Addis Ababa University
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Reading material in Veterinary Diagnostic Protozoology

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Introduction

Protozoology is a branch of zoology that is concerned with the study of protozoa. As such, it may simply be described as the science of protozoa (microscopic eukaryotes that either exist as parasites or free-living organisms). Here, protozoologists focus on the different types of protozoa, their respective characteristics, as well as how they interact with their surrounding (as parasites or free-living organisms). As with any other organism in nature, protozoologists not only consider the pathogenic aspect of these organisms, but also some of the other important roles (in the food chain, degradation etc) they play as components of their respective ecosystems.

Veterinary Protozoology: deals with the study of protozoans of veterinary importance (unicellular organisms that cause disease in animals).

Protozoa are unicellular organisms that are more primitive than animals, and no matter how complex their bodies may be all the different structures are contained in a single cell. Protozoa, like most organisms, are eukaryotic, in that their genetic information is stored in chromosomes contained in a nuclear envelope. In this way they differ from bacteria which do not have a nucleus and whose single chromosome is coiled like a skein of wool in the cytoplasm. This primitive arrangement, found only in bacteria, rickettsia and certain algae, is called prokaryotic and such organisms may be regarded as neither animal nor plant, but as a separate kingdom of prokaryotic organisms, the Monera. Classification of the kingdom Protozoa is extremely complex and undergoing constant revision. This chapter presents the classification, which is intended to give an outline of the basic differences in the structure and life cycles of the main groups.

- 4 sub phylum under the phylum of protozoa:
- ✓ Sarcomastigophora: composed of 2 classes: Sarcodina (Entamoeba) & Mastigophora(Trypanosoma, Leishmania, Trichomonas, Histomonas and Giardia)
- ✓ Sporozoa: consists of the class coccidia (Eimeria, Cryptosporidium, Isospora,Toxoplasma, sarcocystis, Besnoitia, Neospora); class Piroplasmidia(babesia, Theileria) and Haemosporidia(Plasmodium)
- ✓ Ciliophora: Balantidium

- The amoebae (Sarcodina) move by means of pseudopodia.
- The flagellates (Mastigophora) typically move by long, whiplike flagellae.
- The ciliates (Ciliata) are propelled by rows of cilia that beat with a synchronized wavelike motion.
- The sporozoans (Sporozoa) lack specialized organelles of motility.
- There are about 45,000 protozoan species; around 8000 are parasitic, and around 25 species are important to humans.
- Diagnosis – (Laboratory and field), must learn to differentiate between the harmless and the medically important. This is most often based upon the morphology of respective organisms and their developmental stages
- Transmission - mostly person-to-person, via fecal-oral route; fecally contaminated food or water; other means include sexual transmission, insect bites or insect feces.
- Trophozoite - the motile vegetative stage; multiplies via binary fission; colonizes host.
- Cyst - the inactive, non-motile, infective stage; survives the environment due to the presence of a cyst wall. Cysts do not multiply, however, some organisms divide within the cyst wall.

Diagnostic Features: the following features are considered in identifying the parasitic protozoans

- Nuclear structure - important in species differentiation.
- Size - helpful in identifying organisms; must have calibrated objectives on the microscope in order to measure accurately.
- Cytoplasmic inclusions - chromatoid bars (coalesced RNA); red blood cells; food vacuoles containing bacteria, yeast, etc.
- Appearance of cytoplasm - smooth & clean or vacuolated.
- Type of motility - directional or non-directional; sluggish or fast.
- Postmortem lesions

Nuclear Structure:

- Chromatin - nuclear DNA. Present as “peripheral” chromatin and the karyosome.
- Karyosome - a small mass of chromatin within the nuclear space. Also called “endosome” or “centrosome.”
- Peripheral Chromatin - chromatin adhering to the nuclear membrane.
- Nuclear membrane - membrane surrounding all nuclear material.
- Chromatoid body or “bar” - coalesced RNA within the cytoplasm of the cyst stage.

Intestinal Coccidia

- Organisms infecting humans include *Isospora, Sarcocystis, Cryptosporidium, Cyclospora & Toxoplasma.*
- *Sarcocystis, with developmental stages in muscle of an intermediate host, Cryptosporidium which infects certain epithelial cells & Toxoplasma are all coccidian but tese parastes are considered separatyely.*
- Some have 2-host life cycle.
- Sexual & asexual reproduction - Schizogony - asexual binary fission; Sporogony - sexual reproduction.
- Diagnostic stages are often difficult to locate. Acid fast stains are used to visualize. Oocysts do not stain with iodine or permanent stains like trichrome.

Coccidiosis is a protozoan disease of domestic animals caused by a microscopic protozoa called coccidia and is characterized by diarrhea, unthriftiness and variable levels of mortality. Each species of coccidia is host specific and does not infect a wide variety of animals. Affects the intestinal epithelial cells characterized by enteritis. Coccidiosis is transmitted by direct or indirect contact with droppings of infected animals. When an animal ingests coccidia, the organisms invade the lining of the intestine and produce tissue damage as they undergo reproduction. The oocysts shed in the droppings are not capable of infecting another animal unless they pass through a maturation process (sporulation) in the litter. This sporulation occurs within a one to three day.

Diagnosis of Intestinal Coccidia

- Coccidia of the three genera Eimeria, Isospora and Tyzzeria are all primary parasites of epithelial cells. Most of them infect the lining of alimentary tract
- Invasion areas vary with the species of parasite and some infect other organs such as hepatic bile duct (Eimeria stiedai in the rabbit) or kidney tubules (E. Truncata in the goose)
- Coccidia are with few exceptions strictly host specific
- They provoke a marked immune reaction from the host, which is host specific.

Major species of Eimeria affecting animals

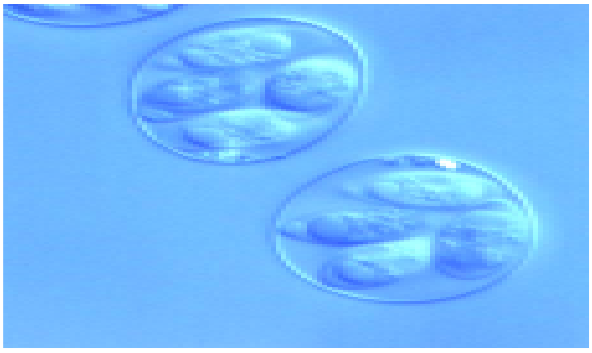
Poultry: *Eimeria tenella , Eimeria necatrix , Eimeria brunetti , Eimeria maxima*
Eimeria acervulina
Cattle: *E. bovis, E. zurnii & E. alabamensis*
Sheep: *E. crandallis, E.ovinoidalis, E. bakensis*
Goats: *E. arlongi, E. ninakohlyakimovae*
Pigs: *E. deblieki*
Horses: *E. leuckarti*
Rabbits*: E. stiedae* (in liver)
Tyzzeria: Protozoa Species specifically affecting Waterfowl. *T. alleni; T. chenicusae; T.pellerdyi; T.perniciosa; T. parvula ...*

Identification of Eimeria species

- ✓ Mainly Based on: Site of infection in the intestine (site specific), Sporulation time, Prepatent period, Morphological features (size, shape, presence or absence of structures like polar cap) of oocysts , Characteristics of the sporulated oocysts
- ✓ Cocidia of different genera may be distinguished by the characteristics of the sporulated oocysts
- ✓ Eimeria: 4 sporocysts each containing 2 sporozoites
- ✓ Isospora: 2 sporocysts each containing 4 sporozoites
- ✓ Tyzzeria: 8 free sporozoites

Sporulated oocyst has 4 sporocysts each containing 2 sporozoites [Eimeria]

- ✓



Avian coccidiosis

- The form of the disease tend to be chronic and associated with many species of Eimeria
- Mortality may not be heavy but morbidity may retard growth significantly or reduced egg production
- **Clinical diagnosis: Signs**
  Clinical signs of coccidiosis usually are present or shortly following stress such as

weather changes; weaning; overcrowding; long automobile or plane rides; relocation to a new home and new owners; and/or unsanitary conditions. Symptoms or signs of coccidiosis will depend on the state of the disease at the time of observation.

- In general, coccidiosis affects the intestinal tract and symptoms are associated with it. In mild cases, only a watery diarrhea may be present, and if blood is present in the feces, it is only in small amounts.
- They are easily transmitted from one house to another on contaminated boots, clothing, free-flying birds, equipment, feed sacks, insects and rodents.
- Coccidiosis usually occurs in growing birds and young adults. It is seldom seen in birds under three weeks or in mature birds.
- Signs of an outbreak include birds that are pale, droopy, tend to huddle, consume less feed and water, have diarrhea, and may become emaciated and dehydrated. Laying hens will experience a reduction in rate of egg production.
- Cecal coccidiosis may produce bloody droppings and anemia that is often followed by death. Intestinal coccidiosis is not as acute and is more chronic in nature. It produces less mortality than the cecal form.

NB: "Nervous coccidiosis" is a nervous system condition associated with coccidial infection. Signs are consistent with central nervous system involvement, and include muscle tremors, convulsions and other central nervous system symptoms. A consistent sign in "nervous cocci" dogs is that stimulation of any type seems to trigger the symptoms

Fecal culture for sporulation of oocysts

- Sporulation is a process of development that takes place in the oocyst
- In fresh feces, oocysts of various species of Coccidia may appear similar to one another; however once sporulation occurs, coccidia of the genus Eimeria can be easily distinguished from those of the genus Isospora. A fully sporulated oocyst of Eimeria contains 4 sporocysts where as a fully sporulated oocyst of Isospora has two sporocysts

Procedures

- When the Coccidian oocysts are found in a fresh fecal sample, place 10 to 20 g of the sample in a beaker or a paper cup and cover with about 60 ml of 2.5% potassium dichromate solution. Mix this solution thoroughly with a tongue depressor
- Pour in to a Petri dish and incubate at room temperature for 3 to 5 days. Open the plate daily and swirl the contents gently to allow air to reach the developing oocysts
- After incubation, centrifuge the plate’s contents as indicated for sedimentation procedure

- Process the fecal sediment by the centrifugal flotation procedure to recover the oocysts, then examine microscopically

Cryptosporidiosis is a diarrheal disease caused by microscopic parasites, Cryptosporidium, that can live in the intestine of humans and animals and is passed in the stool of an infected person or animal.

Cryptosporidiosis, also known as **crypto** is a parasitic disease caused by *Cryptosporidium*, a genus of protozoan parasites in the phylum Apicomplexa, affects the distal small intestine and can affect the respiratory tract in both immunocompetent (i.e., individuals with a normal functioning immune system) and immunocompromised (e.g.,persons with HIV/AIDS or autoimmune disorders) individuals, resulting in watery diarrhea with or without an unexplained cough. In immunosuppressed individuals, the symptoms are particularly severe and can be fatal. It is primarily spread through the fecal-oral route, often through contaminated water; recent evidence suggests that it can also be transmitted via fomites in respiratory secretions. It is associated with municipal water supplies which causes diarrhea. During the past two decades, *Cryptosporidium* has become recognized as one of the most common causes of waterborne illness worldwide.

Species and host diversity

There are many species – not all infect humans. The parasite is found in mammals, birds, fish and reptiles.

- Species: *C. parvum* in domestic animals and man; *C. hominis* (man), *C. baileyi* and *C. meleagridis* in poultry
- *Cryptosporidium parvum* has been recognized as a human pathogen since 1976.
- Definitive Host: Human while reservoir Hosts: kittens, puppies, goats, calves, mice
- It is a zoonotic disease and can travel from animals to humans.

Intestinal cryptosporidiosis

Common signs and symptoms of intestinal cryptosporidiosis include:

- Moderate to severe watery diarrhea sometimes contains mucus and rarely contains blood or leukocytes.
- In very severe cases, diarrhea may be profuse and cholera-like with malabsorption and hypovolemia.

Respiratory cryptosporidiosis

Symptoms of upper respiratory cryptosporidiosis include: Inflammation of the nasal mucosa, sinuses, larynx, or trachea, nasal discharge, Symptoms of lower respiratory cryptosporidiosis include: Cough, Shortness of breath, Fever, Hypoxemia

Life cycle

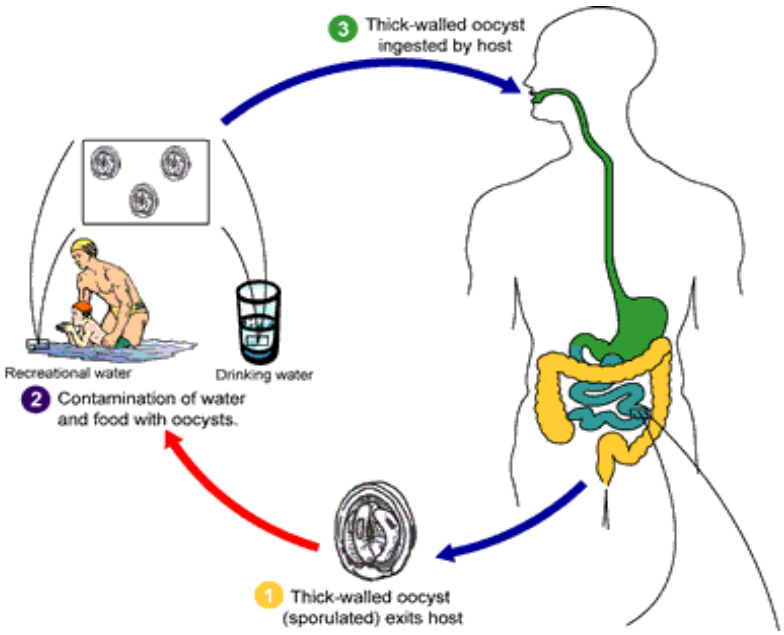
Cryptosporidium spp. exist as multiple cell types which correspond to different stages in an infection (e.g., a sexual and asexual stage). As an oocyst – a type of hardy, thick-walled spore – it can survive in the environment for months and is resistant to many common disinfectants, particularly chlorine-based disinfectants. After being ingested, the sporozoites within oocysts excyst (i.e., are released) in the small intestine. The released sporozoites subsequently attach to the microvilli of the epithelial cells of the small intestine. From there they become trophozoites that reproduce asexually by multiple fission, a process known as schizogony. The trophozoites develop into Type 1 meronts that contain 8 daughter cells.These daughter cells are Type 1 merozoites, which get released by the meronts. Some of these merozoites can cause autoinfection by attaching to epithelial cells. Others of these merozoites become Type II meronts, which contain 4 Type II merozoites. These merozoites get released and they attach to the epithelial cells. From there they become either macrogamonts or microgamonts. These are the female and male sexual forms, respectively. This stage, when sexual forms arise, is called gametogony. Zygotes are formed by microgametes from the microgamont penetrating the macrogamonts. The zygotes develop into oocysts of two types. 20% of oocysts have thin walls and so can reinfect the host by rupturing and releasing sporozoites that start the process over again. The thick-walled oocysts are excreted into the environment. The oocysts are mature and infective upon being excreted.

- Transmission - the endemic cycle is maintained via person-to-person, fecal-oral route transmission; a relatively common finding in “day-care” diarrhea; can be sexually transmitted; big potential for being waterborne due to significant resistance to disinfectants.
- Pathology - most infections cause severe diarrhea. In the immunosuppressed patient, the condition is protracted and life threatening. There is no drug effective against this parasite**.**

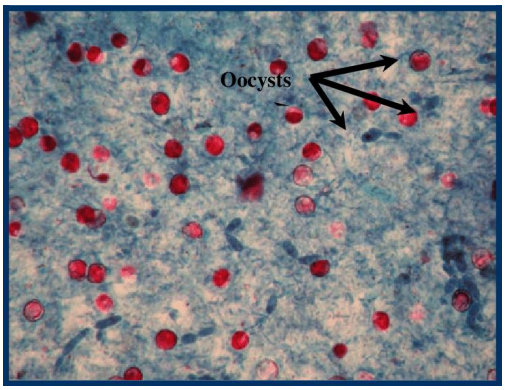
There are many diagnostic tests for *Cryptosporidium*. They include Microscopy with an acid fast stained stool smear, Enzyme immunoassay for greatest sensitivity and specificity and Molecular methods using PCR for detection of antibodies. Microscopy can help identify oocysts in fecal matter. To increase the chance of finding the oocysts, the diagnostician should inspect at least 3 stool samples. There are several techniques to concentrate either the stool sample or the oocysts. The modified formalin-ethyl acetate (FEA) concentration method concentrates the stool. Both the modified zinc sulfate centrifugal flotation technique and the Sheather's sugar flotation procedure can concentrate the oocysts by causing them to float. Another form of microscopy is fluorescent microscopy done by staining with auramine. Other staining techniques include acid-fast staining, which will stain the oocysts red. One type of acid-fast stain is the Kinyoun stain. Giemsa staining can also be performed. Part of the small intestine can be stained with hematoxylin and eosin (H & E), which will show oocysts attached to the epithelial cells.

Detecting antigens is yet another way to diagnose the disease. This can be done with direct fluorescent antibody (DFA) techniques. It can also be achieved through indirect immunofluorescence assay. Enzyme-linked immunosorbent assay (ELISA) also detects antigens.

Polymerase chain reaction (PCR) is another way to diagnose cryptosporidiosis. It can even identify the specific species of *Cryptosporidium*.[1] If the patient is thought to have biliary cryptosporidiosis, then an appropriate diagnostic technique is ultrasonography. If that returns normal results, the next step would be to perform endoscopic retrograde cholangiopancreatography.



People at most risk for this disease are children in day-care centers, child-care workers, parents with infected children, international travelers, backpackers, hikers and campers who drink unfiltered or untreated water, swimmers who swallow water from contaminated sources, people exposed to human feces during sexual contact, and individuals who handle infected cattle.



Oocyst of Cryptosporidium by Modified cold Ziehl-Neelsen stain with magnification 1000X.

Interesting facts: *Cryptosporidium is resistant to chlorine, not protected in chlorinated pool and cannot be infected by blood exposure. The sporocysts are resistant to most chemical disinfectants, but are susceptible to drying and the ultraviolet portion of sunlight.*

Sarcocystis is a genus of parasites, the majority of species infecting mammals, and some infecting reptiles and birds.

The lifecycle of a typical member of this genus involves two host species, a definitive host and an intermediate host. Often, the definitive host is a predator and the intermediate host is its prey. The parasite reproduces sexually in the gut of the definitive host, is passed with the feces, and ingested by the intermediate host. There, it eventually enters muscle tissue. When the intermediate host is eaten by the definitive host, the cycle is completed. The definitive host usually does not show any symptoms of infection, but the intermediate host does.

About 130 recognized species are in this genus. Revision of the taxonomy of the genus is ongoing, and all the currently recognized species may be a much smaller number of species that can infect multiple hosts.

The name *Sarcocystis* is derived from Greek: *sarx* = flesh and *kystis* = bladder.

The taxonomy of this genus and its relationship to other protozoal genera are currently under investigation.

Related genera include: *Besnoitia*, *Caryospora*, *Cystoisospora*, *Frenkelia*, *Isospora*, *Neospora*, *Toxoplasma* etc…

Sarcocystis is the largest genus within the family Sarcocystidae and consists of species that infect a range of animals, including mammals, birds, and reptiles. , another genus within this family, consists of parasites that use rodents as intermediate hosts and birds of prey as definitive hosts.

Besnoitia, *Hammondia*, *Neospora*, and *Toxoplasma* apparently form a single group. Within this clade, *Toxoplasma* and *Neospora* appear to be sister clades. *Isospora* also appears to belong to this clade and this clade is a sister to *Sarcocystis*. *Frenkelia* appears to be very closely related to *Sarcocystis*.

Several molecular studies have suggested that *Frenkelia* is actually a species of *Sarcocystis*. This genus was distinguished from *Sarcocystis* on the basis of its tendency to encyst within the brain rather than within muscle. This distinction may not be taxonomically valid.

Four recognized species infect cattle: *S. bovifelis*, *S. bovihominis* (*S. hominis*), *S. cruzi* (*S. bovicanis*), and *S. hirsuta*. *S. cruzi* is the only species known to be pathogenic in cattle. Several clinical syndromes have been reported in connection with this parasite: eosinophilic

myositis; abortions, stillbirths, and deaths in pregnant cows; two cases of necrotic encephalitis in heifers have also been reported. Typical clinical signs of acute bovine sarcocystosis are: anorexia, pyrexia (42°C or more), anemia, cachexia, enlarged palpable lymph nodes, excessive salivation, and loss of hair at the tip of the tail. Sheep may be infected by four recognized species of *Sarcocystis*: *S. arieticanis* and *S. tenella* (*S. ovicanis*) are pathogenic; *S. gigantea* (*S. ovifelis*) and *S. medusiformis* are nonpathogenic. *S. arieticanis* and *S. tenella* both produce extraintestinal disease. Anemia, anorexia, ataxia, and abortions are the chief clinical signs. Myositis with flaccid paralysis has been reported as a consequence of infection. Ovine protozoan myeloencephalitis is a recognised syndrome that may occur in outbreaks. The usual pathological findings in such cases are multifocal spinal cord white matter oedema and necrosis, glial nodules and mild to moderate nonsuppurative encephalomyelitis.

The diagnosis may be established finding protozoan bodies (12.7–23.0 μm) that stain immunocytochemically for *Sarcocystis* epitopes.

Four recognised species infect pigs: *S. medusiformis*, *S. meischeriana* (*S. suicanis*), *S. porcifelis*, and *S. suihominis*. *S. porcifelis* is pathogenic for pigs causing diarrhea, myositis and lameness.

Five species infect horses: *S. asinus*, *S. bertrami*, *S. equicanis*, *S. fayeri*, and *S. neurona* (*S. falcatula*). All use canids as definitive hosts; transplacental infection has also been reported. *S. neurona* causes equine protozoal myeloencephalitis. Exposure to this parasite appears to be common in the United States, with serological surveys indicating that 50–60% percent of all horses in the Midwest United States have been exposed to it. Clinical signs include gait abnormalities including ataxia, knuckling, and crossing over. Muscle atrophy, usually unilateral, may occur. The lesions are typically focal. Brain stem involvement is common. Depression, weakness, head tilt, and dysphagia also occur. *S. fayeri* may cause myositis in horses.

Pathology

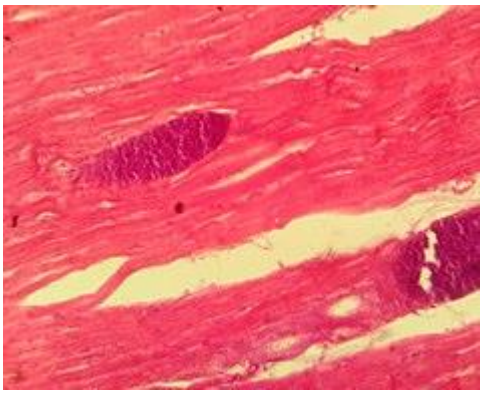
- The pathology is of two types: a rare invasive form with vasculitis and myositis and an intestinal form that presents with nausia, abdominal pain, and diarhea .
- While normally mild and lasting under 48 hours, the intestinal form may occasionally be severe or even life threatening.

- The invasive form may involve a wide variety of tissues including lymph nodes, muscles and the larynx.

S. hemionilatrantis infects mule deer. Death from experimental inoculation has been reported.

These parasites can also infect birds, producing three different clinical forms: an acute pulmonary disease, muscular disease, and neurological disease. Symptoms include lethargy, shortness of breath, tail bobbing, yellow-tinted droppings, and sudden death. The presence of the cysts in the muscle of wild birds is known as "rice breast".

Infection of animals with *Sarcocystis* is common. Rates in pigs vary: 18% to 68%. Camels have a similarly high incidence of infection. Above 80% are known in cattle and goats while the incidence in horses, donkeys, and chickens is much lower.



H&E micrograph showing *Sarcocystis* in a 3-year-old sheep cardiac muscle tissue (40X)

Infection occurs when undercooked meat is ingested. The incubation period is 9–39 days. Human outbreaks have occurred in Europe. Rats are a known carrier. Contaminated water may be able to cause infection, but this remains a theoretical possibility. The pathology is of two types: a rare invasive form with vasculitis and myositis and an intestinal form that presents with nausea, abdominal pain, and diarrhea. While normally mild and lasting under 48 hours, the intestinal form may occasionally be severe or even life-threatening. The invasive form may involve a wide variety of tissues including lymph nodes, muscles, and the larynx.

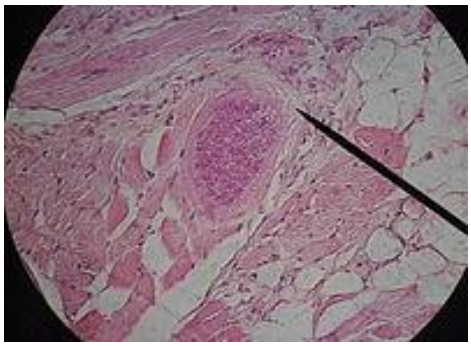
Clinical: Human

- Infection with this parasite is known as sarcosporidiosis. Because of initial confusion over the taxonomy of this parasite it was originally referred to as *Isospora hominis*. The older literature may refer to this organism
- Human infection is considered rare with less than one hundred published cases of invasive disease (approximately 46 cases reported by 1990). These figures represent a gross underestimate of the human burden of disease.

Route of infection

- Infection occurs when undercooked meat is ingested. The incubation period is 9–39 days. Human outbreaks have occurred in Europe. Rats are a known carrier.
- It has been suggested that contaminated water may be able to cause infection but this presently remains a theoretical possibility.

Definitive diagnosis by biopsyof an infected muscle. Sarcocysts are identifiable with hematoxylin and eosin. The PAS stain may be helpful but variable uptake of stain is common. Along with the sarcocysts inflammatory cells may be found. Other findings include myositis, myonecrosis, perivascular and interstitial inflammation, vasculitis and eosinophilic myositis.



Sarcocysts within pig skeletal muscle. Note the readily visible striated border.

Toxoplasmosis

Toxoplasmosis is a parasitic disease caused by Toxoplasma gondii. Infections with toxoplasmosis usually cause no obvious symptoms in adults. Occasionally, people may have a few weeks or months of mild, flu-like illness such as muscle aches and tender lymph nodes. In a small number of people, eye problems may develop. In those with a weak immune system, severe symptoms such as seizures and poor coordination may occur. If infected during pregnancy, a condition known as congenital toxoplasmosis may affect the child.

The disease is caused by a protozoan unicellular parasite T. gondi which Was first discovered in Africa in 1908 in African rodent known as gondii. Two morphological forms occur: (a) Bradyzoites: Proliferative form (b) Cyst: (i) Oocyst (ii) Tachyzoites

Final Host: Felidae. Cats while intermediate hosts are all mammals including man, Sheep, goats, cattle, dogs, horses,Birds.

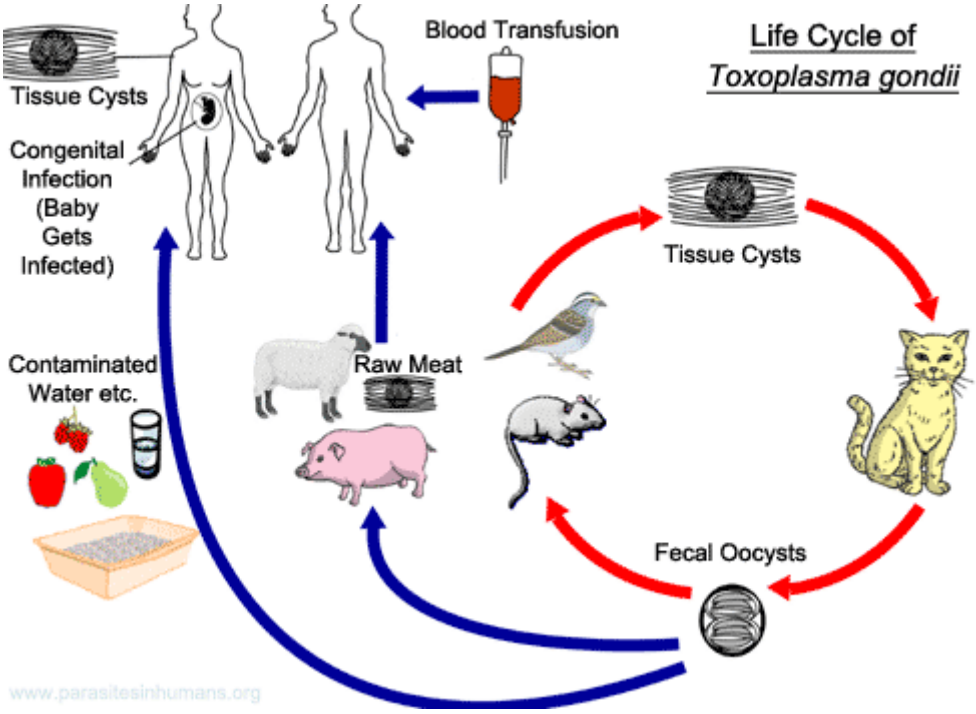
Life cycle

- Cats are infected through ingestion of rodent tissue containing mature bradyzoites.
- Direct transmission between cats through ingestion of cat feces containing oocysts (Sporozyoites) is possible.
- Digestion of the bradyzoites in cats stomach.
- Bradyzoites bore the intestinal wall and multiply sexually (gametogony) and asexually (schizogony). During reproduction in the intestinal mucosa, organisms invade extra – intestinal organs (muscle, liver lymph nodes spleen). Multiplication in these organs takes place.
- Cats Shed oocysts with feces. Mammals get infected by ingestion of oocysts (feces) or bradiozytes (meat).

Infection of intermediate host takes place in 2 ways:

- (a) by ingestion of sporulated oocysts
- (b) by ingestion of bradyzoites ór tachyzoites found in the flesh of another intermediate host.

When antibody develops, the invasiveness of the tachyzoites is arrested. This results in formation of cysts known as bradyzoites. Bradyzoites are produced in muscles. When bradyzoites are ingested, toxoplasma infection ensues.



Sources of infection are: feces, raw or under cooked meat and meat products, milk, urine, Saliva and conjunctival fluid. Cats play a central role in the epidemiology of toxoplasmosis. The disease is absent from areas where cats do not occur. Infection is more frequent in stray cats. Cats shed oocysts for 1 –2 weeks. Cats remain carriers. Pregnant ewes are more frequently infected. coprophagous insects can contaminate vegetables, meat and fodder.

People at risk: Veterinarians and para-veterinary personnel, Lab personnel, Pet attendants, meat handlers, Shepard and Pregnant women.

Incubation Period: varies according to infection dose. Toxoplasmosis appears in (a) acute form: occurs during the proliferation phase. (b) sub-acute form : occurs when Immunity develops c) chronic form is characterized by cyst formation in tissues.

Congenital infection: Congenital infection occurs, when a woman is exposed to infection during pregnancy. The fetus is infected through the placenta as a consequence of maternal parasitemia. Infection of pregnant woman is very serious, while it results in abortion, still birth and damage of the CNS of the neonate. The rate of infection is particularly high during the first trimester of pregnancy. If the child is born, it may suffer from serious mental retardation within few weeks after birth. The neonate may reveal hepathomegaly, splenomegaly, and hydrocephaly.Further clinical signs manifested include convulsive attacks, epilepsy, neuropsychic retardation deafness and blindness.

Clinical signs: non congenital infection in man

- The prominent clinical signs observed in the acute phase include lymphadenopathy, especially the cervical nodes, fever, myocarditis, fever, lymphocytosis and meningo encephalitis.
- Toxoplasmosis affects more immune-suppressed AIDS patients.
- The disease is fatal in AIDS patients, if treatment is not followed.

Toxoplasmosis in Sheep

Source and mode of infection: Ingestion of cat feces. A single cat can shed millions of oocysts that can contaminate the pasture.

Symptoms: The disease in sheep is characterized by placentitis, abortion in the last months of pregnancy or the sheep bears weak lamb. Congenital infected lambs suffer from muscular incoordination. Further clinical signs listed include mummification of the fetus, uterine decompostion, subcutaneous edema of the fetus, and presence of excess fluid in cavities. Enteritis in case large number of toxoplasma are ingested. There may ulceration even. Pneumonitis is common, if there is viral infection.

Diagnosis: humans

Diagnosis based on clinical signs is very difficult. Diagnosis may be achieved by

A.Serological tests 1.ELISA, immunofluorescent anti body test (IFA), CFT

- 2. The skin test: the antigen is prepared by rapidly freezing and thawing toxoplasma obtained from mouse inoculation.

B. Demonstration of the organism in tissues of mice inoculated with suspect material.

- Inoculation of toxoplasma free mice by interperitoneal by intracerebral route and with subsequent demonstration of tachyzoites or bradyzoites in smears of organs.

Tick-born Protozoan diseases: Are transmitted by the different developmental stages of ticks. The major ones are Babesiosis, theileriosis, Cowdriosis and Anaplasmosis. They cause enormous economic loss via death of animals, reduced growth rate and milk production. They also hamper livestock export market (East Cost Fever – *T.Parva* infection).

DISEASE	CAUSATIVE AGENT	VECTORS	DISTRIBUTION
Anaplasmosis	*A. marginale*	*B. decoloratus, R. simus simus*	South, West, East, Central, North
Babesiosis	*B. bigemina, B. bovis*	*B. decoloratus, R.e.evertsi*	South, West, Central, North
Theileriosis	*T.mutans, T.annulata*	*A.variegatum, Hy.anatolicum*	West and Central
Cowdriosis	*C. ruminantium*	*A.variegatum, A.gemma, A.hebreaum, A.pomposmas, A.lepidum*	South, East, West, Central, North

BABESIOSIS

Babesiosis is a tick-borne malaria-like illness caused by species of the intraerythrocytic protozoan *Babesia.* Humans are opportunistic hosts for *Babesia* when bitten by nymph or adult ticks. Currently, *Babesia* infection is transmitted by various tick vectors in different part of the world. Many different species (types) of *Babesia* parasites have been found in animals, only a few of which have been found in people. *Babesia microti* which usually infects white-footed mice and

other small mammalsis the main species that has been found in people. Occasional cases caused by other *Babesia* species have been detected.

How do people get infected with *Babesia*?

The main way is through the bite of an infected tick. *Babesia microti* is spread by *Ixodes scapularis* ticks, which are commonly called blacklegged ticks or deer ticks. (Although white-tailed deer are the most important food source for the adult stage of the tick, deer are not infected with *B.microti*.). The parasite typically is spread by the young nymph stage of the tick. Nymphs are mostly found during warm months (spring and summer) in areas with woods, brush, or grass. Infected people might not recall a tick bite because *I. scapularis* nymphs are very small (about the size of a poppy seed). Other possible ways of becoming infected with *Babesia* include: Receipt of a contaminated blood transfusion (no tests have been licensed yet for donor screening);or transmission from an infected mother to her baby during pregnancy or delivery.

In animals, Babesiosis, also known as Red Water, Texas fever, Malignant Jaundice and Tristeza. It is mainly a disease of cattle and is caused by Babesia spp from class Piroplasmidia, they are strictly intraerythrocytic parasites. Transmitted by hard ticks and pass transversally, via \ the egg, from one tick generation to the other and then *Babesia* are transmitted by any of the three stages of tick (larval, nymphal or adult). Cause anaemia and haemoglobinuria. The disease is sever in native animals introduced into endemic areas. When infection persists from one stage to the next (two-or three-host ticks feeding on different hosts) transmission is said to be transtadial.

Classification of the causative agent Bovine babesiosis (BB) is a tick-borne disease of cattle caused by the protozoan parasites of the genus Babesia, order Piroplasmida, phylum Apicomplexa. The principal species of Babesia that cause BB are: *Babesia bovis, Babesia bigemina* and *Babesia divergens*. Other Babesia that can infect cattle includes *B. major, B. ovata, B. occultans and B. jakimovi*. Resistance to physical and chemical action. This agent does not survive outside its hosts and can only be transmitted through a tick vector. Therefore, parameters associated with resistance to physical and chemical actions (such as temperature, chemical/disinfectants, and environmental survival) are not meaningful.

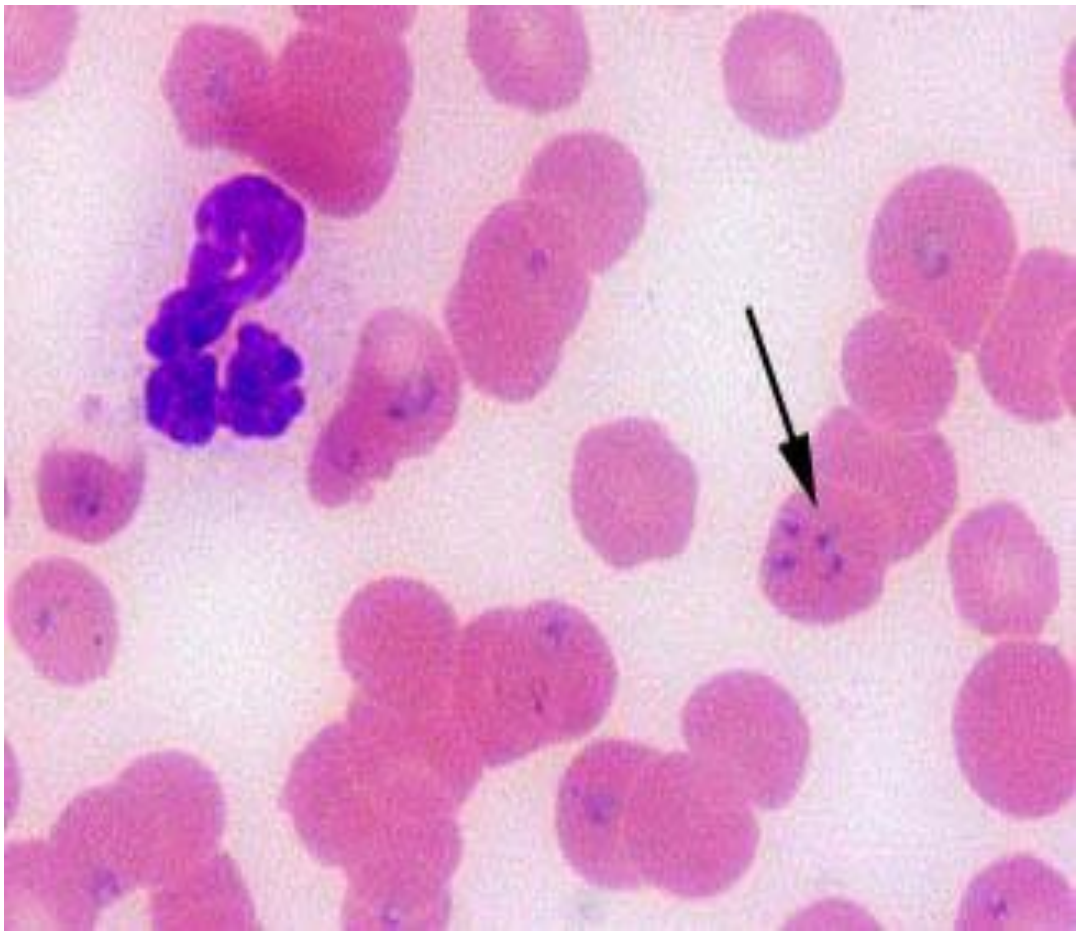
HOST SPECTRUM OF *BABESIA* SPP IN THE TROPICS AND SUBTROPICS

Babesia spp	Host	Vectors
B.major, B. bigemina, B. bovis (B. argentina)	Cattle	*Boophilus decoloratus, B.annulatus*
B. motasi, B. ovis	Sheep/goats	*Rhipicephalus, Ixodes, Dermacentor Haemaphysalis,*
B. cabali, B. equi	Equine	*Hyaloma, Rhipicephalus, Dermacentor*
B. perroncitoi, B. trautmanni	Pigs	*Rhipicephalus, Boophilus Dermacentor*
B. canis, B. gibsoni	Dogs	*Rhipicephalus*
B. felis	Cats	*?*
B. microti, B. divergens	Man	*Unknown*

Temperate zone: *Babesia divergens,* Tropical area: *B.bovis, B.bigemina B.majo*

MORPHOLOGY ANDIDENTIFICATION

- ✓ They are pyriform, may be rounded, elongated or cigar-shaped : small babesia with pyriform 1-2.5 µm long and large ones with 2.5-5.0µm long
- ✓ Blood film stained by Gimsa or Romanowsky dyes show the parasite to be within red cells, the cytoplasm appears blue while nucleus red with latter stain
- ✓ In the blood almost always singly or paired arranged with their characteristic angle with their narrow end opposed
- ✓ Electron microscopy show at their blunt end ‘apical complex’ which is concerned with assisting penetration of the erythrocyte



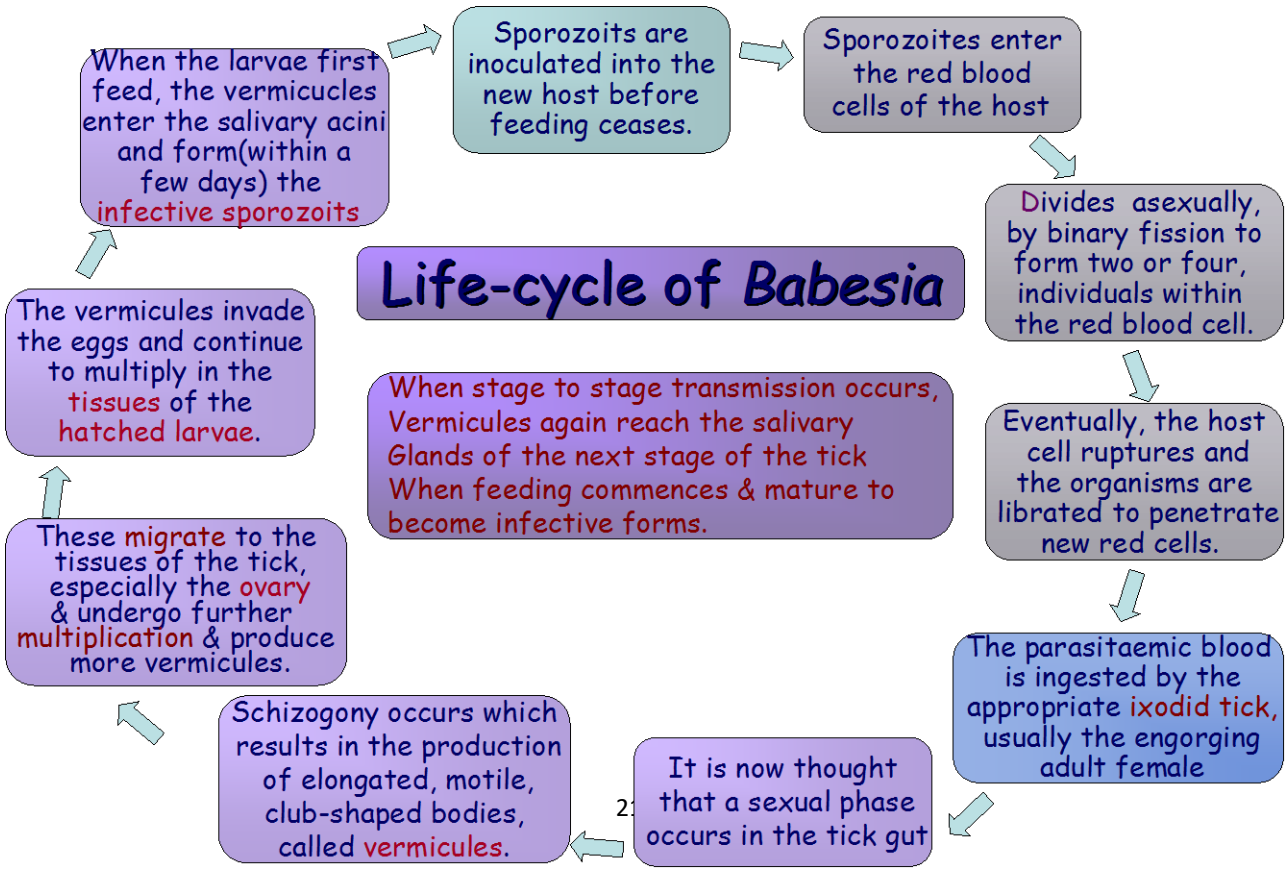
Babesis equi

Babesiosis in the horse is caused by two protozoal piroplasms, *Babesia equi* and *Babesia caballi.* These organisms currently are the only erythroparasites of clinical significance in North American horses. *B. caballi* is a relatively large member of the genus and its appearance has been likened to *B. bigemina*, the species responsible for Texas Cattle Fever. The development of *B. caballi* in the host occurs exclusively in erythrocytes. The trophozoites appear as round, oval, or elliptical basophilic structures that measure 1.5 to 3µm in diameter. The organisms are intraerythrocytic within the cytoplasm of erythrocytes (Fig.1). Pairs of organisms are commonly found in a single erythrocyte oriented such that they form an acute angle. *Babesia equi*, in contrast, is a smaller member of the genus and has been found to have an extraerythrocytic stage of shizogony occurring in host lymphocytes. Similarities to the bovine pathogen *Theileria* have stimulated debate over the rightful taxonomic classification of this organism. Trophozoites of *B. equi* appear as oval, round, elliptical, or spindle-shaped basophilic structures that measure up to 3µm in diameter (Fig. 2). The merozoite stage appears as two or four pyriform parasites together within the erythrocyte, each with a length

of only 1.5 µm on average. When four *Babesia equi* merozoites are present together in one cell, they frequently form a characteristic "Maltese cross"

BIOLOGY

Reproduction: asexually by binary fission to form two or sometimes four individuals within the red cell. Usually the host cell rapture and the parasites are liberated to penetrate new red cells. When the parasitaemic blood is ingested by ticks, a sexual phase takes place in the tick gut followed by schizogony which results in the production of elongated motile, club-shaped bodies, known as vermicules. Vermicule migrate in to the ovary of ticks and multiply and produce more vermicules. In the ovary of the ticks vermicules invade the eggs, multiply in the tissue of the hatched larvae, when larvae first feed; vermicules inter the salivary acini and within a few days, form the infective sporozoites which are inoculated into the new host before feeding of the tick ceases. Some species of *Babesia* may be transmitted via the ovary of two or more generations of female ticks (Vertical transmission)



PATHOGENESIS AND CLINICAL SIGNS

- ✓ The acute disease occurs 1- 2 weeks after the tick commences to feed and is characterized by fever
- ✓ Rapid destruction of red blood cells
- ✓ Haemoglobinuria ('redwater'), fever, increased heart, pulse and respiratory rate, jaundiced mucous membrane, diarrhoea followed by constipation.
- ✓ Loss of weight, milk production and abortion may occur
- ✓ In acute case PCV falls by 20% and death in few days
- ✓ Depending on the spp of *Babesia*, once the clinical sign appear, parasitaemia may involve 0.2% to 45% of the red blood cells
- ✓ In *B. bovis* and *B. canis* clumping of erythrocytes may also occur in the capillaries of the brain, producing nervous signs of hyperxcitability and incoordination
- ✓ Mild form is associated with less pathogenic spp or with relatively resistant hosts. In animals previously exposed to infection or infected with a Babesia sp. of low pathogenicity, clinical signs may be mild or even in-apparent.
- ✓ At necropsy: pale and jaundiced carcass, with thick and granular bile

EPIDEMIOLOGY

All Babesia are transmitted by ticks with a limited host range. The principal vectors of B. bovis and B. bigemina are Rhipicephalus spp. ticks and these are widespread in tropical and subtropical countries. The major arthropod vector of B. divergens is Ixodes ricinus. BB is principally maintained by subclinically infected cattle that have recovered from disease. Morbidity and mortality vary greatly and are influenced by prevailing treatments employed in an area, previous exposure to a species/strain of parasite, and vaccination status. In endemic areas, cattle become infected at a young age and develop a long-term immunity. However, outbreaks can occur in these endemic areas if exposure to ticks by young animals is interrupted or immuno-naïve cattle are introduced. The introduction of Babesia infected ticks into previously tick-free areas may also lead to outbreaks of disease

- ➢ Infection of cattle with *B. bigemina* (vector *B. decoloratus*) and *B. bovis* (vector *B.annulatus*) is are reported in Ethiopia, the former is widespread in the country while *B. bovis* is f recent origin

in the livestock disease scenario of the country and has so far detected in Gambella, south-west Ethiopia

- During a serological survey in 1992 using ELISA test on blood isolates from state and private dairy farm s in Ethiopia indicated 60% and 20% positivity for *B. bigemina*
- In another serological survey by IFAT, 49% showed the presence of antibodies to *B. bigemina,* but few parasites were detected in blood smear collected from indigenous cattle
- *B. motasi, B. equi* (Radley, 1980) and *B. canis have also been reported*
- Clinical cases of babesiosis in Ethiopia encountered rarelyand reported losses are minimal
- Sporadic clinical cases in Debre Zeit among canine (dogs and cats) reported
- Babesiosis in horses is also known to exist in Ethiopia (??)
- To determine the real prevalence of the disease country wide sero-epidemiological survey in different hosts is required

Factors affecting the epidemiology of the disease

1. The age of the host: young animals are less susceptible than adults
2. The immune status of the host: young animals first acquire immunity passively may show mild clinical signs, with persistence of carrier state active immunity may develop, known as premmunity, this immunity remain solid
3. The level of tick challenge: in endemic region with high tick infestation, immunity remains at high level
4. Stress: parturition, presence of another disease may increase the risk of clinical disease
5. The virulence of the particular spp of *Babesia:B. divergens and B.* cais are highly pathogenic, while *B. ovis* in sheep and *B. major* in cattle cause mild form of a disease

Diagnosis

Clinical manifestations of disease associated with BB are typical of a haemolytic anaemia disease process but vary according to agent (i.e. species of parasite) and host factors (i.e. age, immune status). BB is predominantly observed in adult cattle with B. bovis generally being more pathogenic than B. bigemina or B. divergens. Infected animals develop a life-long immunity against re-infection

with the same species and some cross-protection is evident in B. bigemina-immune animals against subsequent B. bovis infections.

A high index of suspicion is necessary to diagnose babesiosis. Babesiosis develops only in patients who live in or travel to an endemic area or receive a contaminated blood transfusion within the preceding 9 weeks, so this aspect of the medical history is vital. Babesiosis may be suspected when a person with such an exposure history develops persistent fevers and hemolytic anemia. The definitive diagnostic test for babesiosis is the identification of parasites on a Giemsa-stained thin blood smear So-called "Maltese cross formations" on the blood film are essentially diagnostic of babesiosis, since they are not seen in malaria, the primary differential diagnosis. Careful examination of multiple blood smears may be necessary, since *Babesia* may infect less than 1% of circulating red blood cells and thus be easily overlooked.

Serologic testing for antibodies against *Babesia* (both IgG and IgM) can detect low-level infection in cases where there is a high clinical suspicion but negative blood film examinations. Serology is also useful for differentiating babesiosis from malaria in cases where people are at risk for both infections. Since detectable antibody responses require approximately one week after infection to develop, serologic testing may be falsely negative early in the disease course.

A polymerase chain reaction (PCR) test has been developed for the detection of *Babesia* from the peripheral blood. PCR may be at least as sensitive and specific as blood film examination in diagnosing babesiosis, though it is also significantly more expensive. Most often, PCR testing is used in conjunction with blood film examination and possibly serologic testing.

Other laboratory findings include decreased numbers of red blood cells and platelets on complete blood count.

In animals Babesiosis is suspected by observation of clinical signs (haemoglobinuria and anaemia) in animals in endemic areas. Diagnosis is confirmed by observation of merozoites on thin film blood smear examined at maximum magnification under oil using Romonovski stains (methylene blue and eosin). This is a routine part of the veterinary examination of dogs and ruminants in regions where babesiosis is endemic.

Babesia canis and *Babesia bigemina* are "large babesias" that form paired merozoites in the erythrocytes , commonly described as resembling "two pears hanging together", rather than the "Maltese Cross" of the "small babesias". Their merozoites are approximately twice the size of small babesias.

Cerebral babesiosis is suspected in-vivo when neurological signs (often severe) are seen in cattle that are positive for babesia bovis on blood smear. Outspoken red discolouration of the grey matter on post-mortem further strengthens suspicion of cerebral babesiosis. Diagnosis is confirmed post-mortem by observation of babesia infected erythrocytes sludged in the cerebral cortical capilaries in a brain smear.

THEILEROSIS (East Coast Fever, Corridor Disease): Theileriases are a group of tickborne diseases caused by *Theileria* spp. A large number of *Theileria* spp are found in domestic and wild animals in tick-infested areas of the Old World. The most important species affecting cattle are *T parva* and *T annulata*, which cause widespread death in tropical and subtropical areas of the Old World. *T lestoquardi*, *T luwenshuni*, and *T uilenbergi* are important causes of mortality in sheep. Both *Theileria* and *Babesia* are members of the suborder Piroplasmorina. Although *Babesia* are primarily parasites of RBCs, *Theileria* use, successively, WBCs and RBCs for completion of their life cycle in mammalian hosts. The infective sporozoite stage of the parasite is transmitted in the saliva of infected ticks as they feed. Sporozoites invade leukocytes and, within a few days, develop to schizonts. In the most pathogenic species of *Theileria* (eg, *T parva* and *T annulata*), parasite multiplication occurs predominantly within the host WBCs, whereas less pathogenic species multiply mainly in RBCs.
The disease is highly prevalent in cattle and sheep in Africa, Asia, Europe and Australia. Their causative agent is a sporozoon of the genus Theileria that infect the lymphocytes, macrophages and red blood cells of cattle. Transmitted only after cyclic development in ticks

Host spectrum, vector and distribution

Minor/ mild pathogenic: *T. mutans, T. taurotragi, T. veliferia, T. orientalis* (cattle: Africa); *T. sergenti* in Asia,*T. ovis*: in sheep.

Species	Host	Vector	Disease	Distribution
T. parva	Cattle	Rhipicephalus	East coast Fever	East and central Africa
T. annulata	Cattle	*Hyalomma*	Meditherranean/ tropical theileriosis	North Africa, South Europe, Asia
T. hirci	Sheep/ goats	*Hyalomma*	Malignant theileriosis	North Africa, South Europe, Asia

***Rhipicephalus appendiculatus* on Ear of Calf**

IDENTIFICATION

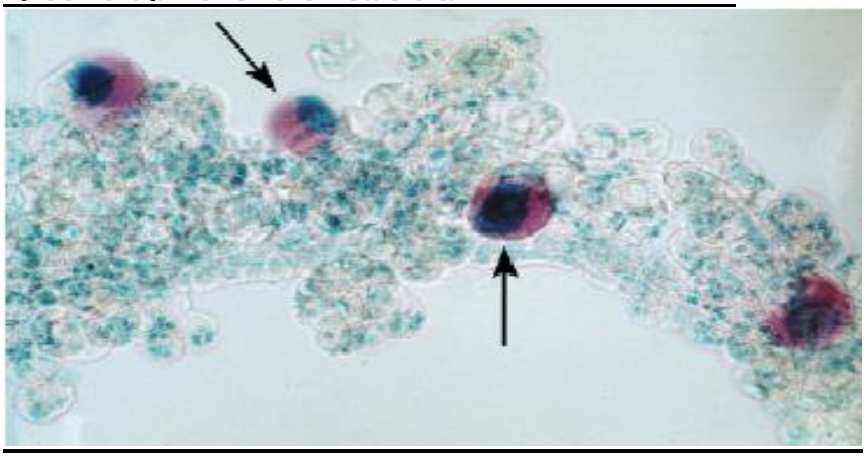
- In red blood cells the piroplasms are rod-shaped (round, oval and ring-shaped can occur) and up to 2 µm and 1µm wide

- With Giemsa stains, the cytoplasm is blue with a red chromatin dot at the end
- Commonly more than one parasite in each erythrocyte, in the cytoplasm of the lymphocytes in the lymph nodes and spleen the schizonts called Koch`s blue bodies (macro/ microschizonts)

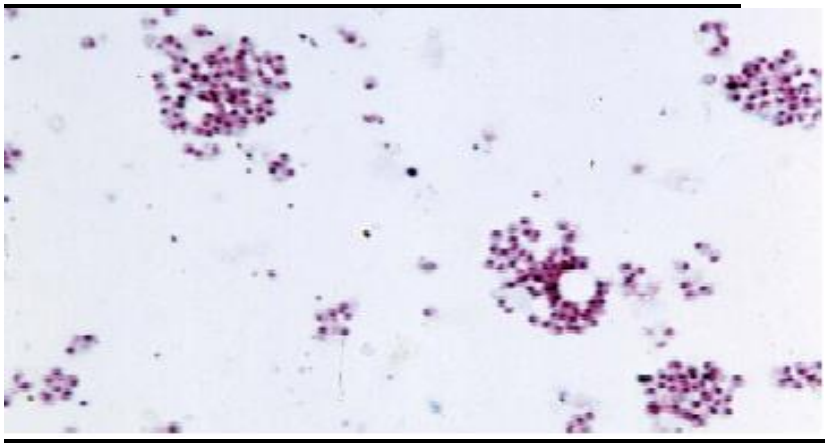
LIFE CYCLE

- The sporozoites are inoculated into cattle by ticks (*Rhipicephalus appendiculatum* (the brown ear tick for *T. Parva*); enter lymphocytes, usually via parotid lymph gland. The lymphocyte transforms to a lymphoblast which divides rapidly, the parasite also divides to form two infected cells
- About 12 days after infection macroschizonts develop into microschizonts, which change into micromerozoits, liberated by rupture of the microschizonts, then invade the red cells to become piroplasms which do not multiply in the RBC
- The piroplasms require to be ingested by the larvae or nymphal stage of the tick vector where a sexual phase occurs in the tick gut then formation of sporoblasts in salivary glands
  The sporoblasts produce infective sporozoites, female ticks infect the host. Transmission is transtadial: by next stage of tick but not transovarian

Sporozoites of *Theileria annulata*, purified from ground up ticks. Giemsa's stain.



***Theileria annulata* sporoblasts in salivary gland of *Hy. a. anatolicum*.** The immature sporoblast stains red for RNA, the enlarged nucleus of the infected salivary gland cell stains green for DNA. Methyl green and pyronin stain.

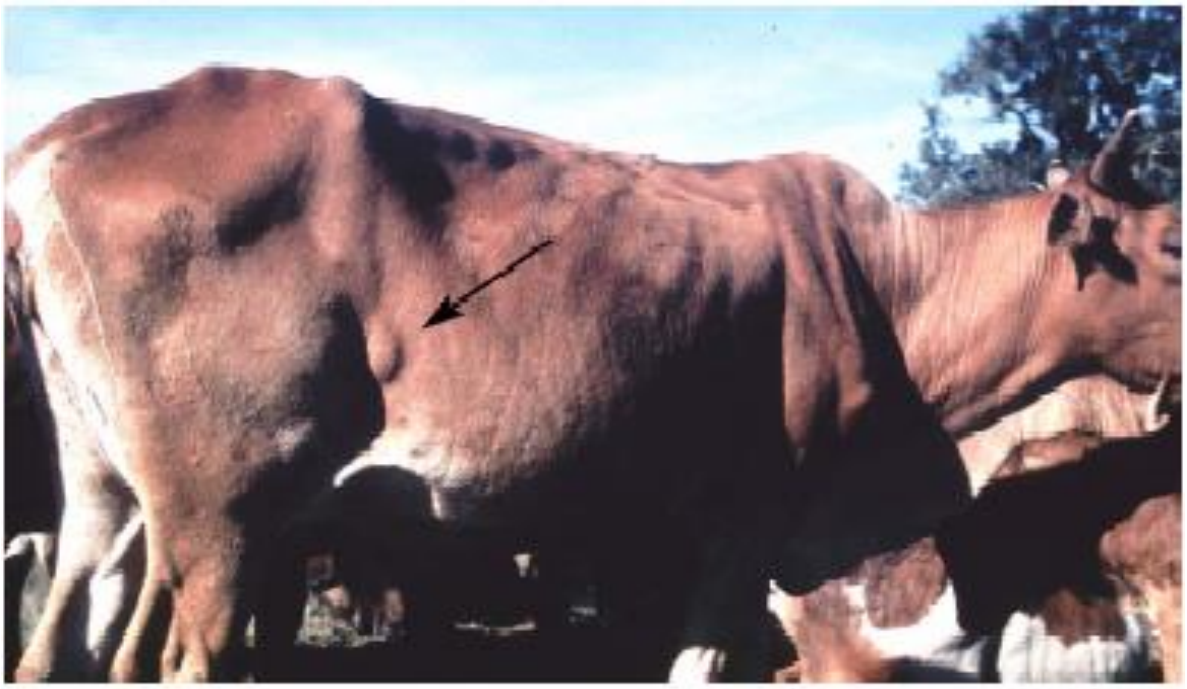


PATHOGENESIS AND CLINICAL SIGNS

- Incubation period is one week, with no lesion and symptom
- On the 2nd week, marked hyperplasia and expansion of infected lymphoblasts, initially in the regional lymph node at the site of tick bite
- Third week:lymphoid depletion and disorganization due to massive lymphocytolysis and depressed leucopoesis, the reason may be due to activation of natural killer cells like macrophages
- Terminal phase: necropsy shows atrophy of the cellular content of the lymph nodes and spleen, lung oedema, emphysema and petechial haemorrhages on the GIT mocosa

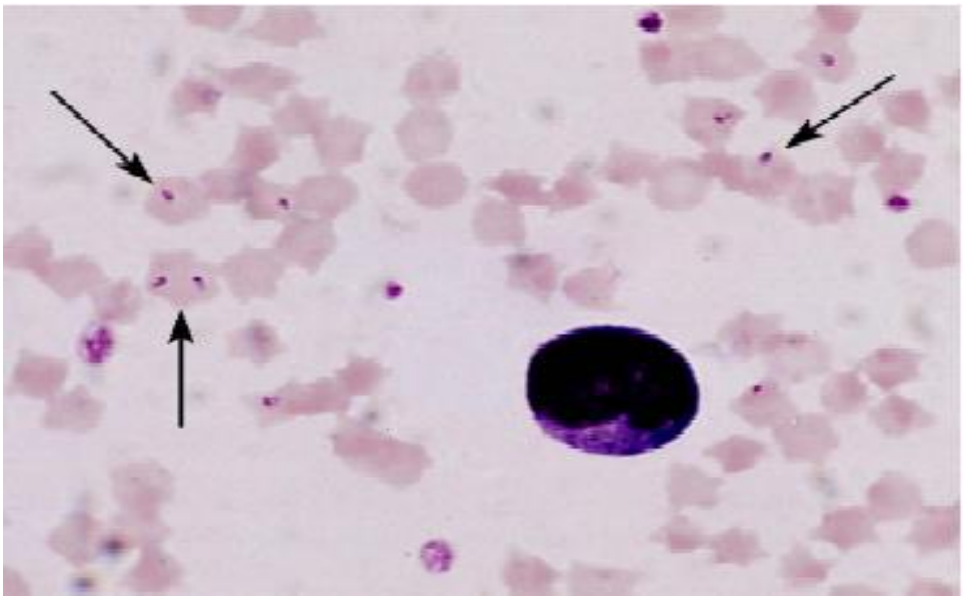
- Occasionally nervous signs, `turning sickness` is observed due to presence of schizonts in cerebral capillaries. Enlarged regional lymph nodes, within few days generalized fever (41-42 oC), loss of condition,

dyspnoe, blood stained diarrhoea and petechial haemorrhages under the tongue. Death in 3 weeks.



Clinical sign of theileriosis - swollen lymph nodes. This cow had a carrier infection of *Th. parva*.

***Theileria parva* piroplasms in cattle blood.**
Giemsa's stain.



EPIDEMIOLOGY

- *Theileria mutans* has been known for a long time to occur in Ethiopia. It is certainly as widespread as its vector *A.Verigatum* (Morel, 1980)
- The disease was reported in \state and private dairy farms. Out of 5208 blood smears collected from different ecological zones of the country, 29.7% were found to be positive for *T.mutans*
- From 700 blood smears collected from western zone of the country, 40% were positive for Theileria spp (Mekonnen *et al*., 1992). *T. orientalis* (*T. buffeli*) in cattle (Becerra *et al*., 1983-west Ethiopia), *T. velifera* (Radeley, 1980)
- An outbreak is seasonal since ticks become active following the onset of rain. Indigenous cattle reared in endemic area are highly resistant but may remain carrier, newly introduced cattle may suffer from high mortality
- An outbreak of East Coast Fever is enhanced by prolonged rainy season which is favourable for proliferation of tick vector
- In East and Central Africa where population of cattle and wild African buffalo overlap the epidemiology is complicated by presence of strain of *T. parva, T. parva lawrenci* which is characterized by high mortality in cattle. In Ethiopia, there are no reported cases of bovine tropical theileriosis (*T. annulata* or East Coast fever (*T. parva*)

DIAGNOSIS

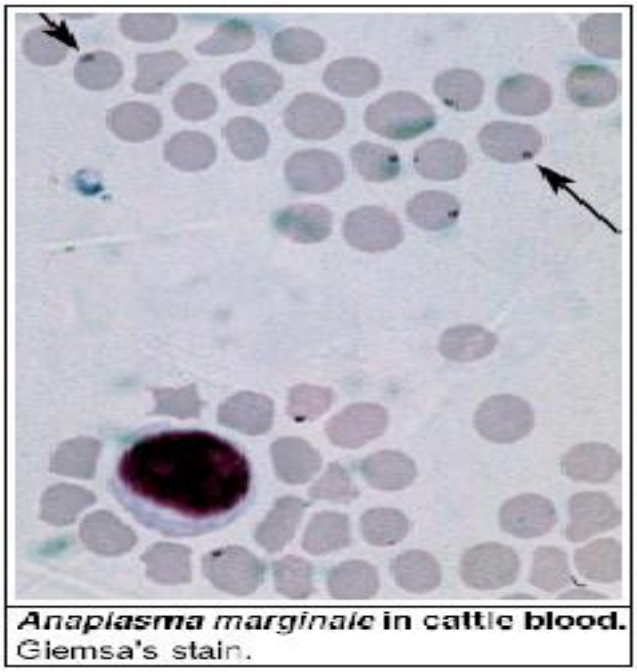
- Detection of schizonts in biopsy smear from lymph nodes and spleen of dead animals
- Giemsa-stained blood smears show piroplasms in the RBC
- Serology: The indirect fluorescent antibody test (IFAT) is used to detect AB in cattle which have recovered from East Coast Fever, AG detection- PCR and ELISA
- Tetracycline if effective at the time of infection but has no value to treat clinical cases, effective drugs are: anti-coccidial such as halofuginone and others as naphthaquinone compounds such as parvaquone and buparvaquone
- Among control methods: restriction of cattle movement during known outbreak of East Coast Fever, prevent access of nomadic cattle and buffalo, treating animals against ticks,
- vaccination with *T. parva* strain and at the same time treating with long acting TTC had shown to be effective preventive measure in endemic areas.

ANAPLASMOSIS [GALL SICKNESS]

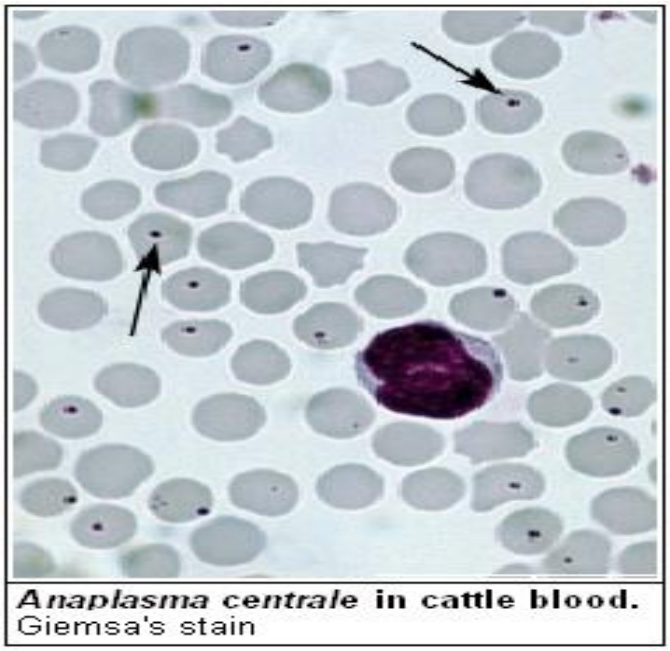
Anaplasmosis is a disease caused by a rickettsial parasite of ruminants, *Anaplasma* spp and is therefore related to rickettsial disease. The microorganisms are Gram-negative, and infect red blood cells. They are transmitted by natural means through a number of haemtophagous species of ticks. The *Ixodes* tick that commonly transmits Lyme disease also spreads anaplasmosis. The disease is also transmitted by a variety of bloods sucking insects, particularly horse fly, *Stomoxys spp* and ticks or mechanically by biting insects or even during blood transfusion or contaminated hypodermal needles or surgical instruments. The disease occurs in tropical and subtropical regions. Hosts: Cattle, wild ruminants, and perhaps sheep, may act as reservoir of infection.

IDENTIFICATION

Main species: *Anaplasma marginale* and *A. centrale*, distributed in tropical and subtropical countries. In properly stained thin blood smear *A.marginale* bodies appear as spherical granules measuring 0.2 to 0.5 microns near periphery of the cell. The bodies have slight haloes surrounding them. In Giemsa stained blood films *A. marginale* are seen as small dark red inclusion bodies within the red cell. Often only one organism in the cell localized marginally and centrally for *A. centrale* (mild pathogenic)



***Anaplasma marginale* in cattle blood.** Giemsa's stain.



***Anaplasma centrale* in cattle blood.** Giemsa's stain

Multiplication and transmission

Once in the blood, the organism inters the red cells by invaginating the cell membrane forming a vacuole, thereafter they divide to form an inclusion body containing up eight 'initial bodies' packed together. The inclusion bodies are numerous during acute infection. Transovarian infection is known to take place but there is a little information on the development of the parasite in the ticks. *Anaplasma* multiply by extracellular binary fission in the lumen of the digestive tract or the malpighian tubules in the ticks' body. They do not grow within the digestive, salivary system or in other cells of the ticks. They can only infect the ruminants through excreta in the attachment lesion during the blood meal. The disease is transmitted from one instar to another but a transovarian infection is considered doubtful.

PATHOGENESIS AND CLINICAL SIGN

After incubation period of around four weeks, fever and parasitaemia appear. The hyperthermia that signals the beginning of the disease lasts for 1-3 weeks. The body temperature reaches up to 40-41 c. The animal walks with unsteady gaits, panting along with salivation drops rapidly. Atony of rumen is revealed by chronic constipation. The continuous haemolysis causes anaemia and emaciation. During parasitaemia, the anaemia becomes more sever, within a week up to 70% of erythrocytes are destroyed.Necropsy: jaundiced carcass, a gross enlarged gall bladder, on section liver is suffused with bile, the spleen and lymph nodes are enlarged, unlike that of Babesiosis the urine is normal in colour. Cattle aged 2-3 years develop typical often fatal anaplasmosis. The major clinical signs include pyrexia, haemolytic anaemia, often jaundice, anorexia, laboured breathing, in cow sever drop of milk yield and abortion. Par-acute forms kill the affected animal in a day showing only severe hyperthermia and nervous signs.

EPIDEMIOLOGY

- Reservoirs of infection are carrier cattle, wild ruminants and sheep
- Adult cattle introduced to endemic area are very susceptible, mortality being up to 80%
- Susceptibility increases with stress or by other diseases such as babesiosis
- Infection by *Anaplasma marginale* is widespread in Ethiopia as its major vector *B. decoloratus.*

- Serological studies carried out in different ecological zones in the country showed that the prevalence of antibodies against *A.marginale* in state and private dairy farms was 99% but only few showed parasitaemia in thin blood smear examination.
- The impact of this disease on the livestock industry in the nation is highly negligible

DIAGNOSIS; Clinical sign supplemented with haematocrit estimation and demonstration of Anaplasma inclusions in the RBC. Detection of immune carriers by complement fixation and serum agglutination tests are used.

Cowdriosis (Hertwater): Is the most important tick-born disease of erxotic and cross bred cattle. *C. ruminantium* is a bacterial gram negative coccal pathogen causing the tickborne disease "Heartwater" in ruminants in Subsaharan Africa. Animals often acquire the disease when moved on to heartwater infected grazing. It is an intracellular bacteria, residing in endothelial cells and affecting the cardiovascular, respiratory and neurological systems. Cowdriosis has a huge economic impact in Africa, both in direct losses and as an obstruction to the improvement of breeding stock due to the susceptibility of introduced high producing breeds.

In 1992 a devastating outbreak has occurred in in Ethiopia Abernosa ranch where Boran X Friesian crosses are kept and was caused by *C. ruminantium.* Outbreaks have occurred in some dairy farms with a mortality rate of 25%. The disease is characterised clinically by pyrexia, nervous signs, diarrhoea and death and; at necropsy by hydropericardium, hydrothorax and oedema of the lungs and brain.

Epidemiology

Heartwater is endemic in many parts of Africa affecting both domestic and wild ruminants. The disease has been reported in Madagascar, Nigeria, Kenya, Tanzania, South Africa, Reunion and Sao Tome islands. The disease is transmitted by *Amblyomma spp* ticks. In most parts of Africa *A. variegatum* is the principal vector. *A. hebraeum* is an important vector of the disease in the Republic of South Africa while *A. gemma* and *A. lepidum* have been reported to be involved in the transmission of heartwater in East Africa. Wild animals maintain the vector ticks and are asymptomatic carriers of the disease. Transmission occurs mainly trans-stadially whereas, transovarial transmission rarely occurs. Increased tick activity which is associated with increased humidity especially during the rainy season may be associated with increased incidence of the disease. Outbreaks occur when susceptible animals

from tick-free areas are moved into endemic areas. Different strains of *C. ruminantium* with differences in virulence exist and animals which are immune to one strain may succumb to others. Kids and lambs possess innate resistance in the first week of their life although sometimes they may succumb to a clinical disease. Goats are more susceptible to heartwater than sheep and the Angora goats are particularly very susceptible. Black Head Persian sheep posses some degree of natural resistance to the disease.

Pathogenesis

The organisms are introduced in the body through the saliva of the infected ticks. Initial multiplication in the regional lymph nodes is followed by colonisation of the endothelial cells of blood vessels in all organs. *C. ruminantium* has a distinct predilection in the endothelial cells of the brain cells. Invasion and colonisation of the endothelial cells causes vascular damage resulting in increased vascular permeability, transudation and effusion of fluids into the body cavities and subsequent development of oedema and hypovolaemia. *C. ruminantium* also produces an endotoxin which, together with increased cerebrospinal fluid pressure are considered to be involved in the pathogenesis of the brain lesions. Progressive pulmonary oedema and hydropericardium results in asphyxia and cardiac insufficiency which terminate into death. Severe renal ischaemia and nephrosis has been reported to occur in goats. The severity of the disease varies depending on the virulence of the infecting organism, breed, age and immune status of the host.

Clinical features

The incubation period of heartwater in goats and sheep is 1-5 weeks and the course of acute disease takes 3-6 days. A peracute, acute, subacute or chronic disease may occur. The peracute syndrome is characterised by sudden death without premonitory signs. Sometimes, animals with a peracute disease may exhibit high fever, prostration and paroxysmal terminal convulsions. The case fatality rate in peracute cases is 100%. The acute disease is characterised by pyrexia, dullness, anorexia and nervous signs. The nervous signs include unsteady or high-stepping gait, ataxia, circling or galloping movements, chewing movements and aggression. Other signs include dullness, bleating, nystagmus, twitching of the tail, blindness, opisthotonus, droopy ears, lowering of the head, increased urination

and forced respiration. Convulsions, prostration and lateral recumbency are observed in terminal stages of the disease. The case fatality rate of the acute disease is 50-90%.

Subacute heartwater develops in 7-10 days and the clinical signs are less pronounced than in the acute syndrome. They include listlessness, inappetence, loss of weight and hair/wool, recumbency, fall in temperature and ruminal atony. The chronic disease which is common in indigenous breeds of goats and sheep may be characterised by transient fever. Natural recovery and subsequent re-infection may be observed.

Pathological features

Little changes are seen in the peracute disease although petechial haemorrhages may be observed in the endocardium and pericardium. The gross pathological features of the acute disease include ascites, hydrothorax and hydropericardium. The fluid in the pericardial sac is turbid, light yellow and clots on exposure to air. Congestion of the liver and distension of the gall bladder occur. Subserosal haemorrhages, splenomegaly and lymphadenopathy are common features. The trachea and bronchi are filled with a serofibrinous foam, congested and have petechiated and ecchymotic mucosae. The lungs are oedematous and a frothy fluid exudes from the cut lung surface. The mediastinal and bronchial lymph nodes are oedematous. There is also oedema of the brain and, the meninges are swollen, congested and odematous. The choroid plexus is dull greyish in colour.

At histopathology there is perivascular infiltration of organs/tissues with macrophages and lymphocytes. Nephrosis, perirenal oedema and petechiation of the renal cortex occur. Lesions in the brain include foci of necrosis and microcavitation in the cerebral cortex, oedema of the axon sheaths, necrotic degeneration and formation of PAS positive granules and globules in the cytoplasm of neurocytes and accumulation of the latter in the perivascular space. Parasitised endothelial cells are distended. *C. ruminantium* colonies can be demonstrated in the cytoplasm of endothelial cells of the brain, lungs and kidneys. At clinical pathology there is oesinophilia, neutrophilia, lymphocytosis, lowered packed cell volume and haemoglobin concentration and, normocytic, normochromic anaemia.

Diagnosis

Presumptive diagnosis can be based on the epidemiological, clinical and pathological features. Demonstration of *C. ruminantium* in the cytoplasm of the endothelial cells of blood vessels or in Giemsa-stained smears of lymph node or brain biopsy samples is confirmatory. In dead animals, the organisms can be demonstrated in brain crush smears prepared from the hippocampus or cerebral cortex. The brain crush smears are air-dried, fixed in methanol for 1 minute and then stained with 10% Giemsa for 30 minutes or 50% Giemsa for 10 minutes. *C. ruminantium* appear as clusters of bluish-purple to reddish-purple cocci in the cytoplasm of vascular endothelial cells. The organisms may also be demonstrated in histological sections of the endothelial cells of renal glomeruli or capillaries of the grey matter of the cerebral cortex.

Other methods of diagnosis include the inoculation of blood from suspected animals in susceptible animals in which reproduction of a clinical disease is a positive diagnosis. Indirect fluorescent antibody test and ELISA are the common serological methods of diagnosis.

Sudden death in peracute heartwater should be differentiated from anthrax and while the nervous signs can also occur in bacterial meningoencephalitis, tetanus, plant and heavy metal poisoning. Demonstration of the causative organisms is necessary in order to differentiate the disease from anthrax, tetanus and bacterial meningoencephalitis. The nervous symptoms observed in tetanus are more severe than those encountered in heartwater. A recent grazing history and presence of poisonous plants or heavy metal contaminants in the animals' environment can be highly suggestive of poisoning. Hydropericardium and hydrothorax may also be observed in bluetongue and pulpy kidney disease. Bluetongue is can be differentiated by the mouth and feet lesions and hydropericardium is not a feature. Virological and serological tests can also be used to differentiate heartwater and bluetongue. Characteristic lesions in the kidney and isolation of C. *perfringens* type D and demonstration of the epsilon toxin will confirm pulpy kidney disease.