or *B. major* infected erythrocytes. The cells were irradiated with a dose sufficient to prevent a parasitemia from developing after injection of the irradiated parasites. Where the irradiation dose only "inactivated" or "killed" the majority of the parasites, the calves were later strongly immune to challenge with viable parasites. The immunizing dose caused

mild clinical reactions and parasitemia followed injection of the irradiated parasites. The immunizing efficiency of irradiated parasites may be related to the fact that even after irradiation there is some residual metabolic activity retained by the organism (Purnell *et al.*, 1978).

Immunization of cattle against babesiosis caused by *B. bovis* using an "avirulent" vaccine strain has been successfully practiced in Australia since 1964. A vaccinal strain is produced by rapid passage of *B. bovis*-infected blood through splenectomized calves (Callow, 1977). The organism appears safe for inoculation into animals with spleen *in situ,* however, subinoculation of blood from vaccinated animals into another susceptible cow was shown to cause the disease in the latter. The mechanism involved in change of virulence of the organism by passage through splenectomized calves appears to be associated with a selection of a less virulent ring form of the organism.

2. Inactivated Vaccines from Infected Animals

All inactivated vaccines were composed of soluble and/or corpuscular babesia immunogens collected at the peak of parasitemia, processed through limited purification steps, lyophilized or used fresh, and inoculated emulsified with an adjuvant, the latter being most frequently Freund's type adjuvant. Ordinarily, more than one injection was recommended for each vaccination period, and there was usually a need for two or more such periods a year.

Cattle have been partially protected against homologous challenge by immunization with inactivated *B. bigemina* and *B. bovis* (Mahoney and Wright, 1976). Infected erythrocyte antigen of *B. bovis* was more effective than the plasma of infected animals (Mahoney and Goodger, 1972).

3. Inactivated Vaccines from Cell Cultures

At least two types of inactivated vaccines can be prepared from the *in vitro* cultures. The first is a merozoite vaccine and the second is a parasite-free soluble merozoite surface coat vaccine. Both of these vaccines may be administered singly or in combination, can be preserved, stored, and used as stable lyophilized products.

Initially the antigens used for production of these vaccines were derived from the spinner flask cell cultures which produced an average of 3% parasitemia (Smith *et al.*, 1979). They were admixed with incomplete Freund's adjuvant and administered subcutaneously in two doses at two-week intervals. Four weeks after the initial inoculation, all principal and control animals were each challenged with 1000 *B. microplus* larvae from *B. bovis*-infected

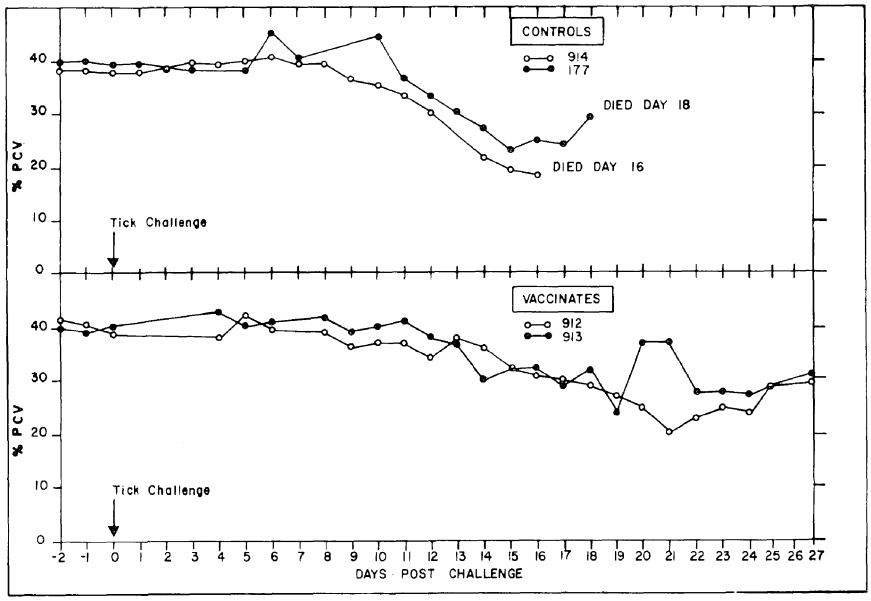

Fig. 8. Pathogenesis of babesiosis exemplified by changes in packed cell volume and mortalities in two non-vaccinated and two vaccinated 19-month-old Holstein cattle. The vaccine consisted of cell culture-derived soluble *B. bovis* antigen fortified by an oil adjuvant. Each animal was challenged with 1000 larvae of *Boophilus microplus* colony infected with *B. bovis*.

Table 1. Summary of early vaccination studies with low concentration of spinner flask culture-derived *Babesia bovis* antigens (parasitemia <5%).

Treatment	*Number of animals*	*Tick challenge mortality (%)*
Corpuscular (merozoite) antigen	8	0
Soluble antigen	14	21
Non-immunized	11	82

colonies (Smith *et al.*, 1978). The results of this initial study are shown in Fig. 8 and summarized in Table 1.

In more recent studies soluble *B. bovis* antigens were derived from the improved MASP culture which produced an average of 29% parasitemia. The antigens were concentrated approximately 7 times by lyophilization, admixed with a new Saponin (Quil-A) adjuvant and administered subcutaneously in two doses on days 1 and 14. Several small experiments were conducted, each consisting of 3 to 4 cattle. All inoculated animals showed a marked rise of IFA antibodies reaching a maximum titer of 1:20 000 at approximately 3 to 4 weeks after inoculation. The antibody titers persisted for 3 to 4 months and

then subsided below detectable levels. Animals challenged at this time mounted a strong anamnestic antibody response which reached IFA titers of 1:350 000 at 10 days after challenge. Vaccinated animals showed no detectable clinical or hematologic signs of infection. In fact, in one case studied, no evidence of even subclinical infection was detected indicating that the challenge infection was fully rejected. Conversely, all control animals developed clinical signs of the disease and died or were treated and recovered (Ristic and Levy, 1979).

4. Prospective Look into the Vaccination Future

Historically, *in vitro* systems for propagation of babesiae signify an important turning point in our research efforts. Henceforth, the speed with which a practicable vaccine for control of babesiosis will be available is only a matter of time needed for scaling up the technology for commercial production of such a product. Steps in this direction are now under way and the bovine industry should benefit in the near future by the availability of an effective deterrent to babesiosis.

The choice of a live versus inactivated vaccine will depend in part upon the type of disease control program in which it is to be used. For control of babesiosis in endemic areas, either live or inactivated vaccines could be used. Manipulation of live vaccines, however, is more difficult since these must be preserved by freezing and refrigeration for long and short-term preservation, respectively. Another disadvantage of live (not stable attenuated mutant) vaccines is that their implementation broadens the source of babesial infection for tick vectors.

Inactivated vaccines are definitely preferred in marginal areas where babesiosis is a seasonal event, for the immunization of animals to be moved from tick-free zones into endemic areas, and for regions where eradication of babesiosis is contemplated. In these situations vaccination would prevent death losses during an occasional unexpected exposure to babesiae but would not disseminate organisms which could be picked up and transmitted by vector ticks.

These new developments and prospects for an early effective vaccine should not mean that it will be a remedy for all ecological occurrences of the disease. We must continue to define the antigenic spectrum of babesia in various geographic regions by isolation of the organism in cultures directly from ticks. Another subject yet to be examined is the role of *B. bigemina* in the total babesiosis syndrome. Finally, an anti-babesia vaccine destined for use in the tropics must be used in combination with an anti-anaplasma vaccine in order to be practically useful.

Treatment

Treatment of babesiosis is usually concerned with moderating the clinical signs of the disease. Some compounds are highly effective and specific, making elimination of the infection by a single dose possible. While this treatment may eliminate a reservoir of infection, it may not be a desirable approach when the animal is in an endemic area, where the exposure to ticks is frequent. If a sterilizing treatment is given early in the course of infection, the persisting sterile immunity is lower than if the treatment is given after a high parasitemia. When animals with good sterile immunity are reexposed, a carrier state is achieved without evidence of overt infection. If reinfection is delayed for 6 to 12 months and the sterile immunity has waned, the infection may be associated with clinical signs and even death.

In general, the small babesias are more refractory to treatment than the large one, i.e., *B. bovis* is less responsibe to treatment than *B. bigemina*. Some of the more important compounds used in treatment of bovine babesiosis are shown below (Kuttler, 1979).

Trypan blue is probably the first specific drug used to successfully treat *B. bigemina* infections. Intravenous injection of trypan blue at the rate of 2–3/ mg/kg effectively eliminated *B. bigemina* but not *B. bovis*. The drug usually produces discoloration of the animal's flesh. For this reason, and in view of availability of new, more effective drugs, it is used less frequently.

Quinoline derivatives, among which Acaprin is the most prominent, are very effective against *B. bigemina* and to a lesser extent against *B. bovis*. The drug is toxic when given in excessive doses, thus the exact formulation is essential. A prescribed dose is 1 mg/kg given subcutaneously.

Acridine derivatives are effective against both *B. bigemina* and *B. bovis*. A 5% solution is administered intravenously in doses of 15 to 20 ml per animal.

Diamidine derivatives, among which the most prominent are amicarbalide (Diampron) and diminazene aceturate (Berenil-Ganaseg) are very effective and safe to administer against most bovine babesias. Amicarbalide is given intramuscularly at the rate of 5 to 10 mg/kg. Diminazene aceturate is also given intramuscularly but at the rate of 3 to 5 mg/kg. There is an indication that even lower doses of this drug may have a good chemotherapeutic effect. There is a high safety margin with either of the above drugs, thus, the dosage can be adjusted to moderate the parasitemia and the clinical response.

Imidocarb, 3,3'-bis-(2-imidazolin-2-yl) carbanilide disproprionate held great promise for the treatment and chemoprophylaxis of bovine babesiosis. It was also shown to prevent infection of ticks with *B. bigemina* and *B. bovis* during the period that these circulated in the blood of inoculated animals. A 5 mg/kg

of Imidocarb given 14 days before and 14 days after exposure to babesia-infected *B. microplus* larvae rendered the next generation of larvae incapable of transmitting babesia infection. The drug has been used prophylactically at a dose of 2 mg/kg intramuscularly. It suppressed the development of acute babesiosis in calves treated 46 days prior to exposure with a lethal dose of *B. bigemina* and *B. bovis* infection. Higher doses of the drug up to 20 mg/kg have been used, however, acute signs of toxicity were noted, particularly when intravenous administrations were practiced.

Residue problems with Imidocarb were responsible for its withdrawal from the market.

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36. BOVINE TRYPANOSOMIASIS

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Abstract. African trypanosomiasis in cattle represents a major constraint to agricultural and socio-economic development in vast areas of Africa. The disease is caused principally by three species of trypanosome (*Trypanosoma congolense, T. vivax* and *T. brucei*) which are transmitted by several species of tsetse flies (*Glossina*). Trypanosomiasis in cattle results in poor productivity and mortality. The widespread nature of the disease is due to the distribution of tsetse, the ability of the trypanosomes to escape host defense mechanisms by undergoing antigenic variation and by their capacity to infect a large variety of other hosts, including wild game. Cattle can also act as carriers of the human pathogen *T. b. rhodesiense*. Following the intradermal inoculation of trypanosomes by the tsetse fly into a susceptible host, there is proliferation of trypanosomes within the skin resulting in a localized skin reaction, known as the chancre, which develops before the appearance of parasites in the bloodstream.

Infected animals suffer from a severe anemia and there is widespread tissue damage affecting organs such as the heart, skeletal muscles, endocrine system and reproductive tract. The lymphoid system undergoes marked changes characterized initially, by intense proliferation associated with hypergammaglobulinemia and later, by depletion. Definitive diagnosis depends on demonstration of the trypanosome in blood samples. No field vaccine is available for bovine trypanosomiasis, and the methods currently employed for control include chemotherapeutic and chemoprophylactic drugs, tsetse eradication or control and the use of trypanotolerant cattle.

Disease

Trypanosomiasis (or Nagana) of cattle is a disease caused by several species of extracellular hemoprotozoan parasites which are cyclically transmitted by tsetse flies. Because of the widespread distribution of the insect vector (Fig. 1) and the limitations of current control measures, it is prevalent throughout vast areas of Africa. As a consequence, trypanosomiasis is regarded economically as the most important disease of cattle on the African continent. In addition to causing clinical disease resulting in mortality and poor productivity of livestock inhabiting tsetse-infested areas, the presence of the organism also renders large areas of Africa totally unsuitable for maintenance of lives-

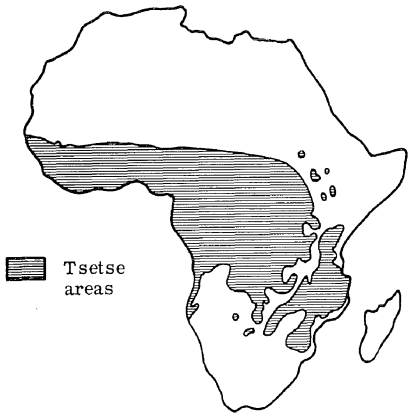

Fig. 1. The distribution of tsetse fly (*Glossina*) in Africa.

tock. Trypanosomiasis, therefore, represents a major obstacle not only to increasing food production but also to the agricultural and socio-economic development of communities within infested areas.

Etiology

The Trypanosome

The trypanosomes of cattle are flagellated blood-borne parasites ranging from 8 to 39 μm (Mulligan, 1970). They are spindle shaped with a centrally placed oval nucleus and they possess a single elongated mitochondrion which extends along much of the length of the cell. Towards the posterior end of the cell is a small densely-staining structure known as the kinetoplast which contains DNA. In close proximity to the kinetoplast is a small pocket (flagellar pocket) from which emerges a single flagellum which is attached along the longitudinal axis of the cell. The trypanosome also possesses a prominent layer of microtubules which lie just beneath the plasma membrane. The organisms are extremely motile by virtue of their flagellum and it is thought that the microtubules help to maintain cell shape during locomotion. Several of the structural features, such as the size and position of the kinetoplast and the presence or absence of a free flagellum at the anterior end of the organisms are used to distinguish the different species of trypanosomes in stained thin smears (Table 1). Perhaps the most important structural feature of the trypanosome, with regard to its ability to induce persistent infections in its mammalian host, is the possession of a surface coat. This cell coat, which is

Table 1. Characteristics of trypanosomes pathogenic for cattle.

Species	*Site of development in tsetse fly*	*Bloodstream forms in cattle*		
		Size (μm)	*Morphology*	*Behavior*
T. congolense	Midgut Proboscis	9–18	Kinetoplast – Posterior end – Undulating membrane – Flagellum –	medium size, marginal blunt poorly defined. no free flagellum	Sluggish movement often attached to red cells
T. vivax	Proboscis	20–27	Kinetoplast – Posterior end – Undulating membrane – Flagellum –	large, terminal rounded usually poorly defined free	Very rapid movement across the microscopic field
T. brucei	Midgut Salivary glands	15–39 *	Kinetoplast – Posterior end – Undulating membrane – Flagellum –	small, subterminal pointed well defined free	Rapid movement in confined areas

* Polymorphic – slender, intermediate and stumpy forms; no free flagellum on stumpy form.

Fig. 2. Trypanosoma congolense in a thin blood smear. Giemsa. ×1600.

approximately 15 nm in depth and is only visible at the ultrastructural level, covers uniformly the entire plasma membrane and is composed predominantly of a single glycoprotein molecule of MW 55 000–65 000K. It is this glycoprotein on the surface of the trypanosome which changes antigenically during the process of antigenic variation (Cross, 1978).

Three distinct species, belonging to different sub-general are pathogenic in cattle. These are *Trypanosoma (Nannomonas) congolense* (Fig. 2), *T. (Duttonella) vivax* and *T. b. brucei* which belongs to the subgenus *Trypanozoon*. All three species are found in cattle throughout the tsetse-infested areas and it is not uncommon to find all three trypanosomes in the same animal. The three species can be distinguished on morphological grounds and by characteristic behavior when observed in a fresh wet blood film (Table 1).

However, *T. b. brucei* is closely related to and morphologically indistinguishable from the human pathogens, *T. b. gambiense* and *T. b. rhodesiense* which belong to the same subgenus. Unlike *T. b. brucei,* these subspecies are not found throughout the tsetse belt but are restricted to localized foci. While the evidence as to whether or not *T. gambiense* can infect cattle is conflicting, infections with *T. rhodesiense* have been confirmed. In such situations, when trypanosomes with the morphological characteristics of *T. b. brucei* are isolated, the only certain way at present of distinguishing the subspecies is to ascertain whether or not the organisms are infective for man. Over the years these organisms have been regarded as separate subspecies, but more recently, it has been suggested that they belong to the same species and that through time *T. b. brucei* may give rise to variants which are infective for humans and

thus by definition have transformed to *T. b. rhodesiense*. It is known that human plasma can be cytotoxic for *T. b. brucei* while certain *T. b. rhodesiense* and *T. b. gambiense* strains are resistant; thus the blood incubation infectivity test was devised in an attempt to distinguish between these subspecies (Rickman and Robson, 1970). Another promising means of identification is by isoenzyme electrophoresis; it has been found that *T. b. gambiense* has a distinct isoenzyme pattern and by using this approach *T. b. gambiense* has been identified in pigs and dogs (Gibson *et al.*, 1978).

When examining blood samples from cattle in endemic areas of trypanosomiasis, *T. congolense* and *T. vivax* are usually found more frequently than *T. brucei* and in general are considered to be more pathogenic. However, by subinoculation of blood into laboratory animals, a much higher incidence of infection with *T. brucei* may be found and results of a number of studies indicate that *T. brucei* can be pathogenic in its own right.

The Insect Vector

The African trypanosomes are cyclically transmitted by tsetse flies, genus *Glossina* (Mulligan, 1970). The tsetse fly becomes infected with trypanosomes when it feeds on an infected animal. The trypanosomes then undergo a number of developmental stages (trypomastigote, epimastigote and metacyclic forms) before they become infective, once more, for the mammalian host. After ingestion by the fly, the organisms lose their surface coat; in addition, there is marked development of the mitochondrion associated with the decrease in available glucose within the fly. Although the developmental stages are similar for the three species of trypanosomes, the sites in which they occur show some differences (Table 1). Thus, the initial stages of development of *T. congolense* and *T. brucei* take place in the midgut from which the organisms migrate to complete their development in the salivary glands in the case of *T. brucei* and in the proboscis in the case of *T. congolense*. By contrast, all stages of development of *T. vivax* occur in the proboscis. The final stage of development to the metacyclic forms is associated with involution of the mitochondrion and re-acquisition of a surface coat. At this stage the organism is again capable of establishing an infection when the fly feeds on a susceptible host. Another important difference between the three life cycles within the tsetse is the length of time taken to complete the trypanosome cycle. *T. vivax* can be as short as five days, while *T. congolense* usually takes two weeks and *T. brucei* three weeks or longer.

There are a number of species of tsetse fly and these can be divided broadly, according to habitat, into three categories, namely forest (Fusca group), riverine (Palpalis group) and savanna (Morsitans group) types. Between

the different groups and among the different species of tsetse fly within each group, habitat preferences, feeding preferences, trypanosome infection rates, efficacy of transmission and virulence of transmitted infections may vary. In general, the savanna and forest species are more efficient vectors for trypanosomiasis of cattle than the riverine species. However, because of their proximity to animal pasture the savanna and riverine species tend to be the major threat to livestock; in particular the savanna species can give rise to severe disease problems even when present in barely detectable numbers.

Mechanical transmission of trypanosomal infections between cattle can also occur although it is relatively much less important than cyclical transmission. The tsetse fly itself may act as a mechanical vector if it is interrupted during feeding on an infected bovine and then completes its feed on another host. This can only occur when animals are herded closely together. Other species of biting fly can also mechanically transmit the infection. In general, although mechanical transmission may occur with all three species of trypanosome, it is considered to be more important for *T. vivax*. Evidence for this was obtained by observing the nature of infection in pastoral herds after they moved away from tsetse fly-infested areas. It was found that with increased time after exposure to tsetse flies, *T. vivax* accounted for an increasing proportion of the detectable trypanosome infections in the herd. This was attributed to *T. vivax* being more readily transmitted by biting flies than either *T. congolense* or *T. brucei*. The potential importance of mechanical transmission of *T. vivax* is emphasized by the widespread presence of this trypanosome species in South America, a continent where the tsetse does not occur.

Antigenic Variation

Antigenic variation is the phenomenon whereby during infection of a mammalian host by a single species of trypanosome, there arises a succession of parasite populations each recognized as antigenically different by the immune response of the host (Cross, 1978). In infected animals this often gives rise to a cyclical pattern of parasitemia consisting of a series of parasitemic peaks. Thus, when one examines trypanosome populations taken from different peaks during the course of infection the surface (or variable) antigens are quite distinct so that neutralizing antibody against one population fails to neutralize another population. Furthermore, each peak of parasitemia in itself also consists of trypanosomes of several variable antigen types (VATs) each comprising a variable proportion of the population. Early workers suggested that this situation might arise due to a process of selection within the initial trypanosome population so that as each dominant population of trypanosomes

was removed by antibody, another population of different VATs multiplied to replace it. However, this was discounted by the finding that antigenic variation occurred in populations of trypanosomes derived from a single organism (clones).

The maximum number of different variable antigens which a clone of trypanosomes may express, i.e., the VAT repertoire, is not known. However, Capbern *et al.* (1977) working with *T. equiperdum,* an organism closely related to *T. brucei,* have identified 101 different VATs in one repertoire. There appears to be a degree of consistency in the order of appearance of different VATs during infection so that certain VATs tend to be found during the early stages of infection while others tend to be present later. Results of recent studies indicate that, following cyclical transmission of a clone of trypanosomes, the population of metacyclics extruded by the tsetse fly contains a mixture of VATs (Barry *et al.*, 1979), and that, while these are always similar each time this clone is transmitted through flies, they differ from those of other unrelated clones.

In recent years, considerable progress has been made in the biochemical characterization of the variable antigen. Although initial studies, based on serological cross-reactivity and amino acid sequencing of the N-terminal end of the molecule, failed to demonstrate any homology between different variant antigens, more recent investigations have demonstrated the existence of cross-reactive determinants in variant surface antigen both within and between trypanosome species and the possibility of common structural regions has been suggested (Barbet and McGuire, 1978). However, it may be that these "common regions" are not exposed on the surface of the living organism. It is still uncertain whether the trypanosome possesses the genetic information necessary to code for the complete VAT repertoire or whether mutation or reassortment of genetic material occurs each time the antigen changes. However, in view of the marked heterogeneity between different variant antigens and the finding of some consistency in the order of appearance of different VATs, the former possibility appears more likely. The question still remains as to what triggers the change in surface antigen. While the disappearance of a particular VAT is associated with the appearance of specific antibody in the serum, it is still uncertain if this acts as the primary stimulus for the surviving trypanosomes to change their variable antigen.

Distribution and epidemiology

The incidence of trypanosomiasis in cattle is directly related to the distribution of the tsetse fly. This in turn is governed by variable habitat which is

related to climatic conditions, in particular temperature and humidity. Consequently, an area of approximately 10 million square kilometers of the African continent, involving 37 countries, is infested by tsetse flies which harbour trypanosomes pathogenic for cattle. A number of factors contribute to the ubiquitous nature of these trypanosomes. Firstly, there is a number of tsetse fly species capable of transmitting the infections. Secondly, all three trypanosomes which are pathogenic for cattle exhibit a wide host range. Thus, they are infective for the majority of domestic animal species as well as wild game species. In particular, high infection rates have been found in waterbuck, kudu, reedbuck, giraffe, bushbuck and eland and since these animals are capable of surviving in areas heavily infested with tsetse flies, they represent a constant reservoir of infection. Thirdly, the phenomenon of antigenic variation and the resultant persistent infections in the bloodstream of infected hosts provide ample opportunity for transmission by tsetse flies.

The impact of bovine trypanosomiasis may be considered as two-fold. Firstly, in a large proportion of the tsetse fly-infested areas, the level of challenge is so high as to totally preclude the maintenance of cattle. Because of the climatic conditions within these areas, they contain abundant pasture which would otherwise be suitable for grazing of cattle. An additional factor to be considered is that, because of the unsuitability of tsetse fly-infested areas for cattle production, there is a much higher stocking density in the semi-arid zones than would otherwise be the case. In these areas, cattle are less productive because of poorer grazing and they may also be a contributory factor to the complex problem of desertification. The second aspect of the problem is the impact which the disease has on the cattle which inhabit the marginal areas of the tsetse fly belt. In these areas, depending on seasonal climatic changes, there may either be a low grade continual challenge or a seasonal challenge. In addition, the herds of nomadic pastoralists may experience a seasonal challenge as they migrate towards the tsetse belt during the dry season. Persistence of infection in these animals may result in the detection of infection in them after they have left the area of tsetse challenge.

The severity of the clinical disease in infected cattle shows considerable variation. Sometimes acute disease with high mortality occurs; more often a chronic debilitating syndrome is the result leading to poor productivity, decreased fertility and sometimes abortion. The extent and severity of the problem in these areas is dependent upon a number of variable factors involving the tsetse fly, the trypanosome and the mammalian host.

Factors Involving the Tsetse Fly

Not only the density of tsetse infestation but also the species of fly, the infection rates, the efficacy of transmission and the feeding preference can

influence the degree of risk to which cattle are exposed. Thus, very small numbers of the savanna species, *Glossina morsitans,* which readily feeds on cattle and efficiently transmits the infection, can result in a major disease problem. It should be emphasized that clinical trypanosomiasis of cattle can be prevalent in areas where it is exceedingly difficult to find tsetse flies.

Important factors which can alter the distribution of the tsetse fly are change in habitat and food supply. In parts of West Africa on the margin of the tsetse belt, there is considerable fluctuation in the extent of infestation by the tsetse between the wet and dry season. This seasonal change is a major factor in governing the migration of the nomadic pastoralist herds. During the wet season, these herds remain in areas outside the tsetse belt. However, as the dry season advances and forage becomes increasingly scarce, the herds are forced to move into tsetse fly infested zones. Fortunately, because of the regression of the tsetse fly at this time, the challenge risk is correspondingly reduced. However, small pockets of tsetse flies, particularly riverine but occasionally savanna species, remain and, when encountered, result in infection of the cattle.

Intensive cultivation accompanied by bush clearance and exclusion of wild game animals, by changing the habitat, can be an effective means of lowering the tsetse population in infested areas. On the other hand, plantation of trees or bushes may provide cover which allows invasion by the tsetse fly; invasion may be encouraged further by the introduction of domestic pigs and cattle. In areas where intensive cultivation is discontinued, there is often a rapid re-invasion by tsetse flies.

Factors Involving the Trypanosome

The severity of disease resulting from challenge with infected tsetse flies depends on the species and strain of trypanosome. There are large numbers of different strains of trypanosome which show widely varying virulence for cattle. Some isolates of *T. vivax* can be extremely virulent while others produce chronic low grade infections. *Trypanosoma congolense* also varies in virulence although it is never as pathogenic as the most highly virulent *T. vivax.* In cattle, *T. brucei* usually produces chronic low grade infections.

Factors Involving the Host

There are differences in susceptibility to trypanosomiasis between breeds of cattle and probably between lines within the same breed. This is best exemplified by the small West African breeds of cattle such as the N'Dama and West African Shorthorn. These animals are less susceptible to the disease

than Zebu or European breeds and are commonly found in endemic areas of trypanosomiasis. They are referred to as trypanotolerant breeds, this term being used to define animals which are able to survive in tsetse infested areas without the aid of chemotherapy.

Recent experimental and field studies carried out in The Gambia, West Africa on N'Dama and Zebu cattle, not previously exposed to trypanosomes, have confirmed that the greater resistance of the N'Dama is an innate characteristic and is related to their superior capacity to control levels of parasitemia and thus develop less severe anemia. However, it must be stressed that N'Dama may become clinically ill with trypanosomiasis, may show stunting or weight loss and may even die (Murray *et al.*, 1979).

In addition to the genetic make-up of the host, certain environmental factors may also affect its susceptibility. The nutritional status of the host undoubtedly influences the severity of the disease. Thus, in areas of poor pasture or during times of drought the poor condition of the cattle renders them more susceptible to the effects of the disease. This is particularly the case when severely anemic animals are required to cover large distances in order to obtain sufficient fodder. In addition, other stress factors such as work load of draft animals and parturition increase the severity of the disease.

Clinical signs and diagnosis

The clinical disease produced in cattle by infection with trypanosomes varies considerably in severity and duration. The disease syndrome is often considered to be either acute or chronic although the line of demarcation between these is poorly defined (Fig. 3). Thus, following infection with some isolates of *T. vivax,* death may occur within 2 weeks. On the other hand, relatively virulent isolates of *T. congolense* may result in death of the host 6-10 weeks after inoculation. In the context of trypanosomiasis, both of these syndromes would be regarded as relatively acute; the chronic disease might, therefore, be arbitrarily defined as occurring in animals infected for longer than 3 months. However, in the field, these definitions are often difficult to apply as, after initial infection of the animal, further infection with antigenically different trypanosomes may occur throughout the duration of tsetse challenge. This undoubtedly results in more severe disease than following a single experimental challenge. However, a study of the latter is instructive in appreciating the clinical course and pathogenesis of the disease.

The extremely acute disease, produced usually by *T. vivax,* resembles a septicemic condition. The animals are febrile, show sustained high levels of parasitemia and often exhibit massive hemorrhage.

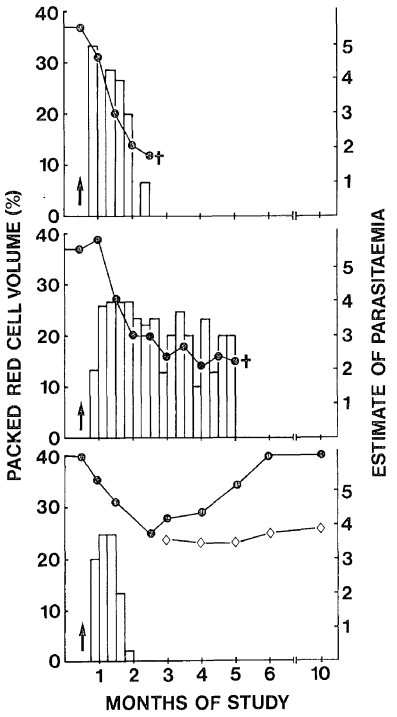

Fig. 3. Some of the different disease patterns in individual cattle infected with *Trypanosoma congolense*. *Top:* An acute syndrome. *Middle:* A more chronic infection. *Bottom:* The outcome of infection in two cattle in which trypanosomes could no longer be detected in the blood. In one animal the anemia resolved, while in the other it persisted.

More commonly, the disease, with all three species of trypanosome, is characterized by lower levels of parasitemia which may persist for many months. This is associated with the development of anemia which is the major contributory factor to the disease. The disease may take one of several courses which, as discussed in the previous section, depend on factors such as level of challenge, strain of trypanosome, the breed of cattle, level of nutrition, etc.

In cattle subjected to a single needle or fly challenge, the packed red cell volume (PCV) progressively decreases by about 40–50% over the first 4–6 weeks. In some instances the PCV continues to fall and the animals die (Fig. 3); while this drop in PCV is usually slow and progressive, on occasions a hemolytic crisis may develop and there is a very rapid and dramatic fall in PCV. In other instances, the PCV stabilizes and is maintained at a low level for a variable period of time (Fig. 3). Some of these animals may eventually die. In others, after a variable length of time, parasitemia gradually disappears, the PCV rises and the animals recover (Fig. 3). However, with

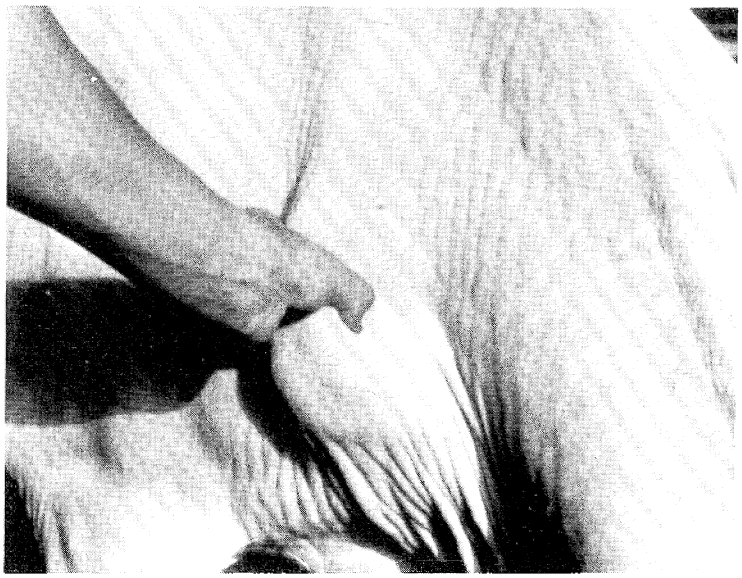

Fig. 4. Enlarged prescapular lymph node in acute bovine trypanosomiasis.

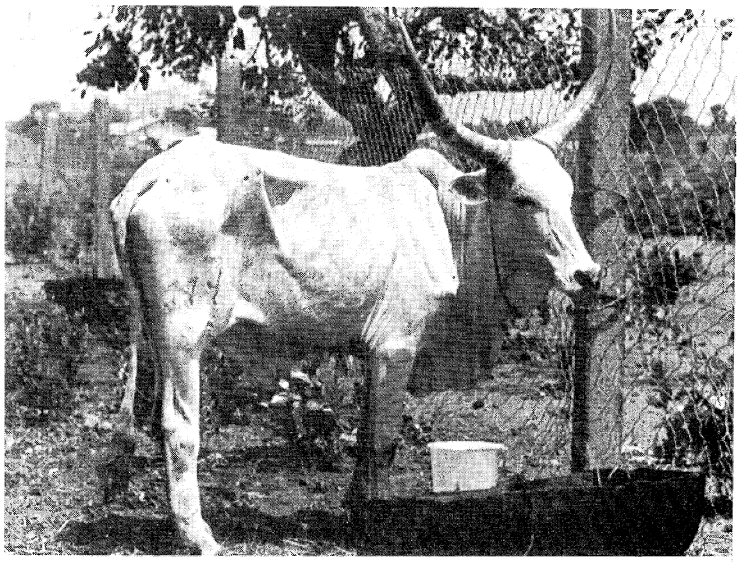

Fig. 5. Marked wasting in a Zebu infected with *Trypanosoma congolense*.

increased duration of infection even when parasites disappear, the increase in PCV is often very slow or insignificant; such animals sometimes respond poorly when treated with trypanocidal drugs.

The major feature of the disease in cattle is anemia which is seen as pallor of the mucous membranes. In the early stages of the infection when para-

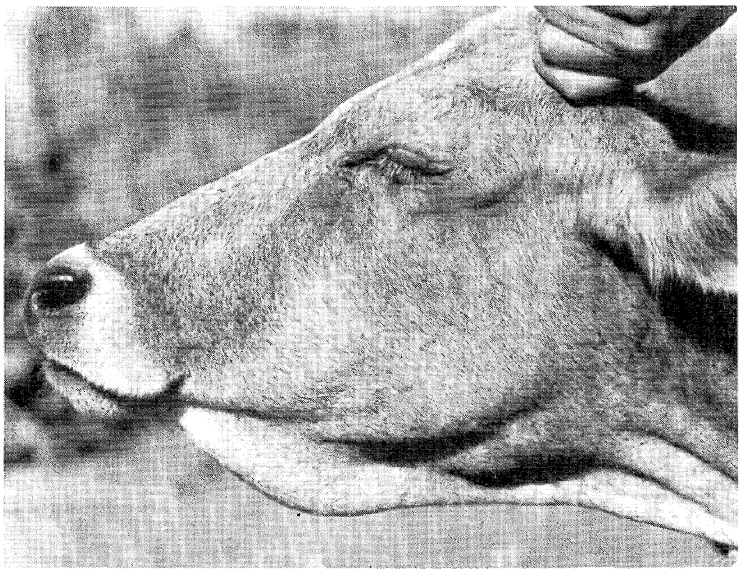

Fig. 6. Congestive heart failure in a trypanosome-infected bovine. There is marked submandibular edema and a prominent jugular vein.

sitemia is readily detected there is intermittent pyrexia. At this time the superficial lymph nodes are palpably enlarged (Fig. 4) although in the chronic phase of the infection they may be normal or reduced in size. While infected animals may occasionally show loss of appetite associated with pyrexia, they usually continue to eat throughout the course of the disease. As anemia becomes more severe, there is a gradual loss of bodily condition (Fig. 5). If these animals are required to forage over large distances they become progressively less able to obtain sufficient food. This in turn leads to further loss of condition. In infected herds, the most severely affected individuals are usually observed trailing at the rear of the herd. They are wasted and lethargic, their coat is dull and they show a "hunched-up" appearance. In the terminal stages of the disease affected animals become extremely weak and are often unable to rise. Cattle die in congestive heart failure which would appear to result from a combination of anemia, myocardial damage and increased vascular permeability. During most of the disease the animals are tachycardic. In the later stages they may show pulsating jugulars (Fig. 6) and as the heart becomes decompensated the pulse becomes progressively weaker. Terminally they may be bradycardic and sometimes they exhibit subcutaneous edema.

Many of the animals which survive remain unproductive for a long period of time and the growth of young animals is often stunted. Adult animals show decreased fertility. In addition, pregnant cows sometimes abort and,

even when calves are born at full term, they are often small and weak so that neonatal mortality is high.

Definitive diagnosis of the disease is ultimately dependent on detection of the trypanosome in blood samples from infected animals. Generally speaking, trypanosomiasis occurs as a herd problem and it is helpful to sample a group of animals from the herd where the disease is suspected. Needless to say, other possible causes of anemia such as piroplasmosis or helminthiasis should be considered. During the acute phase of the disease, trypanosomes can sometimes be detected in stained thin blood smears or in wet blood films. However, usually more sensitive methods need to be used to detect a reasonable proportion of the infected animals. There are 3 methods which are commonly used. Two of these utilize the fact that when infected blood is centrifuged in a microhematocrit tube the trypanosomes sediment at the interface of the buffy coat and the plasma. The capillary tube can then either be examined directly under the microscope (hematocrit tube concentration technique-HCT) or broken just below the buffy coat and the contents of the upper part expressed on to a slide and examined under darkground or phase-contrast microscopy (DG technique). The third method involves making a thick blood smear in which the erythrocytes are lysed on the slide before the smear is stained. A comparative study of these three methods has indicated that, for the detection of *T. congolense* and *T. vivax,* the DG method is the most sensitive, detecting levels down to 5×10^2 organisms per ml. On the other hand, for *T. brucei,* the HCT method was consistently found to be the most sensitive, with the lower level of detection again being 5×10^2/ml. Both of these methods have the added advantage over the thick smear that the PCV can be measured during the procedure, thus providing a monitor of the disease status of the animal. However, a disadvantage of these methods is that the samples must be examined, at least, within 4–6 hours of collection. The DG method has an advantage over the HCT method in that the species of trypanosome may more readily be identified, based on the size and pattern of motility of the organisms (Table 1).

Subinoculation of laboratory rodents with bovine blood may also be used for the detection of low levels of parasitemia. While this is by far the most sensitive method for detection of infections with *T. brucei*, some isolates of *T. congolense* and most *T. vivax* do not grow in laboratory rodents. Furthermore, the method is expensive and does not give an immediate result.

In addition to examining the blood, infections with *T. brucei* and *T. vivax* may also be detected by examination of aspirates from lymph node punctures. However, the sensitivity of this method relative to examination of blood samples has not been investigated critically.

The detection, by serological tests, of antibody directed against the trypa-

nosome has been described for the diagnosis of trypanosomiasis; the most commonly used method is the indirect immunofluorescence test. In comparison with methods for detection of the parasites, the immunofluorescence test is expensive and requires a higher degree of technical expertise. Furthermore, it gives some false positives and it fails to distinguish between animals currently infected and those which have recovered.

Pathogenesis and pathology

The three species of trypanosome which are pathogenic in cattle show differences in their distribution within the host. *Trypanosoma brucei* is found both intra- and extra-vascularly. By contrast, *T. congolense,* apart from the initial site of inoculation, is thought to be restricted to the bloodstream. However, within the circulation many of the *T. congolense* organisms are bound by their anterior end to vascular endothelium. Thus, because the internal surface area of blood vessels increases in relation to volume as the vessels become smaller, the capillary beds contain larger numbers of organisms per volume of blood than do larger blood vessels. Until recently, *T. vivax* was also thought to be restricted to the bloodstream. However, there is now convincing evidence that this organism is also present in the extra-vascular spaces and it has been shown in goats that large numbers of organisms are present in efferent lymph.

The picture usually presented at necropsy of an animal which has died of trypanosomiasis is that of a wet carcass in which the parenchymatous organs are pale; often excess serous fluid is found in the body cavities, particularly the pericardium, and sometimes there is gross edema of the subcutis and viscera. There is serous atrophy of body fat and wasting of skeletal muscles. The heart is often hypertrophied but in advanced cases the ventricles may be dilated due to decompensation giving the heart a globular appearance; the myocardium is pale and irregular streaks of hemorrhage are sometimes observed on the epicardium and occasionally extending into the depth of the myocardium. The liver is enlarged and chronic venous congestion may be visible grossly. In relatively acute cases, there is splenomegaly and enlarged lymph nodes, while in long-standing cases these organs may be normal or reduced in size. In most cases the hemal lymph nodes are markedly enlarged.

In the case of the hyperacute syndrome sometimes produced by *T. vivax,* the animals are often in good bodily condition and the main findings are vascular engorgement and hemorrhage; widespread petechial and ecchymotic

hemorrhages are found on the serosal and mucosal surfaces and there may be frank hemorrhage into the intestinal tract.

The pathogenesis of trypanosomiasis in cattle may be considered under four major headings:

1. the early events following the tsetse fly bite,
2. changes in the lymphoid system,
3. anemia, and
4. tissue lesions.

With the exception of hyperacute infections with *T. vivax,* the pathogenesis of the disease would appear to be similar for all three species of trypanosome. Only the tissue lesions vary due to differences in the distribution of the organisms.

1. The Early Events Following the Tsetse Fly Bite

Within a few days of being bitten by an infected tsetse fly, cattle develop a raised cutaneous swelling several centimeters in diameter which persists for several days (Fig. 7). The term chancre is used to describe this lesion. The bite of an uninfected fly produces no such change. The appearance of the chancre precedes detectable parasitemia by a few days and is accompanied by the development of fever and marked enlargement of the draining lymph node(s).

Fig. 7. Raised indurated swellings (chancres) on the flank of a susceptible Friesian 12 days after being bitten by *Glossina morsitans* infected with *Trypanosoma congolense.*

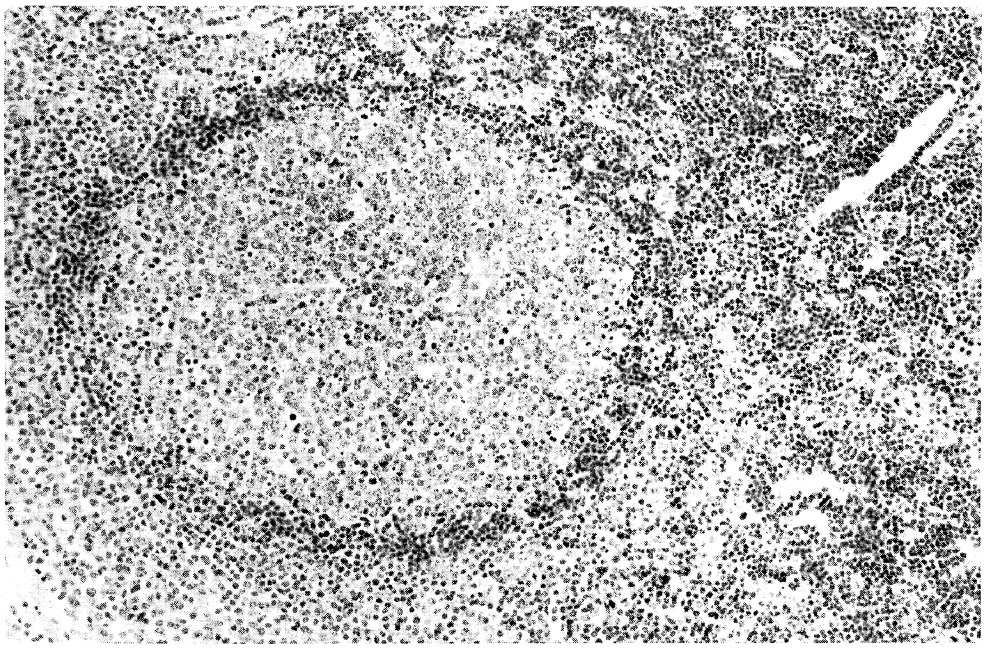

Fig. 8. The follicular cortex of the prescapular lymph node of a bovine 12 weeks after infection with *Trypanosoma congolense*. Note the presence of an active germinal centre. H & E. ×63.

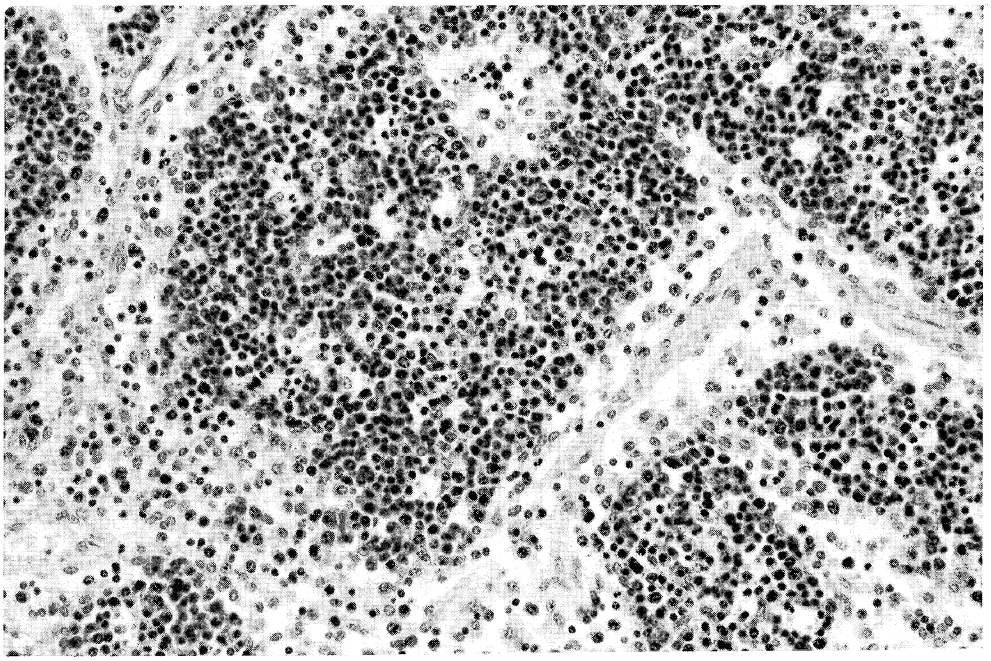

Fig. 9. The medulla of the same lymph node illustrated in Fig. 8. The medullary cords are distended by numerous plasma cells. H & E. ×100.

Trypanosomes are found extravascularly within the chancre. Their presence is associated with an inflammatory response characterized by congestion, edema and extravasation of polymorphonuclear leukocytes. In addition, large numbers of lymphocytes and plasma cells are present. As the lesion declines increased numbers of mature plasma cells, macrophages, eosinophils and mast cells are found.

It is thought that the chancre constitutes the initial site of multiplication of the trypanosomes which then pass either directly or via afferent and efferent lymphatics into the bloodstream. From the point of view of the host, the chancre probably represents the initial site of an immunological response to the trypanosome and may allow priming of the lymph nodes to the first variant antigens which are generated.

2. Changes in the Lymphoid System

Following enlargement of the lymph node(s) draining the chancre, generalized enlargement of lymph nodes and splenomegaly develop. This is associated with marked proliferation of lymphoid cells in these organs. Numerous large active germinal centers are present (Fig. 8) and there is a marked increase in the number of plasma cells in the medullary cords of the lymph nodes (Fig. 9) and in the splenic red pulp. Marked hypergammaglobulinemia accompanies these changes. In addition, in the red pulp of the spleen, there is an increase in numbers of active macrophages, some of which are engaged in erythrophagocytosis. The hepatic Kupffer cells are also increased in number and activity. However, in longstanding infections, the lymphoid organs may be decreased in size associated with a depletion of lymphoid cells.

3. Anemia

Anemia plays a major role in the pathogenesis of bovine African trypanosomiasis and is a reliable indicator of the progress of the disease. Based on the presence or possible absence of the parasite, response to trypanocidal drug treatment, and on clinical and pathological findings, the anemia can be divided into at least two phases. The first phase may last from 3 to 4 months after infection; the onset and severity of the anemia is directly related to the appearance of the parasite in the blood and to the level of parasitemia. During this period the anemia is hemolytic and is the result of increased red cell destruction by phagocytosis in the spleen, liver, lungs, hemal nodes, bone marrow and even in the circulation. Several mechanisms have been incriminated in this process including hemolysins produced by the trypanosome,

immunological factors, fever, and an expanded and active mononuclear phagocytic system. While each of these may function independently, it is possible that they interact, e.g., hemolysins and fever may damage the erythrocyte membrane which then may bind antigen-antibody complexes or complement more readily, thereby facilitating erythrophagocytosis. During this phase, the bone marrow is responsive as judged by ferrokinetic studies; furthermore, although the anemia is usually normocytic normochromic, a macrocytic response can occur. However, even at this time, some workers feel that the erythropoietic response is not as active as might be expected for the degree of anemia (Dargie *et al.*, 1979). While petechiation and ecchymosis are commonly encountered, hemorrhage is not a major feature of bovine African trypanosomiasis, apart from the very striking acute hemorrhagic syndrome produced by *T. vivax*. Hemodilution has also been incriminated as a factor contributing to the anemia although recent evidence would suggest that this is not the case (Dargie *et al.*, 1979); this finding has important clinical implications as it establishes that measurement of packed red cell volume (PCV) provides a reliable index of the degree of anemia.

It would appear that the mechanisms responsible for anemia during this phase of the disease depend on the presence of the parasite, as treatment with trypanocidal drugs usually results in recovery.

Provided cattle survive the initial phase or do not become reinfected, they gradually pass into another stage of the disease syndrome which may be ongoing or may end in the animal's death or recovery (Fig. 3). It is not possible to give an accurate estimation of when the onset of this next phase of the disease occurs but it may be any time between 4 and 6 months after infection. During this time, following progressively decreasing waves of parasitemia, parasites become difficult to detect or disappear completely. Despite the apparent absence of trypanosomes, in many cattle the anemia persists and affected animals usually respond very poorly to chemotherapy. Although the erythrokinetics and ferrokinetics of this chronic aspect of the trypanosomiasis syndrome remain to be investigated, post-mortem findings give some indication of what is going on, namely, continued red cell destruction, iron trapping and dyshemopoiesis. Thus, while splenomegaly is no longer a feature, erythrophagocytosis is still found throughout the body; hemosiderosis is widespread in the spleen, lungs, liver and bone marrow, and the femoral marrow is often yellow, gelatinous and inactive. Ferrokinetic studies on cattle in the earlier phase of the disease suggested a possible defect in iron metabolism (Dargie *et al.*, 1979) with iron retention in the mononuclear phagocytic system; such a situation could lead, in the later stages of the disease, to effective iron starvation of the bone marrow and the development of dyshemopoiesis. This chronic anemic trypanosomiasis syndrome is common in the

field although, because of the absence of detectable trypanosomes, it may be difficult to diagnose.

4. Tissue Lesions

As might be expected from the blood-borne nature of trypanosome infections, most tissues and organs are damaged during the course of infection although some are more consistently and severely affected than others. While necrosis is not a major feature of the disease in cattle, tissue cell damage and degeneration may be marked. The nature of the cellular infiltrate and possibly the mechanisms involved in cell injury would appear to depend on the difference in tissue invasiveness between species of trypanosomes.

One vital organ which is consistently damaged by all three species of trypanosome is the heart. The changes which occur in the heart also reflect to some extent what occurs in other tissues and organs. Initially, lesions predominate beneath the epicardium and the endocardium in both the atria and ventricles; in advanced cases, the entire myocardium may be involved. The

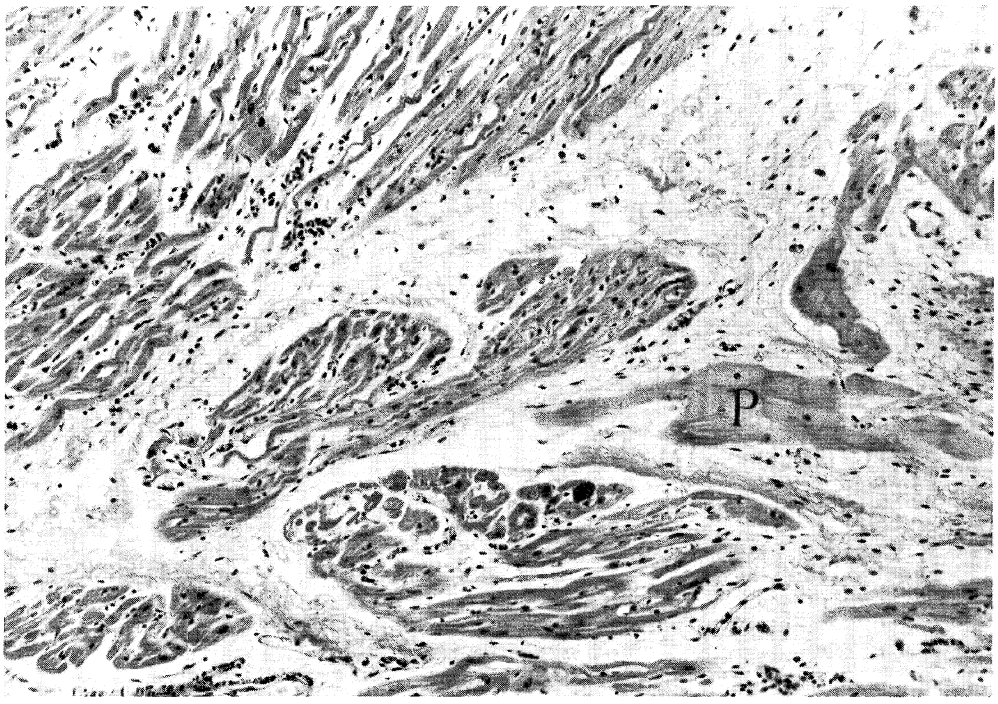

Fig. 10. The myocardium of a bovine in congestive heart failure following infection with *Trypanosoma congolense*. There is marked degeneration of the myocardial fibers which are separated and distorted by severe interstitial and perivascular edema. Note the scanty cellular infiltrate. P = Purkinje fibers. H & E. ×100

lesions produced by *T. brucei* and *T. vivax* are similar; the cellular infiltrate is often marked and composed of lymphoid cells, plasma cells at various stages of maturity, including Russell-body containing cells, macrophages and occasional eosinophils. These infiltrates are found in perivascular and interstitial locations and surrounding Purkinje fibers. Extravascular as well as intravascular trypanosomes may be found. On the other hand, in *T. congolense*-infected cattle where parasites have only been seen in the circulation, the interstitial cellular infiltrate is usually scanty and consists of small lymphocytes and the occasional macrophage and plasma cell (Fig. 10). Although cell death is rare, severe myocardial damage occurs in all three infections and is manifest by distortion and degeneration of myocardial fibers; in addition, the conducting Purkinje fiber system is often widely separated from the myocardium by cellular infiltrates and edema (Fig. 10).

The vessels of the microvasculature in the heart, and also in other organs, are frequently dilated with cells while the vessel walls are occasionally swollen and degenerate but never necrotic and the perivascular space is often distended with fluid; the latter change probably reflects increased vascular permeability. While thrombosis is uncommon, it does occur particularly in *T. vivax* infections.

Other vital organs which are commonly affected include the skeletal musculature, central nervous system, endocrine organs and the reproductive tract.

Possible Mechanisms of Organ and Tissue Damage

While the lesions produced in bovine African trypanosomiasis are well characterized, relatively little is known about the mechanisms by which they arise.

1. *Blood and circulatory disturbances.* It is likely that the anoxia caused by anemia contributes to the widespread cellular degeneration which occurs particularly in the heart, skeletal muscle, liver and kidneys. This situation must be aggravated by cellular infiltration and edema which lead to structural distortion and physiological disturbances. The increased vascular permeability may be due, in part, to the elevated kinin levels which occur in the blood of infected animals; these are thought to be the result of the interaction of antigen-antibody complexes with the Hageman factor (Goodwin 1970). In addition, it has been shown that *T. congolense* elaborates an inflammatory permeability factor, can activate complement and has a selective affinity for endothelial cells of the microvasculature.

Although disseminated intravascular coagulation has been implicated in the

pathogenesis of trypanosomiasis in both humans and laboratory animals, it is not known whether it plays a role in bovine trypanosomiasis. However, thrombocytopenia does occur in trypanosome-infected cattle, the degree being related to the level of parasitemia, and is associated with the development of minor coagulation abnormalities (Wellde *et al.*, 1978).

2. *Biologically-active substances produced by trypanosomes.* Until recently, the hypothesis that trypanosome "toxins" were involved in the pathogenesis of trypanosomiasis has been out of favor especially as the earlier reports were difficult to confirm. However, a range of biologically-active factors have now been demonstrated *in vitro* (Tizard *et al.*, 1978). These include hemolysins, inflammatory and permeability factors, complement activating factors, platelet aggregating factor, immunosuppressive factors and polyclonal B lymphocyte mitogens. Their *in vivo* relevance awaits complete evaluation although there is evidence that hemolysins generated by dying trypanosomes may be operative in trypanosome-infected cattle; phospholipases and free fatty acids have been incriminated as the active substances.

The finding that immunologically incompetent mice infected with *T. brucei* develop little in the way of tissue lesions despite the presence of large numbers of organisms in the tissues (Galvao-Castro *et al.*, 1978) suggests that live trypanosomes by themselves do not produce severe tissue injury; however, it is possible that toxic substances capable of causing cell death may be released following the death of organisms in the tissues.

3. *Immunological mechanisms.* The massive and persistent immunological reaction induced by consecutive waves of trypanosomes has long been considered as likely to contribute to the development of hypersensitivity reactions and cell damage. However, it is only recently that work on *T. brucei*-infected mice has provided direct evidence for this possibility. In intact adult mice, *T. brucei* produces severe myocardial and skeletal muscle damage in the vicinity of extravascular trypanosomes and a cellular infiltrate of lymphocytes, plasma cells, macrophages and a few polymorphonuclear leukocytes; associated with these lesions are granular deposits of immunoglobulin and trypanosomal antigen. However, immunologically incompetent mice, including neonates, sub-lethally irradiated mice and congenitally athymic (nude) mice, show only minimal tissue damage despite the presence of large numbers of trypanosomes; correspondingly, there is a marked decrease in immunoglobulin deposited in the tissues. When *T. brucei*-infected nude mice are given normal syngeneic spleen cells or an Ig-negative fraction of spleen cells, they develop lesions as severe as those in intact mice; at the same time, heavy deposition of immunoglobulin is detected (Galvao-Castro *et al.*, 1978). Thus, it would

appear that the immune response, at least in mice, is important in the development of tissue lesions and Galvao-Castro *et al.* (1978) proposed that the immunological factors involved were most likely to be locally-produced antigen-antibody complexes.

In conclusion, when considering tissue damage, the tissue invasiveness of the organism must influence the nature of the mechanism(s) involved. The invasiveness of *T. brucei* and to a lesser extent of *T. vivax* would make it likely that hypersensitivity reactions are operative, whereas, in *T. congolense* infections most of the tissue damage can probably be attributed to the anoxia, resulting from the anemia, and microcirculatory disturbances.

Immune response

Trypanosome-infected cattle develop hypergammaglobulinemia, involving particularly IgM, soon after infection. Although IgG_1 and IgG_2 levels are not markedly altered, studies on the rates of catabolism of different immunoglobulin classes have shown a 9- and 13-fold increase for IgG_1 and IgG_2 respectively, while there is a 5-fold increase for IgM, indicating a marked increase in production not only of IgM but also of IgG_1 and IgG_2 (Nielsen *et al.*, 1978a).

Antibody activity against the trypanosome is readily demonstrated in these immunoglobulin fractions and there is considerable evidence that antibody is the most important effector mechanism in immunity to trypanosomiasis. Thus, it has been shown in cattle infected with *T. congolense* that if trypanosomes and serum are collected at weekly intervals during infection, serum will react with and usually neutralize trypanosome populations collected one or more weeks previously (Wilson and Cunningham, 1972). Several studies in mice have confirmed that passive transfer of serum or immunoglobulin from immunized animals gives complete protection against homologous challenge. At the same time, it has been shown that protection can be achieved by the adoptive transfer of spleen cells from immunized mice and, furthermore, that while a B-cell enriched fraction gives protection, a T-cell enriched fraction does not (Campbell and Phillips, 1976). In the same way, it is possible to immunize congenitally athymic (nude) mice against homologous challenge but not B-cell deficient mice. Several studies in laboratory animals have indicated that IgM is the important Ig class in controlling the parasitemia and in protection (Campbell *et al.*, 1978) and there is also evidence that this might be the case in cattle (Luckins, 1976). Whether antibody alone is sufficient to kill the parasites *in vivo* or whether complement and/or macrophages are also required is not known.

Hypocomplementemia is known to occur in trypanosome-infected cattle

where there is evidence of activation by the classical pathway as well as properdin activation (Nielsen *et al.*, 1978b). However, no significant changes are found in the amount of C8, indicating that the terminal components are not utilized. This depletion may be the result of several mechanisms including production by the trypanosomes of complement activating substances and increased catabolism. Also in laboratory animals, complement binding antigen-antibody complexes occur and in such cases elevated levels of immunoconglutinin have been recorded, reflecting widespread utilization and fixation of complement (Murray, 1974). It is also likely that complement is consumed in the antibody-mediated lysis of trypanosomes, a reaction well documented *in vitro* which could be important *in vivo*.

Evidence obtained in other species indicates that a proportion of the immunoglobulin being produced is not directed against the trypanosome but contains heterophil antibodies, rheumatoid factor-like substance and a range of auto-antibodies; furthermore increased numbers of background plaque-forming cells to sheep red blood cells are found in the spleens of infected mice (Mansfield, 1978). These observations have led many workers to propose that trypanosome infections induce a polyclonal activation of B cells. A profound immunodepression, as assessed by a number of *in vitro* and *in vivo* assays, has been demonstrated in trypanosome-infected laboratory animals (Mansfield, 1978). Whether these changes also occur in trypanosome-infected cattle has yet to be determined. Several recent studies have indicated that there is suppression of the antibody response of infected cattle to several viral and bacterial vaccines (Whitelaw *et al.*, 1979). However, in none of these instances was the depression of the antibody response of sufficient magnitude to suggest that the vaccines would be ineffective and, in view of the catabolic rate of immunoglobulin in infected animals, there may have been no suppression of the response at the cellular level. It has also been shown that infected cattle continue to generate antibody to new trypanosome populations several months after initiation of infection. Nevertheless, there is circumstantial evidence that trypanosome-infected cattle may be more susceptible to other pathogens, e.g., we have observed recrudescence of *Salmonella* infections in infected cattle. The whole question of immunodepression in cattle and its possible influence on the trypanosome infection itself and on intercurrent infections or vaccination awaits more detailed evaluation.

Prevention and control

The methods currently used to control African trypanosomiasis in cattle include anti-trypanosomal drugs, tsetse eradication or control and the use of trypanotolerant cattle. No field vaccine is available.

Chemotherapy

Several drugs are used for the treatment of trypanosomiasis in cattle, the most commonly used ones being Diminazene aceturate (Berenil, Hoechst) and Homidium chloride (Novidium, May & Baker, Ethidium, Boots). Except in the case of longstanding infections, there is usually a good response to treatment: parasites are rapidly cleared from the blood and this is accompanied by a return to normal hematological values. However, in endemic areas, reinfection can occur from two weeks after treatment, depending on the degree of challenge. Furthermore, experimental work in mice has cast doubt on the ability of the drugs concerned to sterilize the body of parasites (Jennings *et al.*, 1977), results which are similar to observations in cattle (MacLennan *et al.*, 1970; MacLennan, 1971). There comes a stage in the chronic case where the anemia persists despite trypanocidal treatment. Treatment is often used on a herd basis to eliminate infection from a transhumanal herd returning from a tsetse-infested area to its tsetse-free home base, or for infected cattle being moved into a tsetse-free area for ranching purposes.

To obtain the best advantage from treatment of cattle in endemic areas, the animals require regular monitoring to enable the disease to be treated at an early stage. Too often animals receive one treatment without checking its effect on parasitemia or the anemia.

Chemoprophylaxis

In endemic tsetse areas where cattle populations are reasonably stable and can be identified by ear tagging, chemoprophylaxis is by far the best approach to the control of trypanosomiasis. Thus, at present, cattle exist profitably on ranches which otherwise could not maintain cattle. Isometamidium chloride (Samorin, May & Baker) is the drug of choice and in areas of constant heavy challenge, treatment may be required every 6 to 8 weeks. Less rigorous regimes can be worked out only after careful monitoring of each situation. The implementation of efficiently operated chemoprophylaxis should be considered in areas where eradication of tsetse is not feasible.

In both treatment and prophylaxis, drug resistance has been recorded and must continue to be an increasing threat in the future. It should be emphasized, however, that if drugs are properly administered at the correct dosage levels, the risk of drug resistance is greatly reduced.

Eradication of Tsetse

In several countries in Africa, large scale eradication of tsetse has been carried

out in open savanna land containing a large network of small tributaries of rivers with water levels greatly reduced during the dry season. In such areas suitable habitats for tsetse are diminished during the dry season. As a result, it is often necessary to spray only 10% of the land area. Studies on the resting sites of the fly have also shown that selective spraying with residual insecticides such as BHC and DDT on the main trunks and under surfaces of main branches of trees will eliminate *Glossina morsitans* after only one spraying. From the beginning, a large enough area must be selected for such an operation so that it can be expanded each year; careful monitoring for reinvasion by the tsetse fly is essential. During the last 15 years the main successes with this technique have been achieved by large numbers of men using knapsack sprays.

In more recent years, techniques of helicopter and fixed-wing aircraft spraying have been developed successfully for open savanna country. Although these techniques have been successful in riparian forests in the savannas they have not yet been developed for thick tropical forest. The advent of aerial spraying has led to the use of a new generation of insecticides such as endosulfan and the pyrethroids. This technique requires five consecutive sprays from aircraft within short intervals to eliminate successive generations of fly as they emerege from the pupae in the soil.

Eradication programs require to be justified on a cost-effectiveness basis. Nations must be confident that the land from which the fly is eradicated will be adequately used for agriculture. The cost is high but there are many areas in which it can be justified. If eradication does not take place in certain productive areas of Africa, the future is indeed bleak for cattle production in particular, and socio-economic development as a whole. The emotive argument used by some that tsetse eradication will increase desertification due to overstocking is misleading. Overstocking causes erosion of soil and the disappearance of perennial plants from any areas of the world far beyond the tropics. Control and proper use of pasture by cattle is a separate technology from tsetse eradication and there are able practitioners of pasture management available to use sensibly and profitably every hectare cleared of tsetse.

Much has been written about the potential damage which insecticide spraying can cause to the environment. So far the only evidence that this is so has arisen from gross overdosage by certain spraying techniques which have killed fish in tropical forest riverine areas. As far as open savanna is concerned, there is no evidence of damage from highly selective spraying of residual insecticides or by the aerial spraying of insecticides such as endosulfan using techniques which emit droplets as small as 30 m. It should be pointed out that the amount of insecticide used to control or eradicate the tsetse is small in proportion to the quantity of insecticides and herbicides used in agriculture

generally. However, all major eradication programs in the future should incorporate a monitoring system where potential biohazards are evaluated.

Trypanotolerance

The lack of a vaccine and the expense and practical difficulties of implementing the present measures available for the control of African trypanosomiasis in livestock have generated considerable interest in recent years in the exploitation of trypanotolerant breeds of cattle, such as the N'Dama and West African Shorthorn, in tsetse-infested areas. These breeds of cattle would appear to have the capacity to survive in tsetse fly-infested areas where other breeds cannot. However, their importance depends on how productive they are under tsetse challenge. Although in the past observers have tended to regard trypanotolerant breeds, because of their small size, as being poorly productive, recent preliminary work carried out by the International Livestock Center for Africa has demonstrated that there are several million animals of these breeds in West and Central Africa and data are available to suggest that the productivity of such breeds relative to other indigenous types may be much higher than previously assumed. Also, the fact that trypanotolerant breeds are reported to be less susceptible to tick-borne diseases and to streptothricosis is an additional advantage.

Currently, there is evidence that trypanotolerance has a genetic basis and may even be inherited as a dominant trait, although it is known that resistance can be reduced by such factors as the stress of parturition and suckling or by high levels of challenge; on the other hand, it can be supplemented by previous exposure. There is preliminary evidence that the trypanotolerance trait is associated with superior immune responsiveness although it is possible that other factors such as water conservation, ability to forage and skin physiology may be involved (Murray *et al.*, 1979a, b, 1980).

While more data are required on the genetics, mechanisms and productivity of trypanotolerant breeds, there is no doubt that the use of these breeds is one of the most promising approaches available to the problem of trypanosomiasis control.

Vaccination

No field vaccine has yet been produced for African trypanosomiasis, the major constraint being the ability of the parasite to undergo antigenic variation. Thus, it has generally been considered that the possibility of producing a vaccine against trypanosomiasis is remote as it would appear that an effective vaccine would have to contain all variable antigens, a possibly insurmounta-

ble task. However, recent advances in biochemistry, genetic engineering, trypanosome culture and the development of monoclonal antibodies have opened up several new and potentially hopeful avenues of research, particularly with a view to understanding the basis of antigenic variation and how it might be manipulated (Murray *et al.*, 1979a, b, 1980). In addition, field studies in cattle have provided evidence that animals previously exposed to trypanosomiasis may be less susceptible to subsequent challenge. In other groups of cattle, particularly of trypanotolerant breeds, self-cure can sometimes occur. Thus, a combination of fundamental research coupled with an understanding of the field situation in cattle may allow the development of an immunotherapeutic strategy for African trypanosomiasis.

There is little doubt that the availability of an effective field vaccine would make an enormous contribution to the control of African trypanosomiasis and if integrated with properly managed tsetse control and drug strategies would go a long way to opening up to agriculture and socio-economic development the vast areas of Africa dominated by the tsetse fly.

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37. MYCOTIC DISEASES

J.A. Schmitt

Abstract. It can be safely said that an otherwise healthy individual is more resistant to mycotic diseases than is a non-healthy individual. The widespread use of antibacterial antibiotics in animal rations and concomitant suppression of the immune response most probably render the animal more susceptible to invasion by fungi, as has been shown clearly in cases of human mycoses. Thus, any fungus causing disease in an animal or human being may be considered to be an opportunistic fungus. With this concept in mind, the following mycoses were selected and are described and discussed: dermatomycosis, phycomycosis, cryptococcosis, rhinosporidiosis, candidiasis, aspergillosis and histoplasmosis.

A general statement about mycoses needs to be made before proceeding to describe a few of the specific mycoses. In humans, and presumably in animals, only about 60 species of fungi regularly cause disease. There is wide agreement that a healthy person is less susceptible to invasion by fungi than a debilitated person. The intact epidermis is a good barrier to fungal invasion of the skin but a damaged epidermis renders the patient susceptible. Generally speaking, the intragastric route does not lead to the establishment of the fungus in human tissue. Beyond doubt, the respiratory route is involved as the portal of entry in many cases; consequently, it is the route for all primary pulmonary mycoses, and may be involved for most of the deep or systemic mycoses.

The widespread use of antibacterial antibiotics and of immunosuppressive drugs has led to an enhanced degree of susceptibility to mycoses in human beings. Presumably, the use of antibacterials in feed would have a similar effect in cattle. Such compromised individuals may be invaded by fungi routinely considered to be saprobic. Thus, opportunistic fungi not regularly associated with human or animal disease may be recovered as the sole etiologic agent in a compromised patient.

The following sequence of presentation of the mycoses should not be construed as a suggestion of their relative importance or incidence in cattle.

Dermatomycosis

Synonyms: ringworm, dermatophytosis, trichophytosis

Etiology

The dermatomycoses in cattle are a cluster of cutaneous mycoses caused by one or more species of the genus *Trichophyton* (*T. verrucosum* most commonly, *T. mentagrophytes* occasionally, and *T. rubrum* and *T. violaceum* rarely), and occasionally by *Microsporum* sp. There is no one-to-one relationship between a given set of clinical signs and a causal agent.

Distribution

The four species mentioned above have no specific geographic limitations. It is generally accepted that most species of the genus *Trichophyton* have a world-wide existence in soil, the natural reservoir. With *T. verrucosum,* there is evidence of animal-to-man transfer (Kaplan *et al.*, 1978).

Epidemiology

Primary infection in a herd is almost always a result of contamination by fungal propagules (spores or hyphal fragments) from soil. Another common source is by inclusion of already-infected animals into an otherwise clean herd. Animals crowded into a confined area (as in dry-lot feeding have a greater incidence of infection by *T. verrucosum* than those in range-fed herds.

Clinical Signs

The clinical manifestations of ringworm in domestic animals are extremely variable. Lesions of *T. verrucosum* infection in cattle are usually on the head or neck, but may be scattered on the body, legs or tail. In calves, the lesions may be extensive. The lesions usually develop into coin sized or larger distinct plaques with heavy greyish-white crusts. When the crusts are removed, moist, bleeding areas are seen. Old lesions lose the heavy crusts, leaving areas of scaliness and broken-off hair stumps.

Pathology

Dermatomycotic agents are invasive in subcutaneous tissues only in unusual circumstances; they are usually limited to the dead cells of the stratum

corneum, hair and nail. Normally a ringworm fungus grows inwardly at about the same rate that the desquamative process occurs outwardly. In skin, the fungus will appear as hyphal fragments, in or on hairs, hyphae and/or spores can be seen in a potassium hydroxide (KOH) preparation (see technique under Laboratory aids to diagnosis).

Immune Response

Little is known about the immunology of ringworm diseases in cattle. There is evidence in other animal species that the primary lesion is an allergic reaction, representing at least a second encounter by the animal with that fungus allergen (see Schmitt and Miller, 1967).

Laboratory Aids to Diagnosis

Two types of diagnostic tests are available: the microscopic examination of hairs and scales from the infected area, and the cultural demonstration of one of the species of dermatomycotic agents.

For the direct microscopic examination, epidermal scales and/or hair stubs removed from the lesion near the periphery, are placed in 2 to 3 drops of 10% KOH; gentle heating—*no boiling*—will facilitate clearing of the cells for greater ease in recognizing the fungal elements in the host cells. Excessive amounts of scales or hair will obstruct visualization of the fungi. The slide should be scanned using low power (10×); when a suspicious field is seen, change to 43× (it is not usually necessary to use oil immersion) to see the hyphal fragments in epidermal cells and/or chains of spores in or on the hairs.

A "positive KOH" will reveal mycelial fragments intra- and inter-cellularly in the scales for the four species cited above. Additionally, *T. verrucosum* and *T. mentagrophytes* may reveal chains of arthospores (hyphal cells somewhat larger than regular hyphal cells and usually with thicker walls). For hairs, sheats of or isolated spores will be found on the hair surface (ectothrix invasion), or within the hair (endothrix).

A positive KOH preparation is adequate for a presumptive diagnosis of ringworm, and to start chemotherapy. Complete, accurate diagnosis is possible only by obtaining a pure culture of one of the ringworm fungi. Specimens for culture must be taken from the actively advancing periphery of the lesion. Topically disinfect the area to be sampled with 70% ethanol. Aseptically remove scales and hair for planting into agar media; flame-sterilize the surface of two microscope slides, place the flamed surfaces in contact in the laboratory; later, the slides are separated, one to catch the scrappings, the

edge of the other to scrape the specimens from the lesion. The slides are then secured face to face for return to the laboratory.

The ringworm fungi are best isolated on one or more of the following media: Sabourand's dextrose agar (SDA); mycosel® agar (essentially SDA, plus chloramphenicol and cycloheximide, available in dehydrated form) or dermatophyte test medium (DTM, available in dehydrated form from Difco Co., Detroit, Michigan). Dermatophyte test medium is perhaps preferable, since it includes an indicator whose color change is evoked primarily only by ringworm fungi. There are, however, a few saprobic (contaminant) fungi and a few yeasts that also bring about the color change, so DTM is not *per se* an infallible identifying medium.

Sabourand's dextrose agar with and without chloramphenicol and cycloheximide are adequate for primary isolations. Aseptically, scales are transferred from the collection slide to the agar surface. Cool the transfer needle on the agar in the blank slant, then retrieve the scales and hair stubs with the moistened needle for transfer to the spot where the needle was cooled. Use an L-shaped wire, not a loop. The clinical material should be pressed into the agar surface. Incubate at both room temperature (21–23 °C) and at 37 °C (in an incubator if necessary). Although ringworm fungi do not require the higher temperature for continued growth, primary isolation of *T. verrucosum* is more successful if incubated for the first 24–28 hours at the elevated temperature.

Details of the appearance of these fungi in skin and hair, and of the cultural characteristics of the species can be found in Georg (1959).

Prevention and Control

Prevention of an infestation of ringworm in a herd is ideal. Careful inspection of animals being added to the herd can avoid contamination from an external source. Active immunization has been tried as a preventative measure. Care should be exercised in handling cattle with a dermatophytosis since animal-to-man transfer is common also.

Once an animal has ringworm, treatment must be dictated by the circumstances of the individual case, within certain broad guidelines. Since the ringworm fungus is usually limited to the stratum corneum and grows inwardly at about the same rate as desquamation proceeds, the use of keratolytic agents, such as salicyclic acid, may suffice to achieve a cure.

Ringworm lesions of the hairy skin usually are not amenable to treatment with topicals; griseofulvin is the drug of choice. Its action is primarily fungistatic, preventing the normal development of the hyphal. The best results are achieved through use of the microcrystalline form of the drug;

increased absorption and higher blood levels are achieved by administering the drug with a meal high in fat content. The variety of treatment schedules is virtually unlimited. One schedule uses the daily administration of 10–20 mg/lb of animal microcrystalline griseofulvin with concomitant use of a topical agent such as Captan* (as 1:200 solution applied every 4 days for 6 treatments). It has been reported (Jungerman and Schwartzman, 1972) that Captan at the strength above has proved effective in the absence of the use of griseofulvin. Natamycin-S has been used with varying degrees of success. Treatment with local herbs seems to have been effective in Africa; the fruits of *Solanum aculeastrum* ("goat apple", "bitten apple", "bokappel", "bitter-appel", "gifappel", as "murulwa") are used as remedy for ringworm in cattle and horses and "many *Solanum* species are used as ringworm cures by country people" (Palmer and Pitman, 1972).

Phycomycosis

Synonym: Murcomycosis

Etiology

One or more species of the following phycomycetous genera have been reported from mycotic diseases in animals: *Rhizopus, Mucor, Entomophthora, Mortierella* and *Absidia*. The hyphae of these fungi lack regularly placed crosswalls except where a reproductive organ forms. *In vitro,* all phycomycetes reproduce asexually by forming large numbers of sporangiospores in specialized cells, the sporangia. The features of the sporangial mechanism determine generic placement.

Distribution

Phycomycetes occur widely in soil and water, and are commonly found as plant pathogens and food decomposers.

Epidemiology

Fungi involved in phycomycosis in man and animals are opportunistic and succeed in evoking a disease only in a host which is debilitated due to some other cause, ranging from diabetes and prolonged oral antibacterial or corti-

* Orthocide garden fungicide, 50% Captan, Chevron Chemical Company, Ortho Division, San Francisco, California.

costeroid drug use to the trauma of a puncture wound with a contaminated twig.

Clinical Signs

Complete clinical descriptions of animal phycomycosis have been recorded only for the horse. The presenting signs will vary with the site affected and with the circumstances that permit proliferation of the fungus.

These circumstances in animals seem to parallel those in human. For example, one common presenting feature in man is as a rhino-orbital disease in uncontrolled diabetes, paralleled in the rhesus monkey (Martin *et al.*, 1969).

Granulation tissue is usually seen on the surface, and may be hemorrhagic due to self-inflicted trauma. Knudtson *et al.* (1972) reported a case of bovine cerebral phycomycosis in a 6-week-old heifer. The animal had been emaciated since birth, and was anorectic, cachectic and ataxic upon admission, without other signs of disease.

Pathology

The lesion(s) will show varying degrees of tissue destruction due to the presence of draining sinus tracts. The fungus has a predilection for blood vessel walls, proliferating within the walls and extending into the lumen to partially or nearly completely block the flow of blood.

In stained sections, granulation tissue surrounds sinus-containing necrotic tissue within which the fungi are found. In and near blood vessels, the fungi will appear as fragments of wide, non-septate hyphae. Sporulation does not occur in tissue. In the heifer referred to by Knudtson *et al.* (1972), gross lesions were not observed in the parenchymatous organs, the brain was discolored and on sectioning revealed several soft, cream to yellow areas and massive degeneration of the posterior half of the cerebum. Cultures on SDA at 37 °C yielded many colonies of *Mucor pusillus*.

Immune Response

Virtually nothing is known about the immunology of naturally occurring phycomycosis in animals. If the situation known in man and the rhesus monkey case reported by Martin *et al.* (1969) holds true for animals in general, an immune deficiency in the potential host animal may be prerequisite to fungal invasion and the establishment of disease.

Laboratory Aids to Diagnosis

Because phycomycetes are common airborne contaminants, care must be exercised in reporting a phycomycete as the causal agent in a mycosis.

For cultural isolation, the specimens should be plated out on SDA with anti-bacterials; do not include cycloheximide, as phycomycetes are sensitive to it *in vitro*. Plant the specimens near the center of the agar in the petri dish or test tube slant; this may help to decide whether the phycomycete recovered is the etiologic agent or an airborne contaminant.

The recovery of a fungus composed of wide, non-septate hyphae on which ultimately sporangia with sporangiospores form, can be reported as a phycomycete. Identification to genus and species is difficult and the case(s) should be referred to a mycologist for proper identification and reporting.

Prevention and Control

Because the potential etiologic agents are air- and soil-borne fungi, prevention is ruled out. Careful evaluation of feed for the presence of potential causal agents may help. Because of the apparent role of debilitation in the disease, maintaining a herd of otherwise healthy animals may at least reduce the chances of an animal developing phycomycosis.

In the early stages of the disease process, extensive surgical excision may suffice. In animals, as in man, drug therapy is tenuous; sometimes treatment of the underlying predisposing condition will aid in treating the mycosis. No specific anti-fungal drug can be recommended.

Cryptococcosis

Synonym: Cryptococcal mastitis.

Etiology

The causal agent is *Cryptococcus neoformans,* an encapsulated, single-budding yeast which grows at room temperature and incubator temperature (35–37 °C). Synonyms for *C. neoformans* are *Saccharomyces neoformans, Cryptococcus hominis* and *Torula histoytica.*

Distribution

Cryptococcosis has a worldwide distribution. Environments carrying a high

load of pigeon droppings will yield *C. neoformans* usually, but many soils not evidently so contaminated will also culture out the causal agent (Emmons, 1955).

Epidemiology

Cryptococcus neoformans has a wide ecological amplitude: fruit juice, milk, soil, surface of a peach, wasp nests, grass, bodies of insects, butter, tinned milk and the slime flux of the mesquite bush, as well as the surface skin of non-diseased human as part of the "normal skin flora" of that person, and of course, cases of overt cryptococcosis in man and a number of animal species.

Clinical Signs

In man, cryptococcosis is considered to be primary pulmonary mycosis (Pappagianis, 1967) with secondary sites in a variety of organs and tissues, despite the predilection of *C. neoformans* for the central nervous system (CNS).

There is a marked inconsistency in clinical signs in animal host species. Even in cryptococcal bovine mastitis, the clinical signs evoked by invasion of the mammary gland are extremely variable. In outbreaks, the fungus can be recovered from samples when no visible changes are noted in either the gland or milk. The cases with overt signs range from mild and transient swelling of one or more quarters of the udder to severe swelling and distension of the affected glands. The severe case evolves slowly, the glands at first mildly affected with swelling and firmness in their dorsal parts; this progresses in the next several days to extreme swelling and firmness. The subcutaneous tissues of the udder and the anteriorly adjacent area becomes edematous, persisting for several weeks. Palpation of the supramammary lymph nodes commonly reveals enlargement.

Cows with mild disease frequently manifest only the swelling of affected glands, while those with severe infections seemingly suffer notable discomfort – they are reluctant to move and stand with hind legs wide-spread. Body temperature elevation rarely exceeds 2 °C and the depression, anorexia, and dehydration characteristic of severe bacterial mastitis are lacking or are mild. Milk secretion is affected in severe cases, gradually diminishing to complete cessation, even in overtly unaffected quarters. Milk usually shows no change during the early course of infection; first milk changes may be no more than small white flakes in the strip cup. In persistent, severe cases, watery serum containing flakes may characterize the secretion or the milk will appear greyish, highly viscous and mucoid.

Pathology

Gross lesions of cryptococcosis can not be accurately diagnosed using necropsy specimens. Naso- and oropharyngeal lesions may resemble myxomatous nodules. In the lungs and abdominal viscera, granulomatous nodules predominate. With progressive pulmonary involvement, miliary granulomas, small abscesses or large solid or mucoid areas of pneumonitis may involve one or more lobes. Central nervous system involvement will vary from no apparent involvement, through edematous, slimy-feeling meningeal surface, to marked reddening, thickening and obscuring of the underlying brain tissue. Central nervous system lesions (common in man and a number of animal host species) were not found in an outbreak of *C. neoformans* mastitis described by Innes *et al.* (1952); in most cases the infection was confined to the udder, in a few animals iliac or deep inguinal nodes and supramammary glands are affected, with only one cow showing cryptococcal lesions in the lung, suggesting hemotogenous dissemination. Those authors noted differences in the histopathology of different quarters, and in different parts of the same quarter, apparently related to severity and chronicity of the disease. These ranged from large, irregular cysts packed with the yeast cells to fibrous tissue proliferation in intra- and interlobular situations; some foci were granulomas.

In tissue, *C. neoformous* is an ovoid to spherical, thick-walled single-budding yeast with a narrow neck between the bud and parent cell; the mature cell is 5–10 μm in diameter, exclusive of the variably thick capsule. The cells are difficult to detect in a hematoxylin-eosin stained section; Mayer's mucicarmine stain selectively stains the capsule pink to red, while in the periodic acid Schiff (PAS) stain cell wall and capsule stain rose to deep red.

Immune Response

Immunologic evaluation of cases of crystococcosis is not well substantiated. Cross-reactions are common and in the absence of a readily-available, specific antigen, the true prevalence of the disease is unknown. There is a growing sense that it occurs commonly as a benign, self-limited, perhaps even asymptomatic infection. Verification must await development of the sensitive specific antigen on a commercial basis.

Laboratory Aids to Identification

The ultimate procedure for identification of *C. neoformans* is the positive culture. Clinical specimens should be cultured at both room temperature and

37 °C on SDA and BHIA brain, heart infusion agar with an antibacterial if necessary. The vast majority of isolates growing out at both 20–21 °C and 37 °C will be *C. neoformans*. Because the fungus is suceptible *in vitro* to cycloheximide, the antifungal should not be incorporated into isolation medium.

Direct microscopic examination of fluids. Pipette some of the stirred fluid into a drop of half-strength India ink, stir, add a cover glass and observe microscopically. The carbon particles are restricted by the capsule and the negative staining procedure causes seeming halos around the yeast cells.

Selective media have been developed. Shields and Ajello (1966) developed a selective isolation medium with creatinine as the nitrogen source, diphenyl as a mold inhibitor, chloramphenicol as the antibacterial and nigerseed extract as the color marker.

To verify the genus *Cryptococsis* from species of *Candida* and other yeasts, the urease test (slants of Christensen's area agar, incubated at 25 °C) is useful. In 18–48 hours, a red-purple to pink color in the agar is a positive test for an isolate of cryptococcus.

Identification of *C. neoformans* requires recovery of a yellowish mucoid colony of encapsulated, ovate to spherical, single-budding yeast cells. These should be transferred to (1) Christensen's urea agar, incubated at 25 °C, for the urease test and (2) fresh slants of agar incubated at both 20–25 °C and 37 °C, since *C. neoformans* will grow at both temperatures. If necessary, other biochemical tests can be used to confirm *C. neoformans.*

Prevention and Control

Proper sanitation is perhaps the only hope for prevention, and that is not likely. From soil, its natural reservoir, cells of *C. neoformans* become airborne and can be carried moderately great distances without losing viability.

In human cases of cryptococcal infection, some success has been realized by using 5-fluorocytosine. Again from human cases, newer antifungal drugs have been successful in small numbers of cases. It does not necessarily follow that drugs and dosages used in the successful treatment of human systemic mycoses will be equally efficacious in animal mycoses.

Rhinosporidiosis

Etiology

The causal agent is *Rhinosporidium seeberi.*

Distribution

The disease is distributed worldwide, with endemic areas in Argentina, Ceylon and India; sporadic cases have been reported from Australia, Brazil, South Africa and the United States. It has been reported in cattle, horses, mules, dogs, goats, geese and ducks, as well as man. The first case in cattle was reported in 1923.

Epidemiology

Information on the epidemiology of rhinosporidiosis is circumstantial, whether in humans or animals. There is no evidence of transfer of the organism from one host to another; each case arises *de novo*. Many cases of rhinosporidiosis have a relationship to stagnant or semi-stagnant water, yet several kinds of aquatic animals have been evaluated negatively as potential hosts.

Trauma may play a role as a predisposing factor to rhinosporidiosis as suggested by Rao (1938), noting a higher incidence of the disease in male draft oxen whose nasal septum has been punctured for a nose ring or string than in animals not so treated. There is a possibility of an association between rhinosporidiosis and schistosomiasis.

Clinical Signs

The typical clinical sign is a predunculated or sessile polyp, pinkish in color and typically up to 3 cm in diameter. The soft, friable growth bleeds easily and due to its lobulated surface, appears cauliflower-like. The nasal mucosa is the most common site; the conjunctival sac, vagina and the integumentary system of the ear and other sites. Typically only one nasal cavity is involved, but severe dyspnea may occur, together with a blood-stained mucopurulent nasal discharge.

The surface of the polyp usually presents many small white specks, the superficial manifestation of the numerous sporangia (spore containing sacs) seen in stained sections of the polyp.

Differential diagnosis can be accomplished in the following manner: Stertorous breathing or a unilateral mucopurulent nasal discharge should lead to an examination of the nasal cavity. The presence of single or multiple polyps, as described above, should suggest rhinosporidiosis, which can be confirmed by microscopic examination of the exudate or biopsy sections for the presence of spores and sporangia of *R. seeberi*. Nasal granulomatous lesions caused by other fungi or schistosomes must be differentiated from *R. seeberi*.

Pathology

Histopathologically, the polyp reveals a papillomatous epithelium, which may be hyperplastic and contain numerous sporangia. The bulk of the polyp is a stroma of fibrous tissue with numerous large sporangia and with an increased vascularity.

Immune Response

The principal inflammatory cells are lymphocytes and epithelioid cells, but when the sporangial wall bursts, a noticeable increase in neutrophils, eosinophils, red blood cells, giant cells, histiocytes and mast cells is evident. Usually these reactions can be detected in a section stained by the hematoxylin and eosin procedure, although special fungal stains (mentioned earlier) render the maturing sporangia and spores of the causal agent more evident.

Although both man and domestic animals might become infected by *R. seeberi,* virtually nothing is known concerning the immunologic aspects of the disease.

Laboratory Aids to Diagnosis

The report of the cultivation of *R. seeberi* by Datla (1965) has not been confirmed although Grover (1970) reported maturation of spores and sporangia in biopsy material placed in a synthetic liquid medium.

The only method of confirming a diagnosis of rhinosporidiosis is by demonstration of the sporangia and spores in the nasal exudate or in tissue sections. Spores are about 7 μm in diameter, while the sporangia are between 300 and 400 μm in diameter; gentle pressure on biopsy material with a forceps will express the organism into a drop of water for microscopic visualization.

Treatment

Rhinosporidiosis is best treated by surgical incision of the polyps, although they may recur. No drug has been found to have any efficacy.

Candidiasis

Synonym: Moniliasis.

Etiology

The main causal agent is *Candida albicans,* but *C. tropicalis, C. krusei, C. parapsilosis,* and *C. guillermondii* may also cause the condition.

Distribution

The yeasts are endogenous and are part of the normal flora of the skin and intestine in normal healthy humans and animals. Hence, they are worldwide in occurrence.

Epidemiology

Candida spp. may be found at high levels in animal feces. Thus, contamination of meat by feces at slaughter may bring about animal-to-man transfer, or contamination of feed by feces may account for spread of candidiasis in closely housed animals.

Clinical Signs

Presenting symptoms vary with the type of candidiasis. Mammary infections are often mild, transient and frequently self-limiting. When severe mastitis is encountered it usually follows antibiotic infusions; in one outbreak caused by *C. tropicalis,* all treated animals developed acute mastitis to one degree or another. The udders swelled with a spongy consistency and body temperatures ranged from 104 to 107 °F; some individuals were anorectic and lame, and milk production was drastically reduced.

Systemic candidiasis caused by *C. albicans* may occur in cattle, frequently following prolonged antibacterial antibiotic therapy. Clinical signs in calves include watery diarrhea and melena with subsequent anorexia and dehydration, and a gradual progression to prostration and death.

Candidal abortion has been reported, with *C. albicans* and *C. parapsilosis* incriminated.

Pathology

The finding of cells of *Candida* species in a KOH preparation or its isolation from a single cultural attempt should not confirm a case of candidiasis. Because candidas are endogenous, it is necessary to show that the organism has invaded tissue. In tissue sections stained by the PAS or Gomori's methenamine silver (GMS) procedures, the fungus is manifest as pseudohyphae, rarely as the unicellular yeast form.

Immune Response

Because of the ubiquitous nature of *Candida* spp , most humans and presumably other animals have had a sensitizing encounter. Hence, the results of skin tests and the several serologic tests used with other mycoses are of little value as adjunct data for a diagnosis.

Laboratory Aids to Identification

Skin scrapings should be examined microscopically in a KOH preparation; round or oval budding cells, 3–6 μm in diameter and sometimes fragments of hyphae may be seen. Smears of exudates, etc., should be Gram-stained; look for cells, as in KOH preparations.

Cultures must be attempted: use a medium containing an antibacterial antibiotic but use of a medium containing cycloheximide should be avoided since several species of *Candida* are sensitive to it. If media with an antibacterial antibiotic are not available, transfer contaminated yeast colonies to four tubes of SDA to which have been added 1, 2, 3, and 4 drops of 1 N HCL and incubate overnight at 37°C. Subculture from the tube that is free of bacteria (usually the ones with 2 or 3 drops of acid) to a blood agar plate at 37°C; reculture colonies to SDA.

Two tests are used to identify *C. albicans*: the serum filamentation test and chlamydospore formation. In the former, suspect *Candida* cells are inoculated into half-strength human or fetal bovine serum and incubated at 37°C. After 2 hours, check microscopically for the production of "germ tubes" (filamentous outgrowths from the blastospores); only in *C. albicans* do a vast majority of cells produce germ tubes at 2 hours. Both *C. stellatoidea* and *C. tropicalis* may form germ tubes between 3 and 4 hours, but all other species require 7 or more hours at 37°C to form these hyphal outgrowths. Results with this procedure exceed a 95% reliability for *C. albicans.*

The other, older, and more commonly employed test is chlamydospore production on corn meal agar or chlamydospore agar (the latter available commercially in dehydrated form). The suspect culture is cut-inoculated into the agar, part of the inoculated slit should be covered with a sterile coverglass. Plates are incubated at room temperature for 18 hours (chlamydospore agar) or 48 hours (corn meal agar), then examined microscopically for the formation of the terminal and intercalary spherical, thick-walled chlamydospores. If they are produced in abundance, the isolate is *C. albicans,* but slightly less abundant chlamydospore production could indicate *C. stellatoidea* or *C. tropicalis.* This test will detect only 77–80% of the isolates of *C. albicans.* Thus, from the time element and the greater reliability, the serum filamentation test is recommended for the detection of *C. albicans.*

A useful isolation medium is Pagano-Levin medium, an agar including neomycin to inhibit bacteria and tritetrazolium chloride as a color marker. On this, *C. albican* grows as a cream to light pink colored colony, while other candidas and other yeasts will vary from white to deep red.

For complete identification, *Candida* isolates should be confirmed by fermentation and assimilation tests (see Jungerman and Schwartzman, 1972, Table 5-1, p. 70).

Prevention and Control

Because *Candida* spp. are endogenous and usually commensal with the host, prevention of overt candidiasis devolves to the prevention of circumstances that predispose the host: diabetes, malnutrition, irritation of skin-skin interfaces, prolonged antibacterial and corticosteroid drug therapy, and other immuno-suppressive drug therapy.

Once overt candidiasis has become established, treatment is dictated by the site affected. Topical application of nystatin will suffice for cutaneous lesions or for decontamination to the gastrointestinal tract to prevent reinfection with candidal vaginitis from contaminated feces. Amphotericin B. has been used successfully in systemic candidiasis. A wide variety of other drugs have been used to control or cure candidiasis.

Aspergillosis

Etiology

Aspergillus fumigatus, A. flavus, A. niger, A. terreus, and other *Aspergillus* spp. The aspergilli are ubiquitous in nature and are among the most notorious laboratory contaminants. *Aspergillus fumigatus* has been incriminated most commonly, but *A. flavus* and *A. niger* are being recovered more frequently in recent years.

The aspergilli form mold colonies *in vitro*. They are fast-growing organisms, sporulating in 2–3 days after transfer. The sporulating zone varies in color from pale golden-brown to greenish (dark brown in *A. niger*), but never blue-green as in the penicillia. Three features are characteristic of the microscopic appearance of an *Aspergillus*: the conidiophore (spore-bearing hyphal branch) has a terminal enlargement, the vesicle; the conidiophore is nonseptate, and the conidiophore has a foot-cell (the conidiophore is an outgrowth of a somatic hyphal cell).

Distribution

The aspergilli have a worldwide distribution.

Epidemiology

Aspergilli are saprobic organisms, playing an important role in the decomposition of organic debris. They are a common component of the soil mycoflora, and are prolific spore-formers over a wide range of environmental conditions.

There is presumptive evidence in man and experimental evidence in laboratory animals that antibiotics and corticosteroid drug therapies render an individual more susceptible to an *Aspergillus* sp. There is no known, direct animal-to-animal or animal-to-man transmission.

Clinical Signs

Bovine aspergillosis in several forms has been recorded infrequently, although one form seems to be increasing in incidence. Aspergillomas of the skin, yielding *A. terreus,* and the lung, yielding *A. fumigatus,* are known. *Aspergillus*-induced abortions appear to be increasing. *Aspergillus pneumonia* evokes no unusual signs. *Aspergillus*- (and other fungal-) induced abortions usually occur in the 3rd through 8th months, the fetus rarely being alive. The placental lesions show yellow to grey cotyledons which have markedly thickened peripheral areas. Typically, a necrotizing placentitis is seen; edema and hemorrhage are common and extensive (Cordes and Shortridge, 1968).

Pathology

In systemic aspergillosis, any internal organ may be invaded. Multiple discrete pulmonary aspergillomas with necrotic centers are common. Microscopically in the necrotic areas, polymorphonuclear leukocytes may aggregate around clumps of hyphae. In tissue *Aspergillus* branches dichotomously, where branching occurs.

Immune Response

Although skin test antigens and several serologic tests are used in aspergillosis in humans, comparable tests have not evolved for the mycosis in animals (Walter and Jones, 1968).

Laboratory Aids to Diagnosis

Because of the ubiquity of the aspergilli, they can be removed frequently from skin and the upper respiratory tract in the absence of disease. Hence, repeated recovery of an *Aspergillus* spp. in the absence of other pathogenic fungi is presumptive evidence of aspergillosis. When specimens from an area not exposed to the environment can be taken aseptically, recovery of an *Aspergillus* supports the diagnosis. Supporting evidence of the dichotomously branching hyphae in tissue will sustain the diagnosis.

Sabourand's dextrose agar supports the growth of all aspergilli: chloramphenicol, as a bacterial inhibitor, can be used but avoid cycloheximide to which aspergilli are sensitive *in vitro*. Microscopically, the features characterizing an aspergillus (above) can be seen. Speciation is difficult, so isolates should be submitted to a qualified mycologist for proper identification.

Prevention and Control

No effective treatment is available for aspergillosis. Prevention of conditions favoring proliferation of aspergilli in the environment may be attempted, but because of the long distances over which airborne spores can be disseminated, even decontamination of the immediate environment is only an ephemeral approach to control.

Histoplasmosis

Etiology

Histoplasmosis has two causal agents, dependent upon the host species and the geographic location: *Histoplasma capsulatum* and *H. duboisii*. The two species are dimorphic (described later).

Distribution

Histoplasma capsulatum has a world-wide distribution, causing histoplasmosis in man and a variety of other animals. *Histoplasma duboisii* (or *H. capsulatum* var. *duboisii*) is limited to the African continent. Spontaneous histoplasmosis has been found in the dog, cow, cat, rat, mouse, skunk and Kodiak bear, as well as man.

Epidemiology

Histoplasmosis in cattle is not common; since *H. capsulatum* is known to exist saprobically in soils (Emmons, 1949), it is presumed that animals other than man also become contaminated from that source, most probably by the respiratory route. In humans, the organism has a predilection for the reticuloendothelial system.

Clinical Signs

Adler (1950) reported the first case of bovine histplasmosis in a range bull in Hawaii. A second case in cattle was reported in an aged cow in Missouri by Menges and Kintner (1951). The cow had been sick for several months prior to showing terminal signs of dyspnea, diarrhea, swelling of the brisket and grinding of teeth. At necropsy a concurrent traumatic reticulitis was found which may have predisposed the dissemination of the fungus. Menges *et al.* (1962) isolated *H. capsulatum* from bronchial lymph node tissue of a 5-month-old calf which had died suddenly with lesions suggestive of blackleg or a similar clostridial infection.

Pathology

What little is known of the disease in cattle is mentioned under *Clinical Signs* above. Clearly, as more confirmed cases of bovine histoplasmosis are uncovered in standing animals, every attempt should be made to complete immunologic studies and, at autopsy or necropsy, tissue should be taken for a complete histopathologic work-up using special fungal strains (Oshima and Miura, 1972).

Immune Response

The histoplasmin skin test has been found to be helpful as an adjunct to diagnosis. Sharbaugh *et al.* (1973) reported that in experimental histoplasmosis in cattle high titers of latex-agglutinating and complement-fixing antibodies were observed within 3 weeks of inoculation, and after 5 weeks two characteristic precipitin bands (H and M) appeared. Skin tests become positive just prior to formation of precipitins.

Laboratory Aids to Diagnosis

The only valid aid to a proven case of histoplasmosis is obtaining a positive

culture of *Histoplasma capsulatum* or *H. duboisii* from specimens. When a case of histoplasmosis is suspected, specimens should be cultured at room temperature (below 30 °C) on SDA with or without supplements, and at incubator temperature (35–37 °C) on BHIA. The decision to use the supplemented agars should be made on the probability of contamination of the specimen by bacteria and/or saprobic fungi.

The room temperature culture should grow out in 10–14 days as a white to beige mycelial colony which microscopically shows septate hyphae with small, usually round microconidia 1–5 μm in diameter and larger (10–25 μm) macrocondia that are spherical to pyriform and smooth-walled or tuberculate in white and beige strains, respectively. The 37 °C incubated cultures should grow out a yeast colony in 7–10 days, the cells of which are round to ovate and 2–4 μm in diameter. Such yeast cultures, if returned to room temperature, should revert to the mycelial phase of growth.

In *H. duboisii,* the mycelial culture at room temperature is identical to that of *H. capsulatum*. However, the yeast phase cells are significantly larger, being 7–15 μm in diameter and with cell walls 1–1.5 μm thick, intermixed with but not predominating over the smaller size yeast cells of *H. capsulatum*. In tissue also, the yeast cells of *H. duboisii* show the described characteristics and thus resemble the tissue phase (yeast phase) of *Blastomyces dermatitidis,* so the conversion to the diagnostic mycelial phase is crucial.

Prevention and Control

Prevention seems out of the question, especially in cattle. There is no evidence of animal-to-animal direct transfer of the fungus. Seemingly, the infected animal contaminates the soil, where the fungus grows saprobically, producing conidia and hyphal fragments, which serve as the inoculum that contaminates the next animal. There is no evidence of animal-to-man direct transfer, but again, the indirect mode is possible.

No proven effective chemotherapeutic agent is available for the treatment of histoplasmosis or any of the systemic mycoses. Amphotericin B., at an i.v. dose of 1.0 mg/kg body weight daily, has been widely used, but the prolonged course frequently leads to undesirable side effects ranging from emesis, hematesis and anorexia to renal and pulmonary dysfunction. Studies with the newer antimycotic drugs, such as 5-fluorocytosine and serramycin, need to be undertaken in animals. Several imidazoles are available, have shown promise in certain human mycoses, and should be evaluated for veterinary efficacy.

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38. METAZOAL DISEASES

James Armour

Abstract. The etiology, distribution, epidemiology, clinical signs, pathology, diagnosis, treatment and control of four important helminth diseases of cattle, namely, hemonchiasis, osteragiasis, dictyocauliasis and fascioliasis are reviewed. Particular emphasis is placed on the epidemiology, immunity and chemical methods available for preventing these diseases.

The distribution, epidemiology, diagnosis and control of bovine cysticercosis and toxocariasis are also discussed.

Parasitic gastro-enteritis

Parasitic gastro-enteritis (PGE) in cattle in the tropics and sub-tropics is caused by a variety of nematodes of which *Haemonchus* species are particularly important in both climates and *Ostertagia* species in certain subtropical zones. These two diseases are considered separately except for their treatment and control which are discussed together since the principles are similar.

Haemonchiasis

Disease

Haemonchiasis is caused by abomasal nematodes of the genus *Haemonchus* and primarily affects young cattle. The disease is characterized by weight loss, anemia, hypoalbuminemia and in severe cases, sub-mandibular edema.

Etiology

Haemonchus species are among the largest of the ruminant trichostrongyles, the adult males being up to 22 mm and the females up to 34 mm long. The latter are readily recognized by their "barber pole" appearance due to the

white ovary and uterus being twisted around the red, blood-filled intestine. The closely related species *Mecistocirrus digitatus* which occurs principally in India and S.E. Asia is found in the same site but measures up to 45 mm long.

For many years *Heamonchus placei* has been regarded as the main bovine species though *H. concortus* and *H. similis* have also been identified in cattle. These different species can be differentiated morphologically in the males on spicule lengths which are 450-470 μm for *H. placei*, 400-430 μm for *H. contortus* and <340 μm for *H. similis*. Recently, the above classification has been disputed and the suggestion made that *H. placei* is a bovine strain of *H. concortus*.

Distribution

Haemonchiasis occurs in all the continents of the world. It is most prevalent in the tropical savanna areas and the humid sub-tropics with summer rainfall (cf. ostertagiasis). In the latter zones it is the nematode infection which exerts the greatest constraint on productivity, particularly where intensive or semi-intensive systems of grazing management are introduced.

Life Cycle, Epidemiology and Immunity

Haemonchus worms have a high egg-laying capacity and as soon as mature worms are present there is heavy contamination of the pasture via eggs deposited within the feces. Development from the egg through the first and second free-living larval stages (L_1 and L_2) to the infective third stage (L_3) occurs within the feces and is rapid (5 days) at the optimal temperature of 24 °C but slows considerably at lower temperatures and there is no development below 9 °C. Above 30 °C, development is rapid but there is a high mortality. Provided moist conditions are present, the L_3 will migrate onto the pasture from the fecal pat.

Following ingestion of the L_3, infective larval development to the adult stages occurs through the fourth and fifth larval stages (L_4 and L_5). The mature adult stage is usually reached within 4 weeks although under certain circumstances the L_4 stage development is delayed for several months.

Arrested development at the L_4 stage, or hypobiosis as it is frequently referred to, may occur for a variety of reasons. In most countries it has a distinct seasonal distribution; the onset coincides with climatic conditions adverse to the free-living stages and termination parallels the arrival of a favorable climate. In West Africa, for example, hypobiotic L_4 accumulate during the arid dry season, and development of the hypobiotic larvae to the

adult stage coincides with the onset of seasonal rains (Hart, 1964; Fabiyi *et al.*, 1979). It is probable that survival of *Haemonchus* species during adverse climates occurs principally via hypobiotic larvae in the host although the survival of eggs within the feces and the larvae in the soil requires further study.

Resistance to *Haemonchus* develops slowly and animals up to a year old are highly susceptible. Although older stock develop some resistance, haemonchiasis can occur, particularly where nutrition is poor or the animals are under production stress.

The high egg production of *Haemonchus* and warm moist weather created by seasonal rains can precipitate severe outbreaks of bovine haemonchiasis within 3 to 4 weeks of grazing on infected pasture.

Although clinical haemonchiasis principally occurs due to the blood sucking activities of recently acquired worms, it also occurs when hypobiotic larvae mature at a time that haemonchiasis would not be considered a likely occurrence, such as at the end of a long dry season. Since such animals are often on a low plane of nutrition, there is often confusion in the relative roles of malnutrition and haemonchiasis.

Clinical Signs

Cattle suffering from haemonchiasis usually show a progressive loss of weight, anorexia, lethargy and anemic mucous membranes. In those chronic cases, sub-mandibular edema may develop. The feces are of normal consistency but sometimes are dark colored due to the presence of blood. Milk yield of lactating animals is lowered. In young susceptible calves the disease can be more acute with severe anemia developing and death occurring within a few days.

Pathology

The pathogenesis of haemonchiasis is due to the blood-sucking activities of the L_4 and adult worms. At post-mortem the abomasal mucosa is covered with minute hemorrhages where the worms have perforated the mucosa to suck blood. The mucosa is usually edematous and hyperplastic and in heavy infections fresh blood may be present in the abomasum. The carcass often has the characteristic pale and watery appearance associated with malnutrition. If the bone marrow is examined, it seems likely that as in infected sheep, there is depletion of the marrow with resorption of both cancellous and cortical bone; in long-standing cases the marrow may no longer be red.

Hemorrhage depletes the iron reserves and consequently erythropoietic function may be impaired (Allonby and Urquhart, 1972).

Laboratory Aids to Diagnosis

Although diagnosis is based primarily on clinical signs, additional confirmation may be obtained from fecal egg counts and hematological examination. In clinical haemonchiasis the fecal egg counts are very high reaching several thousand eggs per gram (e.p.g.) of feces; sometimes in massive infections disease may be recognized before the adult worms commence egg-laying. In these instances depressed hematological indices (red cell counts, hemaglobin and packed cell volume percentages) provide evidence that an anemia is present.

Prevention and Control

See ostertagiasis.

Ostertagiasis

Disease

Ostertagiasis (parasitic gastritis) is caused by abomasal nematodes of the genus *Ostertagia*; the disease is of particular importance in young cattle and clinically is characterized by weight loss and diarrhea. In older cattle the disease is more insidious in nature but is responsible for lowered productivity in fattening steers and milking cattle.

Etiology

The most common *Ostertagia* in cattle is *O. Ostertagi*; over 90% of infections are due to this species. *Ostertagia lyrata* (S. *skrjabinagia lyrata*, *Grosspiculagia lyrata*) has also been recorded in low numbers from cattle in most parts of the world. The adult worms are 8 mm in length and can be seen by the naked eye on the surface of the abomasal mucosa.

Distribution

The genus *Ostertagia* is much more common in temperate areas of the world but it also causes problems in sub-tropical zones where winter rainfall occurs in important cattle rearing areas.

Life Cycle, Epidemiology and Immunity

The life cycle and epidemiology of bovine *Ostertagia* infections display many of the same characteristics discussed previously in relation to *Haemonchus*. The life-cycle is the direct development of the egg through the free-living L_1 and L_2 stages to the infective L_3 stage. The L_3 stage occurs in the fecal pat where moist conditions are necessary for migration of the L_3 onto the pasture. The optimal temperatures for development of the free-living stages are 15 to 21 °C. The L_3 survive best at low temperatures and rapidly succumb to temperatures above 25 °C, although survival is prolonged if the larvae remain within fecal pats. In the parasitic cycle, development takes place through the L_4 and L_5 stages in the abomasal glands. The L_5 emerge to mature on the surface of the abomasal mucosa. The pre-patent period is usually 21 days but may extend to several months when arrested development or hypobiosis at the L_4 occurs. The latter is common and occurs seasonally at the onset of dry spring weather in winter rainfall areas (Hotson, 1967) and when temperatures decline in autumn and winter in the humid sub-tropics where rainfall occurs throughout the year (Williams and Knox, 1976; Craig, 1979).

Immunity to *Ostertagia* species develops slowly and requires exposure for at least two grazing seasons before a significant resistance develops; even in "immune" cattle it is common to find low numbers of adult worms. Ostertagiasis occurs in two forms:

Type 1 occurs in susceptible grazing cattle during the time when development of the free-living stages is optimal (mild humid weather). Disease occurs 3 to 4 weeks following exposure to heavy infection.

Type 2 occurs in yearlings or adults usually toward the end of the summer/early autumn in winter rainfall areas, or mid summer/early autumn in the humid sub-tropics.

The disease is due to the development of hypobiotic L_4 which were acquired in the spring (winter rainfall areas) or the winter (humid sub-tropics). The release of hypobiotic larvae in cows sometimes coincides with calving.

Pathology

The main pathogenic effect of *Ostertagia* occurs during the emergence of the L_5 stages from gastric glands. At this time the cells lining the glands, particularly the acid secretory parietal cells, are replaced by rapidly dividing undifferentiated cells resulting in a thickened hyperplastic non-functional gastric mucosa. The principal consequences of this are: 1) an elevation of the pH of abomasal fluid resulting in (a) failure to activate pepsinogen to pepsin, (b)

failure to denature proteins, (c) loss of bacteriostatic effect; 2) enhanced permeability of the mucosa to macromolecules leading to a) elevated levels of pepsinogen in the plasma, b) a loss of albumin into the gut.

Macroscopically, the lesion produced in *Ostertagia* infection is a raised nodule with a visible central orifice; in heavy infections these nodules coalesce to resemble morocco leather. In severe infections edema, hyperemia and sloughing of the mucosa occur (Armour, 1974).

Clinical Signs

The main clinical signs are diarrhea and loss of weight. Although the diarrhea is profuse and watery in the severe clinical disease, in some instances, particularly in Type 2 disease, it may be intermittent and episodes occur where only soft feces are observed. Coincident with the diarrhea, anorexia and thirst are present and the coat is dull and heavily soiled. In Type 2, anemia and hypoalbuminemia occur and sub-mandibular edema may be present.

Laboratory Aids to Diagnosis

Diagnosis is based primarily on clinical signs, season and grazing history. Fecal egg counts are of limited value except in Type 1 cases involving young stock where the count exceeds 1000 e.p.g. Plasma pepsinogen values provide a useful diagnostic guide. In clinical cases the pepsinogen level ranged from around 1.0 i.u. to over 3.0 i.u. In Type 1 disease, pasture larval counts provide valuable information on the infectivity of the pasture being grazed. Levels of over 1000 L_3/kg of dried herbage have been associated with a significant loss of production.

Prevention and Control of Parasitic Gastro-Enteritis

The aim of any control program is to prevent or minimize contact between the free-living stages of the parasite and its host. In temperate zones and to a lesser extent in the sub-tropics this can be achieved by the provision of clean grazing as provided by a new grass ley sown after 2 or 3 years of cash crops by which time any surviving larvae will have succumbed. This is seldom practical under tropical conditions and in countries where grazing occurs throughout the year. An alternative which is practiced in some tropical countries is to employ zero grazing whereby the livestock are continuously housed and the grass, either preserved or freshly cut, is transported to the animals. Theoretically, zero grazing should eliminate gastro-intestinal helmin-

thiasis but the unhygienic conditions in which the cattle are often kept and the use of their manure as fertilizer perpetuates the infection, albeit at a low level. Furthermore, where a reversal to more traditional grazing systems has taken place problems have arisen due to the low immune status of the previously housed stock which have had minimal exposure to infection.

In most of the tropics and sub-tropics control is based primarily on anthelmintics combined with possible manipulation of grazing management.

The drugs used should be effective against the wide range of cattle nematodes since it is unlikely that either *Haemonchus* or *Ostertagia* would be the sole nematodes present. The benzimidazoles, preferably one of the newer ones (fenben-, oxfen- and albendazole) thiophanate, levamisole, or morantel tartrate are all satisfactory. At certain times it is important that the drug is effective against hypobiotic larval stages; the newer benzimidazoles appear to be the most effective against these stages. Levamisole is widely used because it may be given by injection or poured onto the skin; both routes are preferable to oral treatment in cattle. Low level medication in feed blocks is not particularly reliable under extensive conditions. Rumen anthelmintic bullets with a slow release mechanism (e.g. Anderson and Laby, 1979), are under development and one containing morantel tartrate is marketed in several countries.

The response to a single treatment for haemonchiasis or Type 1 ostertagiasis is usually dramatic. Hematological status and gastric function are rapidly restored to normal. The response in Type 2 ostertagiasis is not always so good and a second treatment may be necessary in 3 weeks.

Long-term benefits are best obtained from an anthelmintic treatment given during or just prior to a period of minimal reinfection in a safe pasture. The anthelmintic removes the worms and the safe pasture limits the rate of reinfection. Such a procedure minimizes the number of animal treatments required. Safe pastures are those in which resistant eggs or infective larvae are allowed to die off in the absence of grazing cattle. This period can be as short as 2 months in hot dry conditions or up to 6 or even 12 months under cool moist conditions. Alternate grazing with sheep is also useful in controlling ostertagiasis since the respective *Ostertagia* species are host specific; however, with *Haemonchus contortus* cross infection can occur between sheep and young calves (Southcott and Barger, 1975). Once the level of pasture infection has reached high levels, although worm removal is achieved by treatment with anthelmintic, reinfection may follow. Treatments must be given every 3 to 4 weeks and, although this is expensive, it is sometimes necessary and economically advantageous. Unfortunately, the problem of anthelmintic resistance has been increasing, particularly in relation to *Haemonchus* species. Consequently, it is recommended that drugs from different chemical groups be rotated between *Haemonchus* seasons.

Although immuno-prophylaxis using L_3 attenuated by X-rays has produced a good resistance to experimental challenge, such vaccination has failed under field conditions.

Parasitic bronchitis Dictyocauliasis

Disease

Parasitic bronchitis (husk or hoose) is caused by infection with the lungworm *Dictyocaulus viviparus*; it is characterized by increased respiratory rates and coughing.

Etiology

Dictyocaulus viviparus is the only lungworm found in cattle which inhabits the bronchi and bronchioles. They are slender white worms which measure up to 10 cm in the adult stage.

Distribution

Dictyocaulus viviparus occurs primarily in temperate zones, although parasitic bronchitis has also been reported in some parts of the sub-tropics and less often in tropical highlands, e.g., in Argentina, Brazil, Cameroun Republic, Dominican Republic, Mexico and Paraguay.

Life Cycle, Epidemiology and Immunity

The life cycle is direct with L_1 present in the feces developing rapidly through L_2 to the infective L_3 stages in 1 to 2 weeks at the optimal temperatures at 15-21 °C. The L_3 migrate onto the herbage under moist conditions although the sluggish nature of the larvae limits their migration away from the dung pat. Sometimes the spread of L_3 is assisted by migration onto the sporangium of the fungus *Pilobolus* which grows in fecal pats. When the spores are ripe the sporangia carrying the L_3 are liberated into the air and onto the surrounding herbage.

Infection of the host occurs via ingestion of the L_3 and these larvae penetrate the intestinal mucosa and pass to the mesenteric nodes where they moult. The L_4 then travel via the lymph and blood to the lungs where they break out of the capillaries into the alveoli about 7 days after infection. The final moult occurs in the bronchioles a few days later and the young adults

move up the bronchi and mature. The prepatent period is between 3 and 4 weeks.

The epidemiology in parasitic bronchitis is less predictable than that of ostertagiasis and haemonchiasis and it is often difficult to identify the factors which precipitate an outbreak (Duncan *et al.*, 1979).

For all practical purposes, however, the important factors are: a) *the survival of* L_3. These may survive in feces, on pasture, or possibly in soil. Since *D. viviparus* larvae are more labile than those of the gastro-intestinal nematodes, survival is better in a protected environment such as the moist center of a fecal pat. Small numbers of L_3 are capable of surviving the winter in temperature climates but information is lacking on their longevity under tropical conditions. b) *Carrier cattle.* Infected cattle may continue to harbor small numbers of lungworms for several months. Although the numbers of larvae produced by these cattle are low, they are sufficient to "seed" the pasture. In contrast to the gastro-intestinal nematodes, it requires only the ingestion of a few hundred lungworm larvae to cause clinical disease. c) *Arrested larvae.* Recently it has been shown that under temperate conditions, arrested development of lungworms occurs in cattle during autumn and winter, the larvae are being arrested at the L_5 stage (Inderbitzin and Eckert, 1976). By analogy with gut trichostrongyles, it is possible that a similar phenomenon occurs under tropical conditions during periods of prolonged dry weather.

Usually only young cattle are clinically affected with parasitic bronchitis since a good immunity develops following exposure to even a moderate infection. Adult cattle not exposed in calfhood, however, remain susceptible and clinical parasitic bronchitis can occur in older animals.

The good resistance which develops following a primary infection has enabled the development of an X-ray attenuated larval vaccine widely used in Europe.

Pathology

The pathological changes which result from a primary infection may be divided into four stages (Jarrett *et al.*, 1960):

1. *The penetration phase* – penetration by the larvae into the intestine and then migration to the lungs. This occupies the first 7 days after infection and is of little pathological or clinical significance.

2. *The pre-patent phase* – this can occur between 7 and 25 days post-infection. The main lesion is blockage of the small bronchi and brochioles by the eosinophil exudate produced in response to the developing and migrating larvae. The blocked bronchi are not permanently damaged but the obstruction results in collapse of the distal alveoli; as a result, the rate of respiration increases to overcome the loss of lung function in affected areas.

3. *Patent phase* – this occurs from day 25 to 55 after infection. It is associated with the presence of adult worms in the bronchi which produce a bronchitis with resultant exudation into a blocking of air passages. Of more importance is the primary pneumonia which develops as a result of the cellular response to aspirated eggs and larvae. At post-mortem the consolidation is particularly prevalent in the diaphragmatic lobes. This phase is characterized clinically by increased respiratory rates and coughing.

4. *Post-patent phase* – this occurs from day 55 to 90. During this phase most of the adult worms are expelled and the majority of animals gradually recover except a proportion (up to 25%) in which, for some unknown reason, many alveoli show a swelling and proliferation of their epithelium. If this proliferative pneumonia lesion spreads to involve the whole lung then serious clinical consequences ensue. At post-mortem the presence of red or fawn rubbery areas which are not consolidated are suggestive of this parasitic bronchitis stage.

During the pre-patent and patent phases, various complications including pulmonary edema, emphysema and secondary bacterial infection may ensue. Bronchiectasis may occur as a sequel.

Although cattle develop a good immunity to *D. viviparus*, reinfection of immune animals may result in accelerated respiratory rates and coughing may last for a few days. If the challenge infection is heavy, some larvae may reach the bronchioles and the resulting immune response is characterized by lympho-reticular cells around each dead larva resulting in bronchiolar obstruction and a macroscopically visible grey lymphoid nodule.

Clinical Signs

The clinical signs are those associated with a developing bronchitis and pneumonia, i.e., coughing and tachypnea accompanied by varying degrees of pyrexia, anorexia, weight loss and dyspnea. In severe disease, respiratory rates are in excess of 70 per minute and often accompanied by dyspnea in which the calf adopts an 'air hunger' position with extended head and neck.

On auscultation, respiratory and expiratory sounds are harsh and rhonchial and emphysematous crackling are common.

Laboratory Aids to Diagnosis

Diagnosis is usually based on clinical signs, previous grazing history and season. In light infections where the clinical signs are less obvious, examination of feces for the presence of *D. viviparus* L_1 is useful. The L_1 larvae measure 400-500 μm and are characterized by the presence of large food

granules. Successful detection of lungworm larvae in pasture samples requires more sophisticated techniques than for other trichostrongyle larvae. The results obtained with the usual washing and sedimentation methods are unreliable.

Prevention and Control

The ideal method of preventing parasitic bronchitis is by vaccination with X-ray attenuated L_3 of *D. viviparus* which is available commercially in Europe.

Because of the ubiquitous nature of the free-living stages, control by grazing management is unreliable and hence in most areas other than Europe, control is based on anthelmintics. Prophylactic programs such as those described previously for gastro-intestinal nematodes are less reliable because the seasonal fluctuations of lungworm larvae on pasture are less predictable.

Where lungworm infection is diagnosed and treatment has to be instituted, respiratory rates return to normal within 48 hours in most cases. However, due to residual pathology, coughing may persist for several weeks. It is important to use drugs which are effective against adult and larval stages such as levamisole and the three modern benzimidazoles (fenben-, oxfen- and albendazole).

Fascioliasis

Disease

Bovine fascioliasis or liver fluke disease is a chronic wasting disease which primarily affects young cattle although sub-clinical production losses can also occur in older stock. The disease is characterized by loss of weight, anemia and hypoalbuminemia with submandibular edema an occasional feature.

Etiology

Fasciola hepatica and *Fasciola gigantica* are the important liver-fluke species. Both are leaf shaped, although there is considerable overlap and variation in size. *Fasciola gigantica* is usually larger, measuring up to 75 mm long by 12 mm wide compared to 50 mm long by 15 mm wide for *F. hepatica*. The latter species also has a more distinct anterior cone and shoulders.

Distribution

Fasciola hepatica is widespread in temperate areas but also occurs in Australia, South America, North and South Africa and the tropical highlands of Africa and Asia. *Fasciola gigantica* is widely distributed through tropical and sub-tropical areas of Africa and Asia, including many Pacific islands. It has also been recorded from Southern Europe and Southern U.S.A.

Life Cycle, Epidemiology and Immunity

The life cycle is indirect and requires a snail intermediate host. The fluke eggs are passed in the feces and develop into miracidia once free of feces. These motile miracidia penetrate snails of the genus *Lymnaea* where they develop asexually through sporocyst and redial stages to cercarciae which are shed from the snail. The latter encyst to become the infective metacercariae. Under natural conditions the minimum time taken for this part of the life cycle is 6 weeks with *F. hepatica* and about 8 weeks for *F. gigantica*.

Following ingestion by the final host, the metacercariae excyst in the small intestine and migrate throught the gut wall into the peritoneal cavity and eventually to the liver. The young flukes wander in the liver parenchyma for about 6 weeks and then enter the bile duct where they mature to the adult stage. The minimal prepatent period for *F. hepatica* is 10 weeks and for *F. gigantica* is 13 weeks.

The epidemiology of fascioliasis is dependent on the ecology of the snail intermediate host. There are many different species of *Lymnaea* but it is now generally agreed that two main types are involved in the transmission of fluke although there are species variants in different countries. In the case of *F. hepatica*, the most important *Lymnaea* is the *L. truncatula* type. This is a mud snail which prefers moist temperate conditions (15-22 °C) though it appears that variants found in the tropics have adapted to higher temperatures and can breed and survive at 26 °C with sufficient moisture. During the dry season, *L. truncatula* is capable of estivation for at least a year in dry mud. The adult snail measures 1 cm in length.

The intermediate hosts of *F. gigantica* are of the *L. auricularia* type. These are aquatic snails which prefer tropical or sub-tropical conditions and thrive in well oxygenated non-polluted water. These are, however, adaptable to an amphibious environment and can estivate during dry weather. The adult snails measure up to 1.5 cm. Another facultatively amphibious snail *L. tomentosa* is the host for *F. hepatica* in Australasia.

There is a distinct seasonal pattern to infections with liver flukes related to the seasonal activity of the snail intermediate hosts. Optimal conditions for

development of snails and their larval flukes are moderate temperatures and wet weather. Considering the time taken for snail breeding, a minimum of 6 to 8 weeks for larval development, the majority of cercariae are produced 3 or 4 months after the onset of favorable conditions. Therefore, in those countries with distinct wet and dry seasons the cercariae are produced towards the end of the wet season; in the sub-tropics they are shed in autumn, in summer rainfall zones and in late spring, early summer in winter rainfall zones. In irrigation areas, snail breeding and cercarial production is less circumscribed and will continue except for periods when the temperature is extreme.

Clinical fascioliasis occurs 3 to 4 months after infection, i.e., when the flukes become adults. More acute forms of the disease, so common in sheep, are only occasionally seen in young calves.

In cattle there is some evidence of an acquired resistance to reinfection with *Fasciola*. In some situations this is expressed as an absolute resistance, in others as a delayed migration of the challenge infection. The life span of existing burdens is also limited to about 5 months (Doyle, 1971; van Tiggele and Over, 1975; Kendall *et al.*, 1978).

Clinical Signs

The clinical signs are loss of weight, anemia and in severe cases, submandibular edema. Diarrhea is not a feature of chronic bovine fascioliasis except in the terminal stages or where the condition is compilated by the presence of gastro-intestinal nematodes.

Where nutrition is inadequate, as is often the case at the time when infections occur during the dry season, cattle are less able to tolerate fluke infection.

Pathology

The pathogenesis of the disease is principally based on the anemia caused by the blood sucking propensity of the adult flukes. This activity results in anemia, hypoalbuminemia, and a loss of red cells and plasma into the bile ducts.

The major pathological changes encountered at post-mortem are the development of hepatic fibrosis and hyperplastic cholangitis. In *F. hepatica* infections, calcification of the bile ducts occurs but this is not obvious in *F. gigantica* infections. The gall bladder is often enlarged.

Laboratory Aids to Diagnosis

Diagnosis is based on the clinical signs, grazing history and season. In cattle, the disease is associated with the presence of adult flukes. The typical yellow operculated eggs (140×80 µm for *F. hepatica*, 170×90 µm for *F. gigantica*) may be present in the feces although counts are often low, even in high infections.

The presence of anemia, low serum albumin levels and raised serum enzyme levels are helpful diagnostic aids. Gamma glutamyl transpeptidase is probably the best indicator. In older or reinfected cattle the use of serological tests such as the indirect hemagglutination test have proved useful under experimental conditions.

Prevention and Control

The best method to control fascioliasis is to eradicate or reduce the populations of the snail intermediate hosts. In the case of the *L. truncatula* mud snail, this is best accomplished by improving the drainage of the land. Alternatively, areas or ponds suitable for snail colonization may be fenced off, though this is not always practical. Another approach which is also suitable for the control of *L. auricularia* is to apply molluscicides just prior to the snail breeding season. However, it is unlikely that all the snails will be killed and repopulation occurs rapidly unless repeated applications are carried out. A better approach is to apply the molluscicide at a time when the snails have become infected but prior to shedding of cercariae. Such strategic applications have proved economically advantageous.

It is interesting that in developing countries where hydrological developments have taken place the prevalence of fascioliasis has increased. The creation of watering points, dams, etc. , have proved to be ideal reservoirs for *L. auricularia* snails. The use of a potent molluscicide such as N-trityl morpholine at the season when the water level is at its highest has given excellent control and economic benefit (Preston and Castelino, 1977).

In tropical savannas with long dry seasons or occasional droughts, problems can arise when cattle are forced to graze in marshy or swampy areas where metacercarial populations have increased towards the end of the rains. Although it is difficult to control the grazing of such areas in times when good grazing is in short supply, efforts should be made to delay the grazing of marshes as long as possible to allow some mortality of surviving metacercariae and a reduction in the numbers available. A good policy is to admit previously infected and resistant cattle first to such areas (Schillhorn v. Veen, 1977).

Treatment of bovine fascioliasis is costly but several drugs are now available which are very efficient against the adult flukes in the bile ducts and the later parenchymal stages. Those drugs which include nitroxynil, rafoxanide, oxyclozanide and brotianide, are best given routinely 3 months after the animals have first grazed on known infected pasture. If necessary, a second treatment should be given 6 weeks later.

Cysticercosis

Cysticercosis or 'beef measles' is the term given to infection of cattle with *Cysticercus bovis* which is the intermediate stage of the human tapeworm *Taenia saginata*. It is important as a public health problem and under natural conditions there are no clinical signs in cattle.

Etiology

The adult form of *T. saginata* occurs in the small intestine of man and may be recognized by its size (up to 10 m) and the absence of a rostellum or hooks. The larval stage *C. bovis* is a simple cyst or cysticercus. The cysts can measure up to 1 cm in width.

Distribution

These tapeworms are found world-wide. Prevalence is greater in developing countries, particularly where the economy is based on cattle, e.g., many parts of Africa, Central Asia and Latin America. Up to 80% of infected bovine carcasses have been recorded from parts of East Africa and Nepal.

Life Cycle and Epidemiology

The adult *T. saginata* may pass millions of eggs daily and these are capable of survival for several weeks and if optimal conditions of humidity are present, for more than 1 year. After ingestion by a susceptible host, the larva or oncosphere passes via the circulation to striated muscle where it becomes a cysticercus. It takes about 8 weeks for these cysts to become infective to man, who becomes infected by eating raw or poorly cooked beef. Development of the adult tapeworm takes several weeks. Prenatal infection of calves has also been reported.

The epidemiology of cysticercosis in developing countries can often be related to the communal existence of man and animals so that tapeworm eggs

can readily be transmitted to cattle. In many of these countries the eating of poorly cooked meat is a common practice and this perpetuates the problem.

In more developed societies the epidemiology is more complex since cattle are seldom exposed to direct contact with human excreta. The dissemination of infected sewage either by birds or sewage sludge used to fertilize pasture are two routes which have been incriminated in cysticercosis outbreaks. Infected stockmen have also been identified as the source of infection in several 'cysticercosis storms' where contamination occurs either directly during stock feeding of via hay or straw soiled during defecation.

Immunity

If cattle over 6 months old first become infected with *T. saginata* eggs, the infections are usually self-limiting and the cysts degenerate within a year. These cattle are strongly resistant to reinfection. If, however, infection occurs in calfhood, the cysts may persist for years although the calf becomes completely immune to reinfection after 3 to 6 months due to acquired immunity.

Pathology

Cysts first become grossly visible about 2 weeks after infection, appearing as a pale semi-transparent spot in the muscle about 1 mm in diameter. By 8 weeks post-infection, it is a mature fluid-filled cyst about 1 cm in width with a scolex clearly visible and enclosed in a thin fibrous capsule. When mature cysts die, they are replaced by a caseous crumbly mass about 1/2 cm in diameter.

The predilection sites for cysticercosis are the masticatory muscles, heart, tongue and diaphragm but they may be found in a striated muscle, even in light infections (McCool, 1979).

Clinical Signs

Under natural conditions, there are no clinical signs in cattle infected with *C. bovis*.

Laboratory Aids to Diagnosis

Diagnosis is based entirely on examination of the carcass at meat inspection and observation of the cysts. Some authorities find that incision of the

shoulder muscles yields a significantly higher detection rate. At present there is no reliable serodiagnostic test.

Prevention and Control

In countries with a high prevalence of cysticercosis, there is no doubt that indiscriminate defecation is the main factor which perpetuates the infection. The provision of sanitary facilities and controlled disposal of human feces, plus public health education programs are clearly the best measures which limit the spread of infection to cattle. If this is coupled with thorough cooking of beef and regular treatment of the labor force on problem farms, the infection can be suppressed.

A further proviso is, of course, adequate meat inspection to detect infected carcasses and the proper treatment of the latter. Generalized infections should result in total condemnation of the carcass. In lighter infections, the affected part is condemned and the remainder of the carcass deep frozen at $-10\,°C$ for 10-14 days.

Treatment of affected animals is at present unsatisfactory though several of the newer anthelmintics including mebendazole and praziquantel have shown promise at high and possibly expensive dosage rates.

Recently, a vaccine based on the excretions of embryonated eggs (oncospheres) has been developed in Australia and has given very promising results in field trials on farms where cysticercosis is endemic (Rickard and Adolph, 1976).

Toxocariasis

Disease

Toxocoriasis in cattle and buffalo is caused by the small intestinal ascarid *Toxocara vitulorum* (formerly *Neoascaris vitulorum*) and primarily affects animals under 6 months of age. Heavy infections are associated with poor thriving and in some instances intermittent diarrhea.

Etiology

Toxocarra vitulorum is a stout pinkish-white worm, the adult males being up to 25 cm long and the females up to 30 cm.

Distribution

This ascarid is most common in tropical regions though it is also present in parts of Europe, North America and Australia. The prevalence is particularly high in buffalo calves in Egypt, South East Asia and parts of the Indian sub-continent. In West Africa the prevalence in young bovines has reached 98% in some areas.

Life Cycle, Epidemiology and Immunity

Toxocara vitulorum is a migratory ascarid in which the vast majority of infections occur following ingestion of larvae in milk during the early suckling period. Thus the usual cycle is that larvae hatching from ingested eggs (containing an L_2) pass to the tissues where they are retained and constitute a reservoir of infection. In pregnant cows these larvae are mobilized late in the pregnancy and pass via the milk to the calves. Larvae are present in the milk for about 3 weeks and characteristic ascarid eggs appear in the feces 3 weeks after ingestion of the larvae, i.e. when the calves are between 3 and 6 weeks of age.

As immunity to the presence of adult parasites develops, the ascarids are expelled and few if any adults remain by 6 months of age.

Clinical Signs

In many instances the only clinical sign is unthriftiness although loss of appetite, anemia and occasional diarrhea have been reported in very heavy infections.

Pathology

As in all ascarids there is minimal macroscopic evidence of infection in the small intestine though a mild enteritis may be detected. At cellular level there is thought to be an impaired absorption of nutrients which is responsible for the unthriftiness and poor growth rates.

Laboratory Aids to Diagnosis

The minimal clinical signs and the absence of a seasonal prevalence make diagnosis relatively difficult and a greater reliance than usual is placed on the detection in the feces, of large numbers (often >5000 e.p.g.) of the character-

istic round ascarid eggs with a pitted shell. These eggs may be detected by any of the usual flotation techniques and counted in a McMaster chamber.

Prevention and Control

In calves, a single treatment at 4 weeks of age with an anthelmintic efficient against both the developing and mature infection will suffice. By 4 weeks, transmission of larvae in the milk should have terminated and further treatment is unnecessary. Suitable drugs include the piperazine salts and some of the modern benzimidazoles with efficiency against ascarids, e.g. fenbendazole. If the above routine is coupled with an improvement in hygiene, i.e. disposal of feces, regular changing of congregation areas, then infection should become minimal. Complete eradication is difficult because of the resistant nature of the eggs on pasture and the reservoir of larval infection in the tissues for which there is currently no treatment.

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PART III

DISEASES OF MIXED ETIOLOGY OR LESSER FREQUENCY

39. THE CARE OF YOUNG CALVES, NEONATAL CALF DIARRHEA, THE CALF PNEUMONIAS

I.E. Selman

Abstract. Newborn calves are born devoid of any significant resistance to the common calfhood pathogens. To attain a sufficient level of resistance, calves have to suckle or be fed maximum quantities of their dam's colostrum as soon after birth as possible. The immunoglobulins from the colostrum are absorbed intact through the calf's intestinal epithelial cells. If they absorb enough immunoglobulins, they will probably become resistant to the effects of neonatal calf diarrhea, the major hazard calves face during the first month of life. Colostrum may also afford some protection against the other major calf problem, pneumonia, which commonly causes illness between one and six months, particularly in more intensive units.

The care of young calves

Management of the calving cow

Under "natural" conditions, a cow will usually seek out a sheltered, secluded position just prior to parturition. This has three major advantages: first, in areas where cattle are still harassed by wild predators, the cow and her calf will obtain maximum protection at a particularly vulnerable stage in their lives; second, the practice allows ample opportunity for the early formation of a strong dam-offspring bond; third, the choice of an out-of-the-way location should also result in the calf being delivered into a relatively uncontaminated environment. Of course, the major disadvantage of this whole process is that proper surveillance of calving cows is often made more difficult and, in the event of parturition-related problems, handling and treatment are not easy to carry out. Nevertheless, even under modern dairy farming conditions, there is little doubt that major benefits are derived from allowing calving to take place out-of-doors whenever possible, at least from the viewpoint of calf survival (see below).

The selection of the calving area is still most important when calving cannot take place out-of-doors, during periods of adverse weather, or in large

"zero-grazed" dairy herds, or even when a hormone-regulated, batch-calving system is operating and labor considerations dictate that down-calving cattle must be held in nearby paddocks or yards. In the long run, the use of "intensive" calving areas almost inevitably leads to higher neonatal calf losses and may also exacerbate disease problems (e.g., brucellosis) in the adult stock. These factors have to be balanced against labor and other costs. When limited calving areas must be used, they should be changed regularly to avoid infection "build-up" (i.e., *within,* not just *between,* calving seasons) and calving groups should be as small as possible. It is still important to recognize the calving cow's need for relative seclusion in order to minimize "mismothering".

The use of indoor calving accommodation is not usually necessary in tropical areas and is rarely advisable. Indoor calving may lead to high neonatal losses due to infection build-up in calving pens and interference factors which adversely affect early suckling and significant colostral globulin absorption (see below). If absolutely unavoidable, indoor calving should occur in clean, well-littered, well-lighted, loose boxes with surveillance to avoid mismothering and to ensure early suckling.

Finally, the particular problems posed by primiparous females (heifers) should be recognized. Such animals are more likely to experience difficulties at calving and have maternal instincts which have not been strengthened by experience. Often they appear "stunned" or exhausted by the process of calving and, thereafter, frightened of their calves. Thus, the heifers must always receive particular attention at and around calving time.

Management of the newborn calf

The final stage of parturition generally occur with the cow in a recumbent position and the calf is deposited into a large pool of slippery amniotic fluid. This is of little consequence when calving occurs out-of-doors since in its initial attempts to rise, the calf's feet dig into the soil and a firm grip is gained. In indoor parturitions, the calf is often born on a bare or sparsely littered floor and has extreme difficulty in rising. Often the calf becomes exhausted in its attempts and the time of first suckling is markedly delayed (see below). Well-littered calving areas will avoid this problem and calves may rise with a minimum of difficulty.

Once on its feet, a newborn calf almost immediately initiates teat-seeking activities. At this stage, the major difficulties are either an uncooperative or aggressive dam, or a cooperative dam whose only fault is that her udder and/or teats are too low for the calf to find. This latter problem is because the calf's teat-seeking activity is largely directed upwards towards the highest part

of the dam's underbelly. In most beef cows and in dairy heifers, this is usually where the teats are located and early suckling is possible (Selman *et al.*, 1970b). In a large proportion of mature dairy cows, however, the highest point of the underbelly, due to the large size of the abdomen and udder, is the xiphoid region; teat-seeking is thus directly anteriorly (i.e., *away* from the teats) and first suckling is delayed. This is a common problem in dairy herds and approximately 25% of dairy calves born in loose boxes under otherwise ideal conditions may fail to suckle their dams within the first eight hours of life (Selman *et al.*, 1970b). The consequences of this may be considerable and it is essential that animal attendants and veterinarians do not assume that by leaving a cow and calf together in a loose box, suckling will inevitably ensue. Consequently, whenever possible, and when faced with a neonatal diarrhea problem, careful attention is necessary to ensure early suckling. If for any reason the calves are not groomed by their dams it is important for attendants to dry them off as soon as possible. The more vigorous the rubbing, the greater the benefits appear to be. Many experienced cattle attendants advise spraying or otherwise dressing the navels of newborn calves with an antibiotic preparation. If this is to be of any use at all, it should be carried out as soon as possible after birth. While there is little or no scientific evidence to support the practice, the aim of this procedure is to prevent umbilical infections.

Under natural conditions the dam-offspring bond will be established by 12 hours post partum. At this stage the cow and calf usually rejoin the rest of the herd. In beef herds this is assumed to be of benefit to all but is rarely possible in dairy enterprises. In fact, the importance of a successful dam-offspring bond in dairy cattle is either ignored or looked upon as a definite disadvantage. Under certain forms of management, this bond may result in calf feeding difficulties and milk "let down" problems in cows. It is important, however, that calves not only obtain colostrum but also consume it under the right circumstances (see below). The formation of a tight dam-offspring bond (although a temporary one) is vital, particularly in dairy calves, for a newborn calf to acquire resistance to common calfhood infections.

The importance of colostrum

Colostrum is the thick secretion which is normally present in a cow's udder around the time of parturition. It has long been accepted that an early feed of colostrum is essential if a calf is to survive the neonatal period without serious illness. Scientific proof of this fact has been available for only the last 50 years. During this period, a great deal of work involving a vast number of different techniques has shown that the "protective factor" in the colostrum

is immunological and that the colostrum is a rich source of immunoglobulins (antibodies) if only for a limited period (i.e., a day or two) after calving. It is also a rich food source with laxative properties.

The newborn calf is normally born devoid of significant amounts of immunoglobulins and is totally dependent upon acquiring these from colostrum. For a limited period only, its intestinal epithelial cells are capable of absorbing intact immunoglobulin molecules (and many other macromolecules, also). The immunoglobulins eventually reach the systemic circulation via the lacteals and lymphatic duct.

Depriving calves of colostrum regularly results in severe neonatal disease and/or death; however, under farming conditions the mere feeding colostrum to calves is not a guarantee of good health or survival. Colostral immunoglobulin absorption by newborn calves may be a remarkably efficient and consistent phenomenon under carefully controlled experimental conditions (Selman *et al.*, 1971a); under less stringent circumstances marked individual variations may arise in the ability of newborn calves to absorb intact immunoglobulin molecules from colostrum. In fact, immunoglobulin absorption is not an inevitable sequel to colostrum feeding. If due attention is paid to factors which are of prime importance in respect to immunoglobulin absorption in very young calves, it is possible to bring about maximal absorption rates, and hence high post-colostral serum immunoglobulin concentrations, thereby giving a calf maximum chances of survival. A direct relationship exists between the immunoglobulin concentration and the fate of calves during the neonatal period (Gay *et al.*, 1965). The most important factors to be considered in this context are (i) the timing of the first feed of colostrum, (ii) the mass of immunoglobulin consumed and (iii) whether or not a calf receives its colostrum in the presence of its dam. Breed factors also exist but are not usually considered to be of practical significance.

For many years it was believed that immunoglobulin absorption occurred in calves for the first 24 to 48 hours of life, however, this is not the case. More recently it has been shown that the intestine of the newborn calf becomes progressively unable to absorb macromolecules from birth. By 10 hours post partum it has already lost at least 50% of its original absorptive efficiency and "intestinal shutdown" is, for practical purposes, complete by about 16 hours of age. The managemental implications of this are obvious. Suckling should be ensured, or if artificial means are needed, colostrum should be fed within the first few hours of life. Luckily, newborn calves usually have such a strong suckling drive that this does not present a major problem; if artificial feeding is necessary the use of a nipple feeding system is recommended. Using such a system, calves will generally consume large volumes of colostrum (see below) even at one hour post partum.

The widely-held misconception that newborn calves suckle their dams little and often is wrong (Selman *et al.*, 1970b). When newborn calves finally locate a teat, they spend up to 20 minutes suckling and then, obviously full, they retire to a quiet corner and sleep for several hours. Under these circumstances, a dairy calf will consume 5 to 10% of its birthweight (i.e., 2 to 5 liters). A direct linear relationship exists between the mass of immunoglobulin presented to a calf under standard feeding conditions, and the calf's subsequent (48 hours) serum concentration. Usually it is not possible to ascertain the colostral immunoglobulin concentration under farm conditions, therefore, one has to ensure that calves consume maximal *volumes* of colostrum to receive maximal *quantities* of immunoglobulin. Feeding such a quantity of colostrum is likely to precipitate diarrhea. However, this is of no medical consequence.

Finally, the presence of the dam and probably her grooming activities, increase the efficiency of immunoglobulin absorption by a factor of at least 50% in the newborn calf. This has been demonstrated under natural suckling conditions and with strictly standardized conditions using artificial feeding techniques, uniform feeding times, rates and pooled colostrum (Selman, 1973). The basic mechanism involved in this phenomenon is not known. However, a similar situation has been described in other species, notably dogs and rats. Again, whenever possible, calves should obtain colostrum by natural suckling.

From an immunological viewpoint, further colostrum feeding is not necessary since intestinal absorption of intact immunoglobulin molecules is unlikely to occur after 24 hours. One school of thought, however, suggests that further feeding is beneficial; immunoglobulins may exert a "local" effect on the intestinal epithelium. Colostrum is also a highly nutritious product which does no harm and probably does a little good even when fed at a late stage. If for any reason a calf's dam has no colostrum, deep-frozen colostrum or colostrum from another calving cow may be used. Also, there appear to be no objections to storing colostrum at room temperature and feeding it even in a soured and mouldy state at least from the nutritional viewpoint.

Feeding and management to weaning

Little needs to be said about the feeding and management of ranch-type (beef) calves since much of the responsibility for this is taken over by the calf's mother. It is important to ensure that adequate food and water is available to the adult stock for calves to receive a plentiful supply of milk. Weaning is usually between 6 and 10 months. This decision and whether or not to offer supplementary food to calves during the late suckling stage are

commercial considerations based mainly upon local conditions, i.e., availability of foodstuff and/or grazing and on marketing trends.

With dairy calves and dairy-cross calves destined for beef production, the situation is vastly different because such animals are usually reared in isolation from the adult herd. This practice of attempting to rear large numbers of young and highly susceptible calves often creates enormous veterinary problems, particularly with regard to respiratory and enteric diseases (see below) and later, parasitism.

Dairy calves are sometimes reared by single or more commonly, multiple suckling systems. However, this is applicable only to small scale enterprises; more often, this type of calf is reared artificially. The diversity of available systems is considerable and a detailed discussion regarding the advantages and disadvantages of each one is not within the scope and aim of this book. Basically, dairy calves may be reared indoors, outdoors, or on the "shelter-and-run" principle. In each of these systems calves may be housed in single pens, in small numbers in larger pens or in large loose-housed groups of 20 or more animals. Most cattle veterinarians will admit to having seen successes and failures under each method. There is some evidence to show that calves reared in groups are more socially adjusted than calves reared in single pens. On the other hand, certain infectious diseases spread more easily through group-housed calves and it is far easier for them to develop unpleasant and potentially damaging habits, i.e., intersucking and urine drinking. Labor costs are usually higher with single pens but surveillance and individual attention is easier. Irrespective of how many calves share a pen, loose-box, yard or paddock, it is of the utmost importance to ensure their comfort. In hot climates it is important to provide shade and some means of insect control; shelter may be necessary for cold nights or in wet weather. It is essential that calves be provided with a dry resting area and if they are housed, the building should be airy and well ventilated but relatively draught free. If large groups of calves are housed together, size matching is important to minimize bullying. The other major consideration is hygiene. It is very important to ensure that adequate alternative accommodation is available so that indoor pens can be depopulated regularly and disinfected, and outdoor pens and paddocks can be moved or rotated around other parts of the farm. Control of vermin is another important point.

Many different feeding systems are available with the final choice being based on such considerations as personal preference, local supply situations, type of housing or other calf accommodation, and the cost and quality of available labor.

Despite the relatively higher costs involved, many dairy farmers prefer to feed raw cow's milk during the preweaning period; milk is convenient,

home-produced and in regular supply; in addition, if it is fed fresh and undiluted, there is little that can go wrong with it. In certain countries there are stringent regulations regarding the sale of milk from, for example, cattle treated with either local or parenteral antibiotics, thus the feeding of such milk to calves prevents what would otherwise be a total waste during the withdrawal period.

Other farmers prefer to feed cow's milk for a limited period then feed a powdered milk substitute. Many different forms of milk substitutes exist and the decision to use a particular brand must rest with individual farmers in consultation with other brand users. It should be pointed out that inferior products are on the market in some countries and, when in doubt, it is probably best to use cows milk, at least for the first month or so of life. Skimmed milk and whey are also acceptable alternatives but are best used on slightly older calves.

Having settled what to feed, the next question is how to feed it. Again, the final decision is based on housing systems and the availability of labor. Calves in single pens may be fed from pails or nipple feeders of which several different types are on the market. In general, it is easier to introduce newborn calves to nipple feeders, particularly if they have been suckling their dams for a few days and there is the advantage of less spillage. Calves often appear to thrive better when feeding from teats. When calves are housed in groups of 2 to 4, bucket feeding is possible but difficult, spillages are common and intersucking tends to be a problem. Under such conditions, teat feeding in limited quantities from nipple feeders or an ad lib cold acidified milk substitute from a bin is recommended. The high urine output of calves on ad lib diets must be taken into account before a decision is made to convert to this system because it commonly produces drainage and high atmospheric humidity problems.

Finally, when calves are kept in large groups the only acceptable feeding system is ad lib milk substitute delivered through teats. This may be carried out either by using an artificial suckling machine which will mix and deliver hot or cold milk, or ad lib acidified milk substitute which is mixed by hand in a large reservoir every few days and delivered at ambient temperature. These latter systems are expensive to operate because the milk intake of calves on ad lib systems may run two or three times the amount usually fed by pail (i.e., 10% body weight/day). On the other hand, labor costs are considerably lower, particularly with the use of cold acidified powders. The high urine output of calves under ad lib system must be anticipated and contended with. Intersucking is not normally a problem in groups of calves fed ad lib by a teat system.

Calves will normally start nibbling soft hay after a few days and will show

definite interest in concentrate mixtures or calf pencils when they are 2 to 3 weeks of age. However, their intake of dry food will depend largely on their milk intake and, although fresh water should be available from about 2 weeks of age, many calves will not drink appreciable quantities until near weaning time. In view of the high value of milk and the high cost of milk substitutes, there is a tendency to wean calves earlier than used to be the case. Many dairy calves are now weaned at 5 or 6 weeks of age but perhaps the best guide is their dry food intake and a decision to wean should be made when calves are eating about 1 kg calf concentrate/day. This decision must also take local considerations into account, particularly the availability and cost of hay and concentrates.

Neonatal calf diarrhea

Etiology

The etiology of neonatal calf diarrhea will be discussed under the headings "bacteria", "viruses" and "other possible causes". It must be emphasized that the basis for the descriptions is predominantly experimental work, using selected agents under laboratory conditions or limited field studies with extremely expensive and sophisticated laboratory support.

The cause of an outbreak of neonatal calf diarrhea is rarely sought, let alone achieved, even in developed countries with all the benefits of good communications and well-organized veterinary services. Neonatal diarrhea is so common under certain circumstances that farmers are used to seeing diarrheic calves and seek veterinary advice only when a problem reaches what they consider to be an unacceptable level. Another problem for diagnostic laboratories is the submission of dead or dying calves which have already received some form of antibiotic therapy. The most important reason for poor diagnosis, however, is that little is known about the prevalence, relative importance, possible interrelationships and pathogenic effects of the large number of microorganisms which have been shown, or claimed, to be causal. One group of workers (Blood *et al.*, 1979) has developed the term "acute undifferentiated diarrhea of newborn calves" for the vast majority of similar looking outbreaks when a satisfactory diagnosis is either not attempted or reached.

Bacteria. Until recent years, accounts on the subject suggested that almost all calf diarrhea arose as the result of bacterial infections; the causal organism was considered to be the Gram-negative bacterium, *Escherichia coli.* This

subject has been extensively reviewed by Gay (1965) who subdivided calf colibacillosis into three major types (i) colisepticemia, (ii) enteric toxemic (enterotoxic) colibacillosis and (iii) enteric colibacillosis. While there is no doubt as to the pathogenic significance of *E. coli* in the first two forms of the disease, there is still considerable debate as to its role in the third (enteric colibacillosis). It seems likely that future studies will reveal the latter syndrome to be a mixture of disorders, some due to *E. coli* strains of unsuspected pathogenicity, others due to enteric virus infections and other causes.

Colisepticemia may arise following infection by a wide range of so-called invasive serotypes of *E. coli*. Enterotoxic colibacillosis arises as the result of a localized intestinal infection by certain specific serotypes which have the ability to produce enterotoxins.

Other bacteria have also been incriminated in cases and/or outbreaks of neonatal calf diarrhea, i.e., *Salmonella* spp. and, to a lesser extent, *Clostridium perfringens,* types B and C. In most countries the commonest serotypes associated with salmonellosis in cattle are *S. dublin, S. enteritides* and *S. typhimurium* but many others are locally and/or sporadically important. However, *Salmonella* spp. are not specifically pathogens of young cattle and when infections arise they tend to be clinically different from classic neonatal calf diarrhea.

Viruses. Over the last decade, a great deal of knowledge has accumulated regarding enteric virus infections in young animals. Of these, rotavirus infection in young calves is the most significant and has aroused much interest. At the present time it is considered a common cause of diarrhea in calves. However, there is some doubt whether or not this virus is able *per se* to cause severe diarrhea and death. It has been suggested that it is likely to act in concert with other organisms such as *E. coli*. Clearly, this may be so, but it is equally possible with further work that more pathogenic strains of the virus will be discovered. Other viruses found in diarrheic calves are corona, astro and parvoviruses. This subject has been reviewed by Woode (1976) and Blood *et al.* (1979).

Other causes. Many veterinarians feel that quite apart from the infectious diarrheas, there are other causes which arise from faulty feeding and management. While this may be so, it should be emphasized that until the infectious agents involved in calf diarrhea are better defined, "other causes" are probably better considered as possible predisposing factors.

Pathogenesis

Colisepticemia results from systemic invasion usually by one single *E. coli* serotype. The portal of entry may be the nasopharyngeal mucosa, the intestinal tract or perhaps the umbilicus. Rapid collapse ensues, usually followed by death; both events are probably due to the effects of bacterial endotoxin.

Enterotoxic colibacillosis is caused by enterotoxin-producing serotypes located in the small intestine. While anterior proliferation of these organisms occurs and the pathogens adhere to the intestinal epithelial cells, there is no systemic invasion. The pathological and physiological effects of enterotoxin have been studied and it is known that damage occurs to the intestinal epithelial cells of the villi, resulting in fluid and electrolyte loss and diarrhea. If the damage is extensive, severe systemic effects arise such as dehydration, electrolyte imbalance and acidosis; if death occurs it is probably the result of acute cardiovascular collapse (Blood *et al.*, 1979).

In general, the pathophysiological effects of rotavirus infection are similar to those described above, except that severe diarrhea and death do not usually occur in experimentally infected calves. Since rotavirus infection is generally associated with severe diarrhea under field conditions, the presence of other organisms may be required in order to exert severe pathogenic effects. There is some experimental evidence to support this view (Woode, 1976).

Distribution

Calf diarrhea is a major problem in all countries, particularly where there is an intensification of cattle enterprises. Severe losses may occur sporadically in ranch cattle, however, and although largely undefined, the importance to the small farmer or in traditional farming systems must not be underestimated.

Epidemiology

Colisepticemia is a sudden-onset, short-duration and usually fatal disease of calves less than 5 days old. It is the classic cause of death in colostrum-deprived calves or in colostrum-fed calves which have failed to absorb immunologically adequate amounts of immunoglobulin. It is rarely the sole, or major, problem on a farm unless there are outstanding managemental factors to account for widespread agammaglobulinemia in the young calf population. Usually, colisepticemia occurs in a calf when most of its group are suffering from varying degrees of neonatal diarrhea; thus, other aspects of its epidemiology closely parallel that of calf diarrhea.

The classic calf diarrhea syndrome ("acute undifferentiated diarrhea")

occurs only in calves less than one month of age and usually in calves between 5 and 12 days. It has a pronounced seasonal incidence, although this is more likely a reflection of distinct seasonal calving patterns in both beef and dairy herds in most parts of the world. Nevertheless, there are some indications that neonatal calf diarrhea is particularly prevalent during prolonged periods of inclement weather. The problem usually increases as the calving season proceeds and is particularly obvious when the same calf accommodation and/or calving area is used throughout the season.

Apart from the effect of infection build-up when large numbers of highly susceptible and sick calves share the same area, the other major factor in the epidemiology of neonatal calf diarrhea is individual variation in the post-colostral serum immunoglobulin concentrations. When calves have maximal concentrations of serum immunoglobulin, the effects of infection build-up are of little or no practical consequence. The health and fate of a newborn calf during the first month of life is directly related to how much immunoglobulin it absorbs from its dam's colostrum (Gay *et al.*, 1965). Most of the long-recognized epidemiological features are explicable on immunological grounds. Perhaps the most important single determinant is how a calf first obtains colostrum. Under relatively intensive dairy farming conditions in Britain, a calf first obtaining colostrum from a pail stands three times the chances of dying from calf diarrhea than a dairy calf obtaining colostrum by natural suckling (Selman, 1973). Feeding colostrum from a pail is such an inefficient method that on farms where it is practiced during the winter housing period, very few calves are produced which are anything other than agamma or markedly hypogammaglobulinemic (Selman *et al.*, 1971b). The ramifications of this single phenomenon are striking in parts of Scotland where traditionally colostrum was fed by pail during the winter and suckling was allowed during the summer. A marked seasonal decline in mean calf immunoglobulin levels occurred when winter housing (hence indoor calving) commenced, followed by an abrupt rise in mean values immediately when the cows went to grass for the summer (McEwan *et al.*, 1970b). Thus, the period of highest neonatal calf morbidity and mortality occurred at a time when resistance was lowest and pathogenic challenge was probably greatest. Similarly, various surveys have shown that morbidity and mortality are greatest in indoor-born calves (particularly when their dams were neck-tied) and lowest in field-born calves. Significant differences have been shown to exist between the mean serum immunoglobulin concentrations of calves, depending on where they were born. This is demonstrable by comparing calves born to neck-tied dams in byres or stanchions and calves born in loose boxes, where suckling is much more likely to occur.

Breed factors slightly affecting the efficiency of colostral immunoglobulin

absorption are not of practical significance. However, breed differences in susceptibility to calf diarrhea have been described with Friesian or Holstein calves being far more resistant than Ayrshire or Channel Island breeds. A true breed resistance appears to exist even in colostrum-deprived calves although it often merely represents differences in management techniques (Selman *et al.*, 1971b). There is no reliable information suggesting that sex differences exist in susceptibility to calf diarrhea.

Finally, a host of other possible predisposing factors have been described or suggested such as inclement weather, infrequent feeding, milk either too hot or too cold, fatigue, poor housing, and draughts. Little effort or progress has been made in confirming their significant importance. It does seem, though, that certain types of milk substitute may give rise to diarrhea and, while detailed data is not available, many workers feel that raw cows' milk is less likely to cause trouble than certain powdered substitutes.

Clinical signs

Calves with colisepticemia often die so quickly that clinical signs are not observed. If calves are examined they are usually rapidly deteriorating and often unable to stand. Diarrhea is minimal or absent. In the rare event of such a calf living for one or two days it will be febrile (104–106 °F), dull and anorexic; frequently it will have one or more swollen painful joints and may even be showing signs of meningeal irritation and intra-ocular abscessation. If calves do survive, they commonly develop a chronic polyarthritis.

Clinical signs exhibited by calves with neonatal diarrhea depends on the severity of the problem. Mild cases continue to drink milk and usually recover within one to three days. Their feces are yellowish in color, probably loose rather than fluid, smell of sour milk but usually do not contain blood or mucus. Fever is rarely a feature.

In severe calf diarrhea, the animal is usually either sternally or laterally recumbent; it usually has a dry muzzle and mouth although thick saliva may cover the chin; its eyes are sunken and the skin is inelastic. Most often, although this varies markedly, the calf is uninterested in or unable to take milk. There is a very distinct smell from the feces which are often passed continually with a marked fecal staining of the tail and hindquarters. It is not common for the feces to be blood-stained but often there is a great deal of mucus in the feces. Straining quite often occurs along with the occasional passage of flatus. There is no fever in classic neonatal calf diarrhea.

The mortality rate varies enormously from one outbreak to another but in general at least 30% of severe cases die. A careful examination will often

reveal cardiac arrhythmia, tachypnea and hyperpnea, all of which are due to the severe metabolic acidosis.

Calves which recover from the severe form of the disease are often dull, partially anorexic and chronically diarrheic for several weeks. This leads to stunting of growth and frequently calves are prone to respiratory disease in later months. Commonly, such calves lose all the hair from the tail, hindquarters and lower hind legs, which are stained with feces.

Pathology

In calves which have died of colisepticemia, the carcass may appear to be in fairly good condition and there is usually little or no evidence of diarrhea. Frequently, there are no significant abnormalities to be found on gross examination of the viscera, although splenic enlargement may be seen and petechiation can sometimes be found on the spleen epicardium and thymus. The carpal and tarsal joints often contain excessive amounts of fluid, sometimes containing fibrin clots.

Apart from fecal staining of the skin and marked emaciation, the post mortem findings are not dramatic in a calf which died from neonatal diarrhea. The bladder is usually distended with urine. The small intestine in an animal examined within an hour or two of death, is not discolored and a close examination of the lining reveals no ulcers, hemorrhages or any other abnormality. Careful histopathological examination may reveal distortion and stunting of the intestinal villi and morphological abnormalities of their epithelial covering, but it should be emphasized that such changes (i.e., in both enterotoxic colibacillosis and rotavirus infection) have generally been described only in experimental cases.

Immune response

Calves which develop colisepticemia have little or no circulating (colostral) immunoglobulins. Their particular problem is a lack of IgM. Since it is not possible to produce colisepticemia experimentally in calves over 5 days of age (even when such calves have been deprived of colostrum) it is assumed that by this time they have formed enough IgM to withstand challenge with an invasive strain of *E. coli.*

The situation in neonatal diarrhea is complicated. It has been recognized for many years that if calves absorb maximum quantities of colostral immunoglobulins and thereby attain a high level of circulating, passively-derived immunoglobulin, they are likely to survive the neonatal period. They are not necessarily protected from diarrhea, however, only its harmful physiological

effects. Since the dominant immunoglobulin in cow colostrum is IgG since it is also (given optimal conditions) the specific immunoglobulin which attains maximum concentration in a calf's serum, and in view of the fact that a high correlation exists between the level attained and the likelihood of death from diarrhea, IgG is considered to be the important immunoglobulin in the neonatal calf. It probably acts in the small intestine following re-excretion from the systemic circulation until such time as the calf in capable of manufacturing its own local antibody (i.e., at around 3–4 weeks of age).

It is important, however, to understand that this concept of the role of IgG is mainly based upon studies involving total immunoglobulin absorption. Specific investigations with enterotoxigenic strains of *E. coli* and antibodies against them are largely lacking and the relatively few studies which have been carried out with rotavirus infections have produced variable results (McNulty *et al.*, 1976).

Laboratory aid to diagnosis

The proper laboratory investigation of calf diarrhea is both expensive and highly technical. The results are disappointing due to therapy prior to sampling, among other things. Since colisepticemia usually is rapidly fatal, confirmation of diagnosis is generally limited to the isolation of *E. coli* from the blood of the heart, spleen, liver and kidney of a fresh and unheated cadaver.

Prior treatment with antibiotics often results in negative findings. The demonstration of specific (enterotoxigenic) serotypes of *E. coli* and identification of rotavirus (or other viruses) is possible by both electromicroscopical or fluorescent antibody techniques. However, in the absence of a properly conducted background (farm) investigation it is rather naive to assume that the demonstration of either of these agents is proof that they were the causal organism, particularly since their presence may sometimes be established following an examination of stools from non-diarrheic calves. Mixed infections are also said to be relatively common in calves (Blood *et al.*, 1979) and this emphasizes the importance of a comprehensive investigation if any investigation is to be carried out at all.

Prevention and control

In ranch-type (beef) cattle, the prevention of calf diarrhea largely rests upon the avoidance of regular calving areas, the provision of shelter whenever possible for young calves, and the removal of cows and calves to extensive pasture as soon as possible after calving to reduce pathogenic challenge to a minimum. Under certain conditions, however, it is recognised that these

precautions may not be easily possible. If large-scale outbreaks arise in any one year, attempts should be made to identify the causal organism(s), if local circumstances make this possible, in order that specific prophylactic measures may be attempted in subsequent calving seasons. This has been the basis of rotavirus diarrhea control using live oral vaccine (Mebus *et al.*, 1972), although the successes of this particular exercise have been seriously questioned by certain workers mainly on matters of statistical interpretation of epidemiological data (Blood *et al.*, 1979). Other attempts to prevent calf diarrhea, using a variety of different vaccines for both dam and calf vaccination (Blood *et al.*, 1979), are still considered experimental studies. However, dam vaccination as a means of boosting specific colostral immunity, appears to be very attractive for use in ranch cattle since the majority of calves (*ca* 75%) probably attain reasonably high post-colostral serum immunoglobulin levels.

In dairy herds, there is easy access to newborn calves; therefore, it is possible to increase the amount of colostral immunity absorbed by the newborn through the careful management of calving cows and newborn calves described earlier. In addition, it should be possible to decrease pathogenic challenge by regular disinfection, batch-rearing and by using different calf accommodations for different groups of calves. However, these exercises significantly increase the work-load of the animal attendants. The simple zinc sulfate turbidity test (McEwan *et al.*, 1970a) is a valuable means of indicating to farmers just how efficient their colostrum feeding techniques are. Again, when circumstances permit, attempts should be made to identify the organisms which are involved in an outbreak so future prevention attempts are specific. Dairy farm results under temperate conditions, however, suggest that a major problem confronting those who advocate dam vaccination is the relatively poor immunoglobulin absorption. Dam vaccination is therefore only worth considering if the animal attendants are consistently producing high colostral immunoglobulin absorptions in young calves. If this can be arranged, the problem is often overcome anyway.

Perhaps the most common approach to the prevention of calf diarrhea is the oral administration of antibiotics. In many cases, this does not appear to be of great benefit even when based upon an *in vitro* sensitivity test. Other workers claim benefits from dosing calves with freeze-dried lactobacilli in an attempt to confine *E. coli* to the lower alimentary tract. The use of new milk substitute formulations ("acid" milk) presumably has similar aims.

The therapy of neonatal calf diarrhea is discussed by Blood *et al.* (1979), who emphasize the tremendous lack of adequately controlled therapeutic trials. Most of the work and opinions which are cited are totally unacceptable by current scientific standards and almost all seem to ignore the basic truth that the major determinant in deciding whether or not a calf will recover from

diarrhea is its serum concentration of absorbed colostral immunoglobulin. The authors broadly subdivide the forms of therapy into (i) antimicrobial and immunoglobulin therapy, (ii) fluid and electrolyte replacement, (iii) other supportive therapy, and (iv) dietary considerations. However, they do not, nor does this author, advocate a single, foolproof, therapeutic regime.

For many years, oral and parenteral antibiotics and other antimicrobial preparations have been the standard therapeutic procedure in calf diarrhea. Many claim successes but a major problem in the interpretation of this work is the general lack of epidemiological, clinical, immunological, etiological and statistical definition. Often such an approach fails. The administration of large volumes of bovine gammaglobulin intravenously has also been advocated but is difficult to administer under field conditions and is highly expensive.

Fluid therapy (both oral and parenteral) is often recommended and is detailed by Blood *et al.* (1979). In general, the same criticism can be applied to this approach as to the administration of antimicrobials. It is tempting to suggest that with fluid and electrolyte therapy, severe cases might benefit but mild cases would improve without it. The value of fluid therapy has not been demonstrated convincingly. Blood transfusions are often carried out in diarrheic calves but intravenous administration is difficult under farm conditions *and* the collection of blood from a donor cow makes the whole process extremely time-consuming and costly.

The use of such supportive drugs as intestinal protectants (e.g., kaolin) has traditional acceptance but little to commend it, other than that. Many other drugs have also been claimed as effective but on very little sound evidence.

Dietary considerations basically revolve around whether or not to feed milk to diarrheic calves. On balance, it would seem that mildly diarrheic calves should still get milk in reduced quantities for a few days; more severely affected animals, which are nevertheless still interested in drinking (or suckling), should get an oral electrolyte preparation. It is fair to say, however, that there have probably been more arguments about this last point than about any other facet of calf diarrhea.

The calf pneumonias

Etiology

Many of the comments which have already been made regarding the etiology of calf diarrhea could also be made about calf pneumonia. A huge array of many different types of micro-organisms have been isolated from both pneu-

monic and non-pneumonic calf lungs and over the years a large number of these have been suspected of being causal organisms. Unfortunately, the common failure of many of these organisms to produce pneumonia under experimental conditions has led to suggestions that the problem is multifactorial and that either several different agents have to be present together to set up severe lesions or that environmental factors are of major importance. Even a cursory investigation of a number of outbreaks, however, will reveal that epidemiological, clinical and pathological differences do exist. The problem is much more likely to be made up of a number of distinctly different syndromes which might even be caused by different etiological agents.

In order to restrict this discussion, only those pulmonary syndromes of non-parasitic etiology will be dealt with and, unless otherwise stated, the account will be limited to pneumonias of indoor calves.

Bacteria. Of the large number of bacteria which have been isolated, only *Pasteurella haemolytica* and *P. multocida*, *Haemophilus* spp. and *Actinobacillus actinoides* have been tested experimentally for their ability to produce pulmonary lesions in calves. All of these organisms have usually failed in this respect except when administered along with either para-influenza 3 (PI_3) or infectious bovine rhinotracheitis (IBR) viruses. Nevertheless, *P. haemolytica* and *P. multocida* are still widely held to be primary lung pathogens. Early work with *Haemophilus* spp. also proved disappointing but recent studies have shown that *H. somnus* is capable of producing, among other things, severe pneumonia in experimental calves.

Viruses. Over the years a wide variety of different viruses have been isolated from calf lungs; these include adenoviruses 1, 2 and 3, IBR virus (bovid herpes-virus 1), mucosal disease/bovine virus diarrhea (MD/BVD) virus, PI_3 virus, reovirus and bovine respiratory syncytial virus (RSV). In general, attention is gradually focusing upon two of these, PI_3 and RSV, since they appear to be widespread and able to bring about varying degrees of pulmonary disease in experimental calves. (IBR, infectious bovine rhinotracheitis, is a significant disease in cattle but is not generally considered to be a primary pulmonary disorder, nor does it appear to be a major clinical problem in young calves.)

Chlamydia. While one severe outbreak has been attributed to chlamydial infection, the results of infection experiments have generally produced equivical results.

Mycoplasmas. A number of different mycoplasmas have been isolated from pneumonic and non-pneumonic calf lungs; of these, three are considered to

be of pathogenic significance, namely, *Mycoplasma bovis, M. dispar* and *Ureaplasma* sp. (T.-mycoplasmas) (Gourlay and Howard, 1978).

As with calf diarrhea, many other factors are often inferred as causal but it seems much more likely that these are factors which predispose to the above, or other, infections.

Pathogenesis

Little is known about the pathogenesis of the infectious pneumonias of calves since experimental infections are often unsuccessful. It seems likely that infection is acquired by inhalation and that subsequently colonization of the lung initially occurs in the bronchial and bronchiolar epithelium and later to a varying degree within the lung substance. It is often suggested that colonization is aided by environmental factors such as high atmospheric ammonia levels interfering with the normal clearance mechanisms. Sometimes, in certain types of pneumonia (e.g., "cuffing" pneumonia, which is probably of mycoplasmal origin), it seems likely that a massive cellular host response (cuffing) around infected small airways exacerbates the effects of the micro-organisms. The clinical course of many pneumonias is such that it is almost impossible to avoid the conclusion that secondary bacterial infection is responsible for a sudden severe deterioration; however, evidence to support this view is not overwhelming.

Distribution

Wherever cattle units have been intensified, particularly where this means keeping young stock indoors or in close confinement, the prevalence of calf pneumonia is likely to be high.

Epidemiological, clinical and pathological features of pneumonia in young calves

Several different respiratory syndromes may be encountered in young calves but the etiology of most of them is not understood. It is probably best to consider them in epidemiological, clinical and pathological terms and thereafter to discuss their possible cause(s). Detailed discussions regarding the pathological features of several of these syndromes have been presented by Pirie (1978). Basically, the calf pneumonia syndromes can be initially divided into three basic types: (i) chronic and (ii) acute pneumonias of high morbidity and variable mortality, and (iii) a persistent and often progressive chronic pneumonia of individual calves.

Chronic pneumonias. In countries where groups of young calves are housed together for a few months after weaning, it is common for a relatively low-grade pneumonia to arise around 4 to 6 weeks of age and to persist in individuals for at least two months. The disease does not usually give rise to marked dullness, anorexia, or obvious weight-loss but is characterized by widespread coughing and often marked tachypnea (the respiratory rate may range between 60 and 100/min). Often several exacerbations occur within an affected group during the period of illness, as evidenced by an increase in coughing. Fever is uncommon and generally auscultation reveals only harsh respiratory sounds although occasionally there may be squeaks in the antero-ventral parts of the thorax. Usually (and particularly if the stocking-rate is relatively low and ventilation is good) the problem is no worse than this and recovery may occur in about 2 months, although occasional coughing may persist in some calves for much longer than this. Under less favorable environmental conditions (sometimes it would appear to be when sudden cold and/or damp spells of weather occur) the condition of one or more of the calves may suddenly deteriorate. It becomes dull, anorexic and febrile; respiratory signs worsen and the calf may become grossly dyspneic, with mouth breathing, "air-hunger" and an expiratory grunt. It may even die within a day or so.

The milder condition described first has been termed "cuffing pneumonia" due to the presence of a marked lymphocytic cuffing reaction around the smaller airways, particularly in the apical, cardiac and anterior diaphragmatic lung lobes. Macroscopically, most of the lung appears to be normal and the lesions are localized to the aforementioned areas, which are consolidated and usually of a dark plum-red color. It is now thought that such lesions arise as the result of infection with either *M. dispar* or *Ureaplasma* spp. When the lungs of a calf which has undergone sudden deterioration are examined, the lesions are usually far more widespread and more dramatic with areas of congestion and severe inflammation with or without suppuration. Occasionally necrotic areas may also be present. There may also be acute pleurisy. This is usually termed acute bronchopneumonia and is always considered the result of secondary bacterial infection superimposed upon the mycoplasmal pneumonia. Sometimes *Pasteurella* spp. are isolated from such a calf, particularly when the post-mortem picture is more indicative of an acute exudative, interstitial pneumonia.

An occasional calf may also progress from either of the above syndromes into a chronic respiratory syndrome from which recovery does not commonly occur. The calf loses weight, develops a persistent cough and an often mild degree of respiratory distress. This arises as the result of a chronic suppurative pneumonia with or without lung abscessation and bronchiectasis.

The lesions are based mainly in the anterior lobes of the lung and bacteriological findings are varied.

Cuffing pneumonia and its sequelae can be seen in beef calves running with their dams, particularly when they are housed or sheltered with them during the winter. Usually the syndrome is far less dramatic than when large groups of similarly-aged calves are housed together. Occasionally, the cuffing pneumonia syndrome may be seen in older calves, for example when weaned beef calves are housed or penned closely together, but evidence that this is etiologically similar to cuffing pneumonia is lacking.

Acute pneumonias. An acute, febrile pneumonia producing dullness, anorexia, a persistent cough and often severe respiratory distress may occur in calves 3 to 4 weeks of age which have been affected with severe neonatal calf diarrhea. It is not uncommonly fatal, and lesions such as those already described (i.e., an acute exudative or bronchopneumonia) are usually found. A mixed bacterial flora is present and it is assumed that the problem arises mainly as the result of hypostatic congestion following prolonged recumbency during the diarrheic illness.

In older calves, acute, severe pneumonias may arise in the absence of a pre-existing coughing ("cuffing pneumonia") syndrome (Bryson *et al.*, 1979). Often this type of outbreak is heralded by several very sick calves, some of which die within a very short time. Some affected calves may be febrile and usually are grossly dyspneic; there is some coughing and auscultation may reveal areas of crackling in the dorsal, diaphragmatic lung areas. A small portion of these calves may develop subcutaneous emphysema. Recovery may be prolonged in those calves which do not die and some may progress to chronic suppurative pneumonia. Coughing usually arises in the other members of the group over the period of the incident and persists for some weeks.

Post-mortem examination of fatal cases from this type of outbreak reveals widespread pneumonic changes. Lesions range from severe congestion, consolidation, inflammation and acute bronchopneumonia, to severe interstitial emphysema and other changes typical of the acute respiratory distress syndrome (Pirie, 1978) such as pulmonary edema, hyaline membrane formation and even alveolar epithelial hyperplasia. Such a syndrome is generally considered to be due to infection with one of the respiratory viruses, particularly PI_3 or RSV, along with secondary bacterial infection. Many workers now consider RSV to be the specific cause of the acute respiratory distress syndrome (atypical interstitial pneumonia) in calves.

Acute pneumonias may sometimes arise in outdoor ranch-type (beef) calves particularly during periods of inclement weather. These may be acute

bronchopneumonias or acute exudative, interstitial pneumonias.

Finally, it must be emphasized that acute, febrile pneumonias commonly arise (apparently without prior coughing) soon after calves are moved indoors or to more confined quarters. This is often, but not exclusively, seen in weaned beef calves. Such pneumonias may be rapidly fatal but more commonly they affect a relatively large number of calves to a varying degree, most of which recover (with appropriate treatment) within a few days. Post-mortem findings may reveal acute bronchopneumonia but much more commonly a severe exudative interstitial pneumonia is present. The syndrome is usually termed "transit" or "shipping" fever and is generally assumed to be due to infection by *Pasteurella* spp. probably subsequent to viral invasion. This is not always the case, however, even when so-called "typical" histopathological lesions are present (Pirie, 1978).

Immune response

An antibody response may occur, particularly with the pneumonias in which virus infections are etiologically involved. This reaction may be exploited for diagnostic purposes which involves the examination of serum samples from all or a portion of the affected or in-contact animals. Usually the samples are taken when the incident arises (acute sample) and three or four weeks later (convalescent sample). Such an approach is particularly useful in those outbreaks which may be due, for example to PI_3 or RSV infection. While a serological response is likely to occur with other forms of calf pneumonia (e.g., in mycoplasma infections) this is not looked upon as a useful diagnostic test at the present time. The tremendous cellular (lymphocytic) response around infected bronchioles in cuffing pneumonia has already been mentioned.

Laboratory aids to diagnosis

Since the basic etiology of many of the calf pneumonias is still in doubt, any laboratory aids to diagnosis must be looked upon as investigational research at the present time, although this is not the thinking in certain countries. Nevertheless, in addition to clinical and epidemiological studies, any proper investigation of a calf pneumonia problem should include detailed post-mortem investigations and microbiological studies. The examination of nasal swabs from members of the affected group is of doubtful value and if paired serological samples are examined, this should be done on identified individuals and on a large enough scale to make it statistically acceptable.

Prevention and control

In the light of current knowledge, it would seem that with intensification of calf enterprises, respiratory disease of one form or another is almost inevitable. Problems can, however, often be minimized by the proper design of new calf accommodation and modifications to older buildings. The basic aim is to minimize temperature fluctuations, decrease relative humidity and promote an acceptable air-change rate without giving rise to particularly draughty conditions (at least in small calf accommodation). To this end, it is best to obtain specialist advice at the design stage or prior to any structural changes. However, systems which involve the sudden introduction of large numbers of immature cattle into buildings are likely to give rise to respiratory problems no matter how good the buildings are. While it is not possible with young calves, it is advisable to graze mixed groups of weaned beef calves together for a week or two prior to housing.

With the exception of IBR and possibly RSV, there is no convincing evidence available at the moment that vaccination against the so-called respiratory pathogens is of any great value and some studies have indicated their total worthlessness (Stott *et al.*, 1978). New vaccines against certain respiratory viruses are always being developed and the situation may change over the next few years.

In a "cuffing pneumonia" type of problem, treatment is necessary only if individual calves become ill or if coughing reaches severe proportions. A successful regime for either individual or group therapy is a large dose of oxytetracycline administered intravenously on the first day followed by large, intramuscular doses once daily for the following two or three days. Usually, one course of treatment suffices. In fact, the above regime is suitable at least as an initial course of treatment for the vast majority of pneumonias in immature cattle and while the administration of intravenous oxytetracycline might be looked upon as unnecessarily time-consuming, the clinical effects of such an approach make it worthwhile. Some workers also recommend the coincidental administration of either intramuscular corticosteroids or a cardio-respiratory stimulant such as aminophylline. The use of an antibiotic such as oxytetracycline as a water, milk or food supplement is also a commonly-adopted prophylactic or therapeutic approach in certain countries and appears to be of use, particularly in the less-severe syndromes. Sulfonamides may also be administered to pneumonic calves in a similar manner.

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40. ACTINOBACILLOSIS, ACTINOMYCOSIS, NOCARDIOSIS, EPERYTHROZOONOSIS, HEMOBARTONELLOSIS, AND TRICHOMONIASIS

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Abstract. Actinobacillosis is a relatively common, but usually sporadic disease of adult cattle, caused by the Gram-negative bacterium, *Actinobacillus lignieresi*. The lesions are often found in soft tissues around the head and neck, particularly in the oral cavity and pharynx. Lesions, which consist of granulation, suppuration and fibrosis, spread via the lymphatics. Occasionally, the disease may arise in the skin, lymph nodes and body cavities. Treatment with streptomycin is usually highly effective in all forms of the disease.

Actinomycosis, a common sporadic disease of adult cattle, is caused by the Gram-positive organism *Actinomyces bovis* and usually arises in hard tissues, particularly the mandibles and maxillae. Spread of the disease is usually by extension or by the hematogenous route. Except for the difference in tissues affected, the pathological features of actinomycosis are virtually identical to those described for actinobacillosis. Treatment is only occasionally successful.

Nocardiosis is a relatively common, usually benign, sporadic disease of cattle in tropical areas. While there is some controversy as to the actual cause, it is generally believed that the Gram-positive organism, *Nocardia farcinica,* is the causative agent. Infection is thought to arise via skin abrasions and, in some countries, ectoparasites are considered of prime importance in induction of infection. Clinical signs are subcutaneous swellings and, in some cases, eventually marked lymphadenopathy; sometimes the disease progresses further. Pathologically, the disease is characterized by granulation, suppuration, fibrosis occasionally with caseation and calcification; thus, it may be confused with tuberculosis. To date, no effective treatment has been developed.

Eperythrozoonosis and hemobartonellosis of cattle are infections caused by *Eperythrozoan wenyoni* and *Haemobartonella bovis,* respectively. In general, infection with these microorganisms does not result in any clinical disorder. Anemia may rarely occur following primary infection of susceptible cattle, particularly if the animals are debilitated by poor nutrition or concurrent disease. As the parasites are spread by biting arthropods, their control will coincidently aid in control of these parasites. Because the parasites are generally not the cause of disease, however, treatment is usually unnecessary. If treatment should be desired, these parasites are suceptible to the action of tetracyclines and arsenicals.

Bovine trichomonal abortion is caused by *Tritrichomonas foetus*. Infection is common in many areas of the world and the means of spread is sexual intercourse. Bulls become carriers of the parasite but generally show no disease. Cows bred by infected

bulls will usually abort early in gestation. The fetus may die and not be expelled, in which case pyometria may develop. Prevention by elimination of infected bulls and rigid control of breeding stock into the herd is the best course of action for the herdsman to follow. Metronidazole (Flagyl, Seale & Co.) can be used to treat infected cattle and, if given properly, will sterilize the infection in carrier bulls.

Actinobacillosis

Synonyms: timber-tongue, wooden-tongue.

The disease

Actinobacillosis is a relatively common but usually sporadic disease of adult cattle caused by the Gram-negative bacterium, *Actinobacillus lignieresi.* The lesions are usually in soft tissues around the head and neck but particularly in the oral cavity and pharynx. The lesions consist of granulation, suppuration and fibrosis and spread via the lymphatics is common. Occasionally, the disease may arise elsewhere such as in the skin, lymph-nodes and body cavities. Treatment with streptomycin is usually highly effective in all forms of the disease.

Etiology

The causal agent is *Actinobacillus lignieresi,* a bacterium which, on primary isolation, appears as a small (1.2×0.4 μm) rod-shaped organism, although marked pleomorphism may occur. It is Gram negative and sometimes shows bipolar staining. For more detailed information regarding the morphological, cultural, biochemical and antigenic properties of this bacterium, the reader is directed to the work of Soltys (1963).

Pathogenesis

Actinobacillus lignieresi is a common inhabitant of the normal alimentary tract and it is generally accepted that it gains access to tissues through breeches in the epithelial lining such as occur following trauma, ulceration or the eruption of permanent teeth.

The primary lesion is a small purulent focus which gradually enlarges and extends, sometimes with the formation of discharging ulcers. Later, there may be extensive development of granulation and fibrous tissue containing pockets of pus which is usually thick, yellowish-green in colour and contains small (0.4 mm diameter) granules. The associated lymph-nodes are usually also

affected and may show marked enlargement, sometimes with sinus-formation.

Actinobacillus lignieresi shows a marked predilection for soft tissues. Lesions are usually in (or on) the head and neck but cases may be encountered in which affected areas are elsewhere on the skin, in lymph-nodes or within the thoracic or abdominal cavities. Very occasionally, the organism may invade bony structures and give rise to signs and lesions virtually indistinguishable from actinomycosis.

Distribution

Actinobacillosis has a worldwide distribution.

Epidemiology

Actinobacillosis (in all its clinical forms) is mainly a disease of young adult cattle with most cases arising in cattle between one and three years of age.

Cases are usually sporadic but multiple cases may arise locally. The age of cattle with the disease strongly suggests that the eruption of permanent teeth often creates the conditions which are necessary for the organism to penetrate sub-epithelial tissues. Ulcerative disease, such as foot-and-mouth disease, may also allow for invasion by the bacterium. Abrasive foodstuffs may be of importance in this respect. Ectoparasites have sometimes been suspected as predisposing to cutaneous actinobacillosis.

Clinical signs

The classic form of actinobacillosis is "wooden tongue". In this form of the disease, the affected animal is often a heifer or young cow and the history is of oral discomfort and drooling of saliva for several days, coupled with rapid weight-loss.

On closer examination, the animal's tongue may be seen to protrude from the mouth; on palpation it is enlarged and diffusely hardened. Sometimes discharging ulcers may be found on the sides of the tongue. Frequently, the intermandibular space is swollen and hard and the sub-mandibular lymph-nodes are enlarged. Enlargement of the retropharyngeal lymph-nodes sometimes occurs and when present, leads to dyspnoea with respiratory stertor (snoring). Drooling of saliva is a very striking feature, particularly of early cases.

Other forms of the disease may be encountered which are usually not associated with the classic (lingual) syndrome.

Cutaneous actinobacillosis may give rise to lesions anywhere on the body although in most cases the lesions are on the head and neck. Usually, the affected areas are hairless and the lesions are either thick cutaneous plaques or sub-cutaneous nodules. In most cases the affected sites are painless and the associated lymph-nodes are enlarged. Discharging sinuses may arise from sub-cutaneous or lymph-node lesions. If the lesions involve the muzzle it is quite common for facial distortion to be present.

Actinobacillosis may also cause lymphadenopathy in the absence of any other obvious lesions. In such cases marked lymph-node enlargement occurs in a random (non-symmetrical) fashion and affected nodes may discharge pus; sometimes the discharging lymph-nodes are painful. The signs in such cases depend on the site and size of the affected nodes; hence, the syndrome may be upper respiratory distress (enlarged retropharyngeal nodes), chronic ruminal tympany (enlarged mediastinal nodes) or asymmetrical, but widespread, lymphadenopathy. Involvement of the viscera, and in particular the esophageal grove, may give rise to chronic ruminal tympany. Rarely, lesions may be found in the liver.

Pathology

Irrespective of site, the basic lesion of actinobacillosis is a granulomatous process with suppuration and fibrosis. The granules which may be seen on microscopic examination of pus are found on careful examination to be masses containing the causal organism surrounded by rosettes of club-like structures. The true nature of these club-like structures is still in doubt.

Occasionally, necrotic foci are also to be found.

Immune response

Little is known about the immune response to *Actinobacillus lignieresi.*

Laboratory aids to diagnosis

Clinical diagnosis rests upon the examination of pus. In cases of actinobacillosis this examination will reveal relatively small granules (i.e. compared with those found in actinomycosis). The demonstration and identification of the causal organism using the Gram stain will confirm the diagnosis. Serology is not a useful technique as yet in the identification of *Actinobacillosis lignieresi* infection in cattle.

Prevention and control

Usually, actinobacillosis is such a sporadic disease that prevention techniques are not worth consideration. If it occurs locally with high frequency then it is essential to attempt to define (and then to correct) those factors which have encouraged it to assume an unusually high prevalence. Extremely coarse fodder, for example, could be replaced with less coarse feed.

The treatment of actinobacillosis in cattle is dealt with in detail by Blood and Henderson (1979). Basically, the organism is sensitive *in vivo* to a wide range of sulfa-drugs, antibiotics and also to potassium or sodium iodide. The drug of choice would appear to be streptomycin given at the rate of 5 g/day for three days and repeated if necessary.

Actinobacillosis as a zoonosis

Infection has been recorded in man (Soltys, 1963). Infection from animals should be regarded as a hazard, and due care should be taken when handling diseased animals, tissues and preparations.

Actinomycosis

Synonym: lumpy-jaw.

The disease

Actinomycosis is a relatively common sporadic disease of adult cattle. It is caused by the Gram-positive organism *Actinomyces bovis.* The disease usually arises in hard tissues, particularly the mandibles and maxillae and when it occurs, spread is usually by extension or by the hematogenous route. The pathological features of actinomycosis are virtually identical to those described for actinobacillosis. Treatment is only occasionally successful.

Etiology

The causal agent is *Actinomyces bovis,* which is a slender, non-septate filamentous organism showing fine branching; it is Gram positive. Further information regarding this organism, including its classification, can be obtained from Soltys (1963).

Pathogenesis

Actinomyces bovis has been isolated from the alimentary tract of normal cattle. It is thought that the organism invades the sub-epithelial tissues through abrasions, etc.

The organism shows a predilection for hard tissue, particularly bones such as the mandibles and maxillae; the disease rarely spreads to associated lymph nodes.

Distribution

Actinomycosis has a worldwide distribution.

Epidemiology

Actinomycosis is usually a sporadic disease of mature cattle (i.e. over four years of age); it seems unlikely, therefore, that tooth-eruption is a common predisposing factor to infection.

Clinical signs

The classic form of actinomycosis is "lumpy jaw". The course of this disease is usually more protracted than that seen in actinobacillosis. A typical case involves a mature cow in which a mass on the upper or lower jaw has been noted for several weeks or months and which is now giving cause for concern due to distortion, dysphagia and drooling of saliva.

Detailed investigation of the mass often indicates that it is painful and one or more discharging sinuses may be present. In upper jaw lesions there may also be signs of maxillary sinus involvement. In most established cases, an examination of the mouth will reveal that in the affected area there are either loose or missing teeth. Halitosis is common. Drooling of saliva and dysphagia are particularly obvious once the teeth become loose but "hypersalivation" is hardly ever as pronounced in cattle with actinomycosis as in cattle with actinobacillosis. Lymphnodes in the affected region are usually not markedly enlarged.

On rare occasions, actinomycosis has been defined as a cause of a "wood-en-tongue-like" syndrome, chronic mastitis and also thoracic and abdominal disease.

Pathology

The basic lesion in actinomycosis is virtually identical to that seen in actinobacillosis usually with the added effect of destruction of bone and fairly widespread necrosis. The granules which may be found in pus from actinomycotic lesions are usually larger than those found in pus from cattle with actinobacillosis and often measure 3-4 mm in diameter; the granules are often referred to as "sulfur granules"

Immune response

Little is known about the immune response to *Actinomyces bovis* in cattle.

Laboratory aids to diagnosis

Diagnosis is made by examination of pus, "sulfur" granules, and pathological findings. Confirmation of diagnosis is by microbiological identification of *A. bovis* in the "sulfur granules".

Prevention and control

There are no known preventive methods for this disease which, in any case, is usually of a sporadic nature.

Treatment is far less successful than is treatment of actinobacillosis. Sulfadrugs and streptomycin appear to be the durgs of choice and some authorities even advocate surgical procedures such as curettage of infected sinueses. On occasions success has been claimed following the injection of antibiotics into the affected areas. However, established cases rarely respond to any treatment and the results of treating even early cases are often disappointing.

Actinomycosis as a zoonosis

The infection has been recorded in man so the comments made about *Actinobacillus lignieresi* are equally pertinent here.

Nocardiosis

Synonyms: bovine farcy, tropical actinomycosis.

The disease

This is a relatively common, and usually benign sporadic disease of cattle in tropical areas. While there is some controversy as to the actual cause, it is generally accepted that the Gram-positive organism, *Nocardia farcinica,* is the agent involved. Infection is thought to arise via skin abrasions and in some countries, ectoparasites are held to be of prime importance. The clinical signs are subcutaneous swellings and eventually, in some cases, marked lymph-adenopathy; sometimes the disease progresses further. Pathologically, the disease is characterized by granulation, suppuration, fibrosis occasionally with caseation and calcification; thus, it may be confused with tuberculosis. To date, no effective treatment has been developed.

Etiology

For many years, the cause of the disease was thought to be the organism *Nocardia farcinica* but more recently some doubts have been cast upon this and it has been suggested that other similar organisms and also certain mycobacteria are of etiological significance. This question has been discussed in detail by Ainsworth and Austwick (1958) and Soltys (1963).

The nocardia are Gram-positive filamentous organisms, showing irregular staining. Certain species are acid-fast.

Pathogenesis

Transmission is usually held to be via skin wounds and in some countries, ectoparasites (particularly ticks) are held to be of importance.

Early lesions often found subcutaneously may be on almost any area of the body, but the head, neck and limbs are probably the most commonly affected areas. Lesions often coalesce and eventually spread via the lymphatics to the associated lymph-nodes. In some cases the disease may spread internally.

Distribution

Nocardiosis is widely recognized in tropical areas.

Epidemiology

The disease is usually seen only in adult cattle.

Ectoparasites have been incriminated as mechanical vectors for many years on circumstantial evidence, mainly because lesions often arise in areas of

tick attachment. Lice, too, have been suspected as playing a part in the epidemiology of this disease.

Clinical signs

The earliest signs of the disease are single or multiple subcutaneous swellings on the inner surface of the legs, the head, neck or ears. These may remain static or, sometimes, finally disappear; however, in some cases they coalesce and eventually infection spreads to the associated lymph-nodes eventually giving rise to marked enlargement. Sinus formation and pus discharge do not occur.

In many cases – even with marked lymphadenopathy – the health of an affected animal is not markedly impaired.

Rarely, the disease will spread to other organs and it may also produce a chronic respiratory syndrome or even a chronic, indurating mastitis.

Pathology

The basic lesion involves granulation, suppuration and fibrosis. Caseation, and even calcification, may also occur. In India and Ceylon, the respiratory form of disese may be complicated by an ulcerative tracheitis.

In many countries, nocardiosis is only considered to be of importance insofar as it sometimes resembles tuberculosis and therefore causes problems during meat inspection.

Immune response

Certain cases may become hypersensitive to bovine tuberculin.

Attempts have been made to develop cutaneous and serological tests for diagnostic purposes (Blood and Henderson, 1979).

Laboratory aids to diagnosis

In most cases, confirmation of diagnosis rests upon the demonstration of nocardia in material aspirated from affected areas or else in lymph-node biopsies.

As yet, the cutaneous and serological tests referred to above are not widely used.

Prevention and control

Prevention of nocardiosis in areas where it is a relatively common problem generally rests on control of those ectoparasites which are believed to be important in the spread of the disease.

Many different approaches to treatment have been tried, but in most cases, results have been disappointing.

Eperythrozoonosis and hemobartonellosis

The diseases

Microorganisms of the genera *Eperythrozoon* (Schilling) and *Haemobartonella* (Tyzzer and Weinman) infect a wide variety of animals including mice, rats, dogs, cats, swine, sheep, goats, and cattle (Weinman, 1944; Kreier and Ristic, 1968; Gothe and Kreier, 1977). In most of these animals no clinical disorder results from infection of animals with their spleens *in situ*. The only eperythrozoa and hemobartonellae which are considered to commonly produce diseases of economic importance in intact animals are *Eperythrozoon* (*Haemobartonella*) *felis* and *Eperythrozoon suis*. The diseases caused by these microorganisms are feline infectious anemia of cats and icteroanemia or "anaplasmosis like disease" of pigs. There are scattered reports in the literature of diseases, characterized by unthriftiness and anemia, in sheep and cattle which were considered to be caused by eperythrozoa and hemobartonellae.

Etiology

The eperythrozoa found in cattle have been named *Eperythrozoan wenyoni* (Adler and Ellenbogen) and *E. teganodes* (Hoyte). The hemobartonellae found in cattle have been named *Haemobartonella bovis* (Donatien and Lestoquard), *H. magma* (Rodriquez), *H. sergenti* (Adler and Ellenbogen) and *H. wenyoni* (Nieschulz and Boz). *Eperythrozoon ovis* (Nertz, Alexander and Dutoit) occurs in sheep (Kreier and Ristic, 1974).

Eperythrozoa and hemobartonellae are hemotropic bacteria. They occur on or in erythrocytes and free in the plasma. In general, eperythrozoa are more loosely attached to the erythrocytes than are hemobartonellae and are therefore more often observed free in the plasma. In Gram stained blood films prepared from animals undergoing acute, primary infection eperythrozoa and hemobartonellae stain purple-red. In general, hemobartonellae appear to be

rod shaped and are scattered over the surface of the red calls singly or in short chains while eperythrozoa are cocci which in dried, stained, blood films may appear to be small rings.

Distribution

Eperythrozoa and hemobartonellae occur worldwide. Wherever cattle are raised in Europe, Africa, Asia and the Americas these microorganisms have been found if searched for.

Epidemiology

Eperythrozoa and hemobartonellae are spread by blood sucking insects. Lice, fleas, and ticks have been implicated as biological vectors and horse flies (tabanids) as mechanical vectors. Transfer of fresh blood, for example, on needles used for bleeding several animals without cleaning in between, may spread the infection.

Clinical signs

Fever, depression, anemia and loss of condition are the most common signs reported to occur in animals with clinical infection. Most infected cattle if otherwise healthy do not develop clinical disease. Carrier cattle, however, suffering from stress from other parasites or poor nutrition may develop obvious parasitemia, fever and anemia. Exacerbation of infection is particularly common in cattle suffering anaplasmosis, babesiosis, theilerosis, or other blood parasite infections (Merchant and Packer, 1967).

Pathology

The pathology in cattle with disease caused by these parasites is typical of that occurring in animals suffering from a hemotropic parasite. There is anemia, anisocytosis, macrocytosis and reticulocytosis. There may be leukocytosis and generally there is splenic enlargement (Schalm *et al.*, 1975).

Immune response

The eperythrozoa and hemobartonellae establish an infection immunity in the animals they infect. Immune animals appear completely normal clinically but their blood is infectious and will transfer infection to susceptible animals. Antibodies to the parasites can be demonstrated by complement fixation and

fluorescent antibody procedures, but the relation of these antibodies to control of the parasite is unclear.

Laboratory aids to diagnosis

In animals suffering chronic disease and in nonclinical carriers the organisms are not easy to detect in blood films. In such cases injection of blood from the suspect animal into a clean, previously splenectomized animal of the same species will usually permit demonstration of parasites in blood films. Parasites are most easily detected in the early preanemic phase of the infection.

The indirect fluorescent antibody test and the complement fixation test can be used for demonstration of antibody to the parasites in the serum of carrier cattle.

Prevention and control

Sanitation, management and vector control. Control or elimination of blood sucking arthropods will prevent the spread of these microorganisms. The same types of vector control measures used to eliminate the vectors of anaplasmosis and babesiosis will aid the control of the vectors of eperythrozoa and hemobartonellae.

Immunoprophylaxis. There are no immunoprophylactic agents for use against eperythrozoa or hemobartonellae.

Chemotherapy. Antibiotics of the tetracycline group as well as arsenicals are effective against eperythrozoa and hemobartonellae (Gothe and Kreier, 1977). Dosages to be used and routes of administration are those recommended by the manufacturers for treatment of cattle with these compounds.

Trichomoniasis

The disease

The diseases caused by trichomonads are called trichomoniasis (Baker, 1969). Trichomonads infecting the bovine genito-urinary tract cause bovine genital trichomoniasis or bovine trichomonad abortion.

Etiology

Bovine trichomonad abortion is caused by *Tritrichomonas foetus* (Riedmuller) (Levine, 1973).

The trichomonads are flagellate protozoa. The organisms have three, four or five free anterior flagella and one recurrent flagellum which is attached to the body to form an undulating membrane. In appropriately prepared preparations, a nucleus, an axostyle and a filamentous structure called a costa running along the base of the undulating membrane may be seen. Trichomonads are spherical to pear shaped and may be approximately 6 to 19 μm in diameter (Honigberg, 1978a, b).

Distribution

Trichomonads occur world wide. *Tritrichomonas foetus* resides in the vagina and uterus of female animals and in the preputial sheath of males. Incidences of infection of 6 to 7% in the United States in beef cattle and 30% in cattle in Germany and Switzerland have been reported.

Epidemiology

Tritrichomonas foetus of cattle is transmitted by the venereal route almost exclusively. These parasites infect males in which they seldom produce clinically observable disorders. Female cattle do not usually become carriers of *Tritrichomonas foetus* but usually clear their infections often aborting. Maintenance of these parasites is thus by the clinically normal carrier male. *Tritrichomonas suis* is indistinguishable from *Tritrichomonas foetus* and will infect the genital tracts of cattle. In pigs *Tritrichomonas suis* inhabits the nasal cavity and digestive tracts, particularly the caecum. It has been suggested that *Tritrichomonas suis* causes atrophic rhinitis of pigs. Whether pigs acquire infection from cattle or cattle from pigs is uncertain. The relationship between the trichomonads of cattle and pigs is discussed at length by Honigberg (1978a).

Clinical signs

Tritrichomonas foetus infections of cows may cause mild to severe disease. There is usually vaginitis with low grade inflammation. As the disease progresses the uterus is invaded. The organisms cause a low grade endometritis with continuous intermittent discharge. The uterine inflammation interferes with fertilization. If conception occurs abortion follows. Abortion usually

occurs early, between 1 and 6 weeks after conception. If the placental membranes are not expelled along with the foetus, chronic catarrhal or purulent endometritis may develop. The uterus may fill with pus and the animal may become permanently sterile. If the abortion is complete and the membranes are expelled the animal may recover completely, clear its infection and conceive normally later.

In the bull trichomonad infection centers in the preputial cavity and may more rarely involve the urethra and even the testis. On initial infection the prepuce may be inflamed and swollen and there may be a mucopurulent discharge. Orchiditis rarely occurs. Usually all clinical signs of infection cease in about 2 weeks after infection but the bull remains a nonclinical carrier of the trichomonads, generally for life.

Pathology

The pathologic lesions in cattle with bovine trichomoniasis are those one would expect to find in a luminal infection by a noninvasive microorganism. *Tritrichomonas foetus* colonizes the uterus as well as the vagina. In the uterus trichomonads may cause a low grade inflammation and endometritis. Abortion and sterility are the most common consequences of infection and the major pathology in the uterus is determined by the type of abortion which occurs. If the abortion is complete and the foetus and membranes are all expelled, then the prasites die out and the uterus returns to normal; if however the dead foetus is retained or the membranes are retained, then a chronic infection occurs and a pyrometria develops. The uterus may fill up with a relatively odorless, thin, greenish fluid which may swarm with trichomonads. In some cows fluid may drain partially when the cow lies down.

Immune response

An immune response is mounted by the host against *Tritrichomonas foetus*. Cows which recover from infection by *Tritrichomonas foetus* are usually immune to reinfection although reinfection does occur. Antibodies to trichomonads are present in the blood and in the uterine and vaginal secretions of infected and recovered individuals. These antibodies have been demonstrated by most of the standard serological tests, including complement fixation and agglutination tests. Attempts to correlate these antibody responses to immunity and to use serological tests in diagnosis have not been particularly successful. Antibodies to trichomonads are present in many normal, nonimmune individuals with no history of infection with *Tritrichomonas foetus* while in many infected and immune individuals antibodies cannot be demonstrated by

currently available techniques. Tests for cell mediated immunity, such as skin tests, have also given inconsistent results. Trichomonads of distinct species share some antigens. As symbiotic and commensal trichomonads are almost universally present in the alimentary tracts of man and other animals, it is possible that antigens absorbed from the alimentary tract may account for the frequent presence of antibodies in individuals with no history of infection. The poor contact between the parasites in the lumens of organs of the genital tract and the immune system may account for the poor correlation between immunity and antibody in the serum. The hope that the presence of local immunity, indicated for example, by the presence of antibodies in the secretions in the genital tract, would correlate better with infection and immunity than do serum antibody levels has unfortunately not been borne out by experimental work. Despite the poor correlation between antibody in the mucus of the genital tract of females, most who have studied immunity to genital trichomonads apparently consider luminal antibody to contribute to the elimination of trichomonads from the female genital tract.

Laboratory aids to diagnosis

Diagnosis of trichomonad infection is by demonstration of trichomonads in fluids and lesions suggestive of trichomonad caused disease. Absence of trichomonads from fluids and lesions precludes trichomoniasis but as many individuals are healthy carriers of trichomonads it cannot always be assumed that the trichomonads present in an individual are the primary cause of the lesions observed. If however the clinical lesions are characteristic of trichomonad caused disease and trichomonads are present in the lesions it is reasonable to consider the diagnosis positive and then proceed to appropriate treatment.

Diagnosis is best made by examination of wet preparations made from the fluids or lesions. In diagnosis of uro-genital trichomoniasis a cotton swab can be inserted into the vagina or prepuce to pick up mucus. Swabs inserted into the uterine fluid of cattle can also be used to collect material for diagnosis.

On return to the laboratory the swabs are rinsed in a small volume of physiological saline and wet mounts prepared for microscopic examination. Examination of the wet mount may be by bright field, dark field or phase contrast techniques. Movement of the flagella and of the undulating membrane aids in recognition of trichomonads in fresh preparations.

Such material, collected carefully to minimize bacterial contamination, may be inoculated into culture media containing antibiotics and trichomonads looked for after a suitable incubation period.

Material on swabs may be smeared on microscope slides, dried, fixed and stained. Giemsa stain has been generally found satisfactory for preparation of stained smears. This stain has the advantage of being generally available in most laboratories engaged in diagnostic work.

In general at least three consecutive negative tests should be obtained before the animal is considered free of infection.

Immunodiagnostic tests including the mucus agglutination test, are generally considered only useful for herd diagnosis as a high percentage of infected animals give negative tests.

Prevention and control

Sanitation and management. Bovine genital trichomoniasis is a venereal disease. Carrier males and more rarely females maintain the reservoir of infection. Prevention of bovine trichomonad infection requires care in breeding practices. No breeding stock should be introduced into the herd unless tests have been made to assure absence of trichomonads. Mature animals should be held in isolation and tested for trichomonads before introduction into the herd. It has been generally recommended to slaughter infected bulls because treatment is difficult and the results poor. As infected cows usually clean themselves, isolation and sexual rest for not less than 3 months followed by tests for infection before entrance into the herd are usually sufficient safety precautions. If practical, use of artifical insemination rather than natural breeding will aid control of trichomonad abortion.

If the herd is already infected all animals should be examined for infection. Infected cows should not be bred for at least 3 months and should be reexamined before rebreeding.

Infected bulls should be disposed of.

Immunoprophylaxis. No immunoprophylactic agents are available for use against trichomonad infection.

Chemotherapy. Metronidazole (Flagyl, Seale & Co.) can be used to treat cattle. Bulls can be treated orally with metronidazole compounds. Oral administration of dimetridazole, 50 mg/kg, daily for 5 days will apparently cure bulls. A similar treatment can be given to cows but as the treatment is expensive and as the cows will self cure in a fairly short time, treatment may be unnecessary (Honigberg, 1978a, b).

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PART IV

TICKS AND ENVIRONMENTAL EFFECTS

41. TICK TOXICOSES OF CATTLE

Rainer Gothe

Abstract. Of the 800 tick species which have thus far been described, populations or strains of approximately 50 argasids and ixodids are potentially capable of causing pathological and/or pathophysiological changes through inoculation of unknown uninfectious noxes during repletion. These noxes are generally interpreted as toxins.

Only 11 ixodid and two argasid species are considered to cause toxicoses in cattle. These effects are clinically divided into three disease syndromes: (a) tick paralyses, (b) *Hyalomma truncatum* toxicoses and (c) *Rhipicephalus appendiculatus*-toxicosis. A toxicosis is thereby defined as a generalized and experimentally reproducible disease. Only a few "potent" ticks and always during the first infestation suffice for induction of toxicosis. No immunopathological manifestations seem to accompany the toxicosis. The symptom complexes caused by *Boophilus microplus* and *Dermacentor albipictus*, as well as other tick-inducing symptoms (e.g., allergies, skin reactions, etc.) are not interpreted as toxicosis within the parameter of this definition. Likewise the pathophysiological condition caused by *Rhipicephalus appendiculatus* can only be accepted with reservations.

Tick paralyses

Synonyms: (Zeckenparalyse (German); tique paralysis (French); paralisi da zecche (Italian).

Etiology

The capability to induce paralysis in cattle has been demonstrated, described or suggested for 12 tick species belonging to seven genera. These species include *Dermacentor andersoni* Stiles, *D. occidentalis* Marx, *Dermacentor* sp.; *Haemaphysalis punctata* Canestrini and Fanzago, *Hyalomma truncatum* Koch; *Ixodes holocyclus* Neumann, *I. ricinus* (Linnaeus), *I. rubicundus* Neumann; *Rhipicephalus evertsi evertsi* Neumann and *R. simus* Koch from the family Ixodidae and *Ornithodoros savignyi* (Audoin) and *Otobius megnini* (Duges) from the Argasidae.

Although the toxin hypothesis proposed by Gothe (1979) is generally accepted on the basis of experimental investigations with tick saliva and salivary glands material, the chemical structure of the toxin, the principle of the toxic process, the toxin dynamics and kinetics, and the primary point of attack of the toxin remains unsolved.

The functional component of the toxin is believed to be a neurotoxin which has not yet been isolated and chronically defined. It is assumed that this neurotoxin impairs the functions of peripheral nerves and/or the myoneural synapses; additionally, a causal cerebral affection and an interference of the toxin with the energy metabolism of muscle cells, respectively, have been suggested. The toxin etiology is substantiated by negative results obtained following inoculations and transfusions of normal animals with tissues of diseased animals and ticks. It was postulated that the effective volumes of the toxin is produced in tick salivary glands only after a sucking period of approximately 5 days. The incubation is, therefore, exactly temporally fixed and relates directly to the salivary gland activity and thereby to the repletion condition. Further analysis of the action of the toxin showed that a permanent and sufficient toxin secretion is necessary for the persistence of the clinical manifestation. Symptoms seem to be directly related to the presence of the tick. Accordingly, they disappear immediately following engorgement or mechanical removal of the tick (Gothe, 1971a; Gregson, 1973).

The toxin etiology is further substantiated by investigations of tick paralysis by Kaire (1966) who isolated a paralysis-inducing fraction from homogenates of engorged female *I. holocyclus* ticks. Toxic action of this fraction was neutralized *in vivo* and *in vitro* by immune serum. The fraction was found immunogenic by inducing an antitoxic immunity, which was passively transferable and showed a sufficient protection against tick-induced paralysis. Similar experiments with partially engorged female *D. andersoni* ticks, however, were not successful (Gregson, 1973; Gothe, 1979).

In recent investigations it was shown that the maximum toxicity induced by *I. holocyclus* occurs on days 5-6 post infestation and that the toxin is a protein polypeptide moiety of middle to high molecular weight.

Distribution

The most frequent and economically important, as well as the most widely distributed tick paralyses, are caused by female ticks of the species *D. andersoni* in North America and *I. holocyclus* in Australia.

The paralysis of cattle caused by *D. andersoni* is endemic in Canada, particularly in the intermountain region of the western part of British Columbia; in the U.S.A., however, the disease occurs sporadically in Oregon, Mon-

tana and Idaho although this tick species has a greater distribution as cartographically demonstrated by Rich (1971).

The distribution of *I. holocyclus* is limited to a small strip on the Australian east coast, which stretches from Normaton in northern Queensland to the Lake Entrance Bairnsdale district of eastern Victoria. A relationship between distribution of this tick species and the incidence of paralysis in cattle, especially calves, is caused by *I. holocyclus* (Bagnall and Doube, 1975; Doube, 1975; Doube and Kemp, 1975; Doube *et al.*, 1977). Occasionally heavy losses are encountered in adult cattle also, particularly when animals from tick-free regions are brought into endemic areas (Doube, 1975; Doube and Kemp, 1975).

Cattle are not the only host in which the above two ticks induce paralysis. For *D. andersoni* syndrome there is a marked species sensitivity difference. An approximate order of decreasing animal species susceptibility to toxin is man, sheep, dogs, cattle and horses. Wild animals appear to be highly resistant (Rich, 1971; Gregson, 1973).

Especially sensitive to the *I. holocyclus* paralysis are sheep, cats, dogs and human beings; cattle and horses, however, are affected mainly at a very young age. Native animals such as the short-nosed bandicoot *Isoodon marcourus* and the long-nosed bandicoot *Perameles nasuta* are of great importance for the population density and dynamics of this tick paralysis (Doube, 1975).

The tick paralysis caused by *D. occidentalis* has been only occasionally described in cattle in California. Paralysis caused by *I. rubicundus* in South Africa occurs mainly in sheep and goats; cattle are only occasionally affected (Stampa, 1959). The *R. evertsi evertsi* paralysis, primarily also a sheep disease, until now has been observed only rarely in cattle and exclusively in South Africa (Neitz, 1964). On the *I. ricinus* paralysis only one incomplete reference is available describing the disease in Yugoslavia and Turkey; likewise, reports about cattle paralysis caused by *H. punctata* in Bulgaria and by *Dermacentor* in Italy have to be judged accordingly. Information on cattle paralysis induced by *H. truncatum* and *R. simus* ticks in Rhodesia is incomplete. More information is also needed on cattle paralysis caused by *O. savignyi* in Nigeria and Tchad (Neitz, 1964), and by *O. megnini* in British Columbia and California.

Epidemiology

Various biologic parameters on the part of the host and the tick may influence epidemiology of the disease. In the case of the host, these parameters include sensitivity to toxin, age, immune response, behavior, as well as the reaction potential and population density. In the case of tick factors, what must be considered are dynamics and virulence of toxin-inducing ability,

sexual activity, developmental stage, infestation locus and sucking phase, respectively (Gothe, 1979). These biologic aspects, their complexity and interrelations, however, have not been fully investigated. This is especially true with respect to the minimum infestation rate necessary for the development of a paresis or paralysis, the responsible tick state and its repletion phase as well as the population dependent, paralysis-inducing capacity of a tick species. It has been established, however, that the maximum incidence of tick paralyses occurs in the spring and early summer, probably associated with the seasonal activity of female ticks. It is not always possible to establish a relationship between tick quantum and clinical severity of paralysis. In one situation it was shown that a single female tick was sufficient for establishment of paralysis in a 1000-pound bull and a mature steer. Under other circumstances using similar animals, there was a direct correlation between the infestation intensity and clinical manifestation.

Likewise, it is known in *I. rubicundus* that only certain populations are capable of inducing paresis or paralysis whereby some animals of the same species can tolerate up to 100 ticks, others may be paralyzed by a solitary tick. The frequency of occurrence of these potent ticks, however, is difficult to predict with reference to specific geographic location and time. For *I. holocyclus* there likewise has been a species variability in paralysis-inducing capability determined. Consequently, all laboratory studies and field observations suggest that the two most difficult to predict epidemiologic parameters on the part of the host and the vector are sensitive to paralysis and the virulence of the toxin, respectively (Hamel and Gothe, 1978; Gothe, 1979).

Clinical signs

The clinical pictures of tick paralyses in man and animals are fully described by Gregson (1973) and Murnaghan and O'Rourke (1978). The incubation period, because of its dependence on the tick repletion time, is constant and takes an average of 5 to 7 days. During incubation, severe prodromal symptoms such as lassitude, anorexia and heavy vomitus may occur, especially in *I. holocyclus* paralysis.

The first signs of paralysis usually become obvious in the hind extremities and are initially characterized by low to high grade incoordination and muscle weakness. Complete paralysis, which includes the bulbar regions, develops within a few hours thereafter. The trunk, front extremities, throat, esophagus, pharynx and face may be involved. The affected animal is incapable of moving. The typical disease syndrome may be described as ascending, symmetrical, flascid tetraplegia with functional impairment of the superficial and deep tendon and abdominal reflexes as well as nystagmus, chewing-swallow-

ing and breathing difficulties. The skin sensibility is lowered, the temperature is normal, the blood and liquor values are unchanged. If the ticks are removed early enough or repletion is completed, the clinical recovery may take place within a few hours to several days. A persistence of paralytic symptoms over several days after removal of all ticks is observed in *I. holocyclus* paralysis (Doube and Kemp, 1975).

Pathogenic mechanism

The mechanisms of pathogenicity of tick paralysis has not yet been fully investigated. The *Dermacentor*-induced paralyses are now defined essentially as motor polyneuropathies with only limited participation of the afferent pathways. A causal pathogenic involvement of acetylcholine biosynthesis can be excluded. Whether or to what extent the central nervous system is affected cannot be decided from the few contradictory findings. In case of *I. holocyclus*-induced paralyses, it is considered that the toxin may act on the excitation and secretion mechanism at the myoneural synapse, thus preventing acetylcholine liberation at temperatures above 30 °C. Furthermore, the participation of several toxins in this disease syndrome was not excluded. There seems to be a basic physio-pathologic difference in pathogenesis of paralyses induced by *I. holocyclus* and those induced by other ticks.

Pathology

Pathological manifestations caused by tick paralysis have been described and discussed by Gothe *et al.* (1971). In histological sections as well as in electronmicrographs of peripheral nerves of chickens, which at the time of tissue collection were completely paralyzed over a long period as a result of repeated infestations with *A. walkerae*, no morphological abnormalities of the function carrying parenchyma could be demonstrated. Also, no ultrastructural tissue changes could be observed in mice and rats dead or dying from *I. holocyclus* paralysis. A lack of tissue abnormalities in tick paralysis is apparent by the fact that recovery from the disease is rapid and clinically complete.

Immune response

A clear immunologic difference has been demonstrated between the *I. holocyclus* paralysis on the one side and all other tick paralyses on the other side (Gothe, 1971b, 1979). Recovery from *I. holocyclus*-induced paralysis is followed by a relatively long-lasting protective immunity. This immunity is also passively transferrable by way of blood serum or milk. No protective immun-

ity was noted following recovery from the disease caused by other tick species.

Laboratory aids to diagnosis

Diagnosis is made on the basis of consideration of various parameters which include the presence of ticks, the sudden appearance of paralysis, the rapid course of the disease, and the quick clinical recovery after tick removal. As a rule, temperature is normal, blood and liquor values are unchanged. Specific laboratory diagnostic technics are not available.

Prevention and control

Prevention and control of tick paralyses in cattle may be considered under four main headings: (1) Protection of susceptible animals from toxin effects through application of immune serum which, however, has only been possible until now in *I. holocyclus* paralysis. Under field conditions, the application of the method is difficult for economic and technical reasons (Doube, 1975); (2) prevention of the tick repletion time, necessary for the establishment of paralytic symptoms, through development of resistant cattle breeds, and application of acaricides; (3) reduction of ticks on cattle by postponement of grazing and calving periods outside of the tick season as well as materializing on natural behaviors of cows to protect their young. A shifting of the calving in Australia from spring to winter would have two advantages: the calves would be older and larger at first exposure to female *I. holocyclus*, and, furthermore, infestation with larvae and nymphs would have induced a certain pre-resistance to paralysis (Doube and Kemp, 1975). Another method is to select calving areas with a relatively small tick population density for the development of a natural and solid immunity before entering areas with heavy tick challenges (Doube, 1975). Also, certain husbandry practices may greatly reduce the paralysis risk of highly sensitive calves if, as shown with *D. andersoni* paralysis in British Columbia, a system of calf grooming by mother cows and a type of natural kindergarten holding, the "yarding" of calves under the supervision and care of some sentinel cows, which remove the ticks from the calves, is used. (4) Reduction of ticks in the respective ecosystem by combatting the wild tick hosts, use of acaricides and/or biological tick control measures. In Australia the ecological manipulation of tick habitat or the bandicoot population density were shown to be the best method for reducing *I. holocyclus* to safe level for cattle husbandry (Doube, 1975).

Causal therapy methods of tick paralyses in man and animals is limited to the timely removal of responsible ticks (Gothe, 1979). Only in *I. holocyclus*

paralysis is a therapeutically effective immune serum available which has been used successfully in the treatment of man and animals. An etiologically effective chemotherapy or prophylaxis is not available. Symptomatically the dyspnea, the circulatory system and the fluid requirements are to be treated, supported and replenished, respectively.

Toxicosis forms induced by *Hyalomma truncatum*

General and common characteristics of all forms of toxicosis are (a) they can be induced by only a few potent ticks, (b) disease symptoms occur following initial tick infestation without a need for prior sensitizing exposure. Clinical pictures induced by *H. truncatum* are interpreted as three different toxicosis forms: (1) sweating sickness, (2) Mhlosinga, and (3) Magudu. These forms are being differentiated clinically and immunologically (Neitz, 1962).

1. Sweating sickness (Sweetsiekte, nat kalwersiekte (wet calf disease), vuursiekte (fire disease) (Afrikaans); Schwitzkrankheit (German); la dyhydrose tropicale (French); foma (Swaci); 01 macheri (Masai of Kenya))

Etiology. In the absence of a specific etiologic agent, a "dermatropic" toxin was suggested as a possible causal agent (Neitz, 1954, 1956, 1959, 1962, 1964) since the infestation period determines the duration and intensity of symptoms. The removal of responsible ticks results in an immediate clinical recovery. All attempts to transfer the disease from animal to animal by inoculation of blood, tissues and saliva from sick to healthy animals have failed. Recipient animals also remained completely susceptible and became sick or died, respectively, after exposure to potent ticks. The transmission possibility through direct contact between healthy and sick calves is likewise to be excluded.

As further evidence in favor of the toxin hypothesis is the fact that only certain strains of ticks are capable of inducing sweating sickness. This property cannot be acquired by nonpotent ticks through infestation of nonpotent ticks on animals or passaged to other tick species of the same genus. The toxin effect of potent *H. truncatum*, with which both male and female ticks are provided, can persist for at least 16 generations. This persistence is independent of previous infestations on susceptible, immune or refractory hosts.

Distribution. The sweating sickness, widely distributed in Central, East and South Africa (Neitz, 1956, 1964; Konnerup, 1975) with possible occurrence

also in southern India and Sri Lanka, has been recognized as naturally occurring conditions only in cattle. Younger animals up $1\frac{1}{2}$ years are predominately clinically affected. However, animals up to 13 years can become diseased.

Epidemiology. The natural incidence of the disease is regulated by the cyclic periodicity, the density, as well as the sweating sickness inducing capacity of *H. truncatum*. On the basis of the developmental cycle of the responsible tick species, this toxicosis in Africa is a seasonal disease with maximal frequency during the period from January till March. Sporadic cases, however, may appear at any time.

Clinical signs. Under natural conditions, the intensity of sweating sickness of cattle depends on the reaction potential and age of the affected animal and the number and repletion time of potent ticks. Accordingly, peracute, acute, mild or inapparent symptoms of the disease have been described. Incubation lasts from 4 to 11 days with an average of 6 days.

The acute form of sweating sickness occurs mainly in cattle up to 12 months of age. The onset of the disease is sudden with an increase of body temperature to 40-41 °C, which persists over 3-6 days or occurs intermittently over an 8-day period. The animal's general condition is distinctly disturbed, its hair coat is rough. The visible mucous membranes and skin become hyperemic. A wet eccema develops, starting at the cheeks, under the eyes, on the nose, base of the ears, neck and flanks as well as abdominal and/or groin regions and may extend over the entire body surface. The wet eccema appears at 48 hours at the earliest, commonly 72 hours after onset of the disease. The resulting dermatitis is aggravated by a severe hyperesthesia, loss of hair coat and consequent desquamation of the superficial epidermis layers.

Primary symptoms are followed by excessive lacrimation, salivation and nasal discharge which may become mucopurulent. Occasionally the cornea is affected. In the mouth at diphtheroid stomatitis, glossitis, pharyngitis and laryngitis as well as necrotic changes of the lip margins appear. The condition is apparently painful, causing animals to go off feed. The intestinal tract can also be affected; simultaneously, there frequently occurs a diphtheroid vaginitis or posthitis. The central nervous system as a rule is not involved.

This disease may be complicated and further aggravated by concurrent occurrence of anaplasmosis, babesiosis, Nagana, heartwater, salmonellosis and especially skin myiases.

During recovery, a regeneration of the mucous membranes of the digestive tract occurs. The inflammation of the affected skin regions generally subsides

after 3-6 days with superficial epidermis layers sloughing off and rapidly regenerating. After 3-4 weeks, the hair grows anew; however, the hyperemia of the non-pigmented skin may persist up to 8 weeks.

The peracute course of this toxicosis, which occurs in approximately 4% of experimentally infected calves, is difficult to differentiate from similar clinical signs of other diseases. The disease is clinically characterized by a high and sudden rise in temperature, hyperemia and hyperesthesia of the visible mucous membranes and the skin, anorexia, lacrimation as well as rhinitis, salivation, muscle tremor, dysphagia, motility disorders and syspnea. It terminates fatally 48-72 hours after the initial fever.

The subaccute form corresponds in its symptomatology with the acute sweating sickness; however, its clinical signs are less pronounced with no mortality occurring. The mild form is only characterized by a 2- to 3-day pyrexia with middle grade hyperemia of the skin and the visible mucous membranes, which recede after 4-5 days. The inapparent type of this toxicosis occurs occasionally and can only be identified by the existence of a residual immunity following the re-exposure of the animals to potent ticks.

Mortality is extremely variable and directly correlated to the size of the toxin inoculum, which again may be dependent on the potency of the responsible ticks and their repletion time. Under standardized laboratory conditions with 48 calves from 2 to 11 months of age and a potent tick strain, 69% of the animals succumbed. By respective infestation of six older animals, the mortality rate still reached 50% (Neitz, 1959).

Pathology. Pathological changes are dependent upon duration of the disease and the presence of concurrent infections. Changes include an eccematotic dermatitis, inflammation and superficial necrosis of the oral mucous membranes, occurrence of whitish yellow pseudomembranes in the nasal cavity, pharynx, larynx, esophagus and omasum, lung edema and interstitial emphysema, hydrothorax and hydropericarditis, congestion and fatty degeneration of the liver and/or hyperemia of abomasal mucous membranes and of the small and large intestines and the genitalia (Neitz, 1959).

Immune response. A recovery from sweating sickness produced a solid protective immunity which may persist up to 4 years. A passive colostral passage of protective antibodies was not demonstrated (Neitz, 1959, 1962).

Laboratory aids to diagnosis. With the exception of the inapparent form of the disease, all forms of sweating sickness can be diagnosed by ascertaining and evaluating epidemiological data, clinical symptoms and pathological changes. An etiologic diagnosis of the inapparent form of the disease is

difficult. Disease resistance of suspect animals following exposure to potent ticks is the only means for establishing specific diagnosis of the inapparent form of sweating sickness (Neitz, 1959).

Prevention and control. Prevention is based exclusively on the effective control of the responsible ticks with suitable acaricides whereby their use and application must be timed so that a short-term, not clinically manifesting, but immunity-inducing infestation, is made possible (Neitz, 1956).

A causal therapy of sweating sickness is not available. Diseased animals, however, should be protected against adverse climatic conditions, radiation, quietly attended and cared for with water, and high quality and lightly digestable food. An antibiotic treatment against secondary bacterial infections, control of skin myiases and definite therapeutic measures against concurrent infections are mandatory (Neitz, 1959).

2. Mhlosinga and Magudu

Certain *H. truncatum* strains can additionally induce two further forms of toxicosis, which are described as Mhlosinga and Magudo and which occur in cattle, sheep and pigs as a nonfatal mild disease characterized by fever and anorexia. There is no cross immunity between these two diseases. There is also no cross immunity between sweating sickness and Mhlosinga; however, animals that have recovered from sweating sickness are immune to Magudu. Cross-strains of Mhlosinga- and Magudu-ticks appear more pathogenic, but affected animals remain susceptible for sweating sickness (Neitz, 1962, 1964). It is not known whether stomatitis-nephrose-syndrome of cattle occurring in Rhodesia is a toxicosis of a sweating sickness type or is a different *Hyalomma* toxicosis.

Toxicosis caused by *Rhipicephalus appendiculatus*

The disease occurs in cattle in southern Africa and is induced by a massive *R. appendiculatus* infestation. It is proposed that a "leucocytotropic" toxin is involved in induction of this disease. It may cause a paralysis or dysfunction of the reticuloendothelial system with possible consequent reactivation of latent or concurrent infections such as babesiosis, theileriosis, anaplasmosis, borreliosis and/or heartwater disease. The responsible tick stage and its necessary infestation rate for a clinical manifestation of this toxicosis are still unknown.

Clinical signs of this toxicosis were first described by Thomas and Neitz (1958) in 50 approximately 18-month-old cattle in Transvaal/South Africa. Signs included pyrexia, high-grade anemia, cachexis and necrosis of lymph nodes, especially those of the head, throat and prescapular region. The pathomechanism was thereby explained as impairment of the reticuloendothelial system with a possible participation of the bone marrow or else as an aplastic anemia by direct toxic damage to the bone marrow.

The clinical picture of this toxicosis could be experimentally reproduced with the signs of the resulting disease depending upon whether the cattle were previously exposed to a heavy or light infestation with *R. appendiculatus* (Rensburg, 1956). The degree of anemia can be directly correlated to the infestation intensity of the ticks as well as to the severeness of the concurrent blood infections. If ticks are removed, a partial clinical recovery may occur. Evidence for an acute anemia, induced exclusively by the postulated tick toxin, could not be sustained. This toxicosis has also been demonstrated in cattle in Rhodesia and furthermore considered as a possible partial factor in the pathomechanism in bovine cerebral theileriosis.

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PART V

PROBLEMS OF DISEASE CONTROL IN THE TROPICS

42. PROBLEMS OF DISEASE CONTROL IN THE TROPICS

W.I.M. McIntyre and Miodrag Ristic

The tropics cover nearly one-third of the world's total land area, and in those areas live approximately one-third of the human population and approximately one-third of the world's cattle population of one billion. Furthermore, three-quarters of all the cattle in the tropics live in 17 nations with India and Brazil each possessing approximately one-fifth of the total.

The problems of disease control within this enormous population of animals must be reviewed in the broadest possible context before selecting and deciding upon a specific disease control program. Disease control must be governed primarily by the status of the cattle industry within the economy of a respective nation. While the unique ability of cattle to digest and transform the crudest of plants and plant residues into protein fit for human consumption will always justify a place for cattle in the economy of a country, this industry will always be relative to many other socio-economic factors of a respective country.

Many of the non-industrialized nations are in the tropics, and a high proportion of their human population lives in rural villages. To those people, and even more so to nomadic and partially nomadic peoples, cattle have become part of a traditional way of life evolved over thousands of years. Through its government, each community must decide for itself how high a priority the cattle industry should take in the development of the nation. Once development and expansion of the cattle industry has been decided upon, every care must be taken to understand the traditions which have enabled the cattle owners to produce and survive over thousands of years. Often using the most sophisticated methods and devices known only to them, these practices are handed down from generation to generation and seldom are recorded in the classical books on cattle production. All those who would offer advice and help to such people, be they experts from another continent or local professional leaders in animal health and production, must begin by learning from, and respecting, the sophistication of peasantry which has

enabled countless generations of man to survive and rear his families under some of the worst ambient conditions in the world.

Achievements in disease control

It is important to study and draw inspiration from past successes and to consider in detail the methods involved. The most outstanding success in disease control in cattle in the tropics has been the development of vaccination control measures for rinderpest.

After many years of struggle by many different veterinary services using vaccines of limited protection and often dangerous potency, Plowright and Kenya achieved a major breakthrough in producing a vaccine against rinderpest. The virus of this vaccine was grown in a tissue culture system and dispensed in a lyophilized form. Protective immunity induced by this vaccine has been shown to last at least seven years, consequently, this vaccine can be compared with some of the greatest in the world such as those against poliomyelitis, yellow fever and smallpox.

The efficiency of the rinderpest vaccine was so high it led to the control of the disease in many countries within a few years. Moreover, the availability of this effective vaccine has had far-reaching effects on the security of cattle owners and their families. It is said that rinderpest vaccination success may have led to overstocking of cattle in some areas for a temporary period. In reality, the ever increasing demand for meat in the tropics, and the herdsman's skilled sense of the right number of animals for the land have been the dominating factors in deciding cattle numbers. The casual observer has too often ascribed cattle through numbers only, desiring status and wealth, forgetting the calculations which have to be made to protect the owner against several years of severe drought or, in the past, a calamitous outbreak of rinderpest. It must be remembered that only half the cattle population, the females, reproduce themselves and that it takes three or four years to produce a first calf, and that the number of calves produced in a lifetime by a cow varies tremendously under the combined effect of nutrition and disease.

Another less dramatic success story was the development of various types of vaccines for foot and mouth disease. The need and the scale at which these vaccines are used may differ from country to country. In cattle, which takes three to four years to mature and survive in countries where the infection has become endemic, it is often not economic to consider vaccination. On the other hand, those countries which export animals or meat to countries free of the disease have to confront the problems involved. In many countries vaccines have been used to reduce, and in some cases to eradicate, the

incidence of disease. Nonetheless, the problems associated with the multiplicity of these virus strains throughout the world and the relatively short duration of immunity produced are daunting facts which have to be carefully considered by all nations contemplating the control of this disease. In many countries in the tropics the present level of productivity of the cattle industry is insufficient to justify such a program.

Many other viral vaccines in use throughout the tropics play a part in the control of other diseases in cattle but there are still many gaps to be filled and work to be done if the success of rinderpest control is to be emulated.

Within bacterial diseases there are many well-established preventive immunization methods available. With the exception of the vaccine against brucellosis which uses an attenuated live immunogen, most other vaccines are inactivated bacterines. Consequently, the protection conferred by the latter vaccines is relatively short and frequent revaccination is needed. In some of these vaccines, such as those caused by Gram positive agents, the antigens are well established, and dependable protection is expected most of the time. There are other bacterines, such as those against leptospirosis, where the efficacy is good if the field strains are antigenically identical with those contained in the vaccine.

For some other bacterial diseases, e.g., tuberculosis and Johne's disease, no vaccines are available which require the use of more complicated and usually more costly disease control programs. Depending on the country, these diseases may exert a heavy toll on the bovine industry and tuberculosis continues to be a major zoonotic public health problem in some regions. For the foreseeable future, the control of these diseases must continue to be based on detection and quarantining or elimination of diseased animals.

Anaplasmosis is prominent among rickettsial disease by virtue of its worldwide occurrence and the economic impact on the livestock industry. During the last two decades new and safer prophylactic methods have been developed for control of this disease. The inactivated vaccines seem to offer some protection but this effect is overwhelmed by the danger of inducing isoimmunity to blood group antigens in vaccinated animals. A new vaccination approach utilizing soluble cell culture-derived *Anaplasma* antigens has given promising results in recent tests. An effective attenuated live vaccine is now available which is preserved and dispensed in liquid nitrogen. Current efforts to preserve the viability of this vaccine by lyophilization are promising. This successful accomplishment would mean a practical and effectual step towards making the vaccine available to many regions of the tropics where the use of liquid nitrogen preservation method is prohibitive.

Unfortunately there are no available vaccines for prevention of heartwater disease, another rickettsial disease which is limited to Africa. It is, however, a

disease of major consequences in certain regions of that continent and preventive immunization methods are likely to be developed.

Theileria parva was the first protozoan agent of major economic consequence to be cultivated *in vitro*. All expectations that this important finding will be followed by the development of a vaccine for East Coast fever have not yet materialized. However, induction of protection by inoculation of cattle with infected tick homogenates followed by tetracycline treatment have proven effective. This observation suggests that an effective vaccine is a good possibility once appropriate parasite antigens can be produced in cell cultures.

A major breakthrough in a century-long struggle against protozoan babesiosis was recently achieved by development of a cultural method for continuous *in vitro* propagation of *Babesia bovis,* one of the principal agents of bovine babesiosis. The highlight of a series of studies revealed masses of soluble antigens, in all probability merozoite surface coat antigens occurring freely in the culture medium. These antigens proved to be highly effective non-living and cell-free vaccines which induce protective immunity in cattle against challenge exposure with *B. bovis*. Henceforth, the speed with which a commercially praticable vaccine for control of babesiosis will be available appears to be only a matter of time needed for scaling up the technology for mass production of the antigen.

The immunologic principle used in development of babesiosis vaccine in which an antigen component rather than the whole protozoan parasite is used as an immunogen may prove applicable to other agents with a complex life cycle of development. It is apparent that various growth forms of babesia are immunogenically non-eseential and may be excluded from a formulation of inactivated vaccines.

Infections caused by helminths (worms) are inseparable from the grazing of cattle in the tropics. The effects of these worms varies with the species from blood removal, to massive change to liver, lungs, stomach and even arteries. Names such as *Haemonchus, Fasciola, Osteragia, Dictycaulus* are household terms to farmers and veterinarians. They cause death, but the greatest damage caused by infestation with these worms comes from a loss of animal condition and stunting of growth which results in reduced productivity.

Many complex systems of management of grazing, usually combined with anthelmintic drugs, have been evolved in different parts of the tropics as a means of controlling losses caused by the worms. The battle is constant—the worms persist. Once again, drug resistance to certain anthelmintics has developed and the quest for new compounds continues.

Only one commercial vaccine against one worm, *Dictycaulus,* exists today and it is not in use in the tropics despite its widespread use in Europe. Other

vaccines need to be developed if losses from this most insidious group of parasites is to be kept in balance. It is possible that the principle of irradiation used in the development of the above vaccine may be applicable to other helminths. It is certain, however, that immunologic and other biologic research approaches must be strongly considered as a future means of controlling losses caused by worms.

A special problem in disease control caused by a helminth is posed by *Taenia saginata* (man's tapeworm). This parasite deserves special comment as a zoonosis between man and his cattle. The eggs of the tapeworm deposited in man's feces are ingested by cattle and undergo partial development before encysting in the muscles where they remain to be eaten by man in undercooked meat. Such meat is always downgraded in price and subjected to a lengthy maintenance by costly refrigeration before being released for consumption. Treatment of man and rigorous meat inspection have not yet solved this problem in many countries.

Vectors as agents of the disease

The tsetse fly

Glossina, the tsetse fly group, presents a unique and intimidating problem in disease control to some 36 nations of tropical Africa. By transmitting a variety of pathogenic trypanosomes to cattle, which in turn produce acute and chronic disease and often death, the tsetse is the largest single impediment to cattle production in those countries. The areas inhabited by the fly are enormous.

The pioneering work of MacLennan in Nigeria, in which he eradicated the tsetse from some 65 000 square miles by the strategic use of insecticide demonstrated that tsetse control in certain areas was feasible and of economic benefit to farmers in that area.

Today similar programs are being contemplated elsewhere but the cost and human effort involved are prodigious. Nonetheless, the problem must be faced. Wherever tsetse exists, cattle disease and loss will continue. In the meantime, curative and prophylactic drugs have a part to play and selective use of insecticides can reduce the loss in some areas as can the elimination of the natural habitat of the fly. However, all too often man creates tsetse habitats such as new forests for timber and new food-producing tree plantations. The danger to man from cattle, which in some areas can act as a reservoir for the human trypanosomes, has been well-documented.

The tsetse and the disease trypanosomiasis which it transmits, are probably

the most complex biological combinations known to exist among fly and man and domesticated and wild animals, with cattle loss being a dominant factor. Vaccination seems far away, drugs are meeting with resistance and insecticide control and eradication is becoming increasingly more costly. In the meantime, distribution of the tsetse increases yearly despite all man's efforts. A crisis situation exists and must be met by a massive combined effort.

Ticks

Throughout the tropics, diseases of cattle transmitted by ticks present some special control problems. They vary greatly in the morbidity and mortality which they produce. Cattle suffering from East Coast fever in parts of Africa are often beyond effective treatment even before the disease manifestations are noted. Animals affected by anaplasmosis and babesiosis are amiable to treatment and chances for the success of treatment are better if it is initiated during the early phase of the disease.

An important part of any tick-borne disease program is vector control. This has traditionally involved the use of acaricides applied as dusts, sprays, dips or through hand application. Over the years, millions of cattle have been dipped, often twice a week, if any of the above three diseases presented a risk. The magnitude of this operation is difficult to conceive throughout the tropics, however, many herds would not exist today without the regular use of acaricides.

Unfortunately, ticks have developed resistance to arsenicals, DDT, BHC, toxaphene, dieldrin, organophosphates and carbamates. Various hypotheses have been advanced to explain the mechanism of genetic selection for insecticide resistance. Regardless of the nature of this mechanism, the problem is with us and probably will be intensified as it becomes more and more costly to develop new acaricides. It is no exaggeration to state that drug resistance of all kinds, including acaricides, has become one of the great problems of disease control facing stockowners and veterinarians of the future.

Elimination of the disease by various tick eradication programs has generally met with little success. The only true success story in this direction was elimination of babesiosis, "Texas tick fever", from the US by eradication of its tick vector, *Boophilus annulatus*. The success of this program can be attributed to two basic elements: (1) great national expenditure estimated at hundreds of millions of dollars, (2) focus on eradication of the one host tick rather than the more complicated multihost tick system.

This rather precarious situation with vector control suggests a need for more intensified research toward vaccine development. Even then, no vaccine is expected to induce an immunity which would resist all levels of tick

infestation. Accordingly, a reasonable expectation for the future will be a compromise between the use of vaccines and tick control programs.

Resistant animals as a disease deterrent

The concept of breeding for resistance to disease has dominated plant science for a long time. In the animal kingdom there is much circumstantial and some experimental evidence to show that limited resistance to certain diseases exist in cattle. Perhaps one of the best examples is the relative resistance to trypanosomiasis of the N'dama and related breeds of cattle on the West Coast of Africa. This breed of cattle continues to reproduce in areas of tsetse challenge where all other breeds die. In addition, the N'dama is markedly resistant to streptothricosis. Many other examples exist such as relative resistance of *Bos indicus* to certain tick-borne diseases and even to the tick itself.

There is little doubt that such resistance is inherited and has developed over a long period by pressure selection for survival in the constant presence of the tsetse. There is also evidence that such inherited resistance can be enhanced by repeated exposure to infection with trypanosomes.

Some geneticists have shown that inherited resistance can be intensified by selective breeding without endangering other qualities, provided one has a sufficient number of females with which to start. For many years, workers in Australia have led the field of disease-resistant animal selection, accordingly, it would be apropos to test their methods in other parts of the tropics.

Calories for cattle

No discussion on disease control is valid without considering the importance of the total intake of food by cattle, not only for its obvious necessity for growth and milk production, but also for its indirect effect on resistance to disease.

In those countries where there is ample land resources for grazing cattle, the problem is confined to the periodic availability of an abundance of food. Annual climate or excessive drought may change abundance to famine. Although the famine itself is severe enough to cause death on a massive scale, more often it produces its effect by multiplying the loss from disease. Experimental evidence exists to show that animals on a high plane of nutrition will suffer much less from helminth infections, e.g., fascioliasis.

Where calories are scarce, the art of using them at the correct time is paramount, both in relation to the time of the year and to the age of young animals, to milk production, and to work loads in draught oxen. All of these problems become much more difficult when land resource is limited and a choice has to be made between using land for food crops, which man can eat directly, and land for grazing cattle. In these circumstances, the exploitation of any land not suitable for crops must be explored to the full extent if cattle are to justify their place in the economy. Their special qualification is still their ability to eat and digest what others cannot.

Some essential elements for an effective disease control

Much has been done, much is being done, and more is required to be done. The use of this platitude can be justified in the context of the control of disease in cattle in the tropics. If the cattle industry is to prosper by efficient implementation of new research findings and by full utilization of the production skills of cattle owners and herdsmen, an adequate veterinary service must exist to control disease at both the epidemic and endemic levels.

To be effective, the veterinarian must be available to be farmer at all times just as a physician is available to his patient. He must play a full part in rural leadership and be willing to live in the villages and small towns. The distribution of veterinarians should eliminate long-distance traveling as such costs are bound to continue to increase. Constraints placed on enthusiastic young veterinarians are often associated with the problems of housing and transport. Salaries must be proportionate to the responsibility they are carrying for the industry in a particular country. The agricultural industry is often the largest producer in many tropical countries and some non-tropical ones. Thus, for veterinarian and regulatory services to be efficient, they must be well-equipped and provided with essential drugs and vaccines.

For the most part, disease control is dependent on the good will between the livestock owners and the veterinary service, but there comes a time when compulsion by law must take over in the interests of common good. Individual identification of cattle is essential if further progress is to be made in disease control. Under the circumstances, maximum utilization of epidemiologic and preventive vaccination data can be made which in turn should result in an efficient disease control program.

In many countries in the tropics, new assessments need to be made concerning the relative importance of certain diseases with a view to advising

governments on investment priorities. This requires close collaboration between the field veterinarian and the man in the laboratory. They share the same service.

Disease control offers challenge and opportunity. The rewards are great – for both man and beast.

APPENDIX

TOXIC PLANTS GROUPED BY CLINICAL SIGNS AND SYSTEMS AFFECTED

W.B. Buck and R.M. Sharma

Abstract: The effects of toxic plants on livestock can be presented in several ways: e.g., by botanical classification of the plants, by classification of the known toxic principle(s), or by classification of the clinical signs and systems affected. We have chosen the latter method since clinical manifestations are most logical to the herdsman and attending veterinarian.

The accompanying table presents many of the plants known to be toxic to cattle throughout the world. The list is undoubtedly incomplete and many of those listed may be more hazardous to other species of livestock than to cattle. The limited space in this book does not allow a complete listing and discussion of all species of toxic plants. Hopefully, however, most of the important genera are represented. When known, common names are given.

Special diagnostic aids and specific therapeutic measures recommended for certain clinical effects are presented in the last column. One should keep in mind, however, that toxic plants prevention is all important. Management procedures that prevent consumption of plants during their most toxic stage are the best treatment measures available. Before specific therapy is instituted, access to the offending plants should be removed.

Often livestock will not eat toxic plants in hazardous amounts unless forced to do so. Management practices such as overgrazing and starvation probably cause the majority of plant toxicoses in livestock. Accidental mixing of toxic plants in hay or grain or purposefully feeding hazardous forages or grain also account for a high percentage of toxicoses.

General therapeutic measures recommended for animals suffering plant toxicoses include administration of gastrointestinal demulcents or mineral oil. Tannic acid is recommended orally for precipitating many plant alkaloids, but not cocaine, nicotine or atropine. Doses of 5–25 g in 2–4 liters of water are recommended and should be followed with oil or cathartics.

Finally, in diagnosing plant toxicoses it is imperative that evidence be obtained that toxic plants have been eaten in sufficient quantities to produce toxicosis. Unless this can be done, other diseases and toxicoses should be considered in the differential diagnosis.

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
I Autonomic Nervous System					
A. Cholinergic blocker	*Atropa belladona*	Belladonna, deadly nightshade	Tropane alkaloids – atropine scopalamine hyoscyamine.	Acute course; anticholinergic signs; trembling, excitement, rapid heartbeat, weak pulse, dilated pupils, rumen atony, drymouth, incoordination, paralysis, delirium, coma.	Diagnostic aid: drop of urine in eye of laboratory animal causes mydriasis. Rx: KI or tannic acid *per os;* cardiac and respiratory stimulants; Pilocarpine, physostigmine; arecoline.
	Datura metaloides *stramonium* *suaveolens* *tatula* *innoxia*	Jimsonweed, thornapple, Jamestown weed, Apple of Peru, tolguacha			
	Hyoscyamus niger	Black henbane, henbane			
	Cestrum nocturnum *diurnum* *parqui*	Jessamine, night-and-day-blooming Green cestrum, willowleaved jessamine			Atropine-like signs plus fever, gastroenteritis, dyspnea.
B. Nicotinic effects					
1. Steroidal and	*Solanum* spp.	Nightshades	Glycoalkaloid, solanine.	Acute hemorrhagic gastroenteritis.	Pilocarpine, Physostigmine G-I

Saponic Effects	*aurantiacum*				protectants.
	carolinense	Horse nettle, bull nettle.		Acute course; weakness, drowsiness, excess salivation, dyspnea, trembling, progressive paralysis, prostration and unconsciousness.	
	dulcamera	European bittersweet, climbing nightshade			
	eleagnifolium	Silverleaf nightshade, white horse nettle, tropillo			
	gracile	Graceful nightshade		Gastrointestinal irritation, nausea, abdominal pain, vomition, constipation or diarrhea; liver degeneration and icterus (*eleagnifolium*).	
	nigrum	Black nightshade, deadly nightshade, common nightshade			
	rostratum	Buffalo bur, Kansas or Texas thistle			
	sodemeum	Apple of Sodom, popolo			
	triflorum	Three-flowered or cutleaf nightshade			
	tuberosum	Potato		Sprouts and green skin are most toxic.	
	Lycopersicon esculentum	Tomato			
	Veratrum californicum	False hellebore, corn lily, skunk cabbage	Steroidal alkaloids, e.g. veratramine, veratrosine, and numerous others.	Acute course; nausea and vomition; excess salivation, erratic slowed heartbeat, dyspnea, hypermotility of G-I tract, reduced blood pressure,	Respiratory and cardiac stimulants.

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	viride	False hellebore, white hellebore, indian poke		coma; Congenital cyclops in lambs born to ewes that consumed *V. californicum* on 13-14th day of gestation. (See XI B).	
	Ericaceae	Heath family	Resinoid, andromedotoxin (found in all genera); similar effects to *Veratrum* alkaloids.	Common signs are seen in all classes of livestock by all genera in the heath family: Acute course; anorexia, repeated swallowing or repeated eructation and swallowing of cud without mastication, copious salivation, dullness and depression. Vomition is characteristic, accompanied by bloat, straining, abdominal pain, grinding teeth, frequent defecation and increased intestinal motility. As poisoning progresses animals become weak, ataxic and prostrated. Dyspnea with rales, resulting from aspiration of vomitus are common. Death is usually preceded by coma. Postmortem examination reveals undigested rumen contents, ingesta in lungs and nonspecific gastrointestinal hemorrhages.	Laxatives, demulcents and nerve stimulants Atropine may be beneficial.
	Kalmia angustifolia	Lambkill, sheepkill, calfkill, dwarf laurel, wicky			
	latifolia	Mountain laurel, calico bush, ivy bush			
	polifolia	Pale laurel, bog laurel			
	Ledum glandulosum	Western Labrador tea			
	columbianum	Pacific Labrador tea			
	Leucothoe davisiae	Sierra laurel, black laurel			
	Menziesia ferruginea	Mock azalea, rustyleaf			

Pieris japonica	Japanese pieris		Tubular nephrosis has been reported.	
Rhododendron albiflorum	White-flowered rhododendron			
campanulatum				
cinnabarinum				
macrophyllum	California rose bay			
maximum	Rhododendron, great laurel, rose bay			
occidentale	Western azalea			
Aquaria salcifolia	(East Africa and Tanzania)			
Zygadenus spp.	Death camus	Steroidal alkaloids; glycoalkaloids and ester alkaloids, e.g. zygacine.	Acute course; excess salivation, nausea, vomition, muscle weakness, ataxia, trembling, prostration; fast, weak pulse, struggling progressing to coma.	Atropine sulfate subcutaneously; picrotoxin.
densus				
elegans				
fremontii				
gramineus				
nuttallii				
puniculatus				
venenosus				

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Amianthium muscaetoxicum	Staggergrass, fly poison, crow poison	Alkaloid (unidentified) similar to *Zygadenus* alkaloids.	Salivation, nausea, rapid or irregular respiration and weakness. Death from respiratory failure.	
	Lupinis spp.	Lupine, bluebonnet	Quinolizidine alkaloids, over 20 known, e.g. lupinine.	Acute course; heavy, labored breathing, depression, coma with snoring; other cases violent struggle, head pressing, trembling, convulsions, respiratory paralysis, weak pulse. Other species cause chronic liver degeneration and copper toxicosis (*digitatus, auqustifolius, luteus*) probably due to a mycotoxin. Three species (*laxiflorus, sericeus, caudatus*) have produced congenital arthrogryposis when consumed between days 40 and 70 of gestation. (See XI B).	
	leucophyllus	wooly-leafed lupine			
	leucopais	Big Bend lupine			
	argenteus	Silvery lupine			
	sericeus	Silky lupine			
	onustus	Plumus lupine			
	alpestris				
	caudatus	Kellogg's spurrred lupine			
	pusillus	Low lupine			
	Sophora secundiflora	Mescalbean, frijolito	Quinolizidine alkaloid.	Course 1-2 weeks; violent trembling, stiffened gait, falling down unable to rise. Progressing to somnolence. After a few minutes alertness returns and animals return to feed and eat	
	sericea	Silky sophora			
	alopecuroides				

2. Pyridine Effects	*Lobelia cardinalis*	Cardinal flower	Pyridine alkaloids; nicotine, lobeline.	Acute course; sluggishness, salivation, diarrhea, vomition, anorexia, rapid feeble pulse, reduced temperature, paralysis, collapse, coma, death.	Tannic acid orally, stimulants, atropine, has slight effect.
	siphilitica	Great lobelia; blue cardinal flower			
	berlandieri				
	inflata	Indian tobacco			
	Nicotina attenuata	Wild tobacco, coyote tobacco	Alkaloid, nicotine, anabasine?	Peracute course; shaking, localized twitching of muscles, staggering, weakness and eventual prostration; vomition, diarrhea and abdominal pain. Dyspnea; pounding heartbeat becomes rapid and weak. Possible skeletal teratogenesis. (See XI B).	
	glauca	Tree tobacco			
	trigonophylla	Wild tobacco, desert tobacco			
	tabacum	Cultivated tobacco			
	Conium maculatum	Poison hemlock, hemlock, spotted hemlock	At least 5 pyridine alkaloids; coniine, N-methyl coniine, conhydrine, 1-coniceine.	Acute course; nervousness, trembling, ataxia, recumbency; salivation, abdominal pain; slow irregular breathing. Death due to respiratory failure. If consumed by sows during first trimester of gestation produces crooked-legged pigs (arthrogryposis). (See XI B.)	Urine and breath have mousy odor.
3. Curare-like Effects	*Delphinium*	Larkspur, delphinium	Polycyclic diterpenes (delphinine).	Acute course; straddled stance, arched back, muscular collapse usually forelegs first; slightly	Physiostigmine, pilocarpine and strychnine subcutaneously.

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	barbeyi *occidentale* *glaucum* *trollifolium* *scopalorum* *menziesii* *nelsonii* *bicolor* *irrescens* *tricorne* *andersonii* *simplex*	Tall larkspurs Low larkspurs		poisoned animals rest in the sternal position with head erect; struggling attempts to rise followed by quiescence; animals may rise again but rapidly become weak and fall again; Death due to respiratory paralysis or vomition with pulmonary aspiration.	
	ajacis *cheilanthum*	Cultivated larkspurs			
	brunonianum	Common larkspurs in India		Constipation, tympany.	
	Aconitum	Monkshood, aconite	Similar to larkspur alkaloids.	Acute course; restlessness, salivation, weakness, irregular heartbeat, prostration; bloating, constant swallowing, plus other signs of larkspur poisoning.	
	napellus	cultivated aconite, monkshood, wolfbane			
	columbianum	Western monkshood			
	vaccarum				

II Neuromuscular System					
A. Those producing seizures	*Asclepias* spp.	Milkweeds	Resinoid, galitoxin.	Staggering, tetanic convulsions, bloating rapid and weak pulse, rapid breathing, coma and death. Some species cause gastrointestinal and CNS congestion and irritation.	Sedatives, laxatives.
	labriformis	Labriform			
	subverticillata	Whorled			
	eriocarpa	Whooly-pod			
	latifolia	Broadleaf			
	pumila	Low whorled			
	asperula				
	verticillata	Eastern whorled			
	mexicana	Mexican whorled			
	speciosa	Showy			
	syriaca	Common			
	incarnata	Swamp			
	curassavica	(found in Indes, Brazil, India, Queensland)			
	Cicuta	Water hemlocks; cowbane, poison parsnip, wild parsnip, snakeweed, beaver poison, muskrat weed, spotted hemlock, spotted cowbane, musquash root, false parsley, fever root, mockeel root and caratte à moreau	Resinoids, cicutoxin, cicutol (found primarily in the roots, stem base, and young leaves).	Peracute course; Salivation, muscular twitching, clamping jaws, grinding teeth; animal appears in great pain and is seized by muscle spasms and convulsions; running fits continue when animal is prostrate; coma and respiratory failure results in death.	Sedatives, artificial respiration.
	bolanderi				
	bulbifera				
	californica				
	curtissii				
	douglasii				
	maculata				
	occidentalis				
	vagans				
	virosa				

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Corydalis *caseana* *aurea* *flavula*	Fitweed, corydalis, fumatory	Isoquinoline alkaloids; aporphine, protoberberine, protopine.	Acute course; muscular twitching especially about the face; intermittent clonic convulsive seizures; hypersensitive to external stimuli, causing muscle rigidity. Chewing movements, dyspnea, recumbency with running motions, followed by weakness and quiescence before death.	
	Dicentra *cucullaria* *canadensis* *formosa* *exima* *spectabilis*	Dutchman's breeches Squirrel corn Western bleeding heart Wild bleeding heart Garden bleeding heart	Isoquinoline alkaloids as in *Corydalis*	Acute course, rarely fatal; trembling and running back and forth, head held high. Salivation, vomiting, abdominal pain and difficult breathing. Trembling becomes convulsions and opisthotonus. Recovery rapid.	
B. Incoordination and CNS excitement	*Aesculus* *hippocastanum* *pavia* *glabra* *octandra* *californica*	Horsechestnut, buckeye Red buckeye Ohio buckeye Sweet buckeye California buckeye	Glycoside, aesculin, also alkaloids and saponins.	Incoordination, muscle twitching, excitement or sluggishness, paralysis; inflammation of mucous membranes.	Stimulants and purgatives.

Notholena sinuata var. *cochlsensis distans*	Jimmy fern, cloak fern	Unknown (excreted in milk).	Primarily affects sheep; exercise causes nervous syndrome (jimmies); increased incoordination of front and hind legs finally coming to a stop with arched back; tremble violently; increased respiratory rate and heartbeat; become prostrate. If left alone most will recover in 15-20 minutes. Additional exercise brings on second attack. Will die if forced to move.	Allow rest, provide water and food so will not have to exercise.
Gelsemium sempervirens	Carolina jessamine, yellow jessamine, evening trumpetflower	Unidentified indole alkaloid related to strychnine.	Rigid extremities or muscular weakness, staggering, dilated pupils, convulsions and prostration. Death 24–48 hours later.	Relaxants and sedatives.
Atropa (See IA) *Datura* (See IA) *Hyoscyamus* (See IA) *Cestrum* (See IA) *Lupinus* (See IB1) *Sophora* (See IB1)				

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Astragalus spp. *Oxytropis* spp.	Locoweeds, poison-vetches, point locoweeds, pointvetches	Specific toxins unidentified; alkaloids have been isolated; some species accumulate organonitrates (e.g. miserotoxin, 3-nitropropanoic acid, 3-nitro-1-propanol); others are selenium accumulators.	Chronic locoism; highly excitable in some instances but usually depression and emaciation. Animals may be stary-eyed, have head shaking, low head carriage, stiff-clumsy gait, incoordinated and hyperexcitable if handled. Abortions, stillbirths and weak young are common. Lesions are characteristic widespread cytoplasmic vacuolation of neurons from the cerebral cortex to autonomic ganglia. There is also vacuolization in most visceral organs. Animals poisoned by organonitrates display peripheral limb motor paralysis and knuckling. Congenital skeletal deformities are common. (See XI B). Animals develop a craving for locoweeds.	Thiamine hydrochloride injections have been beneficial, rest and quiet, sedatives, laxatives.
	Swainsona spp. *galegifolia* *luteola* *greyana* *canesiens* *procumbens*	Darling pea	Similar to locoweeds.	Similar effects as with locoweed except congenital deformities have not been reported.	

Solanum kwebense	(Northern Transvaal, S. Africa)		Neurological disease, maldronksiekte; temporary loss of balance, transient epileptiform seizures precipitated by external stimuli; Lesions include neuropathy manifested by vacuolar degeneration and necrosis of neurons, especially purkinje cells.	
Cynoctonum capense		Alkaloid, unidentified.	Stiffness of hind legs, moves with short steps and arched back. Animal walks on toes or anterior surface of fetlock; slight external stimuli initiates convulsions. Lesions; fluid in pericardium and congestion of epicardium, meninges, lungs.	
Pseudocalymma elegans	(Brazil)		Acute course; tremors, opisthotonus, instability and decubitus with hind legs splayed backwards.	
Lolium temulentum	Darnel	Mycotoxin, tremorgen, penitrems (*Penicillia*).	Subacute course (1–4 weeks); tremoring syndrome which may be superimposed by head shaking, incoordination, abnormal, staggering gait, stumbling and collapse with severe muscular spasms. Excitement exacerbates the syndrome. After a short rest animals recover and walk off until another attack occurs.	Stimulate hepatic microsomal enzymes, effective preventive in all except claviceps staggers.
perenne	Perennial ryegrass (mold)			
Paspalum dilatatum	Dallis grass (ergot)	Mycotoxin, tremorgen (*Claviceps paspalum*).		
Cynodon dactylon	Bermuda grass			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Polygala klotzchii	(Brazil)		Acute course; animals go down but may regain feet; incoordinated, horizontal movements of head , spasms of generalized muscular contractions, dyspnea, diarrhea. Lesions include congestion of GI mucosa and CNS.	
C. Paralysis with CNS depression or excitement	*Eupatorium rugosum* (*Urticifolium ageratoides*)	Snakeroot, white snakeroot, richweed	Complex benzyl alcohol, tremetol; resin acid.	Subacute course (10–21 days); milksickness; nursing young primarily affected because toxin excreted in milk. Non-lactating animals also affected but lactating animals resistant; reluctance to move; when forced to move will abruptly stand still with feet wide apart and trembling of rear limbs and flank; trembling becomes generalized, animal becomes prostrate in natural recumbent position with head extended. Animals remain prostrate and may die in a coma; other signs include depression, weakness, vomition, labored breathing, and constipation.	Remove young and discard milk; stimulants and laxatives; milk hazardous to man.
	Haplopappus spp. *heterophyllus*	Rayless goldenrod, jimmy weed, burrow weed			

Catharanthus pusilla			CNS depression, incoordination, muscular tremors and weakness, coma death.
Cycas circinalis *revoluta* *media* *Macrozamia* *spiralis* *douglasii* *reidlei* *lucida* *Bowenia* *serraluta* *Zamia* *integrifolia*	Cycad palms Coonties, Florida arrowroot	Glycoside, maerozemin.	Chronic course; wobbles, irreversible paralysis of hind legs, ataxia, knuckling pasterns. Lesions: degenerative changes in spinal tract with characteristic eosinophilic spheroids, especially in the dorsolateral areas. Certain cycads also cause severe acute gastrointestinal disturbance, fatal within a few hours, with severe hepatopathy.
Indigofera hilaris *hololeuca* *endecaphylla*	Indigo		Reported to cause paralysis and delirium. Some may cause seizures; others abortions and reproductive problems; other hepatic and renal damage.
Aconitum spp. *Delphinium* spp.	(See B-3)		
Lobelia spp. *Nictonia* spp.	(See B-2)		
Solanum spp.	(See B-1)		

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Acacia berlandieri	Guajillo	Amine, N-methyl-B-phenylethylamine.	Chronic course; ataxia, (wobbles, limber leg) especially marked when excited; prostration; animal remains alert; death from starvation.	
	Cassia spp.	Sennas	Unknown cathartic principle;	Anorexia, abdominal pain, diarrhea; death rare. *C. occidentalis* and *tora* cause myodegeneration characterized by an afebrile course, dark red urine, incoordination, recumbency and death in 5–7 days; also diarrhea, anorexia, dehydration, lethargy, muscle tremors, altered gait. Lesions: Myodegeneration and centrilobular hepatic necrosis, renal tubular degeneration.	*Rx:* G-I protectants.
	fasciculata	Showy partridge-pea	Unclassified myodegenrative and hepatic toxin.		
	lindheimeriana	Lindheimer senna			
	occidentalis	Coffee senna			
	tora	Sicklepod, sickle senna			
	Karwinskia humboldtiania	Coyotillo	Unidentified.	The whole fruit are most toxic; paralysis but alert; beginning as weakness and incoordination of hind legs, or a dragging motion while walking; superseded by exaggerated high stepping or irregular action of hind legs, later the forelegs;	

				severely affected animals jump backwards; prostration; appetite normal but paralyzed animal dies of starvation. Feeding leaves results in wasting, nausea, progressive weakness and death. No gross or microscopic lesions.	
III Gastrointestinal Irritation					
A. Toxalbumins (phytotoxins)	*Abrus precatorius*	Precatory bean, crabs-eye, rosary pea; jequirity bean	Phytotoxin, abrin, an antigenic protein; nitrogenous cpd., abrine; glucoside, abraline.	Seeds most toxic; Very potent; Acute course; Seeds must be masticated to produce toxicosis; Severe G-I irritation; ruminants more resistant than other species; parenteral administration highly lethal. Has been used to coat spikes for malicious poisoning; salivation, edema at site of injury and loss of hair at the site of injury. Agglutination of RBC.	Antiserum to abrin; arecoline hydrobromide.

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Ricinus communis	Castor bean, palma christi	Phytotoxin, ricin.	Ricin is among most toxic cpds. known. Not readily absorbed via intestinal mucosa. Cattle require about 0.2% of B.W. for poisoning by castor beans. Castor bean meal or cake mixed with other feed common source. Signs include hemorrhagic gastroenteritis; excessive eructation, salivation, diarrhea, swaying gait, tremors and death or recovery within a few hours.	Diagnosis: RBC agglutination; precipitin test for ricin. Rx: specific antiserum; strychnine and lobeline subcutaneously.
	Robina pseudoacacia	Black locust	Phytotoxin, robin; glycoside, robitin.	Anorexia, lassitude, posterior weakness, nausea, dilated pupils, irregular weak pulse, dyspnea, diarrhea (bloody). Lesions include irritation and edema of G-I nucosa.	
B. Saponins	*Agrostemma githago*	corn cockle	Saponins plus oils (glycosides).	Acute course; profuse, watery, sanguinous, diarrhea; inappetence; rumen atony; dullness, general weakness, emaciation; hemoglobinuria; emaciation and death. Lesions include gastroenteritis and congestion of abdominal organs; dark, tarry blood.	*Rx:* Oils and digestive tract protectants; dilute acetic acid orally to neutralize toxins; stimulants; blood transfusion.
	Aleurites fordii montana trisperma	Tung tree			
	Fagus sylvatica	Beech			
	Linium neomexicanum	Yellow pine flax			

	Medicago sativa	Alfalfa			
	Phytolacca americana *dodecandra*	Pokeweed			
	Saponaria officinalis *vaccaria*	Bouncing bet, soapwort, cow cockle			
	Sesbania spp. *vesicara* *drummondii* *punicea*	Coffeeweed, Coffeebean, rattlebox, sesbane, poison bean, bagpod, rattlebrush			
C. **Irritating resins and oils (uncertain chemistry)** (See also XII)	*Armoracia lapathifolia* *cernua* *campestris*	Horeradish	Mustard oil irritants (isothiocyanates).	Acute course; irritant oils produce reddening, vesicle formation and desquamation upon contact; severe gastroenteritis; hemorrhagic gastroenteritis; weakness, inappetence and paralysis (sometimes excitement); salivation and diarrhea; hemoglobinemia (uria) seen with *Raphanus, Thlapsi, B. oleracea* var. *gemmifera* (brussels sprouts). May also be hepatic damage. *B. Napus* produces several syndromes: (1) respiratory, emphysema and edema; (2) digestive, paresis of G-I tract, tympany, black ingesta, hepatic and renal damage; (3) nervous, blindness and pushing against objects,	*Rx:* Protectants such as mineral oil.
	Brassica hirta *integrifolia* *juncea* *kaber* *nigra* *napus*	White mustard Indian mustard Charlock, wild mustard Black mustard Rape			
	Barbarea vulgaris	Yellow rocket, winter-cress			
	Cardaria draba	White top, hoary cress			
	Erysimum cheiranthoides	Wormseed mustard			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects* •	*Diagnostic, prevention and therapeutic measures*
	Raphanus raphanistrum	Wild radish		aggression followed by exhaustion; (4) Hemoglobinuria, anemia, icterus due to hemolysis; (5) Uncontrolled hemorrhage; photosensitization and/or nitrate poisoning (methemoglobinemia); (6) Goiter (also occurs with *B. oleracea* (kale, cabbage) *B. rapa.*	
	Thlaspi arvense	Fanweed, field penny-cress			
	Actaea spp.	Baneberry, dolls-eyes	Glycoside protoanemonin, ranunculin (aglycone).	Gastrointestinal irritation; salivation, diarrhea, signs of abdominal pain; blood stained urine and feces; depression or excitement; Lesions: fiery-red G-I mucosa. Dermal exposure results in vesicle and ulcer formation on skin. (See XII).	*RX:* Protectants and supportive measures. Dilute $KMnO_4$ as skin protectant.
	Anemone spp. *patens*	Windflower, anemone Pasque flower	(anemonin) An irritant volatile oil.		
	Caltha palustris	Marsh marigold, cowslip			
	Ranunculus spp. *acris*	Buttercups Tall field buttercup			
	bulbosus	Bulbous buttercup			
	parviflorus abortivus	Small-flowered buttercup			

repens	Creeping buttercup		
ficaria	Lesser celandine		
sceleratus	Cursed crowfoot		
flammula	Spearwort		
Canavalia ensiformis	Jack bean		Stiffness of hindquarters, diarrhea, loss of condition and debility; lesions include emphysema, hemorrhagic lymph nodes and G-I inflammation.
Calotropis procera	(India, Africa, tropical Asia)	Milky juice, calotoxin, calactin, calotropin, uscharidin, uscharin.	Inflammation of gastrointestinal tract or skin, depending upon exposure; dyspnea, ataxia.
gigantea			
Tanaecium exitosium	(Colombia, S.A.)		Debility, staggering gait, frequent urination, collapse; lesions include hemorrhagic gastritis and cardiac inflammation with petechiation of organs.
Cascuta spp.	Dodder		Digestive upsets; diarrhea
Ipomea fistulosa	Morning glory		Purgation; wasting, depression and other ill-defined pathology after prolonged ingestion.
Convolvulus spp.	Morning glory, bindweed		

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Helleborus niger (See V A)	Christmas rose	Glycoside, irritant and purgative action (unidentified).	Acute course; vomiting severe diarrhea, abdominal distress; secondary nervous effects including convulsions and collapse; digitalis-like effects on the heart. Lesions include G-I inflammation.	*Rx:* demulcents, and cardiac stimulants.
	Mansonia altissima (See XII)	African redwood	Unidentified.	When sawdust used as litter animals develop ulcers on mouth and skin; anorexia, diarrhea, listlessness and death.	
	Lasiosiphon kraussianus	(Nigeria)	Unidentified.	Severe gastroenteritis; hemorrhages, congestion and edema in the brain, liver and myocardium; death.	
	Cassia fistula (See IIC)	Tropical Senna		Cathartic effects.	
	Pimelea trichostachya continua altior	(Australia)	Unidentified, water insoluble, thick, dark, gum-like material (simplexin).	Diarrhea, weakness, anemia due to vascular dilation.	
	Bryonia dioica		Glucosides, bryonin, bryoninidin, bryonetin, bryonol.	Gastroenteritis and diarrhea.	

Cucumis myriocarous		Resinous unidentified material (cucurbitacin).	Caused toxicity in cattle in Australia and Rhodesia.	
Euphorbia spp.	Spurges	Milky irritant sap	Skin blistering on contact with sap; G-I irritation and purgation; some species cause photosensitization; none of the spurges are relished by livestock; dried plants are non-toxic.	*Rx:* G-I protectants and laxatives; skin ointments.
corollata	Flowering spurge	Some species contain cyanogenetic potential.		
cyparissias	Cypress spurge			
esula	Leafy spurge			
helioscopia	Sun spurge			
lathyris	Caper spurge			
maculata	Eyebane, spotted spurge			
marginata	Snow-on-the-mountain			
peplus	Petty spurge			
prostrata				
Croton texensis	Hogwort, croton	Unidentified, acrid, irritant; croton oil.	Intense gastroenteritis	*Rx:* G-I protectants.
capitatus				
tiglium				
Xanthium	Cocklebur	Carboxyatractolicide	Seeds and young plants most toxic; anorexia, depression, nausea, vomition, weak heartbeat, muscular weakness, prostration, dyspnea; opisthotonus, spasmodic running motions and convulsions; Lesions: gastointestinal inflammation, acute hepatitis and nephritis.	*Rx:*, Oral oils and lard (fat). warmth, stimulants, physostigmine given IM.
strumarium	Spiny cocklebur			
spinosum				
Allium cepa	Cultivated onion	Unidentified.	Gastrointestinal inflammation, constipation or diarrhea, vomition, coffee-colored urine. Onion flavor in milk.	
canadense	Wild onion			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
D. Oxalate needle crystals	*Colocasia esculenta antiquorum*	Elephantsear (edible tuber in India)	Acrid juice; oxalate crystal needles.	Subacute course; swollen lips, face, and tongue; drooling saliva; erosions on tongue and buccal mucous membranes; animals are dull and unsteady and urine may be colored.	*Rx:* Intravenous glucose, electrolytes and calcium borogluconate; local treatment of ulcers.
	Arisaema consanquineum triphyllum	Snake cob jack-in-the-pulpit, Indian turnip		All plants cause similar signs due to oral irritation, but death is rarely produced.	
	atrorubens stewardsonii				
	Calla palustris	Wild calla			
	Symplocarpus foetidus	Skunk cabbage			
	Dieffenbachia seguine	Dumbcane			
	Alocasia spp.				
	Caladium spp.				
	Xanthosoma spp.				
E. Alkaloids	*Colchicum autumale luteum*	Autumn crocus	Alkaloid, colchicine.	Alkaloid is cumulative and excreted in the milk; signs include colic, severe	

				diarrhea, foul-smelling, dark feces, collapse and death from respiratory failure.	
	Gloriosa simplex superba	Glory lily Climbing lily	Colchicine.		
IV Respiratory System					
A. Cyanogenetic glycocides	*Acasia greggii*	Catclaw	Glycoside yielding cyanide, e.g. amygdalin.	Cyanide may be released during wilting process or may be hydrolyzed in the rumen. Signs are usually peracute; asphyxiation at cellular level. Death in 15 minutes; mucous membranes bright pink, blood bright red. Excitement is rapidly followed by depression, dyspnea, incoordination and convulsions followed by prostration and death.	*Rx:* Sodium nitrate (20%); sodium thiosulfate (20%) IV in a 1:3 ratio given at rate of 4 ml/100 lbs.b.w. methylene blue, 2-3 g/500 lbs. b.w.
	Bahia oppositifolia	Bahia			
	Cercogarpus spp.	Mountain mahogany			
	Cynodon spp.	Bermuda grass			
	Florestina tripteris	Florestina			
	Glyceria striata	Fowl mannagrass			
	Holcus lanatus	Velvet grass			
	Hydrangea spp.	Hydrangea			
	Kalanchoe integra				
	Linum spp.	Flax			
	Lotus spp.	Birdsfoot trefoil			
	Manihot esculenta utilissinia	Cassava			
	Papauer nudicale	Poppy			
	Pennisetum spp.	Pearl-millet			
	Phaseolus lunatus	Lima bean, Java bean			
	Poa spp.	Bluegrasses			
	Prunus spp.	Cherry, plum, peach, etc.			
	Pyrus malus	Apple			
	Sorghum spp.	Sudan grass, Johnsongrass, etc.			
	Stillingia treculeana	Queen's delight			
	Stipa spp.	Grasses			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Suckleya suckleyana	Poison-suckleye			
	Trifolium repens	White clover			
	Triglochin spp.	Arrowgrass			
	Vicia sativa	Vetch seed			
	Zea mays	Maize, corn			
B. Nitrite, nitrate	Weeds –				
	Amaranthus spp.	Pigweeds	Nitrate (NO_3) reduced in the rumen to nitrite (NO_2) Some plants are both cyanogenetic and nitrate accumulators.	NO_2 forms methemoglobin (MHb) causing acute respiratory embarrassment at 40–70% MHb. Onset of signs usually occur suddenly after several days exposure to nitrate containing forages: cyanosis, dyspnea, trembling and muscular weakness followed by collapse and death. The blood is characteristically chocolate brown colored and is best observed by placing a drop on white filter paper.	*Diagnosis:* MHb filter paper test for chocolate colored blood or chemical test. Diphenylamine test for nitrate in plant material and aqueous humor of eye. *Rx:* Methylene blue, 2–4% solution, IV at a rate of 4–5 mg/kg. Orally give saline cathartic, antibiotics and 3–5 gallons of cold water.
	Astragalus spp. (See IIB)	Locoweeds			
	Chenopodium spp.	Lamb's quarters			
	Cirsium arvense	Canadian thistle			
	Kochia scoparia	Fireball			
	Malva parviflora	Cheeseweed			
	Panicum capillare	Witchgrass			
	Polygonum spp.	Smartweeds			
	Rumex spp.	Dock			
	Solanum spp.	Nightshades			
	Sorghum spp.	Sudan grass, Johnsongrass			
	Tribulus terrestris	Puncture vine			
	Crops –				
	Avena sativa	Oat hay		Abortion may occur after an acute episode of nitrate poisoning.	Vitamin A supplements may be beneficial.
	Beta vulgaris	Beet, mangold			
	Brassica spp.	Rape, broccoli, kale, turnip, etc.			
	Hordeum vulgare	Barley		Chronic signs of nitrate poisoning are less definite but may include poor weight	
	Linum spp.	Flax			
	Medicago sativa	Alfalfa			

	Secale cereale *Triticum aestivum* *Zea mays*	Rye Wheat Corn		gains, decreased milk production, night blindness and other signs of vitamin A deficiency.	
V Cardiotoxic Effects					
A. Digitalis-like effects	*Digitalis purpurea*	Foxglove, digitalis	Glycoside digitoxin.	Acute course; gastric distress, bloody stools, drowsiness, loss of appetite, frequent urination, gross disturbance in heartbeat and pulse, death. Lesions include distended heart auricles and G-I inflammation.	Propranolol (Inderal) has been beneficial in dogs; also dilantin.
	Convallaria majalis	Lily-of-the-valley	Glycoside, convallatoxin.	Similar effects as with *Digitalis;* also purgation.	
	Nearium oleander indicum	Oleander	Glycosides, olendroside and nerioside.	Acute course, highly toxic; severe gastroenteritis; increased pulse rate, cold extremities, mydriasis, sweating, abdominal pain, nausea, vomition, weakness, bloody feces, death. Lesions of G-I inflammation and petechiation of various organs.	
	Thevetia peruviana	Yellow oleander, be-still tree	Glycoside, thevetin.		
	Helleborus niger (See III C)	Christmas rose			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Homeria *bregniana* *miniata*		Glycoside, digitalis-like.	G-I inflammation; nervous prostration and collapse; inappetence, stiffened rear limbs, colic, dysentery, depression, death.	
	Baccharis cordifolia *pteronioides* *glomeruliflora* *halimifolia*	Yerba-de-pasmo	Unidentified cardioactive glycoside.	Acute course; anorexia, bloat, swaying gait, trembling, restlessness, polypnea, tachycardia, death.	
	Acokanthera schimper	African arrow poison	Cardioactive glycoside, ouabain.	Anorexia, abdominal pain, salivation, purgation, irregular heartbeat, progressive debility, collapse, and death.	
B. Cardiac blockage	*Taxus* spp. *baccata* *cuspidata* *brevifolice* *canadensis*	Yew, ground hemlock English yew Japanese yew Western yew Ground hemlock	Alkaloid, taxine.	Acute course; highly toxic; green foliage toxic, also berries; sudden death without other signs; others become depressed, lay down; heartbeat becomes slow and stops in diastole; staggering, recumbency, death without struggle.	Atropine.
	Dichapetalum toxicarium	Gifblaar	Fluoroacetate.	Acute signs of cardiac blockage similar to that of *Taxus*.	
	Acacia georginae	Gidvea	Fluoroacetate.		

Bersama abyssinica		Cardiotoxic bufodiennolides.	Progressive paralysis; subnormal body temperature; weak, irregular heartbeat; tremors, nystagmus, blinking of eyelids; convulsions prior to death.
VI Vasoconstriction			
Claviceps purpurea on various grasses;	Ergot	Alkaloids, Ergotamine and others.	Subacute to chronic effects; ergot sclerotia replaced grain which contaminate feed. Dry gangrene of extremities; swelling and tenderness followed by loss of sensation of affected part. Sloughing of end of tail, tips of ears, teats, and distal phalanges; Abortions; lack of udder development and milk production.
Secale cereale	Rye		
Lolium perenne tumulentum	Perennial wild ryegrass		
Poa pratensis	Kentucky bluegrass		
Agrostis alba	Redtop		
Elymus canadensis	Canada wild ryegrass		
Festuca arundinacea elatior	Tall fescue	Ergot-like alkaloid.	Shivering continuously for several days followed by lameness and swelling of feet; necrosis and dry gangrene occurs after 2–3 weeks; the tail may slough and rough haircoat; agalactia.
	var. *arundinaecea*		
Melica decumbens			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
VII Blood Forming Organs					
A. Coagulation defects	*Melilotus alba* *indica* *officinalis* *Lespedeza stipulacea*	White sweet clover Pale-yellow sweet clover Yellow sweet clover Lespedeza	Coumarin, which is broken down to dicoumarol if hay is improperly cured or moldy.	Hemorrhagic disease, subacute course; internal and subcutaneous hemorrhaging; Prothrombin time increased to 30-90 seconds; factors VII IX and X are inhibited; subcutaneous swelling on ventral part of body; blanched mucous membranes, weakness, death without struggle; normal body temperature. Lesions include generalized frank hemorrhaging.	Vitamin K_1 intravenously; oral menadione; blood transfusion; quiet rest.
B. Bone marrow destruction	*Pteridium aquilinum*	Bracken fern	Radiomimetic * substance, unidentified; Thiaminase (toxic to monogastric animals).	Subacute course; anemia, thrombocytopenia, leukopenia due to bone marrow destruction; generalized hemorrhages at oral, anal and vulval openings, epistaxis and bloody feces; anorexia, ruminal stasis; fever due to septicemia.	Large doses at broad spectrum antibiotic.

* W. T. R. Grimshaw, Department of Veterinary Medicine University Glasgow Veterinary School, Bearsden, Glasgow, GB1 1QH, Personal Communication.

	Cheilanthes sieberi	Mulga or rock fern		Chronic course; enzootic hematuria due to hyperplasia and neoplasia of urinary bladder; animals eventually lose condition, reduced milk production; frequent urination, anemia.
				Chronic course; carcinoma of upper alimentary tract; drooling, coughing, snoring, halitosis, necrotic ulcers in mouth, esophagus, or intestines; difficult swallowing, tympany, diarrhea and wasting.
	Glycine max	Soybean meal	Trichloroethylene extracted soybean oil meal (TCESOM).	Subacute hemorrhaging due to bone marrow destruction; signs and lesions similar to bracken poisoning.
VIII Hepatic Damage				
A. Pyrrolizidine alkaloids (fibrosis)	*Amsinckia intermedia*	Tarweed, fiddleneck	Unidentified pyrrolizidine alkaloid	Chronic course; hepatic necrosis, fibrosis, cirrhosis associated with hepatic and renal megalocytosis.
	Crotalaria sagittalis spectabilis burkena	Rattlebox, crotalaria	Monocrotaline, other unidentified substances.	Progressive loss in condition; icterus; Usually a latent period weeks to months; cumulative and progressive; animal stands alone, depressed, anorexia; skin emits a sweetish odor; weakness, uneasiness; signs
		(associated with 'styfsiekte', 'stijfziekte' or 'stiffsickness' in	Pyrrolizidine alkaloid.	

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Echium plantagineum	S. Africa) Viper's bugloss		of abdominal pain, emaciation, reduced sensibility; diarrhea, dark stained feces; aimless walking, leaning against fences, walking through fences; rough hair coat; ascites; hemorrhages.	
	Heliotropium europeum	Heliotrope	Heliotrine, lassiocarpine.	Lesions: hepatic necrosis, fibrosis and cirrhosis; liver normal sized but yellow and firm; distended gallbladder, bile duct proliferation, parenchymal stenosis, veno-occlusive disease; renal megalocytosis and fibrosis; interstitial pneumonia in some instances.	
	Senecio spp. *integerrimus* *jacobaea* *longilobus* *plattensis* *ridellii*	Groundsel, senecio Stinking willie Tanzy ragwort Wooly groundsel, thread-leaf groundsel Riddell's groundsel	Pyrrolizidine alkaloids; e.g. retrorsine, senecionine, senecine, jacobine; volatile oils and nitrogen oxides.	A condition called 'molten cattle sickness' or 'straining disease' has been attributed to senecio; after a latent period cattle develop a pernicious diarrhea, straining, prolapsed rectum; frenzy, aggression, coma and death result.	

	spartioides	Broom groundsel			
	vulgaris	Common groundsel			
	glabellus	Bitterweed			
	furchellii				
	ilicifoluis				
	erraticus				
	latifolius				
B. Hepatic necrosis	*Lotus corniculatus*	Birdsfoot trefoil,	Unknown.	Sheep primarily affected; Acute course; animals found dead; other depressed, anorectic, recumbent, tachycardia and terminal dyspnea, normal temperature; Photosensitization in 20% of cases. Lesions: Massive hepatic necrosis; pale and dark areas interspersed throughout; hemorrhages on other organs.	
	tenuis	deervetch			
	Helenium hoopesii	Sneezeweed, orange sneezeweed	Sesquiterpene lactones, helenalin, hymenoxin.	Sheep primarily affected, but other species also; subacute course; 'spewing sickness'; depression, weakness, trembling, rapid irregular pulse and respiration; vomition very prominant, associated with slobbering, belching and borborygmi. Lesions: Extremely friable, necrotic liver, G-I inflammation, foreign-body pneumonia; renal degeneration.	*Rx:* Dietary supplement of sodium sulfate (340 mg/kg b.w.) plus high protein before and during exposure.
	autumnale	Bitterweed			
	nudiflorum				
	tenuifoluim				
	microcephalum				
	Hymenoxys odorata	Bitterweed,			
	richardsonii	pingue, rubberweed			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Psilostrophe spp. *Baileya multiradiata* *Viguiera* spp. *Geigeria* spp.	Paper flower Desert baileya Golden eye Vomiting bush		The sesquiterpene lactones probably have antimicrobial action in the rumen and affect vital metabolic function.	
	Gossypium spp. *barbadense*	Cottonseed	Polyphenolic binaphthalene, goossypol pigment.	Swine and other species affected; Progressive weakness, emaciation, dyspnea; widespread congestion, edema; hepatic necrosis and hemorrhage.	*Rx:* ferrous sulfate in diet 1 part to 4 parts of gossypol.
	Vernonia mollissimma	(Brazil)	Unidentified.	Anorexia, constipation, tremors, labored breathing, lateral recumbency; Lesions: nutmeg liver and congestion, hemorrhage of intestines.	
	Encephalartos hildebrandtii			Necrotic hepatic degenerative changes in heart and kidneys of calves.	
	Isofropis spp.			Debility, listlessness, staggering, collapse; hepatic and renal necrosis.	
	Cycas circinalis (See IIC)	Cycads	Glycosides, maerozemin and cycasin.	Acute G-I disturbance, death with severe liver degeneration.	
C. Photosensitization	*Tetrademia canescens*	Spineless horsebrush	Furanoeremophilanes, tetrady-	Animals must be consuming black sage brush (*Artemisia*	Keep animals out of sunlight;

associated with liver degeneration	*glabrata*	Littleleaf horsebrush, spring rabbitbrush, coal oil brush	mol, others.	*nova*) before they are sensitive to the effects of this plant. Photosensitization; big head; dermal erythema, loss of hair and wool, skin ulceration, secondary infection; blindness; Lesions: dermal necrosis and edema; liver enlarged and engorged, degenerative changes; renal degeneration.	antihistamines, skin ointments containing antibiotics; corticosteroids parenterally.
	Lantana camera *aculeata* *sellowiana* *Lippia rehmanni*	Lantana S. American whitebrush	Tripterpenes, lantadene A and B Rehmannic acid.	Acute-subacute course; Photosensitization, jaundice and anorexia; lesions include chloestatic injury to liver, distended gallbladder; renal tubular necrosis. Death due to liver insufficiency, renal failure and myocardial damage.	
	Myoporum deserti *acuminatum* *tetrandum* *laetum* *crassifolium*	Ellangowan poison bush Boobilla Ngaio	Furanosequiterpenoids, myoporone, dehydrongaione, ngaione, dehydroepingaione.	Acute course; photosensitization, jaundice, pale and reddish mottled liver, edema of gallbladder, excessive clear serous fluid in all body cavities, free blood in large and small intestines, intestinal stasis and dehydration; pulmonary edema and hydrothorax. Microscopically, centrilobular, midzonal and periportal hemorrhagic hepatic necrosis with fatty change; fatty infiltration of renal tubules and myocardium.	

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Agave lecheguilla	Lechuguilla	Unidentified hepatotoxin; Saponins, smilagenin.	Subacute course; icterus, yellowish discharge from eyes and nostrils; dark urine; photosensitization; hemorrhagic gastroenteritis; Lesions: hepatic and renal degeneration; G-I inflammation.	
	Nolina texana *microcarpa*	Sacahuista, sacahuiste, bear grass	Unidentified hepatotoxin.	Similar to *Lechugilla* poisoning; commonly seen in cattle.	
	Tribulus terrestris	Puncture vine, caltrop	Unidentified steroidal saponius.	Subacute course; photosensitization and hepatic damage; blindness, necrosis of skin, lips, ears, highest mortality in young.	
	Fagopyrum esculentum *sagittatum*	Buckwheat	Napthrodianthrone fagopyrin.	Primary photosensitization due to circulation of photodynamic agent fagopyrin; also hepatic degeneration; erythema, necrosis of white skin; subdermal edema; nervous signs, hyperexcitable, running, grunting, bellowing, jumping, convulsions, prostration.	
	Polygonum spp.	Smartweeds			
	Brassica spp. (See IIIC)				

	Panicum spp.	Panic-grasses	Unidentified.	Hepatogenic photosensitization in Australia, S. Africa, S. America.	
D. Photo-sensitization without major hepatic necrosis	*Hypericum perforatum*	St. Johnswort Klamath weed, goatweed	Photodynamic pigment, hypericin	Subacute course; classical photosensitization of white-skinned animals; pruritis and erythema of skin followed by edema and necrosis; blindness; convulsions, increased heartbeat and respiration, increased body temperature, diarrhea and hypersensitivity to cold water contact to the skin. Undesirable flavor in milk.	Keep animals out of sunlight, graze during darkness; corticosteroids given parenterally; Dermal antibacterial ointments.
	Trifolium rapens hybridum pratense	White clover Alsike clover Red clover	Cyanogenetic glycoside, lotaustralin; enzyme, linamarase.	Photosensitization and stomatitis when fed in large quantities under certain conditions. May also produce goiter. Colic, diarrhea and other signs of digestive upset. Moldy red clover may cause excessive salivation, bloating, stiff gait, emaciation and abortion due to mycotoxin, slaframine.	
	Medicago sativa	Lucerne, alfalfa			
	Vicia spp.	Vetches, fava bean			
	Cymopterus watsoni longipes	Spring parsley	Furocoumarins, xanthotoxin and bergapten.	Subacute course; severe photosensitization, blistering and peeling of unpigmented areas of the skin; swollen	

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Ammi majus	Bishops weed	Psoralens, other unidentified furocoumarins.	udder, teats and vulva; reduced milk production; keratoconjunctivitis, cloudy cornea, blindness.	
	Heterophylla pustalata	Cegadera	Unidentified.	Photosensitization and blindness.	
	Euphorbia spp. (See III C)	Spurges			
IX Kidney Damage					
A. Nephrotoxins	*Quercus* spp. havardii gambelii breviloba durandii marilandica velutina robur robra prinus stella coccinea others	Oaks Shinnery Gambel's oak Jack oak Yellow-barked oak European oak Red oak Chestnut oak Post oak Scarlet oak	Gallotannin.	Subacute course; buds or young leaves and acorns may be toxic under certain environmental conditions, e.g. swollen or sprouting acorns. Anorexia, rumen stasis, constipation later changing to dark tarry diarrhea, rough hair coat, dry muzzle, dehydration, abdominal pain, excessive thirst, frequent urination; edematous swelling in lower parts of body; rapid, weak pulse, brownish nasal discharge, death. Lesions: Perirenal edema, edema and fluid in retroperitoneal area;	*Diagnosis:* Elevated BUN is an aid. Oral ruminatorics and oils; parenteral fluids prevent with 10–15% calcium hydroxide in protein supplement.

				nephrosis, gastroenteritis; straw-colored fluid in peritoneal and pleural cavities; renal tubular coagulation necrosis and proteinaceous casts in the proximal and ascending tubules.	
	Amaranthus retroflexus (possibly others)	Redwood, pigweed, rough pigweed	Unidentified (plant contains oxalic acid and accumulates NO_3^-).	Subacute course; swine and cattle susceptible; animals that have not previously been on pasture are most susceptible. Signs begin about 7 days after beginning exposure; weakness, knuckling of pasterns, terminating in almost complete paralysis of rear limbs, coma. Death due to hyperkalemic effects on the heart. Lesions: severe blood-trinted or clear perirenal edema, nephrosis and fluid in body cavities. Necrosis and dilation of glomeruli and tubules; oxalate crystals not prominant.	Manage hyperkalemia; mineralcorticoid hormones; 10% calcium gluconate IV to control cardiac irregularities.
B. Oxalate nephrosis	*Halogeton glomeratus*	Halogeton 'barilla'	Oxalic acid, oxalate.	Acute course; rapid, labored respiration, depression, weakness, coma, death. Cattle may become stiff after walking a short distance, then become weak and die. A hypocalcemia develops, accompanied by hyperkalemia, increased phosphorus, magnesium,	Increase water consumption; lime water to precipitate oxalate; IV calcium soln. not very effective; prevent by feeding dicalcium phosphate in salt (3:1 salt: dical) or 5% in
	Oxalis pes-caprae corniculata	Soursorb, Bermuda buttercup, sorrel			
	Bassia hyssopifolia	Bassia			
	Sarcobatus vermiculatus	Black greasewood, chico			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Rumex rhaponticum	Rhubarb		sodium and BUN; atony of G-I tract; some animals develop tetany and incoordination. Lesions: hemorrhages and edema of rumen wall, ascites, swollen kidneys; microscopically, hemorrhages and oxalate crystals in rumen wall, renal tubules; renal tubular necrosis and dilations are predominant lesions.	alfalfa pellets.
	acetosa				
	acetosella	Dock			
	crispis	sorrel			
	Kochia scoparia	Kochia, summer cypress, burning bush, Mexican fireweed			
	Setaria sphacelata	(Australian grass)			
X Pulmonary Edema and/or Emphysema					
	Perilla frutescens	Perilla mint	3-Substituted furans, perilla ketone, egoma-ketone, iso-egomaketone	Onset 2-10 days after placing animals on offending feed. Increase respiratory rate progressing to dyspnea and open-mouth breathing; audible expiratory grunt; normal or slightly elevated temperature; stand with head lowered reluctant to move; exertion may cause rapid death. Lesions: lung fail to collapse, heavy and fluid laden; interstitial emphysema, bubbles, clefts and irregular spaces on lungs are prominant; thick,	
	Ipomoea batatas (Infected with fungi, *Fusarium solani* or *Ceratocystitis fimbriata*)	Sweet potatoes	Ipomeanol, ipomeamarone		
	Myoporum laetum	Ngaio tree	Ngaione		
	deserti	Elangowan bush			

	Zea maize (other green forages and lush pastures)	Maize, corn Indian corn	Tryptophan (These compounds apparently are metabolized to 3-methylindole).	gelatinous fluid throughout lung lobules and interstitial spaces; frothy fluid in bronchial tree; edema and emphysema of bronchial and mediastinal lymph nodes and adjacent body tissues. These compounds are also hepatoxic as well as lung-toxic. (See VIII C).	
	Galega sp.	Professor weed			
	Sphenociatum capitulatum	White top			
	Zieria aborescens		Unidentified.	Massive pulmonary edema and emphysema.	
XI Abortion and Reproductive Problems					
A. Abortions and infertility	*Guterrezia microcephala*	Broomweed, perennial snakeweed, slinkweed, turpentine weed	Saponin.	Cattle grazing on this plant on sandy soil experience abortions, breeding difficulty and even death; some calves born are very small; other signs include listlessness, anorexia, rough coat, diarrhea or constipation, hematuria, vaginal swelling and discharge; liver and kidney degeneration, gastroenteritis may be present.	Supplement grain or hay diet.
	Pinus ponderosa	Yellow ponderosa pine	Pine needles, unidentified principle.	Abortions in last trimester of gestation most common; after 2 weeks of consuming pine needles; other signs	
	taeda	Loblolly pine			

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Cypressus macrocarpa	Monterey cypress, macrocarpa	Unknown.	include edema of vulva and udder, retained placenta, bloody discharge from vulva. Loblolly pine needles have produced death in cattle.	
	Juniperus virginiana	Juniper			
	Trifolium subterraneum	Subclover, subterranean clover	Estrogen, genistein.	Other signs include changes in sex organs, abnormal lactation, infertility, dystocia and prolapse of the uterus; sheep affected more than cattle.	
	Claviceps purpurea (parasitized on numerous grasses, see VI)	Ergot	Alkaloids, ergocryptine, ergocornine, ergocristine.	Abortions may seldom occur but weak, debilitated or dead fetuses are common; reduced milk production.	
B. Fetal death and/or teratogenesis	*Lupinus sericeus caudatus*	Silky lupine	Quinolizidine alkaloid, anagyrine.	Cows consuming plants during days 40–70 of gestation may produce crooked-legged calves (arthrogryposis) and spinal curvature, cleft palate. Greatest hazard when plant is young, least when in late flowering or seed stage.	Avoid grazing animals on plants during susceptible period of gestation.
	laxiflorus (See IB 1)	Kellog's spurred lupine Douglas spurred lupine			
	Conium maculatum (See I B2)	Poison hemlock, spotted hemlock	Piperidine alkaloid, coniine, γ-coniceine.	*Conium* produces similar teratogenic effects as *Lupinus*.	

	Nicotiana tabacum *(See I B2)*	Tobacco	Anabasine	Tobacco roots have caused arthrogryposis in piglets from sows consuming them during gestation.	
	Veratrun californicum (See IB 1)	False hellebore, corn lily, skunk cabbage	Steroidal alkaloid, jervine, cyclopamine, cycloposine.	Congenital cyclops (monkey-faced) lambs produced by ewes fed plants on 14th day of gestation.	
	Astragalus spp. *Oxytropis* spp. (See II B)	Locoweeds	Unidentified.	Birth defects and abortions may occur after exposure during all stages of gestation; reduced fertility in rams; reduced birth size of lambs.	
XII Dermal Inflammation (not photo-sensitization) (See also III C)					
	Calotropis procera gigantea		Milky juice Calactin, calotoxin, calotropin, uscharidin, uscharin.	Causes inflammation of skin or other tissues upon contact.	Dermal ointments, antibiotics.
	Euphorbia spp. *ingens*	Spurges	Unidentified acrid principle.	Irritation and blistering of skin upon contact.	

Primary system affected	*Scientific name*	*Common names*	*Major toxic principle(s)*	*Predominant clinical effects*	*Diagnostic, prevention and therapeutic measures*
	Toxicodendron radicans	Poison ivy poson vine markweed	Resin, penta-decylcatechol.	Dermatitis; reddened, blisters, itchy skin; exudation and secondary infection; not all individuals are susceptible.	
	diversilobum	Western poison oak			
	guercifolium	Eastern poison oak			
	vernix	Poison sumac, poison elder			

XIII Plants Producing Unpleasant Odor in Milk

Achillea millefolium – Yarrow
Alliaria officinalis – Hedge mustard
Allium spp. – Wild garlic and wild onions
Ambrosia spp. – Ragweeds
Anthemis spp. – Chamomile, dog fennel
Artemisia spp. – Wormwood, sagebrush
Brassica spp. – Mustards, turnips
Caltha palustris – Marsh marigold *
Camelina microcarpa – False-flax
Chrysanthemum leucanthemum – Ox-eye daisy
Cichorium intybus – Chicory
Conium maculatum – Poison-hemlock *
Daucus carota – Wild carrot
Equisetum spp. – Horsetail ferns *
Euphorbia. spp. – Spurges *
Helenium tenuifolium – Bitterweed
Hyoscyamus niger – Henbane
Hypericum perforatum – St. Johnswort *
Iva xanthifolia – Marsh-elder
Lupinus spp. – Lupines
Mildbraedia fallax – Hutch
Mercurialis annua – Mercury *
Petiveria alliacea
Quercus spp. – Oaks
Ranunculus spp. – Buttercups *
Rhamnus cathartica – Buckthorn
Rumex spp. – Docks *
Senebiera didyma – Lesser wartcress
Sisymbrium spp. – Hedge mustard
Tanacetum vulgare – Tansy
Thlaspi arvense – Fanweed
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* Affect quality of milk and also cause a falling off in quantity of milk when eaten in large enough amounts by cows.

Supplemental reading

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