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PREFACE

The present volume Herpesvirus Diseases of Cattle, Horses and Pigs in the series "Developments in Veterinary Virology" gives a review on herpesvirus infections in (a) cattle by bovine herpesvirus 1 (BHV-1), BHV-2 and BHV-4, alcelaphine herpesvirus 1 (malignant catarrhal fever) and Aujeszky's disease virus, (b) horses by equine herpesvirus 1 (EHV-1), EHV-2 and EHV-3 and (c) pigs by Aujeszky's disease virus and porcine cytomegalovirus. Some of these viruses also infect small ruminants, therefore sheep and goats are included in this review as far as they are concerned.

The different chapters include the latest knowledge on the viruses and the resulting diseases. Bearing in mind the rapid development of molecular biology and genetechnology in the last years a comprehensive survey on the molecular aspects of the viruses and genetically engineered vaccines is presented, as far as data have been available. However, the other fields have not been neglected. Large space is given to the description of clinical symptoms, pathology, pathogenesis, latent infection, physical, chemical and biological characteristics of the viruses, humoral and cell-mediated immunity, vaccines and vaccination, epizootiology, control, eradication, economics considerations and future aspects.

Therefore, the book does not only apply to scientists working on herpesviruses but also to the veterinary service involved in control of the diseases and to practising veterinarians, who want to improve their knowledge. All of them will find objects of their interest, and 1008 references facilitate the search for more detailed information.

I wish to thank the authors of the different chapters and all the other persons who helped to finish the book.

G. Wittmann

Tubingen

HERPESVIRUS DISEASES OF CATTLE, HORSES, AND PIGS

1

INFECTIOUS BOVINE RHINOTRACHEITIS / VULVOVAGINITIS (BHV1)

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INTRODUCTION

The present review relies upon articles published previously (1-10). Basing upon this it was the authors' intention to consider above all additional newer literature.

Characteristics of the disease

Infectious bovine rhinotracheitis (IBR) caused by bovine herpesvirus type 1 (BHV1) is a worldwide acute, contagious viral disease of bovines characterized by fever, general depression, drop in milk production and emaciation. Primarily involved are the nasal and tracheal turbinates, hence the denomination IBR, but bronchopneumonia may result from secondary bacterial infection. Abortion in infected pregnant females, further meningoencephalitis (predominantly in young calves), conjunctivitis, mastitis and enteritis may be observed.

A second syndrome caused also by a BHV1, which could not be plainly differentiated from IBR-virus biologically and antigenically, is infectious pustular vulvovaginitis (IPV, balanoposthitis in bulls). The infection with this virus leads to pustular lesions of the genital tract of males and females. IPV-virus does not exhibit such a high virulence as the IBR-virus does, and abortions due to IPV-virus are rare or inexistent.

As other herpesviruses of man and animals also BHV1 goes into latency whereby the dormant virus can be reactivated.

BHV1 causes serious economic losses all over the world due to loss of animals, abortions, decreased milk production and loss of weight.

History

In 1841 Rychner, a Swiss veterinarian, was one of the first authors to describe the clinical symptoms of IPV and its nature as a venereal disease

(11). IPV was named later "Bläschenausschlag", a term which was latinized subsequently by Zwick and Gminder (12) to "exanthema vesiculosum/ pustulosum coitale". Already in 1928 Reisinger and Reimann (13) with their filtration experiments succeeded in proving the viral nature and transmissibility of the disease. Thirty years later the virus could be isolated by American and Canadian research groups (14, 15).

To the IBR-virus infection a quite different history applies. According to McKercher (1) this disease was first observed to a limited extent in feedlots of Colorado in 1950. In 1953 it occurred in feedlots as well as in dairy herds on a large scale in California from where it spread to other states and countries. As early as 1956 Madin and coworkers successfully isolated the IBR-virus (16). The first case of IBR in Europe was notified in Germany in 1960 (17) and later on in other European countries.

IBR as a disease with a worldwide geographical distribution is also indigenous in Africa, Asia, Australia and South America. Historical facts concerning IBR were reviewed in more detail by McKercher (1, 325), Kokles (2), Yates (8), Ludwig and Gregersen (18).

CLINICAL SYMPTOMS

Reviews considering thoroughly clinical aspects of IBR/IPV were previously published by McKercher (1), Kokles (2), Gibbs and Rweyemamu (3), Kahrs (4, 7), Straub (5), Yates (8).

Respiratory disease

After experimental BHV1 infection the incubation is 2-3 days, whereas for field cases it may be probably longer, as long as a week (8, 19).

The infection concerns principally the upper respiratory tract but also the lower parts of the lung may be involved. A large variability in the severity of BHV1-infections is described sometimes accentuated by other virus infections (paramyxo-, respiratory syncytial-, adenovirus; 18) or by bacterial superinfection.

IBR is characterized by pyrexia (40,5 to 42,0°C), increased respiratory rate and persisting harsh cough, anorexia, depression, and in milking cows by a severe drop in milk production and emaciation. A clear bilateral nasal discharge develops within a day or two and the mucosa of the nares becomes hyperemic ("red nose") (3). In the early stages the profuse nasal discharge is clear but later becomes mucopurulent. Excessive salivation is noticed in some animals, but oral lesions are uncommon. Some cattle with

IBR have conjunctivitis either uni- or bilateral and excess ocular secretion changing from clear to mucopurulent as the disease progresses (20, 21).

Auscultation reveals the presence of a tracheitis, but apart from the transferred tracheal sound the lung sounds normal. The acute stage of the disease usually lasts from 5 to 10 days after which most animals recover rapidly. In approximately 10% of affected animals the respiratory form of IBR may be complicated with conditions such as secondary bacterial pneumonia or superimposed viral infections. Some of these animals die. These conditions are more likely to occur in the stressful environment of feedlots.

When the respiratory form of IBR develops in a herd that includes pregnant cattle abortions may occur after an incubation time of 3 to 6 weeks, mainly between the 5th and 8th month of pregnancy. Under field conditions about 25% of pregnant cattle may abort after an outbreak of IBR.

Occasionally in calves infection with IBR-virus may lead to non purulent meningitis and encephalitis (see below).

Experimental inoculation of IBR-virus into the bovine udder produces mastitis, and BHV1 has been isolated from cases of acute mastitis. However, BHV1 induced mastitis remains a rather rare event (19).

Disease of the reproductive system

Infectious pustular vulvovaginitis (IPV) and balanoposthitis (IPB) in the bull is observed 1-3 days after mating and leads obviously to a painful inflammation. Frequent micturition and a tail not returning to the normal position are the first characteristic signs. Closer examination of the edematous and hyperemic vulva reveals small pustules (1-2 mm in diameter) disseminated over the mucosal surface accompanied sometimes by mucopurulent vaginal discharge. Secondary bacterial infection is not an uncommon sequel. The acute stage of the disease lasts from two to four days and the lesions heal 10-14 days after the onset of the disease. Outbreaks of combined respiratory and genital disease are rare (3).

Disease of the central nervous system

Occasionally, neurological sequelae were observed in calves suffering from a BHV1 infection. The neurological signs were characterized by incoordination, muscular tremor, recumbency, aimless circling, ataxia, blindness and eventually death was not a rare event (22-25). Sporadic cases of BHV1 encephalitis seem to be more prevalent in Australia and Argentina though this neurological disease exists in other countries, too

(26). BHV1 strains from Australia and Argentina exhibiting a neuropathogenic potential represent an antigenic variant named tentatively BHV1 type 3 (27-30).

Metritis

Large doses of IBR-virus inoculated into the uterus may lead to mild endometritis and temporary failure of conception.

In Belgium a virus resembling BHV1 has been isolated from uterine exudates of cows showing fever, metritis with mucopurulent uterine discharge. The source of this infection (31) could not be detected.

Metritis also may ensue from using BHV1 contaminated semen for artificial insemination (32).

Disease of the alimentary tract

Diarrhea is seldom associated with an outbreak of IBR in adult cattle. BHV1 has been isolated, however, from feces of adult cattle with enteritis and from cattle with IBR but without diarrhea (33, 34). More frequently, diarrhea can be a clinical sign of a generalized and often fatal BHV1 infection of young calves.

Disease of the skin

BHV1 can also incidentally cause dermatitis (35). Dhennin and colleagues described an ulcerative lesion in the interdigital space due to a bovine herpesvirus (36).

Mastitis

BHV1 has been isolated from cattle with mastitis (3, 19).

PATHOLOGY

Respiratory tract

Grossly the spectrum of lesions found mostly in the trachea and the nasal passages ranges from serous, hyperemic and edematous mucous membranes, through mucopurulent exudate, focal necrosis to finally pseudomembranous inflammation in severe cases (37).

Histologically a mild catarrhal inflammation with edema and neutrophil infiltration is observed, the submucosa being infiltrated with lymphocytes, macrophages, and plasma cells. In some cases also diffuse hemorrhages may be observed. With progression epithelial necrosis occurs destroying the mucociliar system leaving cellular debris on the mucosal surfaces and showing nodular mononuclear cell accumulation in the lamina propria as well as in the submucosa. The frequently seen nasal plaques result from

coalescence of discrete pustules and consist of leukocytes, fibrin and necrotic epithelial cells (4).

Scanning electron microscopy revealed that extensive loss of cilia leaving areas of tracheal epithelium covered by microvilli was the main feature. Typical herpesvirus particles were seen in ciliated cells, and tracheal lesions 4 and 7 days after infection were similar (38).

The question whether BHV1 is involved in lung lesions is still a matter of controversy. On the whole, severe pneumonia is probably due to secondary bacterial invasion, but experimental studies have shown that pneumonic lesions are partly due to viral replication (8).

McKercher (1) questions the diagnostic value of intranuclear inclusion bodies which are revealed by histologic examination, first because they are transitory in nature as observed by Crandell and second because nasal smears of experimentally infected cattle did not yield evidence of inclusion bodies (39).

Tonsillitis

Narita et al. described necrotic foci around crypts in the tonsils after inoculation of BHV1. Beside necrotizing tonsillitis also focal necrosis in the nasal and pharyngeal mucosa was observed (40)

Genital tract

Grossly we have to do with a vulvovaginitis and a cervicitis. On the vaginal and vulval mucosa in the initial stage fine pustules may be observed. Later hyperemic nodules appear which persist for approximately a week.

Histologically the pustules consist of compact focal accumulation of inflammatory cells without formation of a hollow space. The damaged epithelial cells are ballonized and their nuclei show caryolysis. At the periphery of the lesions cells with eosinophilic intranuclear inclusion bodies can be found. Fusion of such lesions results in erosions of the mucosal surface and the lesions are delimited towards the deeper layers by massive infiltrates of lymphocytes. Ordinarily there is full restitutio ad integrum of mucosal lesions (5, 7).

Central nervous systems

Carillo et al. (24) in Argentina found grossly congestion of the leptomeninges and petechial hemorrhages in the ventral areas of the brain. The most significant histopathological findings were observed in paraffin embedded brain sections. The lesions consisted of nonpurulent encephalitis

and leptomeningitis. The leptomeninges were congested and infiltrated by mononuclear cells, mainly macrophages and lymphocytes, occasionally also polymorphonuclear cells. Widespread mononuclear perivascular cuffs of variable matter. Disseminated microglial-histiocytic foci with astrocytic edema and malacia were detectable. In such foci astrocytic proliferation subsequently occurred.

Smaller blood vessels displayed necrotizing vasculitis with perivascular infiltration of mononuclear cells. Various degrees of degeneration and cell necrosis combined with neuronophagia and satellitosis were observed in many perikarya and neurons as well as astrocytes embodied eosinophilic inclusion bodies. Similar histological pictures were also described in Refs. 42-46.

Digestive tract

Wellemans and coauthors (34) described an infection of the digestive tract due to BHV1. The layers of the epithelial surface of the abomasum were lacking and were replaced by thick layers of mucus containing cellular debris. The underlaying glandular layer showed hyperplastic mucous cells. The adjacent hyperplastic connective tissue was infiltrated by lymphocytes, macrophages and a few polymorphonuclear leucocytes as well as eosinophils. In places inflammation foci coalesced to form plaques. In the jejunum villi were absent and in their place layers of necrotic cells could be observed. The adjacent connective tissue showed infiltration of lymphocytes, plasmocytes, macrophages, polymorphonuclear neutrophils and eosinophils, involving even partly the submucosal tissue. Also in the large intestine deep necrotic erosions were revealed and some eosinophilic inclusion bodies were detectable. In the liver and kidneys lymphocytic infiltrations and in the mesenteric lymph nodes necrosis was present in the germinative centers.

Abortion

The abortion results from fetal deaths. Neither the aborted fetus nor the placenta show characteristic macroscopic changes but histologically lesions are present in almost every tissue. Microscopic lesions are characterized by tiny focal necrosis and hemorrhages in many organs of the fetus. The center of the lesions often contains necrotic cells and there is a sharp demarcation between healthy and necrotic cells. Lesions in the spleen, liver and lymphnodes are to some extent infiltrated by neutrophils. Intranuclear viral inclusions may be seen in affected cells. The above changes may be

obscured by the autolysis of the fetus occurring between fetal death and abortion (5, 7).

Conjunctivitis

The conjunctiva shows hyperemia and edema as well as papilla-like white prominent lesions.

Histologically the hyperplasia of lymph follicles attracts notice. In the mucous membrane there is a heavy lymphocyte infiltration and capillaries are filled with neutrophils (5).

PATHOGENESIS

Generally speaking a local infection may be followed by a generalization. Such generalization of an infection can be caused by a viremia, by neural spread or by spread through intercellular bridges (47).

After primary infection BHV1 multiplies at the portal of entry in the mucosa of the respiratory and the genital tract. The virus is transported subsequently by monocytes and probably other white blood cells via the blood stream to the target organs such as the central nervous system, the digestive tract, the fetus, or the udder (48).

The viremia taking place is weak and transient, a phenomenon which might be due to a small number of infected leukocytes in the circulation (49).

The type of white blood cells involved in transport via the blood stream is still controversial. Rossi and Kiesel (50) demonstrated that in vitro BHV1 adsorbed on to and penetrated into macrophages but there was no replication. Nyaga and McKercher showed replication of BHV1 in monocytes and other leukocytes but in the latter cell type only after stimulation with phytohemagglutinin. The question of viremia is discussed in detail by Yates (8).

Another route of spread occurs along peripheral nerves. No literature pertaining to neural spread of BHV1 is available, but one may deduce that it behaves like herpes simplex virus (51).

After replication in mucosal cells the virus enters the neural cell at the nerve endings by fusion, and thereafter the naked viral nucleocapsids are probably transported within the axon by retrograde axonal flow to the nucleus in the body of the neuron, where latency is established. After reactivation virus particles are packaged in membranes, usually via the Golgi apparatus and transported towards the periphery, in this case to the mucosa. Two findings point to the probability that such a pathway applies to

BHV1, too. Narita et al. (40) could show that from intranasal inoculation a trigeminal ganglionitis evolves, and that on recrudescence of latent BHV1 also a trigeminal ganglionitis could be observed (52). Additionally we know that during latency BHV1 DNA can be found in neurons of trigeminal and sacral ganglia (53, 54). During this axonal spread viruses are not exposed to the neutralizing influence of antibodies. The neural route of spread probably not only plays a role in establishing latency but also in pathogenesis of meningo-encephalitis of calves (see below).

The significance of viral spread through intercellular bridges is not yet clear. Pastoret et al. (47) presume that this spreading mechanism may be important for viral propagation after reactivation because during this stage cell to cell transmission may be shielded from neutralizing antibodies (55). It may be hypothesized that a spread through intercellular bridges only plays a role in local infections and not in generalizations.

The most important local infection concerns the mucosa of the respiratory tract. In vitro experiments have shown that BHV1 could replicate in epithelial cells but also in cells of the submucosa and of connective tissue (8). In vivo, too, the epithelium of the upper respiratory tract is destroyed by the virus-induced cytopathic effect. Additionally a BHV1 infection leads to an immunosuppression with the consequence of increased susceptibility to secondary bacterial infections resulting in severe pneumonia (56-60). The BHV1 induced suppressive effect on several immune mechanisms was examined more closely by Bielefeldt Ohmann and Babiuk (61). Migration of polymorphonuclear neutrophils (PMN), natural cell-mediated cytotoxicity and mitogen responses of peripheral blood lymphocytes were suppressed as some functional activities of alveolar macrophages. In contrast superoxide anion production by PMN was transiently increased and T helper cell function (IL-2 production) was only marginally impaired.

The authors also ruled out interferon-induced suppression and suppressor cells as decisive factors in the impaired cell function. In summary the mechanisms of virus-induced dysfunction of the host immunological defense system in the respiratory tract are multifactorial.

Conlon and coworkers could demonstrate that BHV1 can cause excessive bronchoconstriction resulting in trapping of secretions in the lower airways thus impairing lung defense mechanisms and favouring bacterial growth (62).

Events leading to abortion are maternal infection, viremia, placental infection, fetal short, generalized, peracute infection and finally fetal death (63). The early embryo is highly susceptible to BHV1 and in vitro death may occur within 24 hours (64).

Bagust and Clark (26) investigated the pathogenesis of BHV1 meningo-encephalitis and could show that the virus passed to the brain from the nasopharyngeal and tonsil regions by the maxillary and mandibular branches of the trigeminal nerve. Once the virus had entered the mid-brain, generalization throughout the brain occurred and development of clinical meningo-encephalitis ensued.

IPV is a typical venereal disease giving BHV1 the chance to directly reach its target cells of the vulvar, vaginal and preputial mucosa.

Intrauterine, intravenous and intramuscular inoculation of heifers during or immediately after estrus resulted in ovarian lesions observed to a greater extent in the corpus luteum than in the stroma and follicular tissue. Such lesions could also be evoked by inoculation of commercially available vaccine strains of modified-live BHV1. In this case oophoritis was similar, and almost as severe, as the one, caused by virulent strains of BHV1. In addition adrenal lesions were induced by inoculating IBR-virus. The authors demonstrated that after intravenous and intramuscular inoculation the virus reached the ovary via the hematogenous route (65-69).

Initial BVD virus infections led to wide dissemination of BHV1 in most tissues of calves, apparently by impairing the ability of animals to clear BHV1 from the lungs (70).

LATENCY

Latency is defined as the silent persistence of the virus in the body, not detectable by conventional virological procedures, with subsequent intermittent episodes of reexcretion. This definition is taken from a recent review on latency of animal herpesviruses (71) and an earlier review dealing mainly with BHV1 latency (47). As the biological and clinical aspects of latency have been well covered in these reviews (see also 4, 7, 72, 73), they are summarized only briefly here. The molecular aspects of latency will merit a more detailed discussion later in this chapter.

Establishment of latency

After multiplication at the local site of infection, the virus enters the peripheral nervous system and is transported, presumably by retrograde

axonal transport, mainly to the trigeminal and sacral ganglia (52, and earlier references therein). Axonal entry is required as the neuronal perikarya seem to lack virus receptors (74). Other possible latency sites have also been considered, e.g. macrophages (58, 75), epithelial cells (71) and (for different herpesviruses) other parts of the nervous system such as the olfactory bulb and medulla oblongata (76). All BHV1 strains including attenuated live vaccines can establish latency, even thermosensitive (77) or thymidine kinase negative mutants (78). None of the inactivated or live vaccines presently available or undergoing clinical trials are able to completely prevent establishment of latency by a superinfecting challenge virus; some vaccines may, however, help to reduce either the incidence of latent superinfection or the amount of reexcreted virus (79, 80).

Maintenance and reactivation

According to one hypothesis, the virus is maintained in the latent state by some form of immune surveillance (81). Alternatively, a property of the host cell (differentiation; physiological state) may determine virus maintenance. A related question is whether the virus undergoes limited multiplication during maintenance or whether it remains completely static, as discussed for other herpesviruses (82).

Reactivation may occur either spontaneously or induced by natural or artificial stimuli, e.g. transport (83), parturition (84, 85), immunosuppressive treatment with glucocorticoids (77, 86-88), superinfection with another virus (90, parainfluenza 3 virus; 91, unsuccessful for pestivirus) or microorganism (92), or treatment with 3-methylindole (93). As shown for other herpesviruses, local irritation of the skin, ultraviolet irradiation (94), or cyclophosphamide treatment (95) can cause reactivation, but the latter does not seem to work for BHV1 (89). In the case of reactivation by glucocorticoids (dexamethasone), a direct effect on latently infected cells has been postulated (47), but an indirect mechanism through suppression of neutrophil and lymphocyte functions appears more likely from in vitro experiments (89a). Reactivation occurs in vitro after explantation of latently infected ganglia, detected by cocultivation with susceptible cells or by examining the maintenance medium (96-99)

Reexcreted virus appears to have unaltered biological and molecular properties (100, 101). Clinical signs during reexcretion are usually mild or nonexistent (102).

An advantage of the BHV1 latency model is that it can be studied experimentally in its natural host. For certain types of experimental work a model involving smaller animals has been sought. Conjunctival inoculation of rabbits with BHV1 appears to cause a latent infection that is restricted to the ipsilateral trigeminal ganglion and optic nerve and that can be reactivated as in cattle (103, 104).

CHARACTERISTICS OF THE VIRUS

Taxonomic status

BHV1 is a member of the family *Herpesviridae* and of the subfamily *Alphaherpesvirinae* (105, 106). The BHV1 genome belongs to group D (105), resembling that of pseudorabies virus, equine herpes virus 1 and 3, caprine herpesvirus 1 (formerly BHV6), and varicella zoster virus. Therefore, BHV1 should be subclassified in the genus *Poikilovirus* (Pseudorabies-like viruses) rather than with BHV2 in the genus *Simplexvirus* (106). The classification of herpesviruses of bovidae has been summarized recently (107).

Morphology and morphogenesis

This topic has been reviewed (3, 9), and we are not aware of any recent contributions. Briefly, BHV1 contains an icosahedral nucleocapsid (diameter 95-110 nm) consisting of 162 capsomeres (each being 12 nm long by 11.5 nm wide with an axial hole of 3.5 nm). The nucleocapsid is surrounded by an electron-dense zone, called the tegument, and by the bilayer of the envelope, forming rather pleiomorphic virions of 150-200 nm diameter. Like other herpesviruses, BHV1 penetrates the host cell by fusion with the plasma membrane and entry of the nucleocapsid; replication occurs in the nucleus; newly assembled nucleocapsids acquire their envelope from the inner lamella of the nuclear membrane, from cytoplasmic membranes, or from the plasma membrane. Tunicamycin blocks transport of viral glycoproteins (gI and gIII) to the cell surface; glycosylation seems to be required for production of infectious virus (108, 109).

Antigenic relationships within BHV1 isolates

In cross-neutralization tests, BHV1 isolates exhibit only one serotype, regardless of their origin from IBR or IPV cases. Indeed, the identity of the IBR and IPV viruses was first demonstrated by serological means (110, 111). Ever since that time, criteria have been sought that would allow to differentiate between virus strains isolated from the respiratory and the

genital forms of the disease. The search was based on the precedent that herpes simplex virus has two antigenic types correlated with different clinical entities. The early literature on BHV1 (reviewed in 3, 9) is about equally divided between those authors that could detect antigenic differences between IBR and IPV strains (e.g. 112, 113) and those that could not (e.g. 114, 115).

At present the tools are available to subdivide BHV1 strains into the five subtypes 1, 2a, 2b, 3a, and 3b according to their molecular properties. The tools are restriction endonuclease analysis on the one hand (Table 1) and selective reactivity of monoclonal antibodies and viral protein patterns on the other (Table 2), discussed here only as far as they pertain to virus typing; more detailed information can be found in "Molecular aspects of the virus". The tables are fairly self-explanatory. Table 1 contains only those features of the restriction endonuclease maps, e.g. fragment size differences or location of cleavage sites, that permit to assign a subtype to a given strain. Similarly, Table 2 contains a selection of monoclonal antibodies that we have found useful for typing. Among the monoclonal antibodies produced in other laboratories, one may also expect a few that are subtype specific, but these would need to be defined using reference strains. The differences in the protein patterns are clear-cut and lend further support to the proposed classification scheme. It would be too cumbersome, however, to rely on protein patterns alone for subtyping of new strains.

Table 3 illustrates with a list of a few selected reference strains that it remains impossible to establish a strict correlation between the clinical origin of BHV1 isolates and their molecular subtype. A partial correlation seems possible, in particular for the subtype 3a and 3b strains which all exhibit neuropathogenic potential, although subtype 1 strains may also occasionally exhibit this property (130). Furthermore, the subtype 1 and 2 strains tend to fall into groups defined earlier as "IBR-like" and "IPV-like", respectively (9, 10, 102, 131). However, the latter names should be discontinued, as there are many exceptions to this rule. In our view, the difference between subtypes 1 and 2 may primarily reflect the evolutionary history and the epidemiology of the virus (i.e. the old European strains vs. the more recent North American strains) rather than the clinical entity.

Antigenic relationships with other herpesviruses

The most convincing relationship has been observed between BHV1 and CapHV1, using cross-neutralization and crossed

Table 1. Definition of BHV 1 subtypes by restriction endonuclease analysis.

Restriction endo-nuclease $) Subtype	HindIII Nr. of sites total	frag.I size (kb) §	frag.K/L size (kb) (cf. Fig.2)	EcoRI Nr. of sites total ‡	site † at m.u. .113	HpaI Nr. of sites total	site at m.u. .022	.404 +.543	.489	BstEII Nr. of sites total	frag.G size (kb)	BamHI Nr. of sites total	site at m.u. .288	.570
1	11-12	12.0	8.5-8.8	4 + 2	+	6	-	+	-	7	8.75	8	-	-
2a	13	12.0	7.25	3 + 2	-	?	?	-	+	7	8.5	9	+	+
2b	14	10.9	7.25	3 + 2	-	6	+	-	+	7	8.5	9	-	+
3 *)	3			1 + 2		?				14 - 15		9 + 4		

The table is a summary of the relevant data from Refs. 28, 29, 116-122; completed by our own unpublished results.

†) Location of subtype-specific cleavage sites in map units (m.u.) according to Engels et al. (122).
‡) (4 + 2) indicates 4 sites in unique regions and a pair of sites in inverted repeats.
§) To the right of HindIII fragment I is an additional cleavage site near m.u. 0.2 in subtype 2 only, but not in subtype 1.
*) Only the number, not the location of cleavage sites is given as the subtype 3 cleavage pattern differs strongly from subtype 1/2 (At most 3 EcoRI, 2HindIII, 4 BstEII and 6 BamHI sites are shared by all 3 subtypes). Subtype 3 isolates may be divided further into subtype 3a having 15 BstEII sites and subtype 3b retaining 14 BstII sites and lacking a BstII site at m.u. 0.420.
$) Other useful enzymes for typing are PstI and Bgl II (28, 123, 124).

Table 2. Definition of BHV 1 subtypes by viral protein patterns and reactivity with monoclonal antibodies.

	Reactivity with monoclonal antibodies									size of selected viral proteins			
Nr.	1240	2002	1111	60	1238	2915 2926	2006	1624	1108	VP9	VP22	VP23	VP25
directed vs.subtype:	1	3	1	1	1	3	3	3	1				
specificity:	gI	gI	gI	gI	VP9 (82K)	gIII	gIII	gII	gIV				
subtype													
BHV 1.1	+	+	+	+	+	-	+	-	+	112K	64K	55K	51K
BHV 1.2 a	+	+	+	+	-	-	-	-	+	116-120K	61-63K	57K	51K
BHV 1.2 b												55K	50K
BHV 1.3 a	+	+	+	-	-	+	+	+	+			59K	50K
BHV 1.3 b												53K	50K
for comparison:													
CapHV 1	+	+	-	-	-	-	-	-	-				

The table is a summary of the relevant data from Refs. 28, 30, 120.

Table 3. Selected BHV 1 reference strains.

Sub-type	Strain	Isolated from	Clinical entity	Geography	Reference
1	LA	nose	IBR	US(Calif.)	16
	Cooper	nose	IBR	US(Colorado)	125
	3156	lung	IBR	Switz.	116 (lane 2)
	Jura	nose	IBR	Switz.	28
2a	Spiel	nose	IBR	FRG	126
	227	prepuce	IPV	Switz.	116 (lane 9)
	B4	vagina	IPV	FRG	127
2b	K22	vagina	IPV	US	15
	Wabu	prepuce	IPV	FRG	128
	739	nose	IBR	Switz.	116 (lane 4)
3a	N569	brain	Neurovir.	Australia	129
3b	A663	brain	Neurovir.	Argentina	24

immunoelectrophoresis (28, 132). Interestingly, the relationship was nonreciprocal, as anti-BHV1 antibodies neutralized CapHV1 far better than anti-CapHV1 antibodies neutralized BHV1. The antigens common to BHV1 and CapHV1 have been identified (133) as VP 7 (gI) and the main capsid protein VP4 (see below).

Limited antigenic relationships of BHV1 have also been reported, using similar methods, with BHV2 (bovine herpes mammillitis), BHV4 (the "Movar" type), as well as with PRV (134-137). In our experience, none of these viruses caused false positive results in diagnostic tests of BHV1 (138-140) despite one report to the contrary (141). The relationship with deer and other wild ruminant herpesviruses will be discussed in "Epidemiology".

Physico-chemical properties

The buoyant density of the virus is d = 1.249 - 1.254 in CsCl (142), d = 1.22 in potassium tartrate (143), and d = 1.21 in 20-65% sucrose (144); its sedimentation coefficient is 1680 - 1830 S (145). The physical : infectious particle ratio is about 1 : 200 (146). The resistance of the virus to physical and chemical agents, as well as its survival in natural environments, has been reviewed in detail (3); the following findings may be added: Some BHV1 strains seem more resistant to ether than might be expected from their enveloped nature (147). Some common inactivation procedures (beta-propiolactone, formalin, heat, ultraviolet light) have been evaluated on BHV1 with a view to vaccine production (148). The influence of temperature, relative humidity, and admixture of nasal secretions on BHV1 stability has been reexamined by Elazhary et al. (149), and the effect of ozone by Bolton et al. (150). Recently the virus has been reported to be stable at room temperature for several days in extended semen, presumably due to a protective effect of added proteins (151).

Biological properties

Host Range. Although cattle are the primary host of BHV1, other animals can be infected naturally or experimentally (3). Thus, *mustelidae* (ferrets, minks; 152), and rabbits (103) can serve as experimental animals. Earlier reports that pigs may be susceptible to BHV1 have been confirmed (140, 153-156), similarly for sheep (157, 158), goats (124), and wild ruminants (see "Epidemiology"). Mice, rats, guinea pigs and chick embryos are not susceptible.

Virus propagation. The virus exhibits a broad host range in cell culture and can be grown to titers of up to 10^9 pfu/ml in a day. Optimal growth is

observed in primary cultures of fetal bovine cells (kidney, testicle, lung) and in established cell lines such as Madin Darby or Georgia Bovine Kidney (MDBK or GBK), Bovine Turbinate (BT), RK-13 (rabbit), MPK (minipig), and Mink Lung (ML). Poorly or not susceptible cell lines include HeLa, BHK 21, PK 15, and mouse L cells (for a more extensive list see 3, 9, 159, 160). The virus induces a focal cytopathic effect with rounding of the cells and subsequent lysis. Infectious particles are quantified by plaque assay or limiting dilution assay. The virus has been reported to transform mouse embryo fibroblasts (161).

Biological differences among virus strains. Numerous groups have attempted to correlate biological properties of virus strains observed in vitro with clinical manifestations. Apart from the differences in DNA and antigens already mentioned above, host cell range, cytopathic effect, growth at elevated temperature, kinetics of serum neutralization and plaque reduction, and plaque morphology have been investigated (162, 163), with the general conclusion that these did not represent useful criteria to distinguish between strains. In vivo, clear virulence differences could be observed between strains, e.g. the Strichen and Colorado strains on the one hand and the relatively less virulent Oxford strain on the other hand (164). The distinct neuropathogenic potential of the subtype 3 strains has already been mentioned; additional literature may be found in Refs. 24, 27, 165, 166.

MOLECULAR ASPECTS OF THE VIRUS

The Genome

The linear double stranded BHV1 genome consists of approximately 135,000 to 140,000 base pairs. Present size estimates are based on restriction endonuclease maps; their range may in part reflect the properties of individual BHV1 strains, in part minor differences in methodology and interpretation. Thus, subtype 2b strains are characterized by a 1000 bp deletion in HindIII fragment I relative to subtype 2a strains (122). The DNA size of strain K22 (subtype 2b) has been estimated to be 138.7 kb, based on HindIII, EcoRI and HpaI maps originally established by J.Skare (117, 167). This is equivalent to a molecular weight of 91.5×10^6 rather than 85.5×10^6 as stated in that paper, assuming that the average molecular weight of a nucleotide is 330 (168). Independent size measurements of the same K22 strain, but based on HindIII, EcoRI and BstEII maps (122) give an average

size of 138.8 kb, which happens to agree even better than may be expected from the accuracy of the method.

In other instances there is some disagreement. For example, the same maps of J.Skare have been summarized elsewhere (9) with a reported molecular weight of 88 x 10^6 for strain K22 DNA, equivalent to about 135 kb, a value that is frequently cited in the literature (see e.g. Ref. 169). Analysis of an IPV isolate similar to strain K22 (121) gave diverging molecular weights: 92.5 x 10^6 from the HindIII map, in good agreement with the data cited above, but only 83 x 10^6 and 81.3 x 10^6 from the EcoRI and BamHI maps, respectively, perhaps due to underestimation of the largest fragment sizes. The Cooper strain (subtype 1) seems to be slightly smaller (136.9 kb) than strain K22 (117), but two other subtype 1 isolates (strains LA and 3156; 122), having 138.7 and 139.1 kb do not exhibit this size reduction.

As shown in Fig.1, the BHV1 genome exhibits the typical arrangement of group D herpesvirus DNA: it is composed of a unique long segment U_L (104 kb), and a unique short segment U_S (11 kb) flanked by inverted repeats IR_S and TR_S (2 x 12 kb). The lengths of the inverted repeats, measured by electron microscopy (122) are 12.15 kb for strain K22 and 11.45 kb for strain LA, in excellent agreement with the lengths calculated from the restriction maps of the K22 and Cooper strains (117). The lengths of U_S, also measured by electron microscopy and confirmed by calculation from the maps, are 10.5 kb for the K22 and Cooper strains, and 11.6 kb for the LA strain. Similarly, strain N569 (subtype 3) has 11.5 kb for IR_S and TR_S and 11.0 kb for U_S.

The genomic termini and the junction between U_L and IR_S from virion DNA, as well as the fused genomic termini from replicative form DNA have been cloned and sequenced (169). Fused genomic termini are formed initially after cell infection by circularization of unit length virion DNA. These circles then serve as templates for the synthesis, by the rolling circle mechanism, of BHV1 concatemers containing much longer than unit length DNA, and therefore also having fused genomic termini. This fusion is illustrated in Fig.2 which displays additional features of the BHV1 short genome segment discussed below. During maturation of virions, the concatemers must be cleaved to unit length by a hypothetical terminase. A model describing this process has to account for the observation that U_S located between IR_S and TR_S can invert relative to U_L, whereas U_L remains fixed.

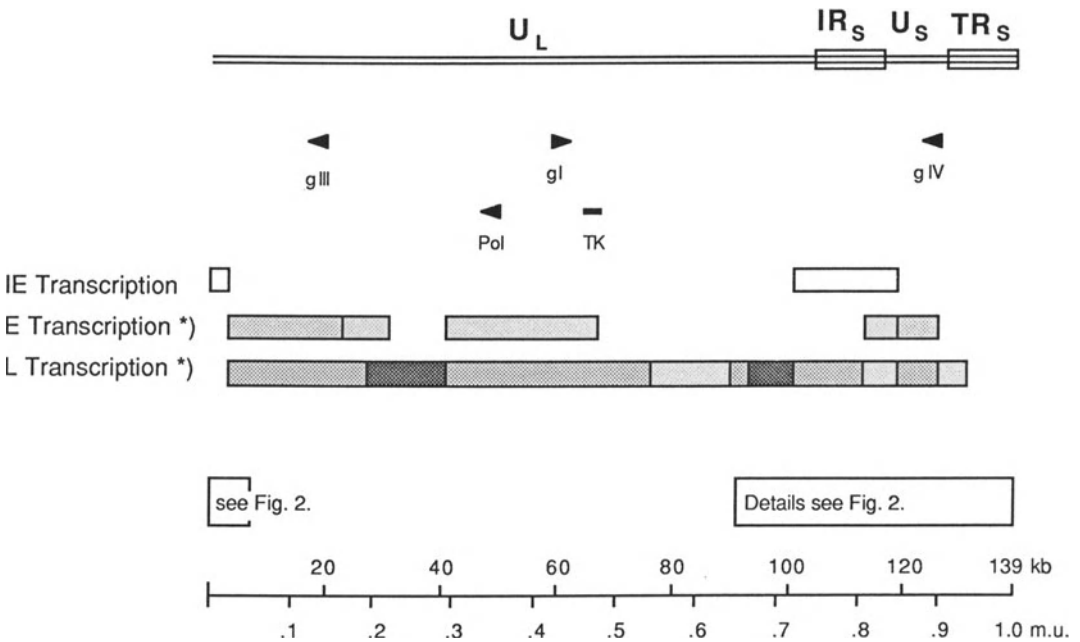

Fig.1. Landmarks on the BHV 1 Genome (see Text).

*) Areas shaded with different intensities as a rough guide to transcriptional activity. The darkest areas correspond to "true late" transcription dependent on viral DNA synthesis.

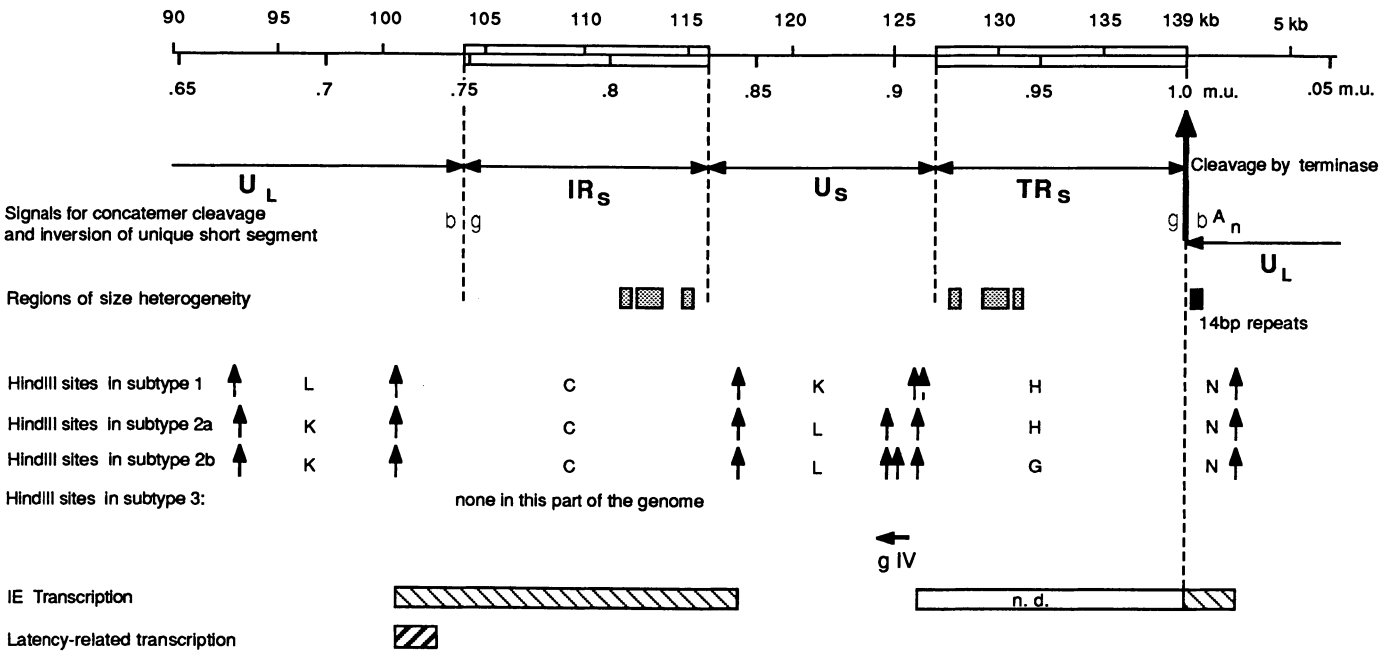

Fig. 2. Features of the S segment of the BHV 1 genome and adjoining regions of the L segment as they would appear in a head-to-tail replicative concatemer

▒ Size heterogeneities detected by electron microscopy (Hammerschmidt et al., submitted)

▮ Variable copy number of 14 bp repeats (127)

▨ Transcriptionally active regions in cycloheximide-treated cells detected by "Northern blotting" (170, and unpublished)

n.d. The terminal repeat has not been tested but is likely to exhibit IE transcription. For other details see text.

Examination of the sequences and comparison with the corresponding regions of other herpesviruses revealed a putative recognition site for the terminase, termed A_n-element, in BHV1 represented by the sequence GAGAAAAAAAAAA located at position 29 to 41 from the left genomic terminus. It was further inferred that the actual cleavage by the terminase occurred after the first 3.5 bp (thus producing a single 3'-base extension) of a 28 bp segment spanning the TR_S - U_L fusion, termed a β-element. Although the same β-element was present at the U_L - IR_S junction, it was not cleaved because the A_n-element was missing there. Yet another recognition site, termed a γ-element, located at position 67 to 93 from the right genomic terminus, and containing a stretch of five adenosines flanked by GC-rich regions, was presumably responsible for inversion (169).

Different BHV1 strains exhibit considerable heterogeneity near the left genomic terminus due to the occurrence of a 14 bp tandem repeat that varies from 8 to 38 copies in reiteration frequency (127). Recently, similar heterogeneities have been observed electron microscopically between the IR_S and TR_S sequences of individual genomes, suggesting the presence of reiterated sequences in the inverted repeats. Furthermore, about 10 % of BHV1 DNA molecules carried at the right-hand terminus a "tail" of cellular DNA (about 40 - 300 bp) demonstrated by electron microscopy as well as cloning, and suggesting that BHV1 may recombine its DNA rather frequently with cellular DNA (Hammerschmidt et al., submitted for publication). Perhaps related to this are the observations that BHV1 DNA may be associated with nucleosomes of the host cell (171), and that genome changes after one host animal passage of BHV1 could be localized roughly to the same areas of heterogeneity (172).

The transcription of the BHV1 genome has been examined by "Northern" blotting (170, 173 and in preparation). The regions of the genome that are transcriptionally active during the IE, E and L phases of the lytic infection are outlined in Figs. 1 and 2.

Some homology (< 8 %) has been detected at the DNA level between BHV1 and PRV, either dispersed throughout the genome (137) or localized in one IR and three U_L regions (174).

Some of the more classical properties of BHV1 DNA are as follows (see the reviews cited above): Density in CsCl, 1.730 g/ml; melting point in 0.1 x SSC, 85.6°; GC-content, 71.5 % rather homogeneously distributed after shearing; sedimentation constant, 59 S; contour length, 46 μm (132).

Genes and gene products

Glycoproteins. The BHV1 genome encodes four unique glycoproteins or glycoprotein complexes, designated gI, gIII, gIV, which have been studied extensively because they are the major immunogenic components of the virus, and gII, which has received much less attention. Some of their properties are summarized in Table 4, based in large part on the work of Babiuk and collaborators, but supplemented with other data including our own.

At the time of this writing, the genes for gI, gIII, and gIV have been identified and sequenced (Zamb, manuscript in preparation, cited from Ref. 177), but unfortunately the sequences have not yet become available to the scientific community as they carry a certain economic potential. At the 12th Int. Herpesvirus Workshop in Philadelphia (August 1987), their map locations have been disclosed (Fig. 1), as well as the number of amino acids they encode (Table 4) and the presumed homology with glycoproteins of pseudorabies virus (PRV) and herpes simplex virus 1 (HSV1). This homology seemed convincing for gI (54 and 44 %, respectively), but marginal for gIII and gIV (on the order of 20 %). However, the unpublished sequences and the derived homologies may still be open for revisions. This may also account for the discrepancy in the number of amino acids in gIII, stated as 521 by the Babiuk group but as 509 by the Kit group, determined in an independent sequencing project (178). At least two other laboratories have analyzed the glycoprotein genes: that of Keil in Tübingen (personal communication) and that of Lawrence in Philadelphia. The latter has mapped the gene for gI (Cooper strain) and sequenced it (180, 180a); the former has mapped and sequenced the genes for gIV and gI (Schoenboeken strain) and cloned them in vaccinia as well as in bacterial expression vectors.

Unlike the genetic information, biochemical and immunological work on the glycoproteins themselves is freely accessible (Table 4). These studies have been greatly helped by the establishment of hybridoma cell lines producing monoclonal antibodies to BHV1 (30, 120, 180-186, 186a). The specificity of the antibodies was tested by these authors with a variety of techniques including immunoprecipitation, immunoblotting, immunofluorescence, and enzyme-linked immunoassay. In this way, the initially recognized number of 11 glycoproteins (176; actually 12 bands because the 74K band later turned out to be a doublet) could be reduced to

Table 4. Properties of BHV1 glycoproteins.

Designation (1)		earlier designations (1)	(2)	apparent MW mature (3)	precursors (1)	number of amino acids	putative glycosylation sites (6)	homology with PRV and HSV1	function	epitopes (7) †N	‡C
g I	(uncleaved)	GVP6	VP7	130K	105K, 117K	932 (8)	N-linked (6 potential sites)	gII PRV 54%	essential	6	3
	(cleaved, disulfide-linked heterodimer)	GVP11a and GVP16	VP17	74K	62K	438 (4)		gB HSV 46%		+	++
			VP23	55K		?				-	-
g II	(monomer)	GVP7	VP10*	108K	90K, 100K	?	N-linked (nr.unknown)	?	?	-	-
g III	(monomer)	GVP9	VP12	91K	61K, 69K	521 (4) 509 (5)	N + O-linked, at least 4 sites	g92 PRV gC HSV	nonessential; hemagglutinin	1	6
	(homodimer)	GVP3	VP3	180K							
g IV	(monomer)	GVP11b	VP17A	71K	58K, 63K	411 (4)	N + O-linked, at least 3 sites	g50 PRV gD HSV	essential	++	++
	(homodimer)		VP5	140K							

†N = epitopes recognized by monoclonal antibodies capable of virus neutralization.
‡C = epitopes recognized by monoclonal antibodies participating in complement-mediated cytolysis.
* using a monoclonal antibody, VP10 was precipitated together with glycoproteins VP17B and VP22A (Ref. 30).
References:
(1) Ref. 175; (2) Ref. 30; (3) Ref. 176; (4) Zamb, manuscript in preparation (see Ref. 177); (5) Ref. 178 ; (6) Ref. 108; (7) Ref. 179; (8) Ref. 180a.

the presently accepted number of four. Eight of the 12 bands can be accounted for by the monomers of the four glycoproteins, and additionally by the dimeric forms of gIII and gIV, as well as by a cleaved form of gI held together by disulfide bonds, resulting in two bands after denaturation under reducing conditions. The four remaining glycoprotein bands have not been characterized well. The band designated GVP1 (176) barely entered the gel and could represent a multimer of some of the other glycoproteins; GVP15, 20, and 21 could be underglycosylated precursor molecules (175) or cellular proteins associated with the virion envelope (184).

The ability of the monoclonal antibodies to neutralize virus in the presence or absence of complement in plaque reduction tests and to lyse BHV1-infected cells was investigated by several authors (179, 184, 184a). In the most detailed study, Van Drunen Littel - Van den Hurk et al. (179), using a competitive antibody binding assay, identified six epitopes on gI and one epitope on gIII involved in virus neutralization, whereas three and six epitopes on gI and gIII, respectively, participated in antibody- and complement-dependent lysis of virus-infected cells. Extending this work, Marshall et al. (184) tested 41 monoclonal antibodies, among them 14 directed against gIV, which had the largest proportion of neutralizing antibodies. The number of epitopes was not determined, but 9 antibodies neutralized completely and 2 partially in the absence of complement, and another 2 antibodies neutralized partially in the presence of complement. From the properties of the remaining antibodies which had a much smaller proportion capable of neutralizing virus and mostly required complement, the authors concluded that gIV is the major glycoprotein involved in virus neutralization, followed by gIII and gI in that order. They did not identify any monoclonal antibodies against gII, and the only two such antibodies that are available from other laboratories (30, 181; the former against gII of BHV1.3) do not neutralize.

Work of other authors (cited above) having tested the neutralizing activity of their monoclonal antibodies is largely consistent with the two studies summarized here. It seems surprising that gIII is not essential for the replicative cycle of BHV1 (178), yet induces neutralizing antibodies. Perhaps this may be attributed to the hemagglutinating activity demonstrated for gIII (186-188, 188a), which suggests that gIII is particularly exposed at the virion surface, and that antibodies bound to it might interfere sterically with the function of neighboring essential glycoproteins. In addition

to the four glycoproteins, a nonglycosylated virion protein (107K), presumably part of the tegument, was found to elicit monoclonal antibodies. Two of these were reported to neutralize virus (182), but this was not the case for antibodies that were identified subsequently (30, 184).

Another benefit of the monoclonal antibodies was the possibility to purify individual virion glycoproteins by immunoadsorbent chromatography and to use the products for induction of monospecific antisera in rabbits (189). Monospecific antisera have also been induced by electrophoretically purified glycoproteins (119, 184, 190), but the former technique is probably more efficient and the glycoproteins purified by immunoadsorbent chromatography seem to retain their immunogenicity better (189).

After all this work with mice and rabbits, the natural host of BHV1 was not forgotten, fortunately. Van Drunen Littel - Van den Hurk & Babiuk (191) analyzed sequential serum samples from cows experimentally infected with whole virus. By determining the levels of antibody to individual glycoproteins they found that gI induced the earliest and most consistent response, whereas antibody responses to gIII and gIV appeared somewhat later and were more variable. In an earlier report, similar conclusions had been reached with regard to gI and gIII (192). In an important step forward, Babiuk et al. (177) then used immunopurified gI, gIII and gIV, alone or in combination, as subunit vaccines in cattle. They observed a protective effect, to be discussed below, and also analyzed the immune responses to the glycoproteins. As expected, sera contained only antibodies against the respective glycoproteins with which the animals had been vaccinated. Surprisingly, gIV as a subunit vaccine seemed to induce the highest serum neutralization and ADCC titers, and gI gave marginal titers, contrary to the preceding study with whole virus.

Enzymes. The thymidine kinases (TK) specified by many herpesviruses have attracted much attention because they are a potential target for chemotherapy or attenuation of vaccine strains and provide an useful selective marker for genetic studies. TK is the only BHV1-specified enzyme that has been characterized biochemically to date (193). The BHV1 enzyme was distinguished from host cell TK by its ability to use CTP in place of ATP as the phosphate donor. Like TKs of other herpesviruses, the BHV1 enzyme exhibited broad substrate specificity and could phosphorylate the bromo-, bromovinyl-, and methylmethoxy derivatives of deoxyuridine.

Following the isolation of TK-negative cell lines (rabbit skin fibroblasts and bovine kidney cells) and TK-negative mutants of BHV1 (194, 195), the TK locus was mapped by marker rescue to a 1.1 kb Bgl II-Sal I fragment at 0.47 to 0.48 map units (195). Since the TK genes of other alphaherpesviruses map at a similar position, this assignment is more likely to be correct than the previously reported map location around 0.14 m.u. (196). The two results are difficult to reconcile and can probably not be attributed to a major rearrangement as the restriction map of the Zee strain (196) seemed indistinguishable from that of the Cooper strain (117).

In recent experiments (197), using a HSV1 DNA polymerase gene probe, cross-hybridization with a 2.5 kb region (0.334 - 0.352 m.u.) of the BHV1 genome was detected. Partial nucleotide sequence analysis (830 nt) revealed a potential homology at the amino acid level with the C-terminal third of HSV1 DNA polymerase (64 % identity). Definitive assignment of this locus must await completion of the sequence and identification of the polymerase gene, e.g. by marker rescue.

Other proteins. Like other herpesviruses, BHV1 exhibits temporal control of viral polypeptides (176), which can be grouped into at least three classes, α (immediate early), β (early; dependent upon prior viral protein synthesis), and γ (late; dependent upon viral DNA replication). According to these criteria, gI and gIV have been grouped as β-proteins and gIII as a γ-protein (198).

Estimates of the total number of virion proteins have been increasing over the years. Pastoret et al. (199) identified 21 proteins by SDS-polyacrylamide gel electrophoresis; Misra et al. (176) found 25 with the same technique, and introducing an additional dimension of isoelectric focusing they observed that at least two glycoproteins (gI and gII) and five nonglycosylated proteins (69K-35K) gave multiple spots, thus putting the total estimate at 33 proteins. Bolton et al. (200) arrived at the same number and showed that 15 of these proteins copurified with nucleocapsids, 13 were envelope-associated, and the remainder could not be assigned. Metzler et al. (28, 120), by pushing the limits of the electrophoretic separation further and by using information from immunoblotting experiments, were able to enumerate 38 proteins. Adding to the number of virion proteins that of nonstructural proteins (15 estimated by Misra; at least 5 by Metzler), a total of 43-48 virus-specified proteins may be presumed. In reality the number could be even higher, considering that the genome size

of BHV1 lies midway between that of varicella zoster virus (124,884 bp; 201) and herpes simplex virus 1 (152,260 bp; McGeoch et al. 12th Int. Herpesvirus Workshop, Philadelphia, August 1987), the two alphaherpesviruses that have been sequenced to date and that contain 67 and 70 genes, respectively.

Molecular aspects of latency

Using in situ hybridization, BHV1 DNA could be detected in the trigeminal ganglia (13 out of 23) of latently infected calves that had been inoculated 1-3 months earlier by the tracheal route (53). A positive signal was detected in only 5% of the sections. In the few neurons that were positive, the signal was restricted to the nucleus and may have represented on the order of 100 copies of viral DNA. In the same way, BHV1 DNA could be detected in sacral ganglia of latently infected calves after intravaginal infection (54). Whereas in these studies hybridization to RNA was excluded by prior treatment with NaOH, viral RNA was specifically sought in another study (202) in trigeminal ganglia of latently BHV1 infected rabbits. Viral transcripts were detected in approximately 0.3 % of all neurons and seemed to be specifically retained in the nucleus. In contrast to the preceding study, no viral DNA was found. This could be due to the different animal system used, or to a partial elimination of viral genomes between the acute phase (when viral DNA was readily detected in the ganglia) and the latent phase, or simply to a difference in sensitivity. It should be noted that the same authors did detect PRV DNA as well as RNA in ganglia of latently infected swine, i.e. in the natural host (203).

The latency-related viral transcripts have been characterized further and shown to map to a 1.9 kb region (0.734 - 0.748 m.u.) of the viral genome (204). This was the only region of the viral genome that gave a positive hybridization signal (2.4 kb at the left end of the genome and two segments in U_S, together about 1 kb,were not examined). Hybridization with a single-stranded RNA probe indicated that the latency-related RNA was transcribed in a rightward direction. This would be opposite to an immediate-early transcript of the acute infection that has been identified in our laboratory (Wirth et al., unpublished) by "Northern" blotting, S1 nuclease analysis and nucleotide sequencing, and that seems to have its 3' end at about 0.740 m.u. in the latency-related region. Thus, the situation may be similar to the latency-related anti-sense RNA that has been observed by several groups (205, and references cited therein) in HSV1-infected mice or rabbits.

However, the latency-related regions of BHV1 and HSV1 do not cross-hybridize. The functional role of these transcripts in establishment or maintenance of latency remains to be established. The following possibilities have been proposed (204): Latency-related RNA might control expression of viral genes critically involved in acute infection (e.g. interference of antisense RNA with immediate-early gene expression), it might encode a protein regulating latency; or it might be a consequence rather than a cause of the latent virus-cell interaction.

DIAGNOSTIC PROCEDURES

Clinical diagnosis

Clinical, pathological and histopathological criteria for diagnosis of BHV1 infections in all known clinical entities, as well as differential diagnosis, have been reviewed by Gibbs and Rweyemamu (3) and Straub (5, 41).

Typical clinical signs and (histo-)pathological lesions may be observed in classical BHV1 diseases, but no real pathognomonic signs are known. In addition, a variety of atypical diseases caused by BHV1 have also been described. Thus, a confirmation of diagnosis by laboratory examinations is compulsory in most cases. Exceptions may be justified when typical IBR outbreaks accompanied by abortions or IPV/IPB cases occur in regions where the infection is endemic (3).

Laboratory diagnosis

Virus isolation: The most common technique still is virus isolation in cell culture and characterization by means of neutralization using a reference BHV1 antiserum. Primary bovine cell cultures are preferable for virus isolation (159, 160). Common virus isolation techniques have been proved to be very sensitive (206). Virus isolation, however, is also dependent on the test material and mode of sample collection, thus gauze swabs yield better results than cotton wool swabs (207).

Since dependence on cell cultures for diagnosis is disadvantageous and time consuming, various attempts have been made to overcome this problem. Electron microscopy may be a good alternative for a rapid diagnosis, but needs confirmation by immunoelectron microscopy.

Several publications deal with the antigen detection by immunofluorescent techniques (IFT) in different variations (165, 208-212). The main advantage of this technique is a rapid diagnosis, which does not

need further virus characterization. However, IFT may be somewhat less sensitive than virus isolation, mainly when nasal swabs are to be tested. Terpstra et al. (211) observed that the presence of antigen can be ascertained by IFT only if nasal discharge is serous and not mucopurulent or hemorrhagic, and samples should be fresh (212). The moment of sample collection in the course of infection may also influence the results of IFT. With decreasing virus replication, antigen detection by IFT is less reliable than virus isolation (211).

Recently the use of immunoperoxidase staining of fixed lung tissue (213) or of impression smears of brain samples and brain sections (165, 166) has been advocated. Smith et al. (213) used a BHV1 specific monoclonal antibody and an avidin-biotin-peroxidase complex kit. This staining proved to be more intense than IFT, probably because of the greater specificity of the monoclonal antibody, and resulted in a better representation of cellular details and tissue morphology. Giavedoni et al. (165) reported an indirect method using rabbit hyperimmune sera, protein A peroxidase conjugate and 3,3´diaminobenzidine with H_2O_2 as substrate and found this method superior to virus isolation. Rodriguez et al. (166) used monoclonal antibodies, rabbit-anti-mouse sera and mouse peroxidase-antiperoxidase staining as a modification and Collins et al. (214, 214a) obtained good results by establishing an antigen-capture enzyme-linked immunosorbent assay (ELISA) and a double-antibody-sandwich ELISA, using monoclonal antibodies, for the detection of BHV1 antigens in nasal swabs. For these tests only virus titers of at least 3.9 log10 TCID50 had to be present. Finally, an amplified ELISA and reverse passive hemagglutination have been reported as sensitive antigen detection procedures (215).

Special emphasis has been attributed to the detection of viral DNA in diagnostic samples by DNA-DNA-hybridization procedures in the recent years (165, 216-220). These methods included dot-blot or slot-blot (219) hybridization with denatured DNA from infected cells or purified virus DNA for test establishment (216, 217, 219) and/or DNA from clinical samples, such as nasal swab material (219), nasal epithelial cells (220) or brain (165), as well as DNA from semen samples (216, 218). Hybridization was carried out using various nick-translated recombinant BHV-1 DNA fragments, labeled with either ^{32}P-dCTP or -dATP (165, 216, 217) or ^{3}H-dTTP and Biotin-11-dUTP, respectively (219). The detection limit was found to be between 2.8 ng DNA/ml (218), 150 pg (216) and 10 pg DNA (217,

219), respectively, using radioactively labeled probes. Dorman et al. (219) found biotin-labeled probes to be less sensitive with a detection limit of maximally 100 ng DNA. The true diagnostic value of these methods still remains to be determined, and improvement is necessary.

The detection of BHV1 in semen needs a special note, since seminal plasma has been shown to be toxic for cell cultures and to contain virus neutralizing activity (32, 151, 216, 221-223). In addition, the elimination of BHV1 infected bulls from artificial insemination centers and commercialization of semen free of BHV1 is more and more demanded, and therefore safer tests for the detection of infected semen have to be established. Several variations of cell culture techniques, with modifications of semen preparation and treatment have been published. These include dilution of semen, extensive washings after adsorption, centrifugation steps and trypsin- or kaolin-pretreatment of semen to eliminate toxicity (32, 216, 221-224). Several methods proved to be useful, but sensitivity remained unsatisfactory. Drew et al. (151) reported that BHV1 remains relatively resistant in semen under various storage conditions. But virus detection may be less effective when milk was used instead of egg yolk citrate as semen extender (222). The most important problem is the fact that infected bulls do not always shed virus, and single straws made from one ejaculate may not contain virus while others do (18, 216, 222, 225-227). In order to establish more sensitive methods, Pacciarini et al. (218) and Brunner et al. (216) introduced hybridization tests for the detection of viral DNA in infected semen. Pacciarini et al. (218) found hybridization of DNA extracted from semen samples with a radioactively labeled specific BHV1 DNA fragment to be a very sensitive method. Brunner et al. (216), however, compared several hybridization methods with immuno-electron microscopy and various cell culture techniques, and came to the conclusion that the most sensitive method was a special cell culture technique, whereby semen was diluted 1:25, inoculated on cell monolayers in 25 cm^2 flasks, adsorbed for 4 hours at 37^oC and replaced with medium without washing. They detected virus concentrations of 5 TCID50 in the first and 2 TCID50 in the second passage, respectively, without encountering toxicity problems.

Serological tests: For a long time the neutralization test has been the most commonly used test for the detection of BHV1 specific antibodies, and still is the reference test in eradication programs. Various techniques and standardization criteria have been described (162, 224, 228-234). The use

of constant virus-varying serum (CVVS) or varying virus-constant serum (VVCS) tests has been investigated by House and Baker (162) and Darcel le Q. (224). They found CVVS, which is more commonly used, to be less sensitive, but by comparing all factors involved, they concluded that CVVS gives accurate results for routine diagnosis. In CVVS usually 25 - 100 TCID50 of virus are used, the serum being diluted 1:4 or remaining undiluted, and IBR titers of 1:2 (1:4 if 25 TCID50 of virus are used) are regarded as significant (224, 228, 231, 234, 235). Undiluted serum, however, may contain unspecific factors that inhibit virus and cannot be eliminated by the commonly used heat inactivation of serum (30 min, 56^{o}C) prior to use (224, 230, 236). Attempts to overcome this problem by kaolin treatment of the sera gave no satisfactory results (224, 236). Complement may enhance the sensitivity of the neutralization test (162), but is usually not used in routine diagnosis. Potgieter (237) reported that the demonstration of complement-dependent 19S globulins in an immune serum may be useful as a marker of recent infection. Bitsch (230) tested the influence of virus-serum incubation time and temperature, and found the neutralization to be most efficient with an incubation at 37^{o}C for 24 hours.

A great disadvantage of the neutralization test is its dependence on cell cultures. Alternative tests have been evaluated, such as immunofluorescent techniques (238, 239), agarose gel diffusion tests (240, 241-243), indirect and direct hemagglutination tests (187, 244-247), RIA (233, 233a) and, most intensely, various ELISA techniques (141, 248-258). Meanwhile the ELISA has replaced nearly all other serological tests, since it does circumvent use of cell cultures and has proved to be sensitive, rapid and economic, and thus is an ideal test for large scale surveys. The correct standardization is a very important point in the establishment and use of an ELISA. Standardized BHV1 ELISA kits are commercially available. Modifications of the common ELISA techniques for antibody detection have been reported (259-262). Riegel et al. (259) established a competitive ELISA on the basis of competition between serum antibody and a virus-neutralizing monoclonal antibody and found this test to be highly reproducible and sensitive. Spirig et al. (262) described the use of filter discs containing dried whole blood in the ELISA. This method was as sensitive as the common ELISA. In the course of eradication programs, requiring rapid, sensitive and economic examination of large numbers of samples, the development of an ELISA for (bulk) milk samples (260-261a)

was of great importance. The use of bulk milk samples, diluted 1:2 in commercial ELISA kits has been shown to be sensitive enough to evaluate one seropositive animal in pooled milk of five animals. This test is now routinely used in countries where eradication of the BHV1 infection is demanded (258, 263, 264).

Darcel le Q. and Dorward (265) were the first to demonstrate a skin reaction in cattle having BHV1 neutralizing antibodies, when material containing inactivated BHV1 was injected intradermally. This has been confirmed by Aguilar-Setién et al. (266-268) and by Straub (269). They found, that although cross-reactions in pseudorabies virus infected animals (267) and modification of the immune status of the animal tested (268) may occur, this test might be useful to detect seronegative, latently infected animals, to evaluate infected herds in the field rapidly, and to distinguish passively acquired from actively produced antibodies in calves. This test, however, is yet at an experimental stage and not used routinely (18, 207, 270, 271).

IMMUNOLOGY

Unspecific immune responses

Bovine interferon (boIFN). The boIFNs are, as in other species, classified into three types on the basis of cellular origin, antigenic specificity, structure and gene organization (272). IFN-gamma (also called IFN type 2 or immune IFN) is produced by activated T-lymphocytes, thereby serving as a specific immunoregulator. IFN-alpha is produced by leukocytes including natural killer (NK) cells and antibody-dependent cell-mediated cytotoxicity (ADCC) effector cells. IFN-beta originates from fibroblasts and other non-immunocompetent cells (collectively IFN-alpha and -beta are also called IFN type 1). IFN type 1 can be induced in cattle by BHV1 infection, either in nasal or genital secretions, or can be found as circulating IFN in serum after intravenous inoculation (268, 273, 274). After intranasal BHV1 infection IFN appears in nasal secretions 5 to 40 hrs post infection (p.i.), reaches a maximum at 72 - 96 hrs p.i. and persists for about 8 days (9, 268, 275, 276). Its rapid production may be responsible for a rapid local protection early in infection (268), and leukocytes at the site of an inflammatory response to viral infection acting as a local source of high levels of IFN may be most important in the recovery process (277).

Although IFN production in the course of a BHV1 infection is detectable there are conflicting experimental results concerning its effect on BHV1 itself. Several reports exist showing that BHV1 is not highly susceptible to IFN in vitro (268, 272, 278, 279), other authors claimed the contrary (58, 273, 280-282). Investigations on the in vivo effect of boIFN-alpha showed reduction of resistance to clinical disease, but without much impairment of BHV1 replication (283-285). Some indirect mechanisms/functions could be attributed to the influence of IFN: By treatment of calves with recombinant boIFN-alpha before challenge with BHV1 and Pasteurella haemolytica Babiuk et al. (283) found reduced clinical signs, number of sick days, lung lesions and weight loss, but no direct antiviral effect in the upper respiratory tract. Their results confirmed previous observations, that IFN(s) can modulate nonspecific effector functions, such as leukocyte migration, phagocytosis and release of potentially bactericidal compounds. The higher susceptibility of BHV1 infected calves to secondary bacterial infections is related to anatomical damage as well as to suppression of a variety of leukocyte functions (272, 286). In IFN-treated animals there is less immunosuppression and leukocytes can respond rapidly and clear bacteria before establishment (283). Babiuk et al. (272) and Lawman et al. (287) found that IFN treatment can either prevent systemic virus replication and thereby reduce the level of polymorphonuclear neutrophil (PMN) paralysis, or alternatively it may activate PMN. Besides affecting neutrophil and monocyte functions, IFN has been shown to mediate enhancement of natural cell-mediated cytotoxicity (283, 288). Rouse et al. (289) could show that bovine PMN themselves are capable to produce IFN, leading to PMN-mediated cytotoxicity (reviewed in 290). When enriched populations of PMN were added in the presence of antiviral antibody to BHV1-infected cell cultures, a marked inhibition, but not a complete virus elimination, was found, which was not due to ADCC, since inhibition was also present when using IgM or $F(ab')_2$ fragments, or when separating effector and target cells by a 0.45 µm pore-membrane. This IFN differed in several aspects from other known IFNs and it was concluded that the IFN produced by bovine PMN may be unique. Another salient killing mechanism involves release of toxic cationic proteins from neutrophil granules, which are known to kill gram-positive and gram-negative bacteria, fungi, helminths and tumor cells. Thorne et al. (291) could demonstrate the activity of such lysosomal cationic protein from bovine neutrophils in ADCC against BHV1 infected target cells.

Macrophages. Bovine alveolar macrophages (BAM) are known to be susceptible to BHV-1 infection in vitro (58, 292), and their functional capacity is thereby altered (56). About 5% of cells in culture have been demonstrated to express viral antigen after infection (58, 293), but less than 0.1% of lung lavage cells from experimentally inoculated calves were found to be infected (57, 292). Pretreatment of BAM with recombinant boIFN-alpha1 in vitro resulted in an increased resistance to infection with BHV1 and in an increased extrinsic antiviral activity of the cells as expressed by inhibition of spread of BHV1 in MDBK cells and by ADCC (292). It was concluded that the primary function of IFNs in recovery from viral infection might be to protect macrophages from those viruses capable of infecting them. Such cells then in turn can act as the principal mediators of specific and non-specific processes. Various factors interact in the macrophage-mediated defense mechanisms. Exogenous boIFN-alpha can modulate macrophage activities in rendering them resistant to infection, but also in lending them the capacity to prevent virus spread (292). Bovine macrophages are able to produce IFN themselves and, additionally, they express Fc-receptors, allowing their participation in ADCC (268). However, only a subpopulation of BAM from normal calves express Fc-receptor activity. After boIFN-alpha1 exposure a numerical increase of receptors per cell as well as an increase in cells bearing Fc-receptors was found (292), thus explaining the increased ADCC activity. Macrophages also display cytostatic activity, and, since BHV1 replicates better in rapidly dividing cells, this effect may additionally be involved in resistance to viral spread (292).

NK cells. NK cells lacking surface immunoglobulin, C3 receptors and phagocytic activity have been described (294). These cells are antibody-independent and their activity may be increased by IFN. NK cells are also found in cattle (288, 295). Following infection with BHV1 a transient increase in NK activity of peripheral blood leukocytes (PBL) was observed (61). By pretreatment of PBL with exogenous boIFN the decrease in NK activity, which occurs usually due to immunosuppression after a BHV1 infection, could be prevented or at least diminished (288). In the bovine the NK activity is exhibited by cells closely related to the mononuclear phagocyte system, and monocytes have been found to be highly cytotoxic (288) . Exposure to IFN may enhance the stimulation of monocyte production and/or prevent the differentiation of monocytes into mature macrophages, thereby preventing virus spread by killing virus-infected cells. Rouse (290) postulated that PMN

could also act as elicitors of NK cell activity by the production of the PMN-specific IFN after stimulation by virus-infected cells. He suggests that this IFN, added to peripheral blood mononuclear cells, would initiate the maturation of NK precursor cells to active NK cells. A further unspecific cytotoxic activity in BHV1 infections can be attributed to the PMN: the complement-dependent neutrophil mediated cytotoxicity (CDNC) (290, 294, 296, 297). The underlying mechanism is activation of complement by the alternative pathway due to infected cells. The PMNs then bind to the infected cell by their C receptor and mediate cytotoxicity (290). Macrophages were also found to act similarly but far less efficiently, and lymphocytes, in this respect, were totally ineffective (290, 294).

Specific Immune Responses

Humoral antibodies and antibody-dependent cytolysis. The role of humoral antibodies is questionable, concerning prevention of virus spread, since they appear too late after primary infection (4, 268, 277), and since BHV1, like other herpesviruses, can escape their activity by spreading through intercellular bridges and by neural spread (47, 268, 277). Systemic humoral immune response relies upon serum antibodies of the immunoglobulin (Ig) classes IgM and IgG (268). The production of antibodies to BHV1, as detected by virus neutralization, begins approximately 8 to 12 days p.i. and may persist for at least 5 1/2 years (3, 298), but the persistence requires occasional restimulation (4). As has been shown by Rossi and Kiesel (299) IgM antibodies are the first to appear, followed by IgG antibodies. During the first month p.i. both require complement for viral neutralization, IgG antibodies becoming complement-independent and predominate in anamnestic responses. Guy and Potgieter (300) examined the kinetics of antibody formation after primary and secondary inoculation of BHV1, and after BHV1 induced abortion. They found IgM and IgG antibodies to appear 7 days after the primary infection. In non-pregnant animals maximal IgG titers were reached at 35 days p.i., in pregnant animals at 14 days p.i., whereas maximal IgM activity was found at 14 days p.i. in both groups. In pregnant animals the IgG antibody activity was restricted to the IgG1 subclass. Secondary infection was characterized by an anamnestic IgG antibody response, primarily of the IgG2 subclass. Reexposure by intranasal inoculation elicited no secondary IgM response. IgM antibody response can therefore serve as an indicator for a recent primary infection, and primary immune responses may be differentiated from

secondary responses by the predominance of IgG1 and IgG2 subclasses, respectively. After reactivation of a latent infection an anamnestic IgG (with both IgG1 *and* IgG2) *and* IgM response was observed (301).

In fetuses humoral antibodies are rare, since fetal infection usually ends in fetal death and abortion (9). Nevertheless, BHV1 antibodies were found in commercially available fetal bovine serum pools (268, 302) and are inducible by experimental fetal infection (303). Newborn calves are provided with maternal antibodies via colostrum mainly in the first 12 hrs post partum (p.p.), and resorption ceases at about 36 hrs p.p. (9). Passively acquired antibodies, essentially of the IgG1 subclass (268), persist during 1 to 6 months, dependent on the amount of colostrum, efficiency of intestinal absorption (4) and on the maternal serum antibody titer (304). Passive immunity affords no absolute protection, but infection results in milder disease and lower lethality rates (268).

Neutralizing activity could also be detected locally in nasal and genital secretions, due to IgA class antibodies (9, 268). The activity of IgA seems to be restricted to the upper part of the respiratory tract, whereas cellular mechanisms predominate in the lower part (18). Bouffard and Derbyshire (282), however, found only marginal reduction and delay of virus replication when treating BHV1 infected fetal bovine tracheal organ cultures with "immune" nasal secretion. A direct humoral antibody mediated neutralization is only functional in case of a reinfection or of a reexcretion after reactivation of a latent infection (4,268).

The major significance of humoral antibodies lies in their co-operation in mechanisms destroying virus-infected cells.

Antibody-complement lysis. Complement-mediated destruction of antibody sensitized virus-infected cells usually seems to occur by the classical pathway with herpesviruses (277). Its role in preventing virus spread early in infection is not clearly elucidated. Babiuk et al. (305) found that viral antigens were detectable on cell membranes at 6 hrs p.i., but cells were not susceptible to antibody-complement lysis until 10 hrs p.i., when intracellular virus and intercellular virus spread was present. In contrast, Rouse et al. (1976) (cited in 3) were able to show by kinetic studies that antibody-complement lysis was functional early enough to prevent virus spread. Activated complement is able to mediate inflammatory response (277). Cellular components of the inflammatory response, e.g. macrophages and PMN, in turn mediate the recovery process. Thus, this mechanism may

at least be important in a late phase of recovery or during recrudescence in latent infections.

ADCC. This mechanism requires effector cells with Fc-receptors, usually of the IgG class, thus antiviral antibodies bind with their Fc portion to the effector cells and with their Fab portions to the viral antigens expressed on the surface of the target cells (268, 277, 294). As shown by means of chemiluminescence this process seems to depend on the antibody Fc-portion (305a, 305b). Several cell types can act as effector cells in ADCC, but in cattle the PMN are postulated to be the most effective ones (268, 277, 289, 290, 294, 306-308), in that they required less antiserum and destroyed the target cells faster and more efficiently than macrophages. Maximum cytotoxicity of BHV1-infected target cells was observed after 18 hrs of incubation with BHV1 antiserum and PMN as effector cells (307). Inactivated antisera were less effective, and fresh antisera also were able to mediate cytotoxicity without PMN, on the grounds of antibody-complement lysis. As observed by Rouse (290) the cell destruction mediated by PMN was due to the production of a specific IFN and was mainly independent of antibodies. Bovine lymphocytes were ineffective in ADCC (268, 294). Experiments carried out with PBL and mammary gland leukocytes showed that mammary leukocytes had greater activity, and within this cell population only the nonadherent, monocyte-
macrophage enriched fraction was able to kill virus-infected target cells by ADCC (294). The role of BAM in ADCC has already been discussed. Since low levels of antibody are needed it may be assumed that "ADCC" with neutrophils as effector cells is the most important primary recovery process (277, 294), but in general there is not much support for ADCC to play an important role in recovery (277).

Complement-facilitated ADCC (ADCC-C). ADCC-C is even more effective than ADCC, since IgM also can participate, and since it functions at limiting conditions, e.g., low effector to target cell ratio, low antibody concentrations and short term assays. Only required is the presence of both, a C- and an Fc-receptor on the surface of the effector cell. PMN seem to be the only cell type with Fc-receptors for IgM. Probably ADCC-C plays a major role at an early stage of recovery, when IgM is predominant, antibody levels are low and effector cells are few (reviewed in 268, 277, 290). All these defense mechanisms have been shown to be effective in vitro, but the idea

that the same mechanisms are also functional in vivo still remains speculative.

Several activities depend upon the binding of antibody to specific viral polypeptides (see "Molecular aspects of the virus").

Cell-mediated immunity (CMI) and immunoregulators. CMI is defined as antibody-*independent* T lymphocyte (T cell) mediated cytotoxicity, and two main functions are essential in CMI: antigen recognition and effector or mediator function. These functions are implemented by T lymphocytes and phagocytic cells (monocyte, macrophage). T lymphocytes can act alone by producing mediator factors (lymphokines), but most essential is their reciprocal interaction with phagocytic cells (for review see 277, 294, 309). Three different subsets of lymphocytes are activated due to respective antigen presenting cells: cytotoxic T cells (CTL), helper T cells (HTL) and suppressor T cells (STL), and the nature of antigen can markedly affect the type of immune response induced. Recovery from infections with intercellular virus spread is particularly based on CMI (277). Detection of CMI is achieved mainly by in vitro tests. The blastogenesis assay (lymphocyte transformation assay) makes information about the capability of immune cells to recognize specific antigen available, and experiments have shown that specifically sensitized lymphocytes appear at about 5 days p.i., peaking at approximately 8 to 10 days. Their capability to act as effector cells can be demonstrated by tests such as virus plaque inhibition assay and ^{51}Cr-release assay (for review see 3, 268, 277). The only in vivo test that is an indicator of specifically sensitized T cells, but not of protective immunity, is the delayed type hypersensitivity skin test (18, 268), which is dealt with in "Diagnostic Procedures".

Mainly three mechanisms of T cell mediated immunity have been identified: a direct cytotoxicity, the activation of macrophages and the release of "immune" IFN and other immunomodulators.

Direct cytolysis of BHV1 infected cells by sensitized T lymphocytes has been demonstrated (294, 310-312), but, unlike in other species, a genetic restriction between effector and target cells was not observed in cattle (294, 306, 313). But CTL are defined by their specificity for lysis of histocompatible cells expressing the appropriate antigens. Therefore the direct cytolysis of BHV1-infected cells without genetic restriction is believed by some to correspond to natural cytotoxicity (313, 314). Recently, Splitter et al. (314)

were able to demonstrate in experiments with BHV1 that antigen specific and genetically restricted CTL clones exist.

As reviewed by Rouse and Babiuk (277), T cells recruit macrophages to the site of viral lesions. When the latter are not permissive for virus replication an abortive infection results. Macrophages are able to disrupt intercellular bridges and thus may inhibit virus spread between susceptible cells. Immune PBL, mainly of the T type, have been shown to inhibit plaque formation in BHV1 infected cells (281), and this could be attributed to the production of IFN-gamma by immune lymphocytes. The inhibitory activity of these lymphocytes declined 3 weeks p.i. (294). Babiuk and Rouse (281) reported that virus infected cells but not free virus were the most effective stimulant for T cells to produce IFN, and virus infected cells are the most likely way in which T cell exposure to viral antigen during the inflammatory process would occur. In addition, macrophages were also required for maximal IFN production.

The characterization of cell populations involved in defense against BHV1 was limited because of lacking phenotypic markers. Splitter and Eskra (315) were the first to make up this deficit. Based on peanut agglutinin (PNA)-binding (= T cell specific) and cell sorting they found the following: only PNA+ cells in the presence of BHV1 responded by proliferation. Based on the reaction of these cells with specific monoclonal antibodies they could show that only a T cell subset could respond to BHV1. They also found that T cells responding to BHV1 undergo phenotypic expression of an MHC class II molecule, either through endogenous expression or acquisition. The same authors showed that phytohemagglutinin-transformed PBL from immunized animals expressed considerable numbers of virus-receptors, while resting lymphocytes and non-activated macrophages bound only small quantities of virus. The suggestion that these activated cells may produce factors that would serve as soluble signals to other cells during an immune response to virus was confirmed by measuring IFN-gamma production.

Viral elimination by lymphocytes may mainly depend on soluble factors, lymphokines, produced by these cells. They have immunoregulatory functions and enhance or suppress immune cell interactions. The role of IFN-gamma has already been discussed. Since IFN activity can be measured directly it is also the best examined lymphokine. Thus, Babiuk and Rouse (316) reported that immune IFN preparations, which have also a

wide range of other lymphokine activities, could enhance the level and speed of ADCC mediated by bovine effector cells against BHV1 targets (316, 317). The same preparations could in addition enhance direct T cell mediated cytotoxicity as well as the phagocytic potential of macrophages (277). The macrophage activation factor is probably identical to immune IFN (315).

Another outstanding lymphokine is interleukin 2 (IL-2). It triggers exponential proliferation of virus-specific T cell clones and induces cytotoxic T cells reactive to viral antigens (294, 318). IL-2 activity requires strictly the presence of antigen. Bovine lymphocytes stimulated in vitro with BHV1 were able to synthesize IL-2. Maximal levels of IL-2 were reached after 2 to 3 days of culture, which resulted in peak cell proliferation on day 5 and 6 after BHV1 stimulation. IL-2 production is ascribed to T helper cells after virus presentation by accessory cells. Further soluble mediator factors of T cells are lymphotoxin, chemotactic factor, migration-inhibition factor (MIF) and prostaglandins (268, 294, 315, 316). Their modes of action during herpesvirus infections are not yet clearly elucidated (294).

On the whole CMI depends on a balanced activation by infectious virus, i.e., non-replicating virus and isolated glycoproteins are weak stimulators of CMI (317). This could explain why newborn calves with immature cellular immune functions are more prone to severe infection, and why inactivated vaccines are less effective than live vaccines (18).

Immunosuppression in the BHV1 infection

Immunosuppression is the first effect of a BHV1 infection and renders animals highly susceptible to secondary bacterial infections. The impaired immune mechanisms are discussed in "Pathogenesis". The optimal time for bacteria to invade the BHV1-infected animal seems to be 4 days p.i. in experimental studies (272, 286). Forman and Babiuk (58) showed that macrophage functions were disturbed as early as several hours p.i., whereas Yates (319) observed that the synergism between BHV1 and Pasteurella haemolytica may last for about 30 days, and he postulated that this certainly corresponds better to the natural situation.

Immunity in latent BHV1-infection

The role of the immune system in latent herpesvirus infections has been reviewed by Hill (320), and the same mechanism may be functional in latent BHV1 infections. The main function of defense mechanisms is attributed to recrudescence. Animals with low specific immune responses

were those showing more severe clinical symptoms and excreting the highest levels of virus after reactivation (10, 321, 322). In primary infections as well as in experimentally induced reactivations the following immunological activities were observed: production of sensitized antibodies involved in ADCC, ADCC-C and antibody-complement lysis was detected 4 to 7 days after primary infection and reached a maximum level after 2 weeks. A slight rise was observed after reactivation (323). Pastoret et al. (322) reported that after primary experimental infection the animals had normal levels of neutralizing antibodies, but lower ADCC activity and a lower blastogenesis index. After the first reactivation an increase in neutralizing antibody titers, in ADCC activity and blastogenesis index followed. After a second reactivation there was no more increase in neutralizing antibody titers and ADCC activity, but still an increase in blastogenesis index, and much smaller amounts of infectious virus were excreted. The authors concluded that reactivation may serve as a booster rendering the immune system capable to control virus reexcretion with time. Decreasing reexcretion rates have also been observed in spontaneous reactivation processes with infected bulls (324).

EPIDEMIOLOGY

Geographic distribution

Descriptions of clinical signs and antibody prevalence studies indicate a worldwide distribution of BHV1 infections (41, 325). Low sporadic to enzootic disease occurrence is reported from many countries in the Americas, in Europe, Asia, Australia and New Zealand but the declarations on the whole do not seem to be precise (326). Worldwide distribution of BHV1 infections does not implicate an uniform spread of the disease in all regions of a given country. Whilst in Switzerland the eastern and central parts showed a high incidence of IBR the western parts were significantly less affected (327). Also in West-Germany (FRG) IBR prevalence rates of antibodies to BHV1 were subject to great variations of 0% - 42% in different regions of the country (328-331). On the other hand van Malderen et al. (332) reported a uniform spread of IBR over Belgium concerning 62% of 8'285 herds examined and recently Bohrmann et al. (333) reported a 55% prevalence of antibodies to BHV1 in cattle in the Djibouti Republic. The prevalence of antibodies to BHV1 in cattle in Scotland was 12% (334).

BHV1 infections may be observed throughout the year with no accumulation in a certain season.

Transmission

BHV1 infections are rather easily transmitted directly from one animal to another because large quantities of virus are shed essentially in respiratory, ocular and reproductive secretions of infected cattle. Dose levels of BHV1 for experimental and natural infection were reviewed by Yates (8). Shedding by latently infected animals does not seem to be as much abundant and additionally not lasting as long as in an acute stage of the infection (19, 335).

A further source of infection is semen. The problems concerning virus contamination of bovine semen and the techniques used to isolate viruses from the semen of bulls have been reviewed by Kahrs (32). An infection of an inseminated cow only takes place if infectious doses 50% of $10^{5,0}$ per straw are used (336). According to Straub (206) an infectious dose for cattle (CID50) of a virulent BHV1 strain amounts to 3,2 $TCID_{50}$. The importation of BHV1 contaminated bovine semen is especially risky for a country such as Switzerland having successfully eradicated IBR (337). Therefore regular serological testing of animals kept in artificial insemination centers is compulsory. For the transfer of embryos BHV1 apparently does not represent a danger as long as adequate precautions are taken (271, 338).

Cattle are the principal reservoir of BHV1 (3, 4) but serologic surveys in North America, Australia and Europe have demonstrated BHV1 antibody in numerous other species of wild ruminants (133, 140, 339-343). Seropositive animals represented the families Bovidae, Cervidae, Giraffidae, Hippopotamidae and Suidae. The prevalence of antibody to BHV1 in wild ruminants captive in United States zoos (3%) poses no epidemiological problem (339) and the same holds true most likely for zoos in other countries.

On the one hand Rosadio, Everman and Müller (344) were able to isolate sheep herpesviruses which were all neutralized by specific BHV-1 antibody. According to this finding Elazhary (345) detected in 22% of collected sheep serum samples antibodies to BHV1 in the Quebec region. On the other hand Lamontagne (346) in Canada and Hasler and Engels (140) in Switzerland found sheep sera exempt from antibodies to BHV1. The role of sheep in BHV1 epidemiology thus remains unclear and there is an urgent need to characterize sheep herpesviruses closer.

Though a large body of literature dealing with BHV1 infection of the caprine species exists (140, 344, 345, 347-349) goat BHV1 infections only play a minor or an even negligible role in IBR epidemiology. Ackermann et al. (133) could show that BHV1 infection of goats is a rare event and that this infection is not easily reactivated. In seroepidemiological surveys using goat sera often only antibody titers to BHV1 are presented. Hasler and Engels (140) were able to demonstrate that by including also antibody titers to CapHV1 a differentiation between goat and bovine herpesvirus infection could be achieved.

In British deers antibodies to BHV1 have been detected in 1978 (350) and consequently Inglis et al. (351) described an ocular disease of red deer calves associated with a herpesvirus infection in Scotland. Further studies revealed that the red deer herpesvirus, tentatively named (CerHV1), was distinct from BHV1 but antigenically related (352, 353). For this reason it was concluded that CerHV1 can be a threat to red deer farming but not to cattle. Nevertheless CerHV1 may interfere with serological IBR diagnosis (352). Also in France, Belgium and East-Germany antibodies to BHV1 in deer sera were detected (341, 343). Thiry and coworkers (340) reviewed in detail BHV1 infections in wild ruminants . The authors came to the conclusion that latency would allow herpesviruses of wild ruminants to persist for long periods in a restricted population. They advocate a better characterization of wild ruminant herpesviruses to better know what epidemiological impact they could have on BHV1-infections in cattle. Additionally they presume a similarity between herpesvirus induced keratoconjunctivitis of chamois and deer. In Finland a herpesvirus serologically related to BHV1 was isolated from a reindeer treated with dexamethasone whereas in the same region cattle sera contained no antibodies to the IBR-virus (342). The same epidemiological situation was observed in Canada where herds of Caribous (Rangifer tarandus caribou) with high percentages of seropositive animals lived in close contact with cattle. The antigenically related reindeer herpesvirus could be distinguished from BHV1.

In Australia herpesviruses from buffaloes have been differentiated by restriction patterns from BHV1. Thus, Brake and Studdert (29) propose that alpha herpesviruses from individual ruminant species are species specific. In the authors' opinion such a classification is highly desirable to avoid confusion concerning measures to be taken in a region or a country where

IBR is successfully eradicated and BHV1 antibodies are found in sera of wild ruminants in close contact with cattle.

The swine does not seem to play a prominent role in IBR epidemiology. On the one hand BHV1 has been isolated from the trigeminal ganglion of a feral pig after dexamethasone treatment and from stillbirth in swine (354). Furthermore, Joo et al. (154) could show that in utero infection of swine fetuses with BHV1 resulted in fetal death and mummification. On the other hand pigs inoculated intranasally with BHV1 did not respond clinically or serologically (155). After an experimental infection, six pigs inoculated with BHV1 developed specific antibodies to BHV1, but not to PRV (156). In Switzerland swine do not represent a reservoir for IBR because only in 0,7% of examined swine sera antibodies to BHV1 could be detected (140). It remains an open question whether a porcine cytomegalovirus interferes with serological IBR diagnosis in swine (355).

The role of rabbits in IBR-epidemiology most probably may be neglected (103, 104, 356-358).

BHV1 is also shed by intranasally infected cows even after reactivation of a latent infection through the milk, detected by feeding seronegative calves (19).

For the sake of completeness it should be mentioned that herpesviruses could possibly also be transmitted by ticks. In mule deer (Odocoileus lemionus) bedding areas in the Sierra Nevada mountains Taylor et al. (359) were able to isolate a herpesvirus related or identical to BHV1 from soft shelled ticks (Ornithodorus coriaceus). As it is known, mule deer are susceptible to BHV1 and mule deer and cattle sometimes occupy the same ranges in the western United States.

VACCINES AND VACCINATION

Conventional BHV1 vaccines with either live modified (MLV) or inactivated virus, and either mono- or polyvalent, are in commercial use in many countries since 30 years. Development, production, modes of attenuation and experiences with the various vaccines have been reviewed in part by Kahrs (4), Gibbs and Rweyemamu (3), Plowright (360) and Lupton and Reed (361). Many opinions exist about the use of vaccines and the efficiency of a vaccination. One fact, however, is accepted by most producers and users: Dependent on the ability of the vaccine to induce an immunity, vaccination against BHV1 may be effective by reducing clinical

disease, duration of virus shedding and titers of excreted virus after an infection with field virus. Therefore economical losses can be diminished and spread of BHV1 controlled to a certain degree, but vaccination does not fully protect against infection (3, 207, 225, 263, 270, 362, 363).

MLV vaccines for parenteral application

The first commercial vaccine (364) has been developed from BHV1 strain "Colorado I", attenuated by rapid serial passage in primary bovine kidney cells, in order to select variants with a rapid multiplication rate and low virulence. The 40th passage of this strain was found to be apathogenic and protective for cattle, and safe for contact animals. Subsequent vaccines were attenuated by multiple passage in bovine kidney cells, by adaptation to porcine or canine cells, by adaptation to cell cultures at 30°C or by selection of heat stable mutants (3, 360, 361).

The most important advantage of this type of vaccine consists in its convenient mode of administration, and the possibility of combination with other viruses such as BVD or PI-3 (4, 178, 362). Usually a single vaccination should be sufficient to afford an effect (4). It can be used for calves of any age (4), but two vaccinations are recommended in this case (365).

The use of MLV vaccines brings about many drawbacks. Most prominent is the fact that numerous products cause fetal infection and death, followed by abortion, when vaccinating pregnant cattle (3, 4, 80, 268, 360). Therefore, vaccination of pregnant cattle by intramuscular application of MLV vaccine should strictly be avoided (268). Furthermore it has been observed that vaccinated calves which were held together with pregnant cows shed vaccine virus leading to infection of the cows and subsequent abortion (366). In addition, MLV vaccines may cause immunosuppression and in this way increase susceptibility of vaccinated individuals to bacterial infections (367).

MLV vaccines for intranasal application

In order to circumvent abortions, several attempts have been made to produce an MLV vaccine for intranasal application, which should induce rapid local protection without generalization of the virus.

Such vaccines have been produced with virus attenuated by serial passage in rabbit cell culture (368), or using virus modified by treatment with nitrous acid followed by selection of temperature-sensitive (ts) mutants (369).

The great advantage of this vaccine type, compared to the MLV vaccine for parenteral application, is its safety for pregnant cattle. It gives

rise to rapid and sound protection by inducing prompt production of IFN and secretory antibodies (IgA) on mucosal surfaces and results in comparable titers of humoral antibodies as well as in comparable CMI responses (3, 4, 361, 370). The use of ts mutants as vaccine strains is even more advantageous, because the temperature-restriction guarantees a strictly local virus replication, and the danger of virus spread through the organism leading finally to abortion can be avoided (72, 360, 361). Furthermore, the ts mutation has been shown to be genetically stable in vivo and in vitro (369), and vaccine strains can readily be distinguished from field virus strains by the ts marker (360, 371).

But this vaccine type, too, has its drawbacks. Intranasal application may bring about problems in large feedlots where animals are held freely. Moreover the vaccine has to be applied deeply enough, and sneezing must be avoided (4, 178). Intranasal application may occasionally provoke adverse clinical reactions, such as fever, nasal discharge, intranasal plaques and temporary drop in milk production (4, 372). Virus shedding after vaccination and transmission of vaccine virus to nonvaccinated animals has also been demonstrated (reviewed in 361).

Diverse opinions about the protective value of different MLV vaccines for intranasal application have been reported (102, 363, 371, 373-375).

The duration of protection is unknown, but annual revaccination is recommended (4). Many MLV vaccines for intranasal application are combined with other viruses involved in respiratory tract disease, mainly with PI-3 (4, 362).

MLV vaccines originating from IBR virus strains are also effective against IPV/IPB and vice versa (3), vaccines originating from IPV eventually being non-abortigenic (131).

One of the major problems still awaiting a solution is, that all types of attenuated vaccine virus may establish latent infection themselves, or do not prevent latent infection by field virus, leading to a permanent threat to non-immune animals (7, 72, 102, 207, 225, 322, 360, 376, 376a). In addition, Nettleton et al. (102) demonstrated that after vaccination with a ts mutant strain and challenge with wildtype virus recombinational events may occur. The authors found a reexcreted isolate with altered characteristics. They suggested that it had originated from the vaccine virus and remained avirulent but had lost its ts marker.

Genetically engineered MLV vaccines

A BHV1 specific thymidine kinase (TK) has been detected and characterized by Weinmaster et al. (193). After the isolation of TK-negative mutants, Kit et al. (78) demonstrated that these mutants are stable during in vivo passage, apathogenic for intranasally or intravenously infected calves and thus efficacious as a vaccine. Further studies confirmed that vaccination of pregnant cattle with a TK-negative mutant, either intramuscularly or intravaginally, did not cause adverse reactions, protected against abortion, reduced clinical signs in the vaccinated animals as well as virus spread after challenge with wildtype virus (80). Recently Kit (178) presented a new generation of TK-negative BHV1 vaccines with following properties: (i) Virulent BHV1 strain "LA" was used as starting material. The principal basis for attenuation was the deletion of TK gene sequences, but during BrdUrd and IdUrd selection steps unknown random alterations were additionally introduced. (ii) Instead of the TK gene a 19-base oligonucleotide sequence with stop signals in all 3 reading frames was inserted to guarantee complete inactivation of TK gene activity. (iii) A selection to temperature-resistance followed by means of plaque purification procedures in order to render intramuscular application possible and to avoid the disadvantage of ts mutants which only replicate in the upper respiratory tract and thus have to be applied by the intranasal route. (iv) Recently a vaccine was developed possessing all the above listed properties, and additionally was deleted in the gIII gene. Such vaccine strains can readily be distinguished from wildtype strains.These vaccine strains have not yet been tested in the field, but the experiences with vaccination of calves and pregnant cows with the afore-mentioned TK-negative mutants may guarantee their safety and efficacy. Problems concerning establishment of latency by vaccine or field virus however are not yet solved.

The U.S.patent of the vaccine containing the mutant deleted in the TK and gIII gene is still pending. Since lawsuits have been initiated against the general use of genetically altered virus vaccines, it is not certain that these products will soon be free for commercial use, although one case has recently been won by the producer.

Although efforts are in progress to construct vaccines by recombination of BHV1 glycoprotein genes with the vaccinia virus genome (177, 377, 378, Keil, pers. commun.) no such vaccine is commercially available at the time of writing.

Inactivated vaccines

Inactivated vaccines have been produced by formalin, ethanol or ethylenimine (379) treatment, and by heat or UV inactivation. Since inactivated vaccines are not efficient without adjuvants, Freunds complete adjuvant, oil, saponin, adsorbed aluminium hydroxide gel and sodium alginate had been successfully used to intensify the immunizing effect (reviewed in 361). Sodium alginate as adjuvant has been reported to induce higher levels of neutralizing antibodies (380), and with a vaccine containing beta-propiolactone inactivated virus in combination with aluminium hydroxide and saponin promising results were obtained (381). But the efficacy of inactivated vaccines is subjected to doubts (3, 382-384), although more recently a combined vaccine has been shown to induce fairly good protection (385, 386).

Advantages of this type of vaccine lie in the fact that it does not induce abortion, that there is no virus spread after vaccination, and that establishment of a latent infection by vaccine virus is not possible (4, 207, 379, 385).

However, fatal hypersensitivity reactions (anaphylaxis), nonfatal urticaria or skin nodules and fever have been observed after vaccination (4, 387).

Levings et al. (148) reported that different inactivation procedures led to selective destruction of BHV1 antigens. They postulated that this may allow antigen-specific serological testing to distinguish vaccinated from naturally infected animals.

The duration of protection afforded by inactivated vaccines is not known, but in any case two vaccination cycles with an interval of 4 weeks and annual revaccination is necessary (4, 361).

Subunit vaccines

Problems encountered when using MLV or inactivated BHV1 vaccines initiated the production of alternative vaccines containing single immunogenic viral components. However, none of the so far tested subunit vaccines is ready for commercial use.

Darcel le Q. et al. (388) produced two types of subunit vaccines. One was a crude envelope antigen extracted from purified virus by Freon 113 treatment, the other one was an antigen extracted by a chloroform-methanol mixture after heat inactivation and concentration of infected cell supernatant. Cattle subcutaneously vaccinated with these preparations (in Freunds

incomplete adjuvant) showed significant humoral immune responses. Vaccination of heifers before breeding and challenge during pregnancy did not completely protect against abortion caused by BHV1, but the abortion rate was reduced compared to the unvaccinated control group.

Lupton and Reed (361) produced a subunit vaccine by solubilizing infected cells with the non-ionic detergents Triton X-100 or NP-40. Experiments in calves, vaccinated intramuscularly with these cell extracts, combined with Freunds complete or incomplete adjuvant, showed a promising protection against challenge infection. When 2 doses were given in an interval of 4 weeks clinical disease and shedding of challenge virus could be prevented, at least with the NP-40 extract.

The nature of the antigen can markedly affect the type of immune response induced (389). For example, in herpes simplex virus infections glycoproteins induce CMI response, whereas in cytomegalovirus infections non-structural early proteins take over that role. Therefore, the antigen presentation, together with the choice of adjuvant, is one of the major points to be considered in the production of subunit vaccines. In the recent years, much work about the role of individual (glyco-)proteins of BHV1 in the immune response has been published, using either monoclonal antibodies or immunizing rabbits or cattle with purified viral components (discussed in "Molecular Aspects of the Virus"). Trudel et al. (390) prepared subunit vaccines from fractions containing the hemagglutinating activity (gIII) of live or Triton X-100 solubilized virus and inoculated rabbits subcutaneously. Both vaccines induced an appreciable antibody response, but, preparations stemming from live virus led to higher titers. In a further approach Trudel et al. (390a) bound the purified hemagglutinin (gIII) and gI to micelles of Quil A glycoside (ISCOMs) and assayed this subunit vaccine in rabbits. ISCOMs were found to induce the most marked immune responses and were praised to have a great potential as a subunit vaccine.

Vaccination in the presence of maternal antibodies

Calves receiving colostrum with high levels of neutralizing antibodies are protected against fatal BHV1 infection within 48 h of life (391).

The general rule that maternal antibodies may interfere with the active production of antibodies after vaccination is true for BHV1, too, especially when using vaccines for parenteral application. A revaccination after 6 months of age therefore is absolutely necessary (4, 268, 392-394). Existing reports show that certain vaccines, mainly intranasally applied, may induce

active immunity although maternal antibodies still are present to a high titer (4, 268, 374).

Antiviral drugs / Treatment

Several known antiviral drugs have been tested against BHV1, mainly in vitro, and most of them have been shown to be ineffective in nontoxic doses (272, 288, 395-404).

The prophylactic and therapeutic use of IFN has experimentally been demonstrated to be effective in reducing dramatically clinical disease by preventing secondary bacterial infections (272, 283, 288), however, economical considerations hinder its practical use (225). Therefore, active stimulation of unspecific defense mechanisms is one of the aims propagated in the recent years mainly in Europe. Biological (IFN-)inducers, prepared from avi- and parapoxviruses, attenuated BHV1 or bacterial components are used in prophylaxis and therapy (225, 276, 331, 362, 365, 405-407).

Restrictions and official recommendations

The use of BHV1 vaccines, type of vaccine as well as general restrictions and legislations differ in various countries, and available informations are scarce (see also "Control and eradication"). In the USA commercial vaccines for intramuscular application must contain at least 4.2 log10 TCID50/dose (3). The vaccines have to be produced from master seed virus, not passaged more than 10 times to produce the final vaccine, and the passage method must conform to the outline of production (123). The use of MLV vaccines for intramuscular application is not recommended for calves suckling pregnant cows, and a warning should appear on vaccine package inserts (4). Van der Maaten et al. (65) recommended not to vaccinate cows during or shortly after the estrus, since ovarian lesions and fertility troubles may occur.

CONTROL AND ERADICATION

The prevalence and severity of BHV1 infections in different countries has a direct impact on control and eradication programs. In the USA, where respiratory tract infections leading to severe economical losses in large feedlots have been common for a long time, control was based on vaccination programs. But, as discussed earlier, the use of MLV vaccines led to abortion or masked infections due to the establishment of latency by vaccine or field virus strains. Since inactivated vaccines proved to be unsatisfactory, too, endeavors to produce better and safer vaccines continue

in countries with large feedlots. But authorities in countries where vaccination is common are more and more confronted with the problem that only seronegative animals, IBR-free semen, and IBR-free embryos can be exported (4, 263, 337, 408, 409).

The situation in Europe is somewhat different. As long as disease outbreaks were rare or caused only minor economic losses, random vaccination or "living with the infection" was usual. After the appearance of severe disease outbreaks in the 1970ies, that were probably due to the introduction of a virulent BHV1 variant, control of the infection was and is managed according to the respective situation and/or governmental strategies.

The aim of every control program must be based on the decision either to control the clinical disease or to eliminate the infection (362). Vaccination only protects against disease but not against infection (18, 277, 360, 362). If vaccination is used as control measure, the following facts have to be considered: (i) Vaccination does not prevent superinfection with field viruses, but the immune status may influence the pattern of virus reexcretion in latently infected animals. This implies that vaccination programs are conducted continuously and consistently (72, 225, 322, 410). The decision to combat IBR by vaccination should also correspond to the epidemic situation in each country. Thus a focal distribution of the infection does not necessitate the general application of vaccination, a measure that is only justified in highly infected populations (360). (ii) Vaccine virus distributed together with field virus within a population may end in recombinational events, and reversion to virulence, although unlikely, cannot absolutely be excluded (72, 102). (iii) Even if vaccine virus may be distinguishable from field virus by ts marker or different DNA restriction patterns (72, 123), antibody resulting from vaccination precludes the use of serological tests in control and eradication programs (264, 360, 411).

Control programs depend also on conditions of animal breeding and management, i.e., different programs may be useful and necessary for dairy cattle farms, calf-breeding farms, fattening farms, artificial insemination and embryo transfer centers (4, 392, 408).

As far as informations are available, vaccination is common in most countries in the world. Only two European countries, Switzerland and Denmark, and one district in France (Bretagne) control BHV1 infection by eradication without vaccination (264, 324, 411). Other countries are running

a combined vaccination/eradication program, and emphasize seronegativity of animals kept in artificial insemination centers and bull breeding herds (4, 207, 263, 331, 337, 360, 392, 407, 408, 412).

In Switzerland BHV1 infections posed no problem until 1977. Before that time only one IBR outbreak occurred, which was restricted to one herd without any further distribution of the virus (235). IPV, however, has been known to occur for decades, but could be controlled by artificial insemination strategies (235). No governmental regulations existed until the time when sudden severe IBR outbreaks threatened to spread throughout the whole country. Immediate regulations were prescribed, such as obligatory notification of disease outbreaks and restrictions for infected farms. Vaccination was prohibited, since vaccine tests were unsatisfactory (413) and serological control would have been impossible. Although economical considerations led to divergent opinions and one could not rely on previous experiences from other countries, the government decided to combat the BHV1 infection by an eradication program. The first steps to this aim were carried out regionally and in a non-uniform manner, but the spread of infection could be successfully stopped, and the rate of infected animals markedly reduced to a level where economical considerations allowed to enact a law (7.7.82) for complete elimination of all seropositive animals as a second step. This uniformly managed program was based on the evaluation and notification of all seropositive animals by serological testing of either bulk milk twice a year or single blood samples once a year. In the case of seropositive results the respective farms were placed under official surveillance, and immediate restrictions were set up, such as strict separation from other holdings, trade embargo or direct separate transportation to the slaughter house. After the elimination of all seropositive animals the farms were only signed "IBR-free" when all remaining animals were seronegative by individual blood sampling, or in the following three official examinations of milk samples. Outside the official serological test periods, each animal assigned for trade, markets and alping had to be proved seronegative by individual testing no longer than 6 weeks before the event. In addition, serological examination after abortion was obligatory, and bulls as well as semen, had to be IBR-negative. This law became effective in January 1983, and presently Switzerland can officially be considered free of BHV-1 infection, a few exceptions being under control. The serological surveillance will be maintained for a yet undefined time.

Although this program has proved to be successful, it must be stated that this model would not have been possible without an ELISA for bulk milk and serum samples, without a massive financial support by the government, a small infection rate and feedlot conditions corresponding to a small country such as Switzerland. For these reasons special directions have been stated by the Federal Veterinary Office (3.6.85) for infection control in fattening farms, which are not a direct epidemiological threat but an economical hurdle. Models how to manage elimination of BHV1 infections have been elaborated (see below). Finally it must be stated that no eradication program can succeed without the agreement and co-operation of the farmers' and breeders' organizations. (Informations are based on Schweiz. Tierseuchenverordnung, Riggenbach, 264 and pers. commun., 337)

In Denmark the situation is comparable to that of Switzerland, and a similar eradication program, on a voluntary basis supported by legislation, has been started in February 1984. This program is based on the choice between complete replacement of infected herds or on strict separation procedures in order to eliminate seropositive animals stepwise (258).

In the Federal Republic of Germany efforts are in progress to establish vaccination/eradication programs with the overall aim to eliminate eventually BHV1 infections. These programs are based on directions for voluntariness without legislation and depend on the individual epidemiological and economic situation of the different regions (reviewed in 414). Generally, the directions are based on seroepidemiological investigations, followed by either elimination of seropositive animals or by a stepwise combat. This includes prohibition of trade with seropositive animals, maintenance of seronegative herds and controlled vaccination procedures in infected herds. Maintenance of seronegative herds is based on serological controls and precaution prescriptions (trade, artificial and natural breeding restrictions) similar to the management in Switzerland. Vaccination of all animals more than 6 months of age with inactivated vaccines is allowed in infected herds. Animals have to be vaccinated twice in an interval of 4 months, and revaccinated every 6 months. This has to be continued until all seropositive animals have left the herd. Calves and proved IBR-free new incomers should not be vaccinated. When only seronegative animals remain in the herd the prescriptions as mentioned above get in force. In the case of acute disease outbreaks MLV vaccines may be used, but to this end governmental allowance is needed. In dairy

cattle and mixed herds the animals have to be revaccinated with inactivated vaccine 4 months after the first vaccination, and thereafter management is the same as with "infected herds". In the case of fattening herds, animals vaccinated with MLV vaccine, are only free for slaughter. Special, but similar control programs are prescribed for breeding bulls. In spring 1988, all breeding farms in Bavaria have affiliated to the voluntary eradication program (Informations are based on 263, 270, 271, 337, 407, 409, 415).

So far, experiences with this procedure have been promising, but failures did also occur. These could be attributed to inconsistent or inadequate management of the control program (337). Metzner et al. (387) reported, however, that the proposed directions have to be interpreted with precaution, since too many factors have to be considered in such a system.

Hitherto the only international prescriptions dealing also with the special control of BHV1 infections, have been outlined by the International Embryo Transfer Society and recommended to the International Office of Epizootics (OIE) (338).

ECONOMIC CONSIDERATION

In all reviews and handbooks IBR is described as a disease causing heavy economic losses, due to general depression, drop in milk production, weight loss and abortions, but precise data are not given (325). In fact, indications of economic losses due to retarded weight gains, cost of treatment of animals and death (325) are scarce in the literature and often generalizing. IBR costs American farmers $ 25'000'000.-- annually (178). Pierson and Vair (416) cited also by Gilbert and Seurat (417) estimated the loss in a Colorado dairy herd of 156 animals to be $ 51.-- per animal, whereby the greatest detriment was due to a drop in milk production following abortion. In Switzerland the economic losses were calculated by Meyer (418, 419). According to his investigations abortions were the main causes of loss of income. In Switzerland, where an eradication program is in its final stage, cost were calculated by Meyer (418, 419). Total cost (laboratory examinations, blood and milk sampling, indemnities etc.) of the eradication program in Switzerland for 51'870 eradicated animals came up to approximately SFr. 110 million (Chr. Riggenbach, pers. communication). The higher the abortion rate in a herd the higher was the loss which was valued SFr. 3'000.-- to 20'000.-- per herd.

Estimates by Thomas indicate that the direct losses to the U.K. beef industry due to respiratory diseases, including IBR, in calves could be as much as £ 5,7 million per annum (383). In the same year Wiseman (420) et al. estimated the cost of IBR on fifteen farms. The average cost in fattening farms was higher, average £ 36.-- per animal, than in dairy farms, average £ 6.-- per animal at risk.

In Switzerland fattening farms might represent actually a reservoir of IBR (262). For such farms Weber (421) worked out a "soft" eradication program with the intention to keep cost as low as possible. By isolating seropositive animals from seronegative animals using a plastic curtain and taking adjuvant measures cost could be lowered by a factor of six compared to a "hard" eradication program comprising eradication of all seropositive animals of a herd simultaneously.

Since sampling of sera from animals in fattening farms is expensive and time consuming, Spirig et al. (262) elaborated a method to sample whole blood from the tail by soaking it on filter disks and by testing the sample directly in an ELISA.

FUTURE ASPECTS

IBR may serve as a general model for herpesvirus infections in a natural host. The increasing body of literature dealing with BHV1 provides a proof of economic and scientific importance. Significant advances have been made particularly in the areas of molecular biology and immunology, where BHV1 research is following the lead of human herpes virology. Perhaps there will be sufficient incentive to determine the nucleotide sequence of BHV1 soon.

According to Kahrs (7), the likelihood of a worldwide IBR eradication is minimal. The reasons brought forward weigh heavily: lack of pathogenicity for humans, control program costly to implement, the vast majority of countries would not cooperate, potential for reactivation of latent infections, potential of other animal reservoirs. In spite of these difficulties, Denmark and Switzerland embarked on an eradication program and in the latter country BHV1 as infectious agent is practically eliminated.

In certain regions of the FRG seronegative herds are established and in seropositive herds clinical disease is prevented by administering inactivated vaccines, aiming at establishing seronegative herds later by eliminating seropositive animals step by step. The aim in view is probably a

stamping out of IBR in this country. By the end of this century Denmark, the FRG and Switzerland may be expected to become free of BHV1 infections in cattle. Hopefully, using new methods for constructing vaccines a preparation will be available that induces a sound complex (humoral and cell mediated) immunity. Such a vaccine should effectively prevent the excretion of virus after a primary infection and even should hinder the establishment of latency by a wild virus strain. The artificially induced immunity has further to be differentiable from an immune status caused by a wild virus strain, as it is the case with a genetically engineered IBR vaccine (178). Preliminary experiments with bovine interferon were promising (283). Once the production cost can be lowered drastically, widespread practical application may be envisaged, possibly combined with vaccine administration.

Whether a chemotherapeutic approach to combat IBR will be possible in the near future remains an open question, but should not be excluded in advance. It is well known that in vitro and in vivo active inhibitors of herpesvirus replication exist, and are even marketed for human use. Advances in this realm are well imaginable and an effective chemotherapeutic agent would probably solve some problems posed by BHV1 infections.

A central problem is the viral DNA resting in nuclei of certain neurones within the respective ganglion. In this form the nucleic acid stays shielded from any attack, whether immunologic or chemotherapeutic. Since our knowledge about mechanisms responsible for reactivation of a latent infection is still rudimental, this process cannot be specifically influenced, though our understanding of latency is growing.

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2

BOVINE HERPESVIRUS 2 INFECTIONS

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INTRODUCTION

Bovine herpesvirus 2 (BHV2) (1) is established as the specific cause of two distinct diseases of cattle. These are pseudo-lumpy skin disease (PLSD) which is caused by Allerton or related strains of BHV2 (2) and Bovine herpes mammillitis (BHM) which is caused by BMV or related strains of BHV2 (3).

Pseudo-lumpy skin disease

PLSD occurs as a generalised skin disease of cattle which is characterised by fever followed by the development of hard nodules in the skin over practically the whole body and limbs. It was formerly confused with classical lumpy skin disease caused by a poxvirus (2) but can now be distinguished clinically and virologically.

Bovine herpes mammillitis

BHM is predominantly a disease of dairy cattle in which lesions are generally confined to the skin of the teats and udder. The first report of the identification and characterisation of BHV2 from cases of mammillitis in cattle was by Martin et al. (4) in 1966. The virus identified in that report had been isolated from a large outbreak of mammillitis in cattle in Scotland in 1964 (5). A detailed clinical description of the disease and identification of the causative virus had not been previously made in Europe. It is possible that the "skin gangrene of the bovine udder" described in 1959 (6) was BHM, although there were several discrepancies in the clinical features (7). The clinical features

of BHM have been confused with other viral infections of the bovine teat such as pseudo-cowpox, cowpox or vaccinia especially when a concurrent infection with one of these viruses is present.

Although BHM usually occurs in dairy cattle it has been recorded also in suckler herds (8, 9) and beef cattle (10).

BHM can be a locally serious disease especially in dairy herds but it is not regarded as a disease of national or international economic importance.

CLINICAL SYMPTOMS

i) PLSD is a non-malignant infection of the skin manifest by a febrile reaction followed by the sudden appearance of raised, circumscribed nodules. In milder cases only a few nodules may be detected but in severe cases they may develop over practically the entire body but especially on the face, neck, back and perineum (11).

Nodules are hard, palpable, raised, circular areas over which the hair tends to be slightly raised. A slight depression in the centre of the flat surface of the nodule is characteristic and can be useful in differentiating between BHV2 infection and classical lumpy skin disease. Necrosis of the affected epidermis follows and as the swelling subsides the necrotic tissue dries centripetally and separates from the regenerating tissue. Approximately 14 days from onset the dry lesions are shed or can be readily plucked from the skin to leave an area devoid of hair. Hair growth slowly ensues and the lesions heal in several wk without scarring.

Ulcerative lesions on the tongue of a Tanzanian buffalo (**Syncerus caffer**), from which BHV2 was isolated, were reported by Schiemann et al. (12) and by Kalunda and Plowright (13). These erosive lesions of the tongue and mucosae were coincidental with a severe disease which probably had a different and unidentified aetiology.

ii) BHM. Systemic illness is not generally associated with BHM and pyrexia is usually noted only if the disease is complicated by severe acute mastitis. The lesions of the teat and udder have been graphically described and well illustrated by

Martin et al. (4). In most cases the first sign of developing infection is irritibility on milking as the teat becomes swollen and painful. Vesicles may precede swelling but are infrequently observed. The skin over the teat becomes translucent due to the separation of the tissue layers and at this stage is hot to touch. A yellow to bluish purple discolouration of the teat may follow and subsequently the skin over the affected area sloughs off to leave a wet, raw clearly demarcated ulcer. Exuded serum and blood dry over the ulcer to form a thin scab. At this stage the lesion is less painful but thickening of the skin persists. Without complications the lesion gradually resolves by granulation and heals without scarring.

Commonly, the disease is seen first in heifers shortly after calving. Subsequently other in-contact heifers and cows may develop lesions before or after calving. Calves sucking teats of affected cows can develop ulcers on the lips, in the mouth and on the nose. These lesions tend to be of a transitory nature and cause little discomfort. Animals of any age may be affected.

The incubation period is from 4-10 days. A single localised teat lesion of a mild nature might ulcerate within 48 hr and heal completely within 2 wk of onset. On the other hand a severe infection involving all teats and the udder may take up to 15 wk to resolve. The extent of lesions varies markedly from part of one teat to the entire surface of all four teats and the udder. In severe cases there may be spread of infection to the adjacent skin of the perineum where hard nodular lesions are formed. Prolonged disease is often a result of a secondary bacterial infection or fly attack. In some extreme cases the udder skin may slough off to reveal a heavy maggot infestation. In such cases, and in others with loss of quarters or mastitis, animals may have to be culled, but direct mortality does not occur. Morbidity can range from one animal in a herd to about 75%. Illustrations of BHM lesions are given in Fig. 1, a-d.

Figure 1

BHM lesions on teats and udder

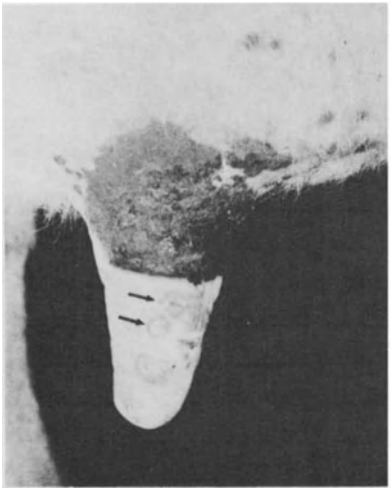


Fig. 1a Ulcerative BHM lesion at teat and udder junction. Note satellite lesions

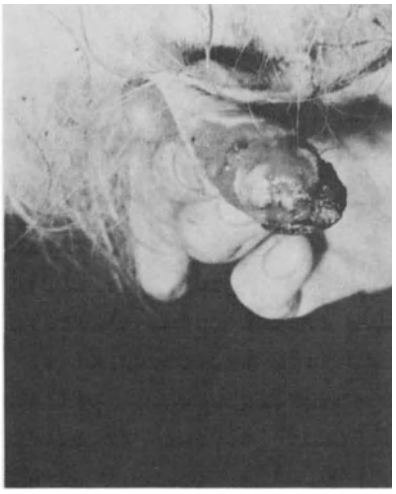


Fig. 1b Extensive raw weeping lesion over most of the teat surface

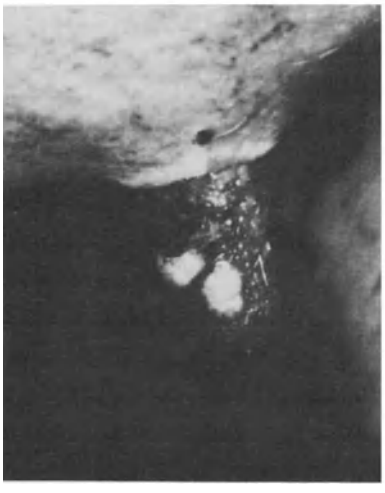


Fig. 1c Severe lesion on teat

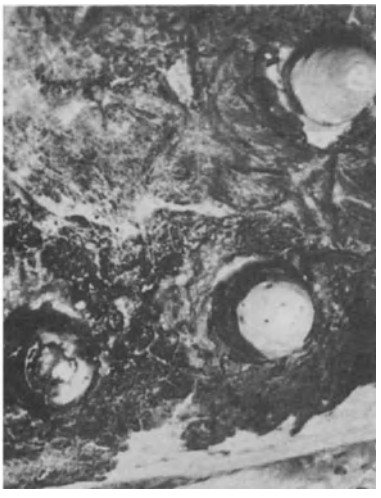


Fig. 1d Severe lesions with ulceration and scabbing on teats and udder

Figures courtesy of The Veterinary Record. Martin et al 1966, 78, 494-7

MICROSCOPIC LESIONS

Much of the knowledge which has been gained on the nature of the lesions is based on observations on experimentally infected cattle (3, 14, 15, 16). Essentially lesions of the teat and udder exhibit the same pattern of change as lesions of the skin on other parts of the body.

The initial reaction in the dermal epithelium is characterised by accumulations in the tissue of polymorphonuclear leucocytes, eosinophils, mast cells and lymphocytes. Eosinophilic intranuclear inclusion bodies are formed in the cells in the stratum germinativum and stratum spinosum. Numerous syncytia form in the non-keratinised cells and are especially prevalent in the inner part of the stratum spinosum and in the stratum germinativum. The sebaceous but not the apocrine glands may be involved. In less affected areas mild intercellular oedema and hydropic degeneration are consistently observed. Necrosis of cells and the formation of microvesicles may be noted also.

Within 24 hr of the initial reaction necrosis of the epidermis becomes severe and there is cell loss from all layers. This is accompanied by an extensive infiltration of polymorphonuclear leucocytes. Numerous intranuclear inclusion bodies are present and syncytial formation is common. Necrosis of the epithelial cells of the hair follicles and sebaceous glands occurs also accompanied by cellular infiltration and the production of intranuclear inclusion bodies and syncytia. Sweat glands are not involved. Perivascular cuffing by mononuclear cells is evident, mast cells are present in large numbers and there is oedema of the connective tissue layer.

Between 3 and 5 days after the appearance of the lesion necrosis of the epidermis progresses and involves hair follicles and sebaceous glands. Dilation of the sweat glands occurs and in some instances the lumen can be seen to contain polymorphonuclear leucocytes. Intranuclear inclusion bodies and syncytia become less numerous. The ulceration of the dermis caused by necrosis is accompanied by haemorrhage and fibrinous exudation into the base of the ulcer. Oedema and cellular infiltration are still obvious.

Scab formation ensues and by day 10 haemorrhages related to scab formation become obvious. Most of the epidermis heals and appears normal without the presence of inclusion bodies in the cells. The sebaceous glands return to normal but there is still oedema present in the deeper areas of the epidermis. At this stage there is an obvious reduction in the number of hair follicles and sebaceous glands, capillaries might still be dilated and there is still some evidence of cellular infiltration mainly composed of mononuclear cells.

By day 28 slight oedema persists in the dermis and infiltrates of polymorphs, lymphocytes and pigment-containing cells are obvious. In the epithelium, although superficial cell debris and necrotic epidermal cells remain, the skin has regenerated and is mainly normal.

PATHOGENESIS

Certain aspects of the pathogenesis of BHV2 have been established by experimental infection of cattle but the initial route of infection and the mode of spread remain enigmatic. Experimental evidence has shown that cattle can harbour BHV2 in a latent form for many months after infection (17) which has led to the hypothesis that an inapparent infection may occur many months or even years before the virus is reactivated in vivo resulting in disease.

Once in the host, BHV2 disseminates like other herpesviruses by haematogenous and neural routes (5, 16, 17, 18, 19). Viraemia however, is not a consistent feature in BHV2 infections and the only successful isolations of virus from blood have been in the first 3 days after inoculation from animals inoculated by the intravenous (16, 17) or the intradermal-lingual routes (13) although this may be due to low sensitivity of detection (20). Experimental infection of susceptible cattle with either PLSD or BMV strains of BHV2 administered intravenously results in a generalised skin infection similar to PLSD, whereas intradermal or subcutaneous injection of BHV2 isolated from BHM-affected cattle consistently produces localised lesions which are

indistinguishable from those of the natural disease.

BHV2 infects cells in the dermis, where the viral envelope adsorbs to receptors on the plasma membrane of target cells. Normal viropexis occurs and the viral capsid is released into the cytoplasm. A DNA-protein complex is then translocated to the nucleus where viral replication occurs. Viral nucleocapsids mature by budding through the inner lamella of the nuclear membrane thus acquiring their envelope. Accumulations of virus gather in the inner and outer lamellae of the nuclear membrane and are transported to the cell surface through the modified endoplasmic reticulum.

Following the eclipse phase of approximately 15 hr a period of exponential virus replication occurs up to 120 hr. BHV2 is first detectable in the skin 48-72 hr after experimental infection and will reach its maximum several days later (15). After intradermal infection BHV2 can be recovered from skin lesions for up to 15 days (17) and following intravenous infection can be re-isolated for up to 20 days (16). By day 30 no virus is recoverable (21).

During the first wk after experimental intravenous inoculation BHV2 has been recovered from skin, lymph nodes, nerve tissues, spinal and basal ganglia and brain (16, 19, 21, 22). After experimental intradermal inoculation the Allerton strain has been recovered from semen, urine and faeces (11). Persistence of infectious virus is not a feature of BHV2 and re-isolations of virus are less frequently made as systemic antibody titres rise.

LATENT INFECTION

One of the most important characteristics of the **Herpesviridae** is the ability of the viral genome to integrate with host cell DNA and induce a latent carrier state. Reactivation of herpesviruses can be stimulated by a wide range of stress conditions including exposure to UV light or immunosuppressive therapy.

The ability of BHV2 to become latent has been demonstrated experimentally (17, 19, 23, 24), and confirmed by the isolation of

BHV2 from a naturally infected cow following corticosteroid treatment to reactivate a supposed BHV-1 infection (25).

Reactivation of BHV2, following an intravenous course of dexamethasone, from skin, buccal ulcers, lips, ganglia, central nervous system and lymph glands (17, 19, 23) has led to the hypothesis that the epithelial cells of the skin and the neural and glial cells are possible sites for latent infection (23). No virus was isolated from oral and nasal swabs and skin biopsies taken between recovery from primary inoculation and challenge with corticosteroid, thus strengthening the argument that BHV2 becomes latent and does not simply persist in host tissue cells.

CHARACTERISTICS OF THE VIRUS

Taxonomic status

The taxonomic description of BHV2 complies with the guidelines of the International Committee on Taxonomy of Viruses (ICTV) as described in 1982 (26). The family name is thus **Bovid Herpesvirus** and the subfamily name bovine herpesvirus 2. Members of the **Bovid herpesviridae** include the herpesviruses of sheep and goats whereas the subfamily of bovine herpesviruses specifically excludes herpesviruses of those species. Sheep and goat herpesviruses are designated caprine herpesvirus 1 and 2 (CHV1 & 2) respectively. Although this description is taxonomically correct it might be less confusing to call the herpesvirus of sheep, ovine herpesvirus 1 and that of goats, caprine herpesvirus 1.

Confusion over the taxonomic description of members of the family **Bovid herpesvirus** continues and Ludwig (20) prefers to retain the title of bovine herpesvirus for the viruses of cattle, sheep and goats. To add to the confusion Tisdall et al. and Engels et al. (27, 28) referred to the herpesvirus of goats as CHV1, the title formally assigned to the sheep herpesvirus (26).

Herpesviruses are subdivided biologically into alpha-, beta- and gammaherpesvirinae. As BHV2 has a wide host range, a short reproductive cycle, a rapid lytic cytopathogenicity in cell culture and can establish a latent infection in vivo it is

assigned a place in the subfamily alphaherpesvirinae.

Morphology

The general morphology of BHV2 is indistinguishable from that of other herpesviruses. The virion is spherical, measures between 120-200 nm in diameter and is composed of four structural components. These are a DNA core on a fibrillar spool, an icosohedral capsid 100-110 nm with equilateral triangular facets, 5 capsomeres on each edge and a total of 162 capsomeres, the tegument surrounding the capsid and a double envelope with minute projections.

Serological relationships

All BHV2 isolates from whatever country or disease are antigenically related (3, 29). Within this single strain different isolates have minor antigenic and immunologic differences. Infection with Allerton for example will not totally protect against heterologous challenge with virus isolated from localised lesions (29). Further studies at a molecular level may ultimately show clear differences between isolates.

Restriction endonuclease analysis of BHV2 DNA has shown minor differences between DNA profiles of field isolates from localised and generalised infections (30). A similar comparison of six isolates from localised lesions from dairy cattle in Scotland showed no distinguishable differences (31).

Serological relationships among BHV2 isolates have been demonstrated by cross neutralisation, gel diffusion and fluorescent antibody tests (32, 33, 34, 35). No serological relationship with other bovid herpesviruses has been demonstrated but Storz et al. (36) have shown that BHV2 and HSV1 and 2 share common immunologic properties and that infection of mice with BHV2 will protect against a lethal challenge of HSV. Similarly, the protective effect of immunising cattle with HSV against challenge with BHV2 has been demonstrated (37) thus confirming a two way cross immunologic relationship.

A common antigen involving proteins of BHV2, HSV1 and 2 and B virus has been demonstrated by immunodiffusion (36), indirect precipitation (38) and immunoelectrophoresis together with PAGE

analysis of the precipitate (39). These studies have shown that the HSV1 and BHV2 antigens recognised by heterologous sera share only partial identity. The locus of this common antigen is on a glycoprotein of molecular weight 125,000 which has been named A/B (40).

Recent evidence has shown that hyperimmune sera to BHV2 will recognise polypeptide antigens described in 1C35a-f polypeptides in cells infected with HSV1 and 2 (41). Additionally three epitopes were detected on BHV2 strutural protein gp 130 one of which was common to all three viruses, one shared only by HSV1 and BHV2 and the third specific for BHV2 only.

Monoclonal antibodies prepared against BHV2 and HSV1 cross-reacted and recognised glycoprotein gB in HSV1 and a related glycoprotein in BHV2. Immunoprecipitation of HSV1 gB by BHV2 monoclonal antibodies and the reverse were also demonstrated (42).

The high degree of homology between the DNA of HSV1 and BHV2, the distinction of their genomes from other herpesviruses, and the close serological relatedness has led to speculation about the common ancestry of these 2 viruses (43, 44). The hypothesis that the locus of the gA/B glycoprotein may have been partially conserved during evolutionary development of these two viruses (27) accords with this theory.

Physicochemical properties

BHV2 is sensitive to lipid solvents (44), is inactivated at pH3 (44, 45) and by heating at 50^{o}C for thirty minutes (44). The titre of the virus is not significantly reduced by three cycles of freezing and thawing (33) and the virus will survive in culture medium for at least 3 months at room temperature. At -70^{o}C or lyophilised BHV2 is stable for years. Rifampicin treatment at concentrations in excess of 100 ug ml^{-1} will destroy infectivity (44).

Little is known of the ability of the virus to survive in field situations but it is extremely sensitive to UV light. It is assumed that the main method of survival is in a latent form within the host. Disinfection with iodophores is rapid and virus is inactivated with 20 seconds (46).

Biological properties

Host range. Cattle and buffalo are the natural hosts of BHV2 and infection in these species can be manifest by disease of either a local or generalised nature. Neutralising antibodies to BHV2 have been detected in sera from giraffe, waterbuck, hippopotamus, impala, eland, bushbuck and oryx (53). Sheep and goats are not natural hosts of BHV2 and do not appear to be important as reservoirs of the virus. In one study neutralising antibodies were detected in 2/114 feral goat sera but not in any of 200 sheep sera examined (54).

Experimental inoculation of neonatal mice, rats and hamsters by intradermal, subcutaneous or intraperitoneal routes results in a progressive infection characterised by inflamed lesions especially around eyes, ears, nose, toes, legs and tail (32, 55, 56). Mortality is high and survivors are stunted in their development. Adult mice, rats and hamsters are not susceptible. A slight local reaction may develop in the foot pad of guinea pigs 4-5 days after scarification and inoculation at this site (56). Following intradermal inoculation of rabbits a mild inflammatory reaction at the site of injection may develop but serial passage in rabbits has been unsuccessful (32, 55, 56). Intradermal injection of virus in the wattle and comb of chickens has not produced a reaction or lesions (44).

Sheep and goats can be infected experimentally with BHV2 by intradermal, subcutaneous or intravenous injection (54, 56). In goats local, circumscribed lesions are produced 5-8 days after inoculation. These resolve quickly by granulation and scabbing and heal within a few days without scarring. In sheep the lesions may be more necrotic and can cause damage to the epidermal tissue resulting in scar formation. BHV2 can be isolated from the lesions, but more readily from sheep than goats. Following intravenous inoculation of virus a period of viraemia of 6-7 days occurs, followed by establishment of a latent infection (54).

Intravenous inoculation of specific pathogen-free piglets causes slight pyrexia 3-4 days after inoculation followed by the development of red papules and raised plaques in the skin of the

ears, face, body and scrotum (35).

In cattle, variation in pathogenicity of different isolates of BHV2 has been reported (29, 57). It was demonstrated that the Scottish isolate BMV from the teat of a cow with mammillitis was more pathogenic than the Italian isolate 69/160 from the oral mucosa of a calf and the African isolate Allerton BA from the tongue of a buffalo. The Allerton BA virus was the least pathogenic and it is hypothesised that in vivo passage in cattle may increase its virulence. Infection with Allerton BA induced less protection against subsequent challenge with the other isolates but this relationship was not reflected in the cross serum neutralisation test results (57).

There are anecdotal but unconfirmed reports of hard, raised lesions on the hands of farm workers coincident with natural BHV2 infection of cattle on the same farm but it is more likely that these lesions resulted from infection with parapox virus.

Growth in cell cultures. Most cell cultures of bovine origin are susceptible to infection with BHV2 and secondary cultures of bovine embryonic kidney or testis cells are commonly used for the isolation and replication of virus. Lamb testis and BHK-21/C13 will also support the growth of BHV2 (32). BHV2 has been propagated in organ cultures of bovine teat skin for up to 165 days (48). Embryonated hen eggs are not susceptible to infection (32, 44).

There is some variation of plaque size when different isolates are tested in the same system. Dardiri (35) demonstrated that BHV2 isolates from Africa, Europe and America produce plaques in bovine cell cultures with mean diameters of 8.56, 5.49 and 6.12 mm respectively. Other workers have confirmed that uniform plaques are produced by isolates of BHV2 from different animals with the same disease (35, 45, 49).

Persistent infection of bovine kidney cell cultures showing cycles of cytopathic effects and regeneration has been reported (50). The authors considered that deionised water in the medium was involved in inducing this effect which was overcome when double glass-distilled water was substituted. There are no

confirmatory reports of this effect but persistent infection with BHV2 of bovine cell cultures cultivated at 40°C has been reported (51).

The effect of temperature on virus replication appears to be critical and peak titres of BHV2 from localised disease lesions have been obtained between 32°C and 35°C (51, 52). It is reasonable to assume that this dermotropic virus will replicate more efficiently at temperatures likely to be found in the readily cooled skin of the teat and udder. It is not known if BHV2 from generalised infections reacts in a similar manner.

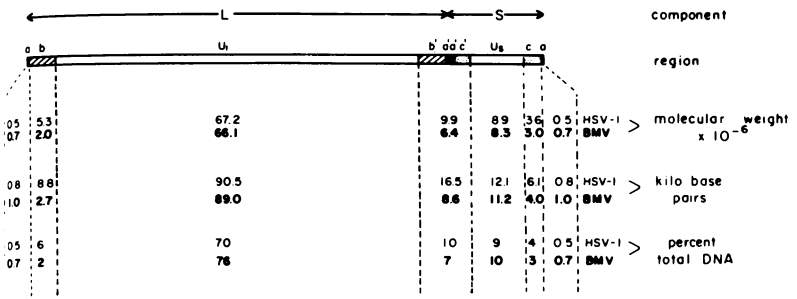
MOLECULAR ASPECTS OF THE VIRUS

The nucleic acid of BHV2 is double stranded DNA with a buoyant density of 1.723 g ml^{-1} in CsCl. It has a G+C content of 63.5 moles % and a molecular weight calculated to be 88 x 10^6 (20).

BHV2 DNA has been shown to consist of two covalently linked components designated Long (L) and Short (S). Component L consists of a unique sequence bracketed by sequences ab and its inverted repeat b′ a′ with molecular weights of 66.1 x 10^6, 2.7 x 10^6 and 2.7 x 10^6 respectively. The short region has a unique sequence bracketed by terminal repeats ca and a′ c′ with molecular weights of 8.3 x 10^6, 3.7 x 10^6 and 3.7 x 10^6 respectively (47). The a sequence of the termini of the molecule is duplicated in tandem and thus DNA can circularize after limited digestion with lambda 5′-exonuclease. BHV2 DNA consists of four equimolar portions which differ only in the relative orientation of the L and S components (47). These characteristics are similar to those of HSV DNA and thus the hypothesis on the origins of four populations of HSV DNA may apply also to BHV2 DNA. A comparison of HSV 1 and BMV DNA and a comparison of the sequence arrangements of five herpesviral genomes is illustrated in Fig. 2.

The buoyant density of BHV2 is estimated to be 1.235-1.250 g ml^{-1} in CsCl and that of the nucleocapsids to be 1.30 g ml^{-1} (43).

Figure 2.



virus	sequence arrangement of DNA	molecular weight x 10^{-6} total	U_l	U_s	U_l+U_s	Complexity of reiterated sequences x 10^{-6}
HSV-1		96	67.2	8.9	76.1	≤ 9.4
BMV		88	66.1	8.3	74.4	≤ 5.7
PrV		92	60	5.4	65.4	≤ 13.3
HVA		89.8	70	–	70	1.0
HVS		103	71	–	71	0.8

Figure 2. Top: Comparison of HSV-1 and BHV-2 DNAs. The results of BHV2-DNA analyses are given in boldface. Sizes of the regions are in molecular weight (x 10^6) of double-stranded DNA and in kilo base pairs.
Bottom: Comparison of the sequence arrangements of different herpesviral genomes including BHV-2. BHV-1 would be similar to PsR virus. Figures courtesy of Journal of Virology, Buchman and Roizman, 1978, 27, 239-254.

DIAGNOSTIC PROCEDURES

BHV2 infection causing BHM is suspected when clinical signs appear on the teats or udder, particularly in late summer and early winter.

Although BHM has distinct clinical features it can be difficult to diagnose especially when secondary bacterial infection alters the appearance of the lesions. In areas where the disease is not generally observed confusion with other viral infections of the bovine teat may occur. The most common of these is probably pseudo-cowpox which, although having distinct pathognomonic features, can in severe cases and depending on the stage of the infection be mistaken for BHM. However, as parapox virus infection is more common there are probably more instances of mild BHM being mistaken for pseudo-cowpox than the converse. Characteristically, infection with parapox virus gives rise to horseshoe shaped, rough yellowish-brown sores which gradually extend, progress and form raised circinate scabs. These should not be missed on critical examination.

Cowpox and vaccinia viruses are members of the **Orthopoxvirus** genus and cause similar lesions when injected into the skin of cows' teats (58). The lesions are raised with central necrosis edged by pale vesicles and surrounded by an inflamed zone. Scabs develop and separate after about 10 days thereafter healing with scarring.

Flat or raised warts caused by bovine papilloma viruses are readily recognised and cannot be confused with BHV2 infection.

The essential features of BHM are its seasonal occurrence, the involvement of newly calved heifers or bought-in cows and its rapid appearance followed by the development of necrotic painful lesions causing affected cattle to become extremely fractious especially when being milked.

Histopathological diagnosis is unnecessary and the disease is commonly confirmed by isolation of BHV2 from affected lesions and by the demonstration of rising serum antibody titres. Isolated virus can be identified by neutralisation with standard reference anti-BHV2 serum. In the early stages of infection herpesvirus

particles may be observed by electron microscopy in sloughed tissue or serous exudates.

Pseudo-lumpy skin disease is most likely to be confused only with lumpy skin disease but the characteristic lesions of PLSD make a differential diagnosis possible. Confirmation of PLSD can be achieved by isolation of BHV2 from excised biopsy tissue and by the demonstration of rising antibody titres to BHV2 in paired sera. PLSD usually occurs in the summer months and frequently affects cattle grazing along river banks which adds strength to the hypothesis that biting flies may be involved in transmission.

IMMUNOLOGY

The humoral immune response to BHV2 infection generally follows the pattern of responses to other herpesviruses. Initially there is a pronounced antibody response which is detectable in serum by neutralisation test about 7 days after infection. Antibody concentration continues to rise to maximum 1-2 wk later and antibody titres frequently reach values in excess of 1/100 following a natural infection. Calves infected experimentally respond similarly but antibody titres may not reach such high values. Early antibody in primary infections is IgM followed by a rise in IgG antibodies which may persist for more than two years in natural infections (59, 60, 61). Following experimental infection antibodies can be detected up to eight months after inoculation (62) and in some instances up to 16 months (19).

Experimental evidence has shown that serum antibody is protective or partially protective against challenge (37, 57), and naturally infected cattle seldom experience a recurrence of the disease. This may be due to persistent antibody or anamnestic responses following re-infection or reactivation of latent virus.

Circulating antibody absorbed from colostrum (59) may confer protection to sucking calves for several months.

There are no reports on the function of local or cell-mediated immune responses in BHV2 infections although these may play an important role in both diseases. However, it has been demonstrated that at parturition in the bovine there is depression

of cell-mediated immune responses (63) which might permit replication of latent virus reactivated at this time. This offers an explanation for the sudden appearance of BHM in a herd but does not explain the method and route of original infection. The detection of antibodies to BHV2 in sera of pregnant heifers does not necessarily correlate with the emergence of clinical disease in these animals (64).

EPIZOOTIOLOGY

PLSD caused by BHV2 was first reported in South Africa in 1957 (2). Since that time generalised skin disease of cattle has been reported in Kenya (65), USA (34) and Australia (66). Localised bovine herpes mammillitis has been reported in Britain (5), Ruanda-Urundi (now Rwanda and Burundi) (56), USA (67), Australia (68), Bulgaria (69), Italy (44), France (70), Zambia (71) and Brazil (72). Serological evidence has confirmed BHV2 infection in cattle in The Netherlands (73) the German Democratic Republic (74) and Somalia (75) although there is no report of the presence of herpes mammillitis or of the isolation of virus in these countries.

One of the main remaining enigmatic features of BHV2 infection is the source of the virus. It is simple to postulate that cattle become latently infected and are therefore a potential source of virus. However, it is not understood how the virus enters a herd with no history of infection and where no cattle have been brought on to the premises. Fly borne transmission has been suggested and it may be that biting flies are important in mechanical transmission. Gibbs et al. (76) have shown that BHV2 can be reisolated from **Stomoxys calcitrans** which have been fed on a solution containing the virus and Weiss (11) was able to isolate BHV2 from **Musca fusciata** which had been caught on cattle with PLSD.

Once clinical BHM occurs in a herd lateral transmission appears to take place and in some instances many animals in a herd may be affected over a period of several wk. The virus content in early lesions is high and may exceed 10^6 $TCID_{50}ml^{-1}$ thus

providing a potential source of infectious virus which may be transmitted readily on cloths, milking machinery and the hands of dairy workers. Frequently, herds on contiguous properties develop BHM but the method of spread between herds is not known.

VACCINES

There is no commercial vaccine available against BHV2 and it seems unlikely that such a vaccine would be economically feasible.

The only successful experimental vaccines developed have been live unattenuated virus vaccines which were shown to be effective against challenge with BHV2 (33, 77, 78). This type of vaccine has the disadvantage of spreading infection and as BHV2 has the potential to become latent its use is inadvisable. Under exceptional circumstances, however, it may be considered for use in infected herds.

CONTROL

During the course of an outbreak of BHM, affected cows should be separated from the herd where practicable and if milking is still possible these animals should be milked separately or after those that are unaffected. The use of iodophore based teat dips is recommended and milking machine teat clusters should be disinfected between cows.

Recent experimental evidence has shown that certain antiviral compounds effectively inactivate BHV2 in vitro (79, 80). These compounds have not been fully tested in vivo but the application of 5-iodo-2′-deoxyuridine, phosphonoacetic acid, cytosine arabinoside and acyclovir to early experimentally induced lesions on cows′ teats did not alter the course of the disease (81).

For both BHM and PLSD insecticidal preparations should be applied to cattle at risk to prevent possible transmission by biting flies.

Neither disease is notifiable but milk contaminated with blood is not permitted to be taken into bulk collection tanks.

ECONOMIC FACTORS

When BHM affects a herd milk yields may be reduced substantially and the skins of cattle affected with PLSD have little commercial value. Although disadvantaging the individual farmer, neither is of national significance.

FUTURE ASPECTS

One of the possible methods of transmission of BHV2 causing PLSD is by biting insects. It is possible that treatment of cattle with pyrethroids may be an effective method of control.

The situation with BHM is less clear and several problems remain to be addressed. The seasonal nature of BHM defies the argument that flies may be important vectors as at this time of the year fly activity is reduced. However, it may be that animals are infected by biting flies in the summer months and that the virus becomes latent in the host. Subsequent stress such as parturition, may cause reactivation of virus and the development of disease. It is not understood why there is no apparent spontaneous recrudescence of BHM lesions or why spring calving heifers do not become affected.

The stress factors causing reactivation of BHV2 are not known but circumstantial evidence suggests that these factors are associated with calving. There are no reports of the isolation of BHV2 from unaffected cattle and virus has not been recovered from experimentally infected cattle subsequent to recovery from primary infection and without immunosuppressive treatment.

The method of spread of disease between animals and especially between farms is not understood. Mechanical transmission causing lateral spread on a farm is likely but cannot explain the pattern of spread to neighbouring farms. In the outbreaks of BHM reported by Scott and Holliman (9), in which more than one hundred herds were involved in an area of over 1000 square miles, the pattern of spread was entirely random and apparently was not affected by climatic conditions, fly activity or movement of stock.

The role of cell-mediated immune responses in BHV2 infections

has not been fully studied although it is probably important in the control of infection. Clearly further work is required on this aspect of immunity and may lead to more effective means of control.

With recent advances in vaccine technology it is possible that a novel vaccine directed at HSV may prove to be protective against BHV2. Other forms of control, such as the use of antiviral drugs might also be considered although these might prove to be costly and difficult to implement. The cardinal feature in effective control of HSV is that the antiviral drug must be applied at onset of disease and preferably at the prodromal period, with regular and frequent application. These criteria would be difficult to fulfil in diseases of cattle such as BHM. An effective, single application, anti-BHV2 drug would be very useful in the control of BHM.

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3

BOVINE HERPESVIRUS-4 (BHV-4) INFECTIONS OF CATTLE*

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ABSTRACT

The bovine herpesvirus-4 (BHV-4) group comprises several antigenically related herpesviruses isolated from a variety of clinical syndromes as well as from apparently healthy cattle. Except for a few isolates, they are mildly or not pathogenic for cattle. Mononuclear blood cells and lymphoid organs are the sites of acute and latent infections in cattle and rabbits. A site of latency in nervous tissue also has been described in cattle. From some of its biological characteristics, such as the morphogenesis in infected cells and a slow viral growth curve, BHV-4 was tentatively classified as cytomegalovirus. Recent molecular data have allowed BHV-4 to be classified as a group B herpesvirus. Natural and experimental infections do not induce high levels of neutralizing antibodies so that serological diagnosis is best achieved by ELISA or immunofluorescence antibody tests. BHV-4 infection has a worldwide distribution: it has been already diagnosed in Europe, America and Africa.

INTRODUCTION

The bovine herpesvirus-4 (BHV-4) group is made up of a collection of antigenically related viruses, distinct from the other bovine herpesviruses. While some were recovered from diseased cattle, others were isolated incidentally from apparently healthy cattle or from primary cell cultures. Few appear to be pathogenic, the majority

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inducing only mild clinical signs in experimentally infected cattle.

The designation of this virus group has been confusing. The International Committee on Taxonomy of Viruses designated it as bovine herpesvirus 3 (1), a name already used by Gibbs and Rweyemamu (2) in the first extensive review of this group of bovine herpesviruses. Nevertheless, it is usually referred to in the literature as BHV-4 and this designation has been conserved in the new classification of bovine herpesviruses proposed by Ludwig (3). Some American authors have also named it bovine herpesvirus-5, but this designation was used only in a few articles. More recently, an agreement has been reached on the name bovine herpesvirus-4 (4).

BHV-4 remains latent in its natural host and also in rabbits which provide an excellent laboratory animal model of the infection.

Another feature that we would report here is that restriction endonuclease and serological analysis have revealed that feline herpesvirus-2 (FHV-2), the virus isolated from cats suffering from urolithiasis, is in fact a strain of BHV-4 (5).

BHV-4 infection associated with respiratory and ocular diseases was identified first in Europe by Bartha et al. (6), and later in the USA by Mohanty et al. (7). Numerous viruses isolated in Africa almost certainly belong to the BHV-4 group. Theodoridis (8) has indeed characterized several herpesvirus strains isolated between 1957 and 1970 from the "epivag" syndrome, a genital syndrome first described in 1938 in Africa, but he failed to study the serological relationship between the African strains and the BHV-4 reference strains. Since 1957, "orphan" herpesviruses have also been isolated from other syndromes in Africa (2), but, without proof of their identity with BHV-4 other than their behaviour in cell cultures, they cannot be classified definitely in this bovine herpesvirus group.

The continuing isolation of other BHV-4 strains has demonstrated the apparent world-wide distribution of this virus and aroused further interest in its contribution to clinical diseases in cattle.

CLINICAL SYMPTOMS AND PATHOLOGY

BHV-4 has been isolated from five groups of clinical entities:

(1) ocular or respiratory disease: conjunctivitis, rhinitis, tracheitis, pneumonia;
(2) genital disease: orchitis, epididymitis, vaginitis, abortion, metritis;
(3) skin lesions: lumpy skin disease, mammary pustular dermatitis;
(4) malignant catarrhal fever;
(5) enteric disease.

Ocular and respiratory diseases

The two reference strains were isolated from animals suffering from keratoconjunctivitis (Movar 33/63) (6) or from calves showing a respiratory disease characterized by nasal discharge, cough, dyspnoea and pulmonary lesions (DN599) (7). Experimental infection of the respiratory tract usually does not reproduce severe clinical signs. No clinical sign was produced by the experimental inoculation of cattle with the Movar 33/63 strain (9). On the other hand, intranasal and intratracheal inoculations of DN599 strain produced a respiratory illness with pneumonia and conjunctivitis. Three calves died, but the role of BHV-4 in this pathology remains questionable, because Pasteurella multocida was isolated from a control calf and two infected calves (10).

Intranasal inoculation of a strain isolated from cows with vulvovaginitis and postpartum metritis induced fever, nasal discharge, anorexia and depression (11). Intratracheal infection of strain FTC isolated from the respiratory tract produced only a mild tracheitis in young calves (12).

Genital disease

A bull infected via the prepuce with an African herpesvirus strain isolated from "epivag" syndrome failed to develop any symptoms. Cows infected intravaginally with another strain isolated from abortion showed only a mild vaginitis (8, 13). Other African strains have been isolated from cases of orchitis (8, 13), but they have not yet been serologically identified as BHV-4. A BHV-4 strain (V.Test) isolated in Belgium from a case of oedematous orchitis and azoospermia (14, 15) produced inconstant lesions by intratesticular inoculation: infiltration of interstitial tissue by mononuclear cells with lesions more frequently observed in the epididymes than in the

testicle. Despite this, virus was excreted by ocular and nasal secretions (16). Other strains of "non-syncytia forming" herpesviruses have been isolated from the semen (17), but they are not pathogenic for calves (2).

Several isolates were associated with abortion. Schiefer (18) reported a case of abortion where inclusion bodies typical of cytomegalovirus infections were observed in various organs of the foetus, but no attempt was made to reisolate the virus. Kendrick et al. (19) inoculated a strain isolated from metritis (20) to foetuses at various stages of gestation. Two foetuses died at 3 to 4 months of gestation showing lymphoreticular activation in the lung and the lymph nodes. No signs were seen in cows infected at 7 months of gestation. A BHV-4 strain and BVD virus were also isolated together from aborted foetuses (21).

BHV-4 has been associated with metritis (20) and especially postpartum metritis with or without peritonitis (22), as well as other symptoms: diarrhea, neonatal mortality, respiratory signs, mammitis (23). Experimental reproduction of the disease was attempted by intravenous infection with strain LVR140 of both pregnant and non pregnant cows. In pregnant animals, metritis occurred at various intervals after infection, but, in every case, about one week after parturition. Symptoms were accompanied by an increase in detectable antibody. The death of inoculated cows was observed, preceded by various symptoms (24).

Intravenous infection of heifers was followed by reisolation of virus from the vagina, the infundibulum and the corpus luteum without any clinical signs (25).

Skin lesions

"Orphan" herpesviruses which share the biological properties of BHV-4 have been isolated from cases of lumpy skin disease in Africa (26), as well as from tumors of urinary bladder and rumen (27). Experimental infection was attempted with BHV-4 strain 3374, isolated from a case of mammary pustular dermatitis (28). A febrile response was observed 4 and 5 days post infection. Intradermal inoculation in the udder produced vesicular lesions, but no definite role in this disease could be attributed to BHV-4 (29). Antigens specific of BHV-4

have also been detected by indirect immunofluorescence in cells derived from bovine ocular squamous cell carcinoma, but no infectious virus was recovered (30).

Malignant catarrhal fever

BHV-4 has a great affinity for lymphoid organs and this is probably why the virus has been isolated several times from animals showing malignant catarrhal fever (31, 32, 33). No clinical signs resembling those of malignant catarrhal fever were observed after experimental inoculation of BHV-4. An association between BHV-4 and BVD virus has been suggested in U.S.A. as an etiological factor in the European form of malignant catarrhal fever (34), but there is no experimental evidence to support this hypothesis.

Enteric disease

In USA, BHV-4 is frequently isolated from the digestive tract (34). Experimental inoculation with a strain isolated from the feces of a diarrheic cow failed to reproduce the symptoms (35).

PATHOGENESIS

In experimentally infected rabbits, BHV-4 replicates actively in the spleen and to some extent in peripheral blood leukocytes. Virus is propagated throughout the body in infected leukocytes, but no further viral multiplication can be detected in other organs. There is also evidence that splenic macrophages can be the site of acute and latent infections by BHV-4 (36, 37).

In the bovine, virus is associated with the mononuclear blood cells (29). It is probable that BHV-4 enters the body via the oronasal route. Virus multiplication then occurs in mononuclear cells and virus is disseminated throughout the animal by these cells. At that time, it can be isolated from various tissues and organs. There may also be multiplication in conjunctiva, anterior respiratory and genital mucosa, producing nasal, ocular or vaginal excretion or metritis in postparturient cows. Postpartum metritis may be provoked by reactivated BHV-4 virus, since it was produced in cows infected at various times before parturition (24); no signs were seen after the primary infection, but metritis was observed after parturition, which could be considered as a stimulus of reactivation as in the case of

bovine herpesvirus-1 (BHV-1) infection (38). Moreover, a secondary antibody response was detected at this time. There is, therefore, a need to examine whether or not diseases induced by BHV-4 infection are, in certain cases, the consequence of reactivation of latent virus.

LATENT INFECTION

BHV-4 is able to persist in a latent state following primary infection. The biological features of BHV-4 latency have been described only recently, although, in 1972, Van der Maaten and Boothe (39) reported the reisolation of a herpesvirus in leukocytes of calves experimentally infected 9 months to one year before. Nevertheless, this strain of herpesvirus, designated as Pennsylvania 47, shares only a partial antigenic relationship with BHV-4 and its DNA presents a different restriction pattern (40).

BHV-4 is frequently isolated from culture of organs of clinically healthy cattle. This strongly supports the hypothesis that virus can persist in a latent state. Thiry et al. (15) isolated a BHV-4 strain in coculture of testicle cells with Georgia Bovine Kidney cells. Testicle cells were obtained from a bull showing oedematous orchitis and azoospermia. The method used for virus isolation and the failure to isolate virus from triturated organs suggest that BHV-4 was latent in the testicle. Other authors have also reported the reisolation of BHV-4 from kidney cell cultures of healthy cattle. In one experiment devoted to the reisolation of BHV-1 from trigeminal ganglia of normal cattle, two isolates of BHV-4 were incidentally recovered (43).

The definitive proof of the establishment of BHV-4 in a latent state was experimentally given by Osorio and Reed (29) and Krogman and McAdaragh (44). Calves infected intranasally with BHV-4 were treated with dexamethasone 2.5 months later. Virus was reisolated from nasal swabs on only one occasion, but all calves showed a rise in specific antibody titres. Virus was reisolated from explant cultures of spinal cord and trigeminal ganglion of one calf (44). In another experiment, BHV-4 was also successfully reactivated from calves by dexamethasone treatment. Reactivation was provoked three

months after experimental intranasal infection and was asymptomatic. Virus was detected in nasal secretions for 8 days. BHV-4 was also reisolated from nervous tissues, especially in calves killed at the beginning of dexamethasone treatment. No booster antibody response was demonstrated by sero-neutralization (45).

As for several herpesviruses (46), latent BHV-4 is therefore reactivated in cattle by the use of glucocorticoids. In the rabbit model of the infection, virus cannot be reactivated by dexamethasone (36). The nervous system, and especially the trigeminal ganglion, appears to be a site of latency in cattle (11, 43, 44, 45). Another site of latency is now well established: the mononuclear blood cells and lymphoid organs (11, 29, 45). Cattle were inoculated intradermally, intravenously and into mammary gland with the strain 3374 (29). Virus was associated with the mononuclear fraction of the blood and was reisolated from coculture and explant culture of spleen from all latently infected cattle. The authors (29) concluded that the association of BHV-4 with mononuclear cells could explain the diversity of tissues and organs from which BHV-4 has been isolated.

Latent infection of BHV-4 can be induced in rabbits (36, 37). Spleen seems to be the organ of primary replication and also the site of virus persistence. Cell-free virus was detected in conjunctival swabs, buffy coats and spleen for up to seven days after infection. Afterwards, coculture of spleen cells or explant culture was required to reisolate the virus. During the latent period, virus has been isolated not only from the spleen, but also, at lower titres, from bone marrow, lung, kidney, salivary gland and liver (37).

CHARACTERISTICS OF THE VIRUS

Taxonomic status

BHV-4 is still officially named bovine herpesvirus 3 (1), but this designation will most probably be replaced in the near future (4). On the basis of its biological properties, BHV-4 was tentatively classified as cytomegalovirus and therefore could be included in the betaherpesvirinae subfamily (47). The structure of its genome, with reiterated sequences at both ends in the same orientation, is characteristic of group B herpesvirus (5). Its genomic organization

resembles that of herpesvirus saimiri (saimiriine herpesvirus 2) (48) and alcelaphine herpesvirus-1 (AHV-1), the causal agent of the African form of malignant catarrhal fever (Bridgen and Reid, personal communication).

The size of its genome and the relatively broad spectrum of susceptible species are not in favour of its classification as a betaherpesvirus. These properties are more in accord with those of members of the gammaherpesvirinae, such as herpesvirus saimiri and also probably AHV-1 (49).

Morphology and morphogenesis

The morphology of BHV-4 is typical of a herpesvirus (Fig. 1). The nucleocapsid is icosahedral, with a dense core within the capsid which is made up of a regular arrangement of short tubular capsomeres (33). The diameter of a naked nucleocapsid is about 90-100 nm while

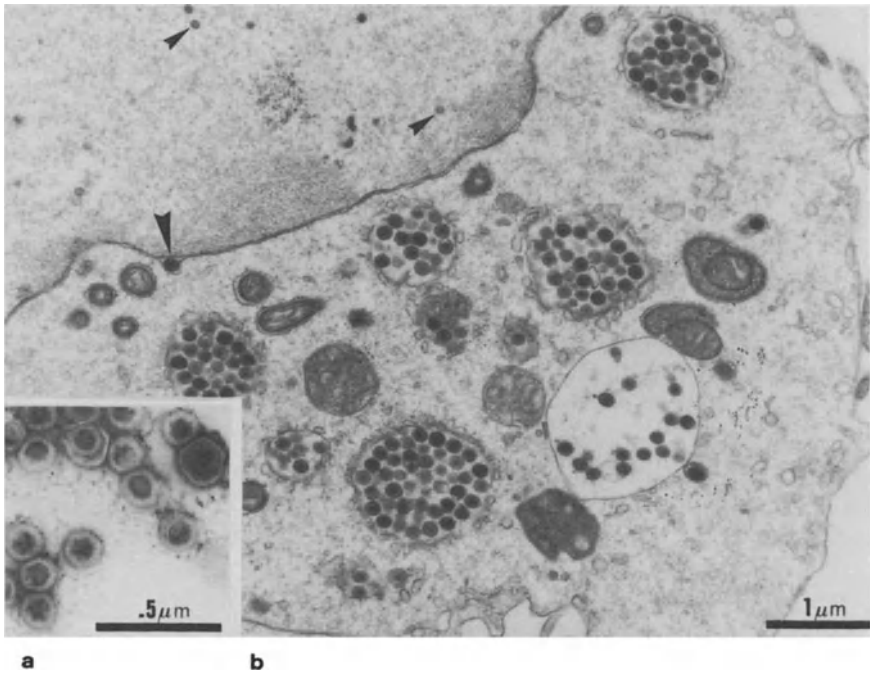

Fig. 1. Electron micrographs of BHV-4 (V.Test strain).
a) Extracellular nucleocapsids and enveloped viruses.
b) Intracellular virus: virions in cytoplasmic vesicles; ► nucleocapsids in the nucleus; ► virion in the perinuclear space.
(Courtesy of D. Dekegel, Pasteur Institute of Brabant, Belgium).

enveloped virions have a diameter ranging from 115 to 150 nm (12, 50, 51). Nucleocapsids are formed in the cell nucleus, the virus acquiring its envelope by budding either at the inner nuclear membrane or at a cytoplasmic vesicle membrane. Many virions are observed in cytoplasmic vesicles which migrate to the cytoplasmic membrane and release virions to the extracellular medium (Fig. 1) (31, 33, 50, 52, 53). Dense bodies consisting of electron dense material, nucleocapsids and enveloped viruses are observed in the cytoplasm of cells 48 to 72 hr after infection. These features are characteristic of cytomegaloviruses (33).

Antigenicity

BHV-4 strains are indistinguishable by immunofluorescence antibody tests (IFAT) (40, 54, 55, 56). Neutralizing antibodies are difficult to detect, but reciprocal neutralization between BHV-4 strains indicate that they are serologically indistinguishable (22, 54). By conventional reciprocal serological tests, no antigenic relationship is demonstrated between BHV-4 and BHV-1 (40, 54) or between BHV-4 and bovine herpesvirus-2 (BHV-2) (40). Mohanty et al. (57) reported a cross-reaction between BHV-1 and BHV-4 by ELISA, but another study showed that cross-reacting sera were probably origi-

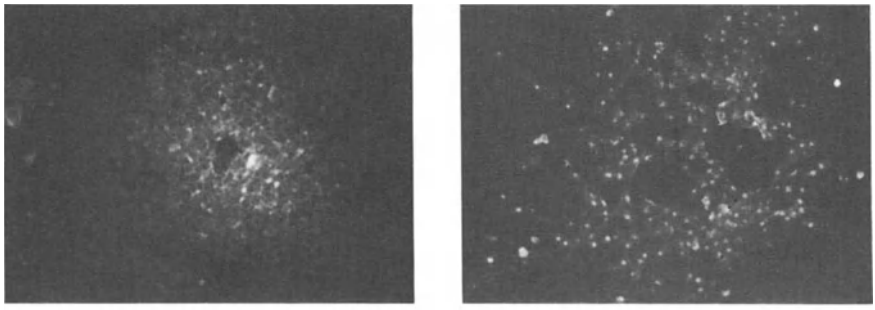

Fig. 2. Indirect immunofluorescence staining of infected Georgia Bovine Kidney cells with monoclonal antibodies directed against BHV-4 (V.Test strain).
a) Cells infected with BHV-4 showing intranuclear and intracytoplasmic fluorescence.
b) Cells infected with strain WC11 of AHV-1 showing intranuclear fluorescence. (Reprinted with permission from ref. 60).

nated from animals infected by both BHV-1 and BHV-4 (58). Two antigens detected by immunoelectrophoresis are common to BHV-4, BHV-1, BHV-2 and suid herpesvirus-1; two other antigens are also shared by BHV-1 and BHV-4 (59). On the other hand, monoclonal antibodies (Mabs) prepared against BHV-4 do not react with BHV-1 and BHV-2 (60). Further studies are therefore needed to clarify the antigenic relationships of BHV-4 with BHV-1 as well as with the other bovine herpesviruses. A serological relationship was also shown with AHV-1 (40, 61). This partial relationship was further proven by the isolation of a Mab directed against BHV-4 which recognizes cells infected with the WC11 strain of AHV-1 by IFAT (Fig. 2) (60). Nevertheless, 6 Mabs directed against AHV-1 do not recognize BHV-4 (Reid and Dubuisson, unpublished results). The use of a panel of Mabs allows distinctions to be made between BHV-4 isolates (60).

Physico-chemical properties

In 20-65% (wt/vol) sucrose gradients, BHV-4 has an equilibrium density of 1.2 g/cm^3 (60). The virus is sensitive to pH 3, 20% ether, 20% chloroform; it is heat-labile (50°C, 30 min.) and its multiplication is inhibited by BUDR (0.1 μM) (6, 22, 41). As with other herpesviruses, its survival in the environment must be limited and lipid solvents will be active as disinfectant.

Biological properties

Domestic cattle are the most likely natural host of BHV-4. No evidence of infection of wildlife has been detected (62, 63). The rabbit provides an experimental host system where BHV-4 persists in a latent state, probably at the same site as in its natural host (37). The infection of rabbit is successfully achieved by intravaginal, conjunctival or intravenous route (36). BHV-4 has also been isolated from sheep; this isolate was experimentally inoculated to sheep and reisolated after dexamethasone treatment (64). Experimental inoculation of mice, guinea pigs, hamster, rats and chickens with BHV-4 does not reproduce a clinical disease (2). The susceptibility of cat for bovine isolates has not been studied: FHV-2 could be originated from cats or was a tissue culture contaminant (5).

BHV-4 grows in both primary and established bovine cell cultures: primary kidney and testicle cells, Madin Darby Bovine Kidney

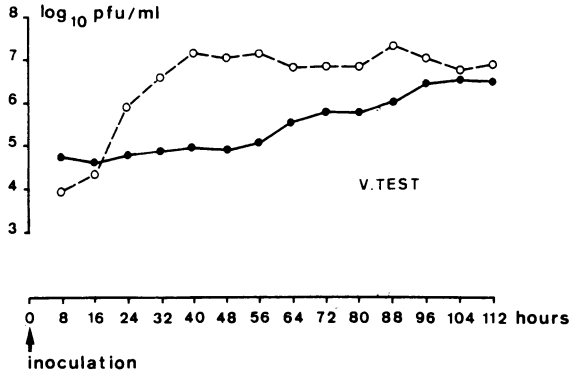

Fig. 3. One step multiplication curve of BHV-4 (V.Test strain) on confluent Georgia Bovine Kidney cells; O cell-associated virus; ● extracellular virus. (Reprinted with permission from ref. 56).

cells, Georgia Bovine Kidney (GBK) cells (6, 15, 41, 47). It also grows in kidney cells of various origins: sheep, goat, dog, cat, rabbit and chicken (6, 41). Cytopathic effect (CPE) is characterized by the presence of rounded cells, dispersed throughout the monolayer. It rarely appears before 48 to 72 hr after infection. Cytolysis is then observed and CPE is total after approximately 5 days, depending on the type of cells and the multiplicity of infection. Cowdry type A inclusions are present in the nuclei of infected cells (6). Viral plaques are visible within 7 to 9 days in bovine fetal spleen cells, in 5 days in actively growing GBK cells (22, 47, 54) and in 9 to 11 days in confluent GBK cells (56). The mean plaque diameter varies from 0.3 to 0.8 mm (22, 47, 56). The range of the plaque sizes is 0.03 to 0.07 mm^2 (56), depending on the viral isolate. This size is much smaller than the area of plaques produced by BHV-1 (47). Plaques possess an irregular contour and may be produced under carboxymethyl-cellulose or agarose overlay. In one step growth experiments, BHV-4 is mainly cell-associated for 48 hr in freshly seeded cells and for 48 to 88 hr in conflent cells (Fig. 3) (47, 56).

No extensive studies have been undertaken to compare the effects of various strains of BHV-4 in experimental infection of cattle and rabbit. In cell culture, representative BHV-4 strains exhibit the same growth curve (56). Significant differences in the mean plaque

size are observed between viral strains (56), but plaque size is very small and this criterion cannot be retained as valuable strain marker.

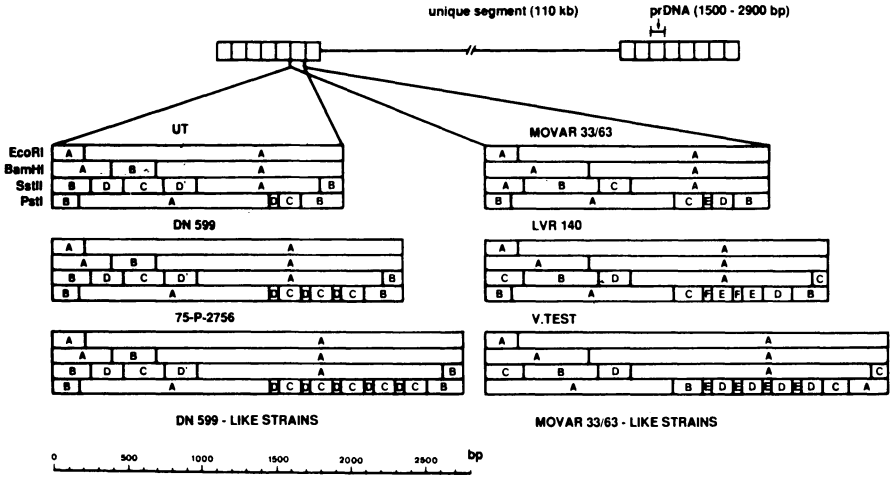

Fig. 4. Organization of BHV-4 genome and restriction maps of prDNA of representative strains: strains UT, DN599 and 75-P-2756 according to ref. 48; strain Movar 33/63 according to ref. 48 and Bublot, unpublished results; strains LVR140 and V.Test according to Bublot, unpublished results.

MOLECULAR ASPECTS OF THE VIRUS

Genome

The BHV-4 genome is a double-stranded linear DNA of 144±6 kb, which consists of a unique segment of 110 kb, flanked at both ends by tandem repeats (Fig. 4). The number of tandem repeats, called polyrepetitive DNA (prDNA) varies at each genomic end, but the overall number of prDNA is about 15 per genome (48) (Fig. 4). Blot hybridization shows a high degree of genetic relatedness between BHV-4 isolates (65). Restriction profiles of BHV-4 DNA completely differ from those of other bovine herpesviruses (3, 40).

BHV-4 isolates are closely related by DNA restriction patterns. The differences between BHV-4 isolates can be detected both in the unique segment and the prDNA. In the unique segment, the variations

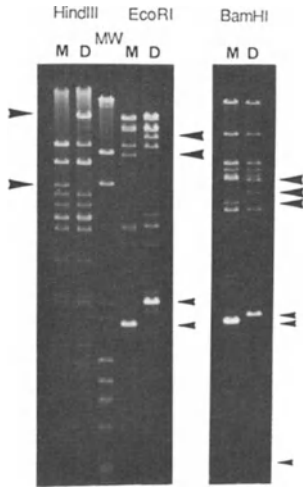

Fig. 5. Restriction endonuclease patterns of BHV-4 strains Movar 33/63 (M) and DN599 (D);
► indicates variations of large restriction fragments;
► indicates variations of prDNA (Bublot, unpublished results); MW: λ DNA cleaved by HindIII and ϕX174 DNA cleaved by HaeIII.

mainly concern the largest unimolar fragments obtained by digestion with restriction endonucleases EcoRI and HindIII (47, 48, 56). This distinguishes between two types of strains: the DN599-like strains comprising the American isolates and the European UT strain, and the Movar 33/63-like strains comprising most of the European isolates (Fig. 5) (47, 48, Bublot et al., unpublished results). Several restriction endonucleases cleave inside the prDNA: ApaI, BamHI, BanI, BglI, EcoRI, HinfI, NaeI, PstI, PvuII, SamI, SstII, StuI (47, 48, Bublot et al., unpublished results). The size of the prDNA varies depending on the viral isolate. This variation is provoked by a sequence which can be several times reiterated inside the prDNA (48). Up to 8 different sizes of prDNA, varying by a multiple of 200 bp from 1500 to 2900 bp have already been observed (Fig. 6). Moreover, the restriction profiles of some isolates exhibit two prDNA of different sizes, suggesting a heterogenous viral population (Fig. 6). (Bublot et al., unpublished results). PrDNA of DN599-like and Movar 33/63-like strains have different restriction maps for endonucleases BamHI, SstII and PstI (Fig. 4).

Thymidine kinase

BHV-4 induces a thymidine kinase (TK) activity in infected TK^- cells. The kinetics of TK activity induction are slower with BHV-4 than with other herpesviruses; this observation may be correlated with the slow replication cycle of the virus (5).

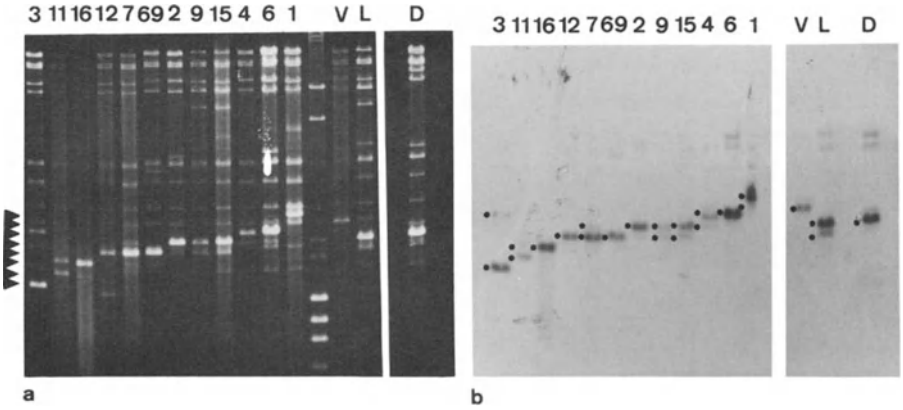

Fig. 6. EcoRI restriction endonuclease patterns of several Belgian isolates compared with BHV-4 strains V.Test (V), LVR140 (L) and DN599 (D).
a) the arrows indicate the 8 different sizes of prDNA.
b) Southern blot hybridization of the same DNA with a subcloned fragment of PstI A of V.Test strain prDNA. Dots indicate the prDNA. Restriction patterns of isolates 3, 11, 7, 9, 15, 4, 1 and LVR140 exhibit two prDNA. The other larger restriction fragments hybridizing with the probe are junction fragments (48). Right terminal fragments are situated 250 bp below the prDNA (eventually mixed with the second prDNA). Left terminal fragments are too small and are not visible (Bublot, unpublished results).

DIAGNOSTIC PROCEDURES

BHV-4 has been isolated from a variety of symptoms. No clear clinical signs are associated with the infection, except a febrile response, mild respiratory signs and postpartum metritis (10, 11, 12, 24). BHV-4 can be isolated from mononuclear blood cells and lymphoid organs. Cows clinically affected by postpartum metritis excrete the virus for long periods in uterine exudates (23). BHV-4 has been successfully isolated from nasal swabs (10, 16, 66). Isolates are easily characterized as BHV-4 by immunofluorescence (Fig. 2). Latent infection is biologically demonstrated by coculture of mononuclear blood cells with susceptible cells (29).

IFAT and ELISA are the best serological tests to titrate anti-BHV-4 antibodies. A good correlation between the two methods has been demonstrated (67). Alternatively, complement-dependent neutralization can also be used, but antibodies are detected later and titres are

usually lower (68). The majority of seropositive animals have probably experienced a subclinical respiratory infection.

Preliminary experiments have failed to demonstrate positive delayed hypersensitivity reaction (skin test) (Dubuisson and Bublot, unpublished results).

Restriction endonuclease analysis is the best tool to characterize BHV-4 isolates, because their restriction patterns are sharply different from those of other bovine herpesviruses (3).

DNA probes have not yet been used in diagnostic tests. The prDNA of at least two strains has been cloned (Fig. 6) (48, Bublot et al., unpublished results). It hybridizes with cellular repetitive DNA and therefore is not entirely specific. Moreover, cross-hybridization of prDNA with BHV-1 DNA inverted repeats has also been observed (Bublot et al., unpublished results).

As stated previously, partial antigenic relationships between BHV-4 and AHV-1, and maybe between BHV-4 and BHV-1 must be taken into account for the interpretation of diagnostic results.

IMMUNOLOGY

The immune response of cattle following BHV-4 infection is characterized by a low production, or in certain cases by an absence of neutralizing antibodies (10, 24, 55, 67). When a weak response is demonstrated, neutralizing antibodies appear 22 to 34 days after primary infection (11, 16). The presence of complement markedly increases the neutralizing antibody titres and antibodies are detectable earlier (18 days after infection) (68). By IFAT and ELISA, specific antibodies are present 14 to 20 days after primary infection (16, 29, 67).

After experimental reactivation of latent virus, an anamnestic immune response is demonstrated by a rise in specific antibodies 7 to 15 days after the first injection of dexamethasone, but no increase in neutralizing antibodies is detected (44, Dubuisson et al., unpublished results).

The association of BHV-4 with mononuclear blood cells and lymphoid tissues both in acute and latent infections could have some implications on the regulation of the immune response in cattle. The

consequences of such infections need to be studied (37).

The role of cell-mediated immunity has not yet been investigated.

EPIZOOTIOLOGY

Several BHV-4 strains have been identified in America and Europe. Herpesvirus strains biologically similar to BHV-4 have been isolated in East Germany (69). In Africa, strains isolated from cutaneous diseases or "epivag" syndrome probably belong to this bovine herpesvirus group. They were isolated in Kenya, Tanzania and South Africa (13, 27, 70). A recent serological survey carried out in Zaire revealed that 70% of cattle were seropositive for BHV-4 (71).

The prevalence of the infection is very different depending on the country where it has been investigated. In 1977, 2% of Oklahoma cattle were serologically positive (72). In 1986, 8.7% of sera from the U.S.A. were seropositive (73). Recent studies revealed that 4.2% of cattle were seropositive in Switzerland (58). Thirty % of sera were positive in Northern Italy, indicating that 50% of the herds were infected (74). In West Germany, 18.4% of sera harboured antibodies against BHV-4 (73). The situation is markedly different in artificial insemination (A.I.) centres: 0 to 69% of seropositive bulls were identified in German A.I. centres (73); 30% of bulls were seropositive in Belgian A.I. centres (Dubuisson et al., unpublished results). This is similar to the prevalence of BHV-4 seropositive animals in Belgian cattle: 28.7% in the southern part of the country and 15% in the northern part (75). In Belgium, 38% of veal calves in two fattening units possessed maternal antibodies against BHV-4 (76).

FUTURE ASPECTS

BHV-4 infection is well established in several countries, but its real pathogenicity remains to be demonstrated, for example, the interaction of BHV-4 with cells involved in the regulation of the immune response. The control of the infection by vaccination or eradication is therefore not a major concern in sanitary policy.

Its lack of pathogenicity confers it two advantages. BHV-4 is a good model for the study of latency in homologous (bovine) system. The infection does not kill the animal and at least one site of

latency is easily and repeatedly accessible: the mononuclear blood cells. Moreover, preliminary experiments may be carried out in the rabbit.

BHV-4 may be suitable for use as a viral vector for recombinant bovine vaccines, as suggested by Kit et al. (5). For this purpose, a better knowledge of its molecular biology is needed. Further research should be devoted to cloning the genome, obtaining restriction maps of BHV-4 DNA, localization of the important genes and analysis of the viral proteins.

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MALIGNANT CATARRHAL FEVER AND THE GAMMAHERPESVIRINAE OF BOVIDAE

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INTRODUCTION

Malignant catarrhal fever (MCF) is a dramatic, fatal disease affecting many species of bovidae and cervidae, characterised by widespread lymphoproliferation and degenerative changes affecting most tissues. There are several different viruses that can induce MCF but only one, designated alcelaphine herpesvirus-1 (AHV-1), has been partially characterised (1-3). The normal host of this virus is the wildebeest (Connochaetes taurinus) in which infection is subclinical (4). However when certain other ruminant species become infected with AHV-1 they develop MCF (5). Where this virus is not the aetiological agent, circumstantial evidence suggests that domestic sheep are the source of infection (6). An increasing body of evidence supports the concept that sheep are infected in a similar way to wildebeest with an antigenically related herpesvirus which can also cause MCF (7, 8). In this review that agent will be referred to as the "sheep-associated" (SA) agent.

In addition, antibody which cross-reacts with AHV-1 can be detected in most species of three sub-families of bovidae: Hippotraginae, Alcelaphinae and Caprinae (9-11), suggesting that antigenically related gammaherpesviruses are widely distributed in these animals and may have the ability to induce MCF in other ruminants. Several of these viruses have been isolated but subjected to only limited characterisation (12-16).

MCF therefore cannot be defined in terms of aetiology but is a specific clinico-pathological response to a variety of related virus agents.

HISTORY

Plowright (17) cites two references that indicate the presence of MCF in Central Europe for well over a century. However, contemporary definitions of MCF are based on the work of Gotze (18) who classified the disease into four categories, 1. head and eye, 2. peracute, 3. intestinal and 4. mild forms. While there can be little doubt of the validity of the first three forms of the disease the definition of a mild reaction in a normally fatal disease that can be established only by histopathological examination of tissues would appear to be unwise until there are alternative ways of confirming infection.

The association between sheep contact and the development of the disease first demonstrated by Gotze and Leiss (19) has been confirmed frequently. That MCF could occur in cattle following contact with black wildebeest (Connochaetes gnu) was recognised by the early colonists in Southern Africa and confirmed by Mettam (20), who transmitted disease to cattle with blood from black wildebeest. Daubney and Hudson (21) subsequently confirmed that the blue wildebeest (C. taurinus) also could infect cattle and they concluded that MCF acquired from sheep or wildebeest was essentially the same disease, a point which had been controversial in the early years of the 20th century.

Whereas the disease in cattle has been recognised for some time it has become apparent only relatively recently that MCF may affect other ruminant species (22-37). Most incidents involving exotic species have occurred in zoological collections of captive animals while on deer farms MCF is at present the most frequently recognised infectious disease (6). Although a specific

diagnosis of MCF in deer was first reported affecting Pere David's deer (Elaphurus davidianus) (28) a report by Lupke (38) in 1906 of a condition diagnosed as periarteritis nodosa (see footnote) which decimated the Kaiser's herd of Axis deer (Axis axis) is on retrospective appraisal likely to have been MCF.

Investigations of the SA form of the disease were severely hampered by the extremely variable success rate in transmitting the disease experimentally and the inability (18, 34, 39-50) until recently (51, 53) to adapt the causal agent to laboratory animals. The transmission of the wildebeest form of the disease to laboratory rabbits (21) was thus an important achievement, although detailed analysis of its pathogenesis was not reported for over 40 years (53). Of great significance was the isolation in tissue culture of a herpesvirus (2) subsequently designated AHV-1 (9) from wildebeest (C. taurinus) blood which was shown to induce MCF in cattle. The definition of the cultural requirements for the virus provided the essential prerequisite for examining the epidemiology of infection in wildebeest (4, 54) and other free-living ungulates in East Africa (9). It became evident from these studies that infection spread very efficiently in wildebeest herds and that related antelope were infected with antigenically cross-reactive viruses. These observations were subsequently extended by examination of animals in zoological parks (16, 55). Thus it is now apparent that most, if not all, antelope belonging to the subfamilies Alcelaphinae and Hippotraginae are infected with their own species-specific gammaherpesvirus, but with the exception of the viruses of wildebeest they do not

Footnote

Periarteritis nodosa is a condition of man of obscure aetiology with histological changes similar to those of MCF, and would be a logical description of MCF in the light of knowledge prevailing at that time.

appear, under natural conditions, to spread to other species and cause MCF.

Initial attempts which employed a serum neutralising antibody test to demonstrate that sheep were infected with a virus related to AHV-1 failed (56). However, when an indirect immunofluorescent (IIF) antibody test was developed antibody was found to be prevalent in all domestic sheep populations examined (7) as well as in exotic sheep and goats (16). These findings have recently been confirmed by examining the reaction of sheep serum to AHV-1 antigens by immunoblotting (57). Thus, there is now compelling evidence for the widespread presence of gammaherpesviruses in the subfamily Caprinae. However, antibody in this subfamily does not generally neutralise AHV-1 so it is likely that these viruses are less closely related to AHV-1 than those of the antelope species.

The pathology of MCF has intrigued many investigators and several have speculated on the pathogenesis of the disease (5, 8, 26, 53, 58-62). Although a few have considered an oncogenic component (26, 53, 59) the majority have favoured autoimmune mechanisms as being responsible, (5, 50, 58, 60, 61) but convincing supportive evidence for the process has been lacking. The recent implication of natural killer (NK) cell dysfunction as a central component of the pathogenesis does however explain many of the enigmas of the disease (8, 58, 63, 64). Furthermore, the development of techniques for culturing NK cells from animals affected with the SA form of MCF (63-65) and the identification of DNA in these cells which cross-hybridises with that of AHV-1 (66), is likely to provide the reagents necessary for resolving the nature of the SA agent.

IMPORTANCE

In assessing the importance of MCF five aspects of

the disease must be considered separately: 1. as a disease of cattle where sheep are also raised, 2. as a disease of farmed deer, 3. a disease of water buffalo and Bali cattle, 4. a disease of cattle where they have contact with wildebeest, 5. a disease in zoological collections

Disease of cattle where sheep are raised

This form of disease caused by the SA-agent occurs on every continent with the exception of Antartica (67). It is generally described as a sporadic condition affecting only one or two animals in each incident (5). Recently however several incidents in which >10 and even >100 animals have succumbed have been reported or have been brought to our attention (55, 68-76). In addition these outbreaks are not restricted geographically as they have occurred in the USA (70, 72), UK (68, 74), Africa (72), New Zealand (69), Australia (75) and Malaysia (76). These high-morbidity outbreaks do not appear to arise through exceptionally intense sheep/cattle contact and no contributory factors have been identified. Furthermore as the diagnosis of MCF can only be made by extensive histopathological examination it is likely that the disease is grossly underdiagnosed (77). The importance of this form of MCF is therefore greater than is generally perceived and the occurrence of serious outbreaks, for reasons that are not understood, is a cause for concern.

Disease of farmed deer

Epizootics of SA-MCF have been reported to have affected farmed deer in the UK (30, 78), Australia (26) and New Zealand (79, 80) where it is recognised as the most serious infectious disease of farmed deer (79). Following the initial outbreak in which 9/15 red deer (Cervus elaphus) died (30) there have been several reports of outbreaks with mortality in excess of 50% and sika deer (C. nippon) (79), rusa deer (C. timorensis) (26) and Pere David's deer (78) have all been affected.

In recent attempts to develop this latter species for commercial venison production in New Zealand and the UK, most animals have died from MCF and it has been recommended that no further attempts to exploit this species should be made until the disease is more fully understood (78).

MCF is thus the most important infectious disease of farmed deer and failure to control the condition could jeopardise the development of this new livestock industry.

Disease of water buffalo and Bali cattle

Documentation available in Europe about MCF in this region is poor but it is becoming clear that Bali cattle (Bos javanicus) (81), and to a lesser extent domestic buffalo (Bubalus bubalis) (76, 82, 83) are very much more susceptible to the SA form of MCF than are other cattle species. In this region sheep and goats provide an essential cash crop while cattle and buffalo are kept for meat and milk as well as being vital draft animals. So great is the problem in Indonesia that in some areas of the country there is legislation to control the disease through the exclusion of small ruminants. MCF is thus one of the most serious animal disease problems of this region.

Disease of cattle where they have contact with wildebeest

The disease in Southern Africa may be of less importance than previously following the decline of the herds of wildebeest, but it may well increase with the trend to ranch "game" species together with cattle (84). In East Africa, where vast herds of wildebeest still exist, mortality of approximately 7% may occur in the cattle population following the wildebeest calving season (85). Although the local cattlemen are generally aware of the risk and seek to limit the contact that cattle have with pasture grazed by wildebeest it is not always practical to do so, particularly in times of drought when

grazing is limited, and in these circumstances heavy losses may be experienced (86).

MCF thus presents an important animal disease problem to these pastoralists and can lead to conflict between the interests of the farming community and wildlife conservation and tourism.

Disease in zoological collections

The survival of many wild ruminants depends on the development of conservation areas where numerous species are maintained in relatively close contact. Already in zoological parks worldwide, MCF has caused serious losses on numerous occasions (24, 27, 28, 34, 87-89). While AHV-1 has been implicated most frequently, in some cases the source of infection has not been obvious. That antibody to AHV-1 is prevalent in three subfamilies of Bovidae indicates that antigenically related gammaherpesviruses are common in these animals and may have the potential of causing MCF if cross species infection were to occur. Failure to recognise the problems of MCF when devising strategies for preserving rare, large, ruminant species could thus be catastrophic for these animals.

FATAL INFECTION

Infection of the natural host with a ruminant gammaherpesvirus has not been associated with any clinical or pathological changes, thus aspects of this host virus interaction will be considered in the section on Latent Infection. In dramatic contrast transmission of virus to other species results in the profound pathological changes known as MCF, the subject of this section.

Clinical signs and symptoms

MCF is a fatal disease, the clinical signs of which are highly variable, although they may be broadly characterised into head and eye, intestinal and

neurological forms (18), which can have a peracute, acute, subacute or chronic course. All susceptible species may exhibit any of the clinical manifestations whether caused by the SA-agent or AHV-1. Thus in this review a single composite description of the most commonly encountered reaction is given and only those responses which diverge significantly are mentioned individually.

Following an incubation period which may vary from a few weeks to several months the animal is usually first noticed as being dull and inappetant and milk yields of lactating animals rapidly decline. Initially the rectal temperature is elevated to 41 or 42^{o}C (21, 30, 90, 91) and the pulse rate is raised, although both can return to normal in chronically affected animals. Bilateral lachrymation, initially limited to the inner canthus, salivation and nasal secretion of clear watery material progresses in a few days to a profuse muco-purulent, greeny-yellow or brownish discharge (50, 92, 93).

With the onset of lachrymation, bilateral congestion of the conjunctiva and sclera also are noticeable, and a progressive opacity of the cornea, which starts at the limbus and can proceed centripetally until the whole cornea is clouded, is frequently detected (92, 50). The density and rate of development of the opacity are variable. The process can be complete by four days but may take considerably longer (93). In chronically affected animals the cornea can become eroded and ulcerated (21) while in the peracute disease there may be no discernible opacity (78), although microscopic lesions will be present. At the same time as these changes develop bilateral hypopyon can commence, although it is not always easy to detect through the corneal opacity (50). Photophobia may develop and progressive swelling of the eyelids together with catarrhal matting of the eye lashes can result in virtual closure of the eyes.

Initially the muzzle is dry and hot, with hyperaemia of the lining of the nares, but as the discharge becomes catarrhal the nares are occluded with sticky, encrusting secretion causing snuffling sounds. In severe cases, blockage of the nares results in mouth breathing. The epidermis of the muzzle may crack and with time extensive sloughing and bleeding can occur (25, 50, 92-94).

With the onset of pyrexia saliva accumulates in the mouth, the oral mucosa is hyperaemic and numerous shallow focal erosions become visible over the next couple of days. Although they are most readily found at the commissures of the lips, erosions also occur on the dorsum of the tongue, on the hard palate and characteristically on the tips of the buccal papillae. In peracute cases macroscopic lesions may be seen only at this last site. The vulval mucosa may become reddened and develop foci of yellowish encrustation which subsequently may slough (50, 90, 92, 94). In cattle constipation is common and can persist, or after 2 or 3 days give way to diarrhoea sometimes blood-tinged, which continues until death. Deer frequently present with acute diarrhoea or dysentery from the onset of illness (95). In cattle, but more commonly in deer the urine may appear dark and contain albumin and even some blood.

Lymph node enlargement preceedes the onset of fever and superficial nodes, particularly the submandibular, prescapular and prefemoral nodes are readily palpated (21, 50, 93). However, in some peracute cases, enlargement of peripheral lymph nodes may not be marked. Scabby lesions occur in the skin and with the associated dried exudate in the hair are rough to the touch. While they can be widespread these lesions are found most commonly in the infracervical and scapular regions, and in the escutcheon, groin and udder (91). The animals' joints are sometimes "puffy" and swollen.

Nervous signs are not uncommon with hyperaesthesia and inco-ordination most often encountered but nystagmus, muscular tremors and head pressing also occur (21, 25, 50, 94).

Haematological investigations indicate an initial rise in circulating white blood cells (wbc) followed by a marked leucopaenia (96). At the same time the proportion of neutrophils rises (92) and they in turn show a "shift to the left". The red blood cell count, packed cell volume and haemoglobin concentration increase in cases with diarrhoea and dysentery due to dehydration and resultant haemoconcentration.

In general, clinical signs are more florid in long standing chronic cases while in peracute MCF, such as is encountered in deer, few of the above signs develop. Thus clinical signs in acutely affected deer may include only slightly enlarged superficial lymph nodes, mildly reddened tips to the buccal papillae, moisture around the eyes and diarrhoea/dysentery (26, 28, 30, 96). Sometimes death occurs without ante-mortem clinical signs being noticed (37, 78).

Macroscopic lesions

In addition to the lesions seen clinically, at necropsy macroscopic changes are widespread. Within the buccal cavity and pharynx and involving the soft palate and the tongue, lesions can be found which vary from distinct red erosions and ulcers to more diffuse patches of necrosis. Sometimes similar but generally milder lesions are also found in the oesophagus and forestomachs while the abomasal mucosa can appear reddened, and occasionally haemorrhagic stripes are associated with the mucosal folds (21, 50, 92, 93).

In cattle, the small intestine is usually congested with a reddened mucosa and sometimes also with petechial haemorrhages, whereas the large intestines usually exhibit more pronounced mucosal haemorrhages and erosions

(94). In deer, the changes are generally much more severe, with congestion and oedema of the intestinal wall extending from the duodenum to the rectum, and with haemorrhagic, watery contents. The mesenteric lymph nodes in deer are characteristically grossly enlarged and often surrounded by translucent yellowish oedema (26, 30, 96). In both cattle and deer lymph nodes are often firm and white on cross section but can contain haemorrhagic zones also. The latter finding is more common in the retropharyngeal lymph nodes which often are soft and necrotic (26, 50, 91, 93, 96). The spleen can be twice its normal size with prominent white pulp in AHV-1-induced MCF (93, 97), but it is usually less markedly affected in cases of bovine SA-MCF (90). In red deer with SA-MCF and in cattle that have suffered protracted illness the spleen may appear shrunken (30, 93).

The liver is usually swollen (21) and congested with pale areas on its surface. In cattle, the wall of the gall bladder may appear normal but petechiation can be present on the mucosa and the bile may be thick and dark green (21).

Lesions in the respiratory system are often more severe in MCF caused by AHV-1 than in disease caused by the SA-agent. They range from congestion to extensive ulceration, diphtheritic deposits and haemorrhages of the mucous membranes covering the nasal septum, turbinates, frontal sinuses and, in cattle, the horn cores (93). Congestion of the larynx, trachea and bronchi is common, and sometimes epithelial erosions, fibrin deposits and mucopurulent plugs may be seen. In cattle, the anterior lobes of the lungs can be oedematous and contain consolidated lobules (21, 93).

Frequently, characteristic lesions occur in the kidneys where, under the capsule and throughout the cortex there are raised white foci one to four mm in

diameter, sometimes surrounded by a thin haemorrhagic zone and the epithelial surface of the urinary bladder haemorrhagic foci of irregular size and shape are commonly found (30, 50, 90).

Genital tract lesions are generally confined to superficial erosions of the vaginal mucosa. The joints can contain excess fluid and the synovial membranes appear swollen and reddened (90) but, in experiments with isolates of virus from hartebeest (Alcelaphus buselaphus), gross accumulations of coagulated material were found in joints of affected cattle (14). The brain may be congested and bathed in excess and cloudy CSF (21).

Microscopic lesions

The microscopic lesions can be divided into epithelial degeneration, vasculitis, hyperplasia and necrosis of lymphoid organs, and interstitial infiltrations and accumulations of lymphoid cells in non-lymphoid tissues.

Epithelial lesions are essentially similar whether they occur in the buccal or nasal cavities, lungs, alimentary tract (Figure 1), gall bladder, urinary bladder, skin, or conjunctiva. They are frequently associated with subepithelial and intraepithelial lymphoid cell infiltrates and sometimes also with vasculitis and haemorrhages. With stratified squamous epithelia, foci of acantholysis and ortho- and para-keratotic hyperkeratosis develop which can give way to erosions and ulcerations (30, 50, 61, 90, 93, 97, 98). Microvesicle formation also has been recorded (50, 98). In respiratory and intestinal epithelia there can be cellular degeneration and sloughing and also superficial accretions of fibrinonecrotic exudate (98).

Vasculitis affects arteries (Figure 2), arterioles, veins and venules and the severity of the lesion may correlate with the duration of illness. The principal

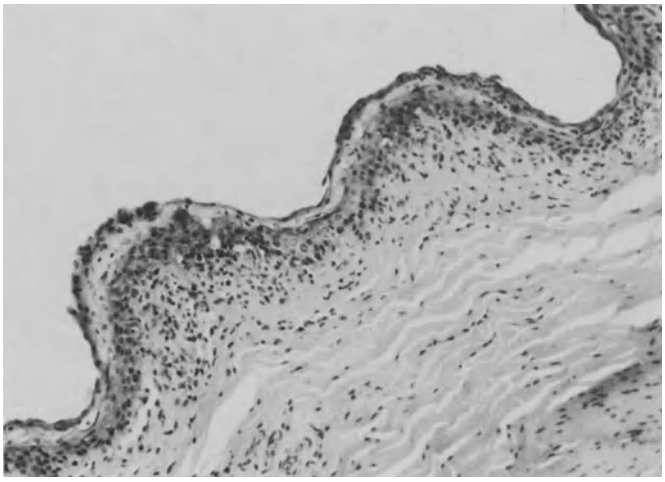

Figure 1. Stratified squamous epithelium in the rumen of a 16 months old Charolais bullock with SA-MCF. Note the parakeratotic hyperkeratosis and subepithelial and intraepithelial lymphoid cell infiltrate. H and E.

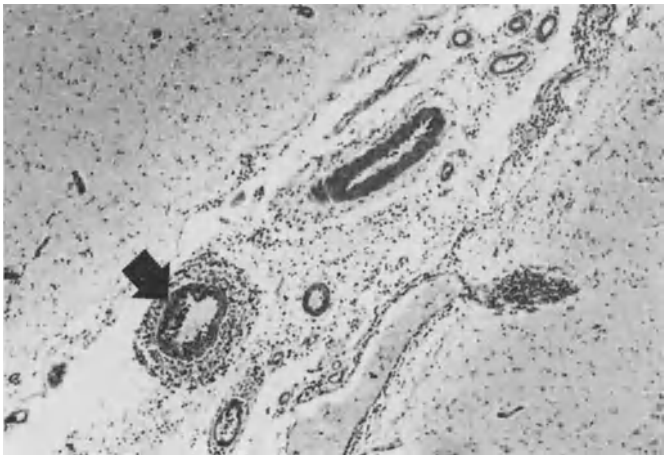

Figure 2. Meninges and associated cerebral cortex in the brain of a 1 year old red deer (Cervus elaphus) with SA-MCF, showing lymphoid inflammation. Note anteritis (arrow). H and E.

inflammatory cell is lymphoid in appearance, and indistinguishable from those found either in the paracortex of the lymph node or in the interstitium of other tissues. Thus lymphoid cells are found in the tunica adventitia and tunica media, in which there is often fibrinoid degeneration. The intima is usually affected also with endothelial cell hypertrophy and degeneration, pavementing of lymphoid cells on the luminal surface, and subendothelial inflammatory cell accumulations. In severe cases occlusion of the lumen by lymphoid cells and hyperplastic endothelial cells can occur (26, 30, 50, 61, 98-100). Haemorrhages are often associated with affected vessels.

Lymph node hyperplasia results from marked expansion in numbers of lymphoblastoid cells in the paracortex, where mitotic figures are readily observed (50, 91). The cortex also is hyperplastic, although to a lesser extent, and there is generally little follicular development (50) although exceptions have been reported (98). Necrosis when present, appears to be follicular in origin (26) but in advanced cases most structures in the node can be involved. Haemorrhages also occur, perhaps as a result of vasculitis. In the medulla the cords are thickened and the sinuses packed with macrophages (50) and lymphoid cells (37). Periglandular oedema and lymphoid inflammation are also common.

The spleen may be enlarged, with marked hyperplasia of the periarteriolar lymphoid sheaths (PALS), but with relatively little follicle development. In contrast, the tissue of the shrunken spleen is substantially depleted of cells and only small 'islands' of lymphoid cells remain to represent the PALS (30).

Interstitial infiltrations and accumulations of lymphoid cells in non lymphoid tissues, such as the periportal areas of the liver and interstitium of the renal cortex (Figure 3), are characteristic of MCF (30,

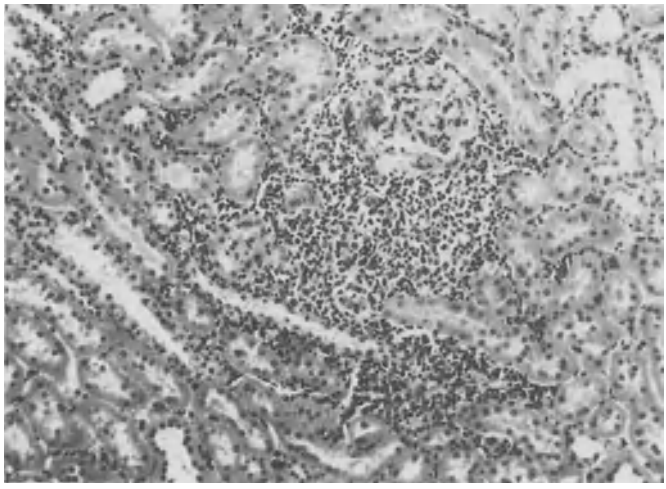

Figure. 3. Kidney from bovine case of SA-MCF. Note interstitial accumulations of lymphoid cells between cortical tubules. H and E.

90, 98). In the renal cortex, accumulations are often so large that they are readily appreciated as macroscopically visible white foci on the surface of the kidney. Other commonly affected tissues include salivary and lachrymal glands, pancreas and cardiac and skeletal muscle (26, 89, 95, 97).

In the brain there is often a non-suppurative meningoencephalitis (Figure 2) with perivascular cuffing by lymphoid cells and some associated small foci of microglial proliferation and periaxonal oedema. The choroid plexus is often infiltrated by lymphoid cells and the cerebrospinal fluid contains unusually large numbers of mononuclear cells (30, 49, 90).

One site of special diagnostic significance, recorded in most reports, is the eye where the macroscopically visible corneal opacity is usually an indication of more severe and widespread lesions. The principal ocular lesion is a lymphoid cell, interstitial

keratitis originating at the limbus and progressing centrally. Depending on the duration of illness lesions may be mild or progress to affect the cornea grossly with oedema, lymphoid infiltrates and erosions of the epithelium. Neutrophil infiltrates occur when lesions have advanced to corneal ulceration. Vasculitis, hypopyon and iridocyclitis also occur (26, 28, 50, 101).

Pathogenesis

The two main components of the pathology of MCF are T-lymphocyte proliferation and tissue necrosis, the latter including terminal destruction of lymphoid tissues, epithelial surfaces, blood vessels, liver and other tissues.

As already stated the incubation period varies from a few weeks to several months and the course of the disease is unpredictable. However it seems likely that the degenerative changes commence at, or around, the start of clinical illness and are probably largely responsible for the symptoms observed, whereas the time of onset of the lymphoproliferation and its role in the disease are unclear but almost certainly commence prior to the onset of symptoms.

Laboratory animal studies of both forms of MCF have helped clarify the pathogenesis. The disease in rabbits is not only a good model of ruminant MCF but also accentuates differences between the diseases due to AHV-1 and the SA-agent.

In SA-MCF of rabbits lymphoid tissues such as the submandibular and mesenteric lymph nodes, appendix and spleen become significantly enlarged prior to the onset of clinical signs (Table 1). The start of clinical disease coincides with and is probably caused by the onset of tissue necrosis. This particularly affects follicles within the appendix and certain lymph nodes (35, 51, 58). Epithelial lesions are less prominent than in affected ruminants. Thus oral changes are not common

and while mucoid diarrhoea is not infrequent, dysentery is rare and the urinary bladder is not commonly affected. Arteritis is uncommon but most readily found in the lungs (58) and lymphoid accumulations in the kidneys are also rare.

AHV-1 induced MCF in rabbits is essentially similar although arteritis is more common as are lymphoid accumulations in the kidneys (53). Lymphoid tissues respond in a similar manner although the spleen and submandibular lymph nodes are significantly larger even than those in rabbits with SA-MCF. The popliteal lymph nodes are also enlarged but the mesenteric lymph node, while significantly enlarged is also significantly less so than in SA-MCF. Rabbits with AHV-1 MCF also develop degenerative changes in the thymus (53).

Table 1. Comparison of the weights (gm) of lymphoid tissues from uninfected, clinically normal, control rabbits and rabbits with clinical signs of MCF, experimentally induced with either AHV-1 or the SA-agent.

Treatment (n)	Live weight (± SE)	P	Lymph nodes 1	2	3	Appendix	Spleen
Control (5)	2869 (± 342)		0.047	0.150	0.323	3.921	0.899
		*	<0.05	NS	<0.001	<0.001	<0.005
SA-agent (16)	2518 (±203)		0.085	0.116	2.935	5.633	1.471
		**	<0.001	<0.001	<0.001	NS	<0.05
AHV-1 (8)	2784 (±255)		0.319	0.780	1.487	5.179	3.376
		***	<0.001	<0.001	<0.001	<0.05	<0.001

P values calculated with Student's t-test, with data expressed as % body weight.
* Comparison between Control and SA-agent infected rabbits
** Comparison between SA-agent and AHV-1 infected rabbits
*** Comparison between AHV-1 infected and Control rabbits
1 Submandibular; 2 Popliteal; 3 Mesenteric.

It has been suggested that different lymphocyte populations exhibit tissue specific tropism through the expression of receptors to high endothelial venules of either peripheral lymph nodes or gut associated lymphoid tissues (102). It is thus tempting to speculate that the differences observed in the two forms of MCF are based on involvement of specific lymphocyte subsets expressing different receptors.

Studies of rabbit MCF, due to the SA-agent, using specific anti T-lymphocyte serum have shown that proliferation of these cells commences soon after infection and progresses until the onset of clinical signs (58). Changes are visible three days after infection in both the mesenteric lymph nodes and the appendix. In the lymph nodes the T-dependent paracortex and interfollicular cortical zones expand but in the appendix both marked follicular stimulation and expansion of the inter-follicular T lymphocyte areas are observed (Figure 4). In contrast, the incidence of immunoglobulin-positive cells shows a relative decrease in numbers with time (58). In non-lymphoid tissues such as the liver, the majority of accumulating lymphoid cells have been identified as T lymphocytes (58).

In rabbits infected with AHV-1 the development of lymphoproliferation affects the same T-dependent regions of lymphoid organs but appears to be slower in onset with relatively little expansion before the start of clinical signs (53, 60, 103).

In addition, it has been shown that cyclosporin-A (Cs-A), a potent T lymphocyte suppressor, given daily (20 mg per kg per day by intramuscular injection) from 1 day before infection can prevent the lymphoproliferative response of both forms of the disease (55, 58). However if administration of Cs-A is initiated one day after infection limited lymphoproliferation occurs (58).

It is significant that Cs-A did not extend the incubation period or prevent the terminal necrosis which commenced with the onset of fever (Figure 4). Thus the T lymphocyte proliferation is believed to be a benign event less directly involved in the outcome of infection, than the terminal necrosis.

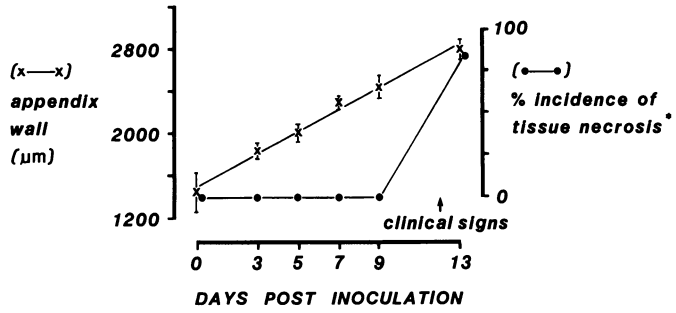

Figure 4. Development of pathology in rabbits experimentally infected with SA-MCF. Rabbits were inoculated with a standard dose of infected cells (58) and two were killed at each of the times indicated except on day 13 when three were sampled. Clinical signs only developed in the three killed on day 13 all of which reacted on day 12. Following exsanguination tissues were fixed in formalin and sections cut from paraffin embedded blocks. Lymphoid cell hyperplasia was assessed on sections of appendix stained by the Gordon and Sweet's method for reticulin. The thickness of each appendix was measured (μm) five times and the results for each day expressed as the mean (± SE) x ----- x. The development of tissue necrosis was measured in H and E stained sections of mesenteric lymph node, appendix, ileal Peyer's patch and the liver from each rabbit. If necrosis was encountered in one of these tissues then it was given a score of 1, regardless of its extent. If no necrosis was found the tissue was given a score of zero. The sum of the values was then expressed as a percentage of the tissues examined (0 --- 0).

In support of these histopathological observations, cell suspensions derived from lymph nodes of rabbits, reacting with MCF due to the SA-agent, were found (in ^{51}Cr release assays for cytoxicity) to kill normal cultured foetal or newborn rabbit cells. Such activity resembles Natural Killer (NK) cell cytotoxicity, and could not be detected in lymphocyte suspensions prepared from normal rabbits or infected rabbits during the incubation period, and correlates with the onset of the necrotising component of the disease (64).

The feasibility of transmitting AHV-1 to hamsters and guinea pigs was suggested by Kalunda and others (104) and subsequently confirmed (105). AHV-1 has been transmitted also to rats and the SA-agent to hamsters (52, 106). The disease produced in hamsters by either agent and that in guinea pigs by AHV-1 closely resembles MCF in ruminants, whereas MCF induced in rats by AHV-1 primarily affected the lymph nodes, heart and kidneys and resembled those lymphomatous conditions of rabbits and primates caused by other gammaherpesviruses (106, 107).

MCF-like disease has been produced in sheep also. Kalunda and co-workers (104) reported that one of three newly born lambs injected with AHV-1 developed disease 17 days later while a proportion of lambs injected intravenously with the SA-agent as 40 to 60 day old conceptuses developed disease (108). The inoculum consisted of viable lymph node or spleen cells from red deer or rabbits with clinical SA-MCF and the lesions produced were histopathologically indistinguishable from those of MCF in cattle and deer. However an explanation as to why disease can be produced in sheep, in these special circumstances, must wait until MCF is better understood.

Because of the apparent absence of viral antigen and viral cytopathic effects in affected tissues (53, 60)

the initiation and progress of the lymphoproliferative lesions have been the subject of considerable debate and several authors have suggested, with varying degrees of conviction, that MCF results from the neoplastic transformation of lymphoid cells (26, 53, 59, 103).

However most descriptions of the lesions of MCF suggest a hyperplastic rather than a neoplastic response and only limited success has been achieved in propagating lymphoblastoid cell lines from affected animals. Cell lines that have been cultured generally have required an exogenous source of Interleukin-2 (IL-2) and/or feeder cells for continued propagation, thus differing from previously described virus-transformed lymphoblastoid cells. An exception would however appear to be the reaction of rats to infection with AHV-1, in which the extensive lymphoid cell accumulations appear neoplastic and from which lymphoblastoid cells can be propagated without IL-2 and/or feeder cells (107). Thus while AHV-1 may be able on occasions to produce a neoplastic response the normal MCF reaction has the characteristics of a T-lymphocyte hyperplasia. As stated earlier this view is supported by the observation that Cs-A, administered before infection, can prevent lymphoproliferation in both forms of MCF but not other aspects of the disease (58).

Among the mechanisms suggested responsible for the degenerative lesions are graft-versus-host rejection (61), direct virus-induced cytolysis (99), and cell-mediated responses to virus-infected vascular endothelium (50, 61). None of these hypotheses were entirely satisfactory. That hypersensitivity to virus or virus-induced antigens might be the cause of the lesions in MCF rather than direct viral damage was proposed by Plowright (5) but the paucity of viral antigens (53, 60) did not support this proposal. The suggestion that immune-mediated damage had a central role in MCF was developed further when it was postulated that the

underlying mechanism was a virus-induced dysfunction of immunoregulatory mechanisms, resulting in uncontrolled lymphoproliferation (62). Subsequently Denholm and Westbury (26) suggested that the T lymphocyte proliferation could be due to viral destruction or inactivation of T suppressor cells with accompanying B lymphocyte destruction. However in rabbits with MCF induced by the SA-agent, lymphoproliferation commenced soon after inoculation but tissue necrosis was not detected until clinical symptoms were apparent (58). Thus we suggest, as a working hypothesis, that the proliferation of T-lymphocytes is a non-specific, benign, polyclonal response driven by excessive IL-2 production resulting from the deregulation of natural killer (NK) cells, while the necrotising process, associated with the terminal phase of the disease, arises through more profound NK cell dysfunction resulting in the destruction of normal host cells.

LATENT INFECTION

Latency is an essential strategy for the perpetuation of the gammaherpesviruses of ruminants. Serological studies imply that infection can be maintained in isolated groups of animals (16) and that infection results in the persistence of virus in individual animals for life with highly efficient transmission to their offspring in the absence of recognised clinical reactions (5, 54). It should be stressed that these observations refer to the natural host. In contrast latent infection in a cow that survived infection with AHV-1 resulted in in utero infection of subsequent conceptuses, one of which had clinical MCF at birth while another developed MCF at 120 days of age (109). Virus could not be detected in the mother's blood or in a variety of tissues when she was killed 85 months after initial challenge.

Infection of natural hosts has been investigated in detail only in wildebeest (2, 4, 54, 110). In the free living populations of C. taurinu in East Africa, Plowright (54) found that all individuals over seven months of age had neutralising antibody to AHV-1 and that viraemia could not be demostrated in animals more than six months of age. In addition, a proportion of animals were viraemic in the first week of life and probably had been infected in utero (4, 111). Moreover, virus was isolated from the blood of 3/7 adult wildebeest in late pregnancy but not from 10 other adults, suggesting that limited productive virus expression may occur during this phase of gestation. Virus was recovered also from the spleen of one wildebeest foetus providing "unequivocal evidence" for in utero infection and supporting the findings derived from examining calves under 1 week of age. However, it should be noted that as all wildebeest become seropositive it would appear likely that infection during gestation occurs subsequent to the development of full immunological reactivity of the conceptus.

Virus could not be recovered from the blood of two seropositive wildebeest following splenectomy (55) but contact transmission between wildebeest subjected to heat stress did occur (17). In addition Rweymamu and others (112) reported the transient excretion of virus in nasal secretions in 2/11 adults given 50 mg betamethasone daily for 7 days. "Stress" associated with transportation may also precipitate virus shedding. Not only has MCF occurred in susceptible species following only relatively brief contact with the presumptive sources of infection during transportation (33, 55) but virus has been isolated from the nasal secretions of a pregnant wildebeest cow immediately following transportation (112).

Evidence has been obtained also for latent infection of hartebeest (A. buselaphus) (9). A survey

for antibody to AHV-1 in serum from free living hartebeest suggested that the epidemiology of a unique hartebeest virus was similar to that of AHV-1 in wildebeest in that animals tended to be infected during their first six months of life. The recovery of six isolates of a herpesvirus in autologous cultures of tissues from adult hartebeest (12, 13, 14) suggests that infection of this species also results in latent infection which persists for life.

By examination of sera from other species, for antibody to AHV-1, a similar epidemiology may be implied both for the viruses of topi and the SA-agent in sheep (13, 7).

CHARACTERISTICS OF THE VIRUS

Taxonomic status

That there is an extensive group of antigenically related bovid gammaherpesviruses is suggested by the detection of antibody that cross reacts with AHV-1, in a large number of species belonging to the subfamilies Alcelaphinae, Hippotraginae and Caprinae (79, 16, 113). However, AHV-1 remains the only agent to have been partially characterised and the evidence suggests that it has the properties of a virus belonging to the subfamily gammaherpesvirinae to which it can be assigned provisionally.

The initial isolation of AHV-1 in tissue culture was achieved in autologous thyroid cell monolayers prepared from cattle reacting with MCF (2), but subsequently virus has been recovered directly from the blood of wildebeest (4) as well as the nasal and ocular secretions of wildebeest calves in bovine cell cultures (110).

Morphology of AHV-1

Plowright (3) described typical herpesvirus particles in the supernatant fluids of infected cultures

in which both naked and enveloped particles were present. The enveloped particles were 140-220 nm in diameter with a loose irregular external membrane which enclosed a central 100 nm capsid similar to that of the naked particles. The latter appeared hexagonal and consisted of subunits 9.5 nm in diameter and 12.5 nm long.

Antigenic relationship

The literature on this subject is both confusing and contradictory due in part to lack of standardisation of the tests employed. In addition ,it is only now becoming evident that there is probably a large group of distinct but related gammaherpesviruses which infect many species of the family Bovidae. It is therefore advisable at this stage to consider the native gammaherpesvirus of each bovid species as being distinct rather than to designate them as subtypes of AHV-1.

Evidence of infection has been generated largely using the prototype WC11 isolate of AHV-1 and initially only neutralising antibody tests were employed (54, 9). The presence of antigenically related viruses in African antelope, other than wildebeest, was first indicated by the detection of neutralising antibody to AHV-1 in the majority of hartebeest and topi sera examined, although at titres lower than those of wildebeest (9). Such antibodies were found in populations that shared common grazing with wildebeest as well as in those entirely isolated from such contact. It was concluded therefore that distinct but related viruses were present in those species, a conclusion subsequently corroborated by the isolation of herpesviruses, with properties distinct from AHV-1, from both hartebeest and topi.

The detection of neutralising antibody in 3/3 Beisa oryx (Oryx beisa) also suggested that these antelope were infected with a related virus and this was subsequently confirmed by Mushi and Karstad (13) who found all of 50 Beisa oryx tested to be positive. Similar antibody has

been found to be prevalent in six other species of antelope belonging to the subfamily Hippotraginae (11).

Neutralising antibody to AHV-1 could not be detected in sera collected from sheep associated with outbreaks of MCF in the USA, Greece, Australia and the UK (7, 55, 56). However, Harkness (16) found neutralising antibody, albeit at low titres, in 6/19 sheep and Heuschele and others (16) reported that 30% of several types of sheep and goats had low titres of antibody. These latter authors also found antibody in sera from species belonging to the subfamily Bovinae and family Cervidae, a finding at variance with the experience of others. The specificity of the reactions detected in these tests should therefore not be accepted uncritically until confirmatory results are available.

It is concluded that within the subfamilies Alcelaphinae and Hippotraginae most species are infected with antigenically closely related gammaherpesviruses which cross neutralise, while reactions detected in other species require to be confirmed.

However using an indirect immunofluorescent (IIF) test Rossiter (7) found antibody to AHV-1 in 162/167 sheep sera examined. The only sera that were negative in this study were five of fourteen derived from gnotobiotic and specific-pathogen-free animals. Thus on the basis of IIF antibody tests, he concluded that an antigenically related agent was prevalent in sheep. We have analysed the reaction of sheep sera to AHV-1 antigens by immunoblotting and found that most sheep sera react with many of the components recognised by wildebeest sera in the same test.

There is therefore compelling evidence for a gammaherpesvirus, antigenically related to AHV-1, being prevalent in sheep. However, on the basis of neutralising antibody tests it would appear to be less closely related to AHV-1 than are the viruses of large antelope.

Heuschele (10) suggested, on the evidence of results derived from an IIF test that Bovine herpesvirus (BHV) 1, 2 and 4 cross reacted with AHV-1 but as the reaction of those antisera with uninfected cells, at the low dilutions employed, was equally intense the significance of this report is difficult to assess. In addition, Sterz and co-workers (117) using complement fixation tests and Ludwig (118) using fluorescent-antibody tests failed to show cross reactivity between BHV-1 and AHV-1. Thus cross reactivity between the bovid gammaherpesviruses and other viruses of ruminants requires clarification.

Physico-chemical properties

As with other herpesviruses, infectivity of the WC11 strain following 50 to 64 serial passages in bovine cell culture was entirely destroyed by treatment with ether or chloroform (3). In one study of thermostability the half-life of cell-free AHV-1, derived from cultures and held at 32°C, was found to be 195 hours and at 37°C, 33 hours (118), while in another report infectivity was totally lost within one or two hours of exposure to pasture conditions in East Africa (120).

The latter authors considered that ultraviolet irradiation probably contributed to the rapid inactivation. Thus infectivity of cell-free virus shed by young wildebeest and not exposed to direct sunlight may persist for several days a characteristic compatible with the observed spread of infection to cattle in the absence of close contact with wildebeest.

Recovery of virus from animals affected with MCF is dependent on the processing of viable cells through to tissue culture or experimental animals. Thus, the apparent fragility of the cell-associated form of virus is a function of cell viability.

BIOLOGICAL PROPERTIES

Host range

It is vital that the host range of each virus is defined in terms of those species which maintain the virus and do not react clinically to infection, as opposed to infections of other species which culminate in MCF and from which infection can be transmitted only by parenteral inoculation of viable cells.

Thus there is only one essential host of AHV-1, namely C. taurinus (2), although infection of 12 other ruminant species in which MCF occurs has been reported (22-37). In addition, experimental disease can be transmitted to rabbits, rats, hamsters and guinea pigs (21, 107).

Although substantial losses due to MCF may occur in cattle sharing pasture with wildebeest herds the disease has not been recorded in any free living ruminant exposed to pastures used by wildebeest. In contrast, some native ruminants can succumb in captivity (10, 24, 31, 83). Whether this apparent difference is due to removal of affected animals in the wild by predators or because contact between free living animals is minimal or whether there is increased susceptibility associated with captivity is uncertain. It is also possible that the discriminating diet of the native species minimises the risk of contact with contaminated pasture in the wild.

With the exception of the SA-agent there is no evidence for the spread of ruminant gammaherpesviruses other than AHV-1 from their essential hosts. Thus, despite the capacity of the virus of hartebeest to induce MCF in cattle and the frequent abundance of hartebeest on some cattle pastures in areas of Africa, there are no reports of disease in cattle due to infection with this virus.

In contrast, the SA-agent may infect and cause MCF in a variety of other ruminants (25, 26, 30, 32, 33, 37,

48, 81, 82). Farmed deer in particular appear to be extremely susceptible to infection and heavy losses have been experienced (6). Free living deer, like free living, native, African ruminants that share pasture with wildebeest, often graze the same areas as sheep but as yet MCF in them has not been recorded.

Although the SA-agent has not been identified, disease can be transmitted experimentally to rabbits and hamsters with cells from cattle and deer reacting with this form of MCF (51, 106).

Virus propagation

Infectivity, which is strictly cell-associated, can be recovered from cattle and rabbits experimentally infected with AHV-1, but titres are generally low (2, 121, 122). Titres of between <1 to 3.5 infected cells per 10^6 have been reported from infected rabbit lymph nodes (121). In contrast, approximately 1 per 10^3 cells in affected cattle lymph nodes were found to be infected (122). These findings may not represent true differences in virus titre but greater efficiency in recovery from bovine lymphocytes (122). Virus antigen in tissues of animals affected with MCF is either not detected or is present in only a few tissues (34, 121-123). In addition, following adaptation to hamsters and rats, virus cannot be recovered either in tissue culture or by inoculation of rabbits, although intraspecies transmission is readily achieved with viable cells from affected animals (107).

Since the original report of the isolation of AHV-1 in bovine thyroid cell cultures (12), virus has been propagated in a variety of cell monolayers including autologous kidney (13), turbinate and corneal cell cultures (124), foetal audad (Ammotragus lervia) (125), bovine testes, adrenal kidney (2) and turbinate (55) as well as rabbit kidney (2). Thus it seems likely that the virus will replicate in most ruminant primary and low

pass cells. Following isolation the cytopathic effects produced by AHV-1 are characterised by focal degeneration and formation of syncytia and the latter may contain numerous nuclei with type A nuclear inclusions (2, 3). Infectivity of all isolates was initially cell-associated but after repeated passage the cytopathogenicity of the WC11 isolate altered. Foci of rounded refractile cells were produced and cell-free virus was released. Similar cell-free virus preparations have been achieved subsequently with other isolates (119, 126), the latter authors recommending a reduced incubation temperature to accelerate this process.

In contrast, isolates of virus from topi (15) and hartebeest (12, 13, 14) that were made in autologous cell cultures could be propagated only by serial passage to cultures of the homologous species. The K/30 isolate from hartebeest (12) which could be propagated in a variety of bovine cells was unlike other hartebeest isolates and it was suggested that it may represent an isolate of AHV-1. This restricted cell tropism may explain why these viruses do not appear to spread to cattle under natural conditions. Insufficient data are available on isolates of virus from hartebeest, topi and scimitar oryx in foetal audad cells (16) for critical assessment.

Propagation of gammaherpesviruses from ruminant species other than the large antelope has not been achieved despite numerous attempts to isolate the SA-agent from affected animals. However, it has been found possible, recently, to propagate lymphoblastoid cell lines from both cattle, deer and rabbits with SA-MCF (55, 63-65). That the disease can be transmitted with some of these cultured cell lines, provided they are viable, strongly suggests that the sheep virus is intimately associated with these cells either as integrated DNA or in an episomal form.

MOLECULAR ASPECTS

Analysis of AHV-1 DNA purified from WC11 virions by fractionation on CsCl density gradients indicates that there are two components consisting of a major peak with a density of 1.71 gm per c.c. and a minor one with a density of 1.73 which corresponds to analysis of the DNA of the two simian gammaherpesviruses H. saimiri and H. ateles (126). Restriction enzyme analysis of AHV-1 also suggests that the viral genome is organised similarly to that of the simian herpesviruses although the percentage of repeated DNA is only 7-10% compared to 30% of the simian herpesvirus genomes (66, 128). Thus it is probable that the organisation of AHV-1 can be summarised as indicated in Figure 5.

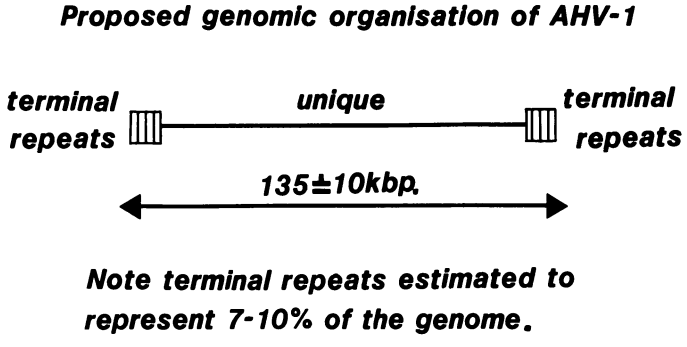

Figure 5

The restriction enzyme profiles of the WC11 strain which is adapted to tissue culture and the low passage, virulent C500 isolate of AHV-1 have only minor differences, indicating that there is little heterogeneity between these isolates. This finding is at variance with the report of Osorio and others (129) who compared, by restriction enzyme analysis, the strain WC11 and an isolate designated 'Oklahoma strain'. These authors found significant differences in the migration patterns of the DNA fragments of the two viruses and concluded that strain diversity existed. The Oklahoma strain of virus was derived from a "gaur and a greater kudu" affected with MCF at Oklahoma City Zoo where the presumed source of infection was white tailed wildebeest (C. gnu) (24). As WC11 was derived from a blue wildebeest (C. taurinus) (111) the reported findings support the view that gammaherpesviruses of different species should be accorded species status and not considered strains of the same virus.

Variation between the culture adapted strain WC11 and the virulent C500 isolate was however detected in the repeat region which in WC11 was composed of 700, 1000 and 1600 base pairs (bp) while in C500 the repeats were of a regular 1000 bp (66). Variation in this region between wild type virus and variants derived in the laboratory have already been reported for two other gammaherpesviruses, Mareks Disease Virus (130) and H. saimiri (131), further confirming the similarities of AHV-1 to other gammaherpesviruses.

DIAGNOSTIC PROCEDURES

Clinical and pathological diagnosis

Clinical diagnosis is relevant only to the MCF reaction and not to the subclinical infection of the natural host. Clinical and post-mortem changes described above can vary from severe to very mild and thus

diagnosis by those criteria alone can be unreliable, firm diagnosis depending on the detection of characteristic histopathological lesions.

Virus isolation

Virus isolation is appropriate only in cases arising from infection with AHV-1 and possibly the gammaherpesviruses of other species of wildebeest. Virus may be recovered in a variety of culture systems but probably the most satisfactory are monolayer cultures of bovine thyroid cells (2). Inoculum must consist of viable cells as otherwise infectivity is lost. Suitable inoculum may be prepared from buffy coat cells or lymphoid tissues and typical syncytial CPE should develop in 10 to 20 days. Identification of isolates may be made by detecting specific antigen in these cultures by IIF or an immunoperoxidase technique (132, 133).

Serology

Serology may be employed either in diagnosis of clinical disease or in identifying latently infected carriers. However as most tests to date have employed the WC11 strain of AHV-1 as antigen, results must be interpreted with caution.

Neutralising antibody is unreliable as an aid to diagnosis of MCF induced by AHV-1, as low titres are detected in only approximately 50% of affected animals (134). However, most animals with this form of the disease do develop antibody detectable by IIF but such tests may be complicated by non-specific reactions at low serum dilutions and only titres in excess of 1/32 can be regarded as specific (116, 134). Antibody responses detectable by complement fixation and precipitation have been described also but have little utility as diagnostic aids (135, 136).

Although antibody to AHV-1 has been detected by IIF in cases of MCF induced by the SA-agent the test cannot be advocated as a diagnostic aid as only approximately

50% of affected cattle (116, 137) and none of 20 affected deer were seropositive (55).

On the other hand, serological tests have been of great value in studying the epidemiology of AHV-1 in wildebeest populations and in identifying species infected with cross-reacting viruses (9, 11, 16, 54).

Virus neutralisation tests have been employed extensively to identify infection in wildebeest and related antelope (9, 11, 54) while the IIF test has proved of greatest value in identifying infection in species such as sheep (7, 16). Additional analysis of the cross-reaction between these viruses has been achieved by immunoblotting (57).

IMMUNOLOGY

Humoral Immunity

The humoral immune response of wildebeest to AHV-1 infection has been examined by testing sera collected from free living animals. It has been concluded that virtually all wildebeest acquire neutralising antibody to AHV-1 from colostrum (54). Such antibody is replaced through an active immune response that occurs some time during the first six months of life and thus few seronegative calves are ever detected. A very similar pattern of infection appears to occur in hartebeest and topi (9) while the distribution of IIF antibody in domestic sheep sera (7) suggests that infection in this species also occurs at a young age while maternal antibody is still present.

The recovery of virus from ocular and nasal secretions of free living wildebeest calves aged two to five months (110) suggests that these secretions are likely sources of contagion for cattle. Virus was recovered from wildebeest calves despite high titres of serum neutralisating antibody, presumably derived from colostrum. In older wildebeest calves antibody could be

demonstrated in nasal secretions when, with one exception, virus could not be isolated. It was suggested that cessation of virus shedding was due to active immunity and secretion of virus-specific IgA antibody in the nasal cavity (138).

The response of wildebeest to AHV-1 has been analysed by Western blotting also (57). Solubilised, purified WC11 antigen was used and all sera examined reacted with 6 major components. Sheep sera also reacted with the same major viral antigens although individual sera tended to recognise only a proportion of those viral components.

Humoral antibody responses may be detected also in other species reacting with MCF. Rossiter and co-workers (136) found low titres of neutralising antibody in approximately 50% of sera from cattle that had been presumptively infected with AHV-1, an observation consistent with development of such antibody in a proportion of experimentally infected animals (135). Neutralising antibody was produced also by rabbits experimentally infected with AHV-1 but in neither rabbits nor cattle was there any evidence that antibody influenced the course of the disease.

Cattle infected with AHV-1 regularly develop serum antibodies, detectable by either IIF or immunoperoxidase (IP) tests (133), which react with both particulate and diffuse antigens in the cytoplasm and nuclei of infected cells. Complement-fixing and precipitating antibodies also have been detected in the sera of cattle infected with AHV-1 (135, 136).

In both rabbits and cattle experimentally infected with AHV-1, IgG and IgM antibodies to the virus appear simultaneously, with IgG_2 antibody becoming detectable some 2-4 days later (139, 140).

To date, only one bovine serum from a case of MCF caused by AHV-1 has been available for immunoblotting and

this was found to react with only three of the viral polypeptides detected by wildebeest serum (57). Likewise sera from cattle with SA-MCF reacted strongly with only one AHV-1 viral polypeptide, which was distinct from the above three antigens recognised by the serum from the bovine with AHV-1 induced disease.

Thus the humoral immune response of cattle affected with either form of MCF is directed at only a few of the viral epitopes which suggests that only limited expression of viral antigens occurs in such animals or that they can react to only a proportion of viral products. In view of the absence of detectable antigen or virus particles in affected cattle the former is the more probable explanation.

Cell-mediated immunity

Cell-mediated immune mechanisms, which may be involved in the maintenance of latency in the natural hosts, have not been investigated while the role that virus-induced autoimmune cellular destruction by NK cells plays in precipitation of the MCF reaction in susceptible animals has been discussed in detail (in the section on pathogenesis).

EPIZOOTIOLOGY OF MALIGNANT CATARRHAL FEVER

As the epizootiology of the gammaherpesviruses in their natural hosts has been described, only aspects of the MCF syndrome will be considered here. The disease occurs in two distinct circumstances following direct or indirect contact either with wildebeest or with sheep and it is therefore necessary to consider the two separately.

Wildebeest-associated disease

MCF, due to infection from wildebeest, is not restricted to areas of Africa where either C. gnu or C. taurinus are present but also occurs in zoological parks worldwide where these species are kept (5, 24, 27, 28, 34, 87-89).

Early reports suggested that MCF occurred in cattle following contact with wildebeest foetal membranes but the evidence that has now emerged suggests that wildebeest calves are a significant source of infection for cattle where the two species share a common grazing (10). Plowright estimated that in such areas in East Africa the annual mortality could reach 7% but as the pastoralists of Africa readily recognise the disease not all cases are reported to the veterinary authorities (85). Furthermore herdsmen avoid grazings where wildebeest are present although when pasture is restricted as in times of drought this may not be possible and substantial losses due to MCF can occur (86).

Transmission from wildebeest to cattle has occurred experimentally following close contact and in the field even when separated by over 100 metres (89). Reports from zoological parks also indicate that close contact is not always necessary for transmission of the virus (28, 34). Such observations imply that substantial aerosol spread can occur and that virus excreted by the natural host may be more resistant than is generally recognised.

Sheep-associated disease

In contrast to the wildebeest-derived form of the disease the SA form is distributed worldwide and probably occurs wherever cattle and sheep are reared (67). The disease normally occurs sporadically affecting only a few animals at a time, although incidents in which many cattle, buffalo and deer become affected are being recognised more commonly (26, 30, 55, 68-80, 82). As no explanation for these multiple case incidents has been identified, reducing the risk or controlling outbreaks is difficult.

However, certain sheep flocks transmit infection to cattle more readily. Gotze (18) reported that of 50 cattle exposed experimentally to a flock of sheep

obtained from an MCF-affected farm, 16 developed the disease and Kock and Neitz (43) made a similar observation in South Africa. Piercy (71) described a flock of Red Masai sheep which were dispersed after 88 cattle developed the disease. Subsequently, multiple cases of MCF occurred on farms to which the sheep had been sent. Likewise Snowdon (75) identified a flock that transmitted disease to 29/36 cattle over a period of five years, before they ceased to induce further cases. From these reports it would appear that certain flocks can be particularly infective to cattle. It should however be noted that on the basis of serological tests, employing AHV-1 as antigen, all sheep become infected with the SA-agent at a young age (7). Thus the SA-agent transmits efficiently between sheep but only occasionally does inter-species transmission to cattle also appear to occur readily.

As with MCF induced by AHV-1, intimate contact between sheep and the target species is not always required for transmission of the SA-agent to occur. Thus Hoffman (82) reported an incident in which 50 water buffaloes died, although separated from lambing sheep by 30 metres. Likewise, outbreaks involving farmed deer have occurred in which no close contact between the deer or sheep can be identified (78, 95) and it must be assumed at this stage in our understanding of the disease that efficient aerosol transmission can occur.

The relative efficiency with which the SA-agent transmits to different categories of animal is also important. Thus Bos taurus and B. indicus would appear relatively resistant to infection compared to domestic buffalo (82) and red (30), sika (79) and rusa deer (26) while Bali cattle (18) and Pere David's deer (78) are even more susceptible to infection. These observations suggest that where the most sensitive hosts are exposed to sheep, as sentinels of infection with the SA-agent,

inter-species transmission is a frequent event. The intensity of exposure rather than non-exposure is therefore likely to dictate the frequency of MCF in the more resistant of species and it can be concluded that high titres of the SA-agent are required to induce MCF in those species. Sub-clinical immunising infections do not however appear to occur as antibody is not detected in unaffected cattle associated with outbreaks of MCF due either to AHV-1 or to the SA-agent (136, 137).

Certain authors have suggested that involvement of sheep is not essential for MCF to occur and that other causes may have to be considered (80, 141). However, with an incubation period of up to six months and where intimate contact is not required for transmission, proof of the non-involvement of sheep is difficult to obtain.

VACCINATION

Vaccination has been attempted only against the AHV-1 induced form of the disease and in no case has it been successful. Piercy (142) employed formalinised tissues from affected cattle with apparently good results. However, in view of the almost certain absence of antigen in such tissue and the difficulty of executing controlled trials a cautious appraisal of these studies is required. An isolate of virus from hartebeest, following prolonged in vitro cultivation, provided cell-free virus which protected cattle from virulent homologous virus challenge but not from AHV-1 challenge, despite the fact that all cattle developed neutralising antibody to AHV-1 (12). Likewise, Plowright and co-workers (85) found that cattle immunised with the WC11 isolate of AHV-1 regularly produced high and persistent neutralising antibody titres, but none was protected from either cell-free or cell-associated virulent virus challenge.

The limited evidence available does not encourage hope for the development of vaccines which will immunise animals against MCF. An alternative strategy worthy of investigation would be to immunise the carrier hosts as young animals in the hope that virus excretion may be reduced and that the risk of in-contact animals developing MCF be thus diminished.

CONTROL AND ERADICATION

Control of MCF relies on prevention based on the separation of carrier species from susceptible hosts. Disease arising from AHV-1 could be eliminated in Africa by excluding wildebeest from pasture to which cattle have access, a strategy which is frequently impracticable (see above). It would appear wise to segregate, as far as possible, all species of wildebeest from other ruminants in captivity, particularly during the wildebeest calving period and during the first six months of life. Control based on the elimination of seropositive animals would appear to be inappropriate as all animals probably become infected at a young age. It should however theoretically be possible to derive virus-negative animals by rearing calves individually from birth and eliminating those that become sero-positive through in utero infection. As other species of Alcelaphinae, Hippotraginae and Caprinae (see above) could act as reservoirs of infection in zoological collections segregation of animals in these categories may also be appropriate.

Control of the SA form of MCF poses even greater problems as the agent has not yet been isolated and recommendations are thus based on extrapolation from our knowledge of AHV-1. In the face of an outbreak segregation of cattle from sheep has been reported (71) to be associated with the termination of cases but the degree of segregation necessary is not clear and greater precautions are required for the more susceptible target

species. As serum antibody to AHV-1 can be detected by IIF tests in species of the subfamily Caprinae other than domestic sheep (16), they too may be able to act as sources of infection and this should be considered in mixed collections of ruminants.

Legislation for the control of MCF is generally considered inappropriate due to difficulty in establishing a diagnosis, although in New Zealand the disease in cattle is notifiable (143). Legislation against the keeping of small ruminants in areas of Indonesia has been introduced to prevent the spread of the SA-agent from sheep to B. javanicus.

Screening of animals for serum antibody to AHV-1 prior to their importation would appear of limited value as tests have not been standardised and, except for sera of C. taurinus only heterologous reactions are likely to be detected. Such heterologous reactions may be poor indicators of the true carrier status of other species as in general the ruminant gammaherpesviruses appear to infect all individuals of their normal host. Sero-positive, clinically normal indicator hosts of MCF have not been recorded as infection is probably invariably fatal.

FUTURE PROSPECTS

Our knowledge of this group of viruses is still rudimentary. Although the epidemiology of AHV-1 in free living wildebeest populations in East Africa has been examined, factors contributing to transmission of the virus to other species are poorly understood. That antigenically related viruses spread in a similar manner within other species of antelope, but would appear not to transmit to other species, is intriguing. Further study of these viruses and their hosts could suggest new methods of control of MCF.

The application of molecular biological techniques to identify the SA-agent is an exciting prospect and should provide fundamentally important information on host-virus interactions and immune-regulation. With the achievement of those goals improved diagnostic methods may be developed and rational strategies for the control of MCF become available.

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5

AUJESZKY'S DISEASE (PSEUDORABIES) IN RUMINANTS

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INTRODUCTION

Aujeszky's disease (AD) in cattle, sheep and goats is characterized by a fatal, non-purulent encephalomyelitis caused by the porcine herpesvirus, type 1.

From 1813 to 1931, AD was predominantly a disease in cattle. Single outbreaks were recorded in the USA, Switzerland, Rumania, France, Russia and Brazil. Enzootics in pigs did not occur before 1931. Thereafter, the pig conquered the first place in host range and cattle the second one (for review see ref. 1).

The main characteristics of AD and of AD virus (ADV) are dealt with in the article on AD in pigs in this book. For this reason only characteristics of the disease specific for ruminants are given in this article.

CLINICAL SYMPTOMS

Several authors have described the clinical symptoms of AD in cattle (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17).

The incubation period varies from 3 to 6 days. Nasal discharge can be the first sign, followed by serious symptoms 2 or 3 days later, namely restlessness, dyspnoe, salivation, foaming and tympany. Loss of appetite does not generally occur, but the animals drink excessively. Muscle tremor is often seen. The animals paddle with their legs when lying in a lateral position and spasms of head, neck and abdominal muscles occur. Intense pruritus is the most characteristic sign of AD, but it is not present in every case (2, 4, 10, 16, 17). The animals bite and

lick their shoulders and their fore- and hind-legs almost continuously; they scratch their heads with the hind-legs and rub the irritated parts and the perineum against walls or other objects, inflicting swelling and open wounds. The animals groan and bellow and can be aggressive. High fever can occur. Quite suddenly the animals may fall to the ground and die, usually 1 to 3 days after the onset of the serious symptoms. In calves death can occur so fast that no typical symptoms of AD are able to develop (16). Recovery of cows from the disease is rare (18, 19), but in Germany at least, the number of recoveries increase.

The location of pruritus and other nervous symptoms depend apparently on the place of virus entry and subsequent virus spread (4, 9). A cranial pattern develops after respiratory infection. ADV is found predominantly in the different brain compartments and in the spinal cord cranial to the 7th cervical vertebra. Death frequently occurs within 24 hrs after the onset of symptoms. A caudal pattern develops after rectal or vaginal infection. ADV is isolated mainly from the spinal cord caudally from the 1st costal vertebra. Death occurs on day 2 or 3. After oral infection either the cranial (12) or the caudal pattern (9) is found.

The clinical symptoms of AD in sheep and goats are rather similar to those in cattle (20, 21, 54). After oral, nasal or tracheal infection the incubation period ranges from 78 to 108 hrs. The main symptoms are pyrexia, pruritus of the head region, restlessness, shaking of the head, facial contractions, chorea, dyspnoe, excessive salivation and mild tympanites. Shortly before death, which occurs within 12 hrs, the body temperature becomes subnormal and the recumbent sheep kick and struggle.

PATHOLOGY

Post-mortem does not reveal alterations specific for AD in ruminants. Gross lesions in cattle (16, 17, 22) are predominantly skin lesions and hemorrhage evoked by pruritus, congestion of lymph nodes partly connected with enlargement, interstitial emphysema and alveolar edema of the lung, subepi- and subendocardial hemorrhages, congestion of the spleen, meninges and

brain and frequently exsiccation of the mucosa of the abomasus, the jejunum and the caecum. Some animals show inflammation of the nasal, pharyngeal and tracheal mucosa, hemorrhage of the pleura and of the bronchial lymph nodes, small necrotic foci in the liver (23), congestion of the abomasum with hemorrhages in the mucosa, and inflammation and ulcera of the gall-bladder.

In sheep (20) the gross findings are skin alterations caused by pruritus of the head, enlargement, edema and congestion of the submaxillary and parotoid lymph nodes, pulmonary edema, epicardial petechiation and meningal congestion.

The histological findings in the nervous system of cattle (16, 17, 22, 24) are rather similar to those in pigs, but in contrast, very prominent in the spinal cord, especially in the cervical, lumbal and sacral regions, with ganglions also being affected. In the brain the olfactory bulb, the adjacent cerebral cortex, the brain stem and the medulla are predominantly involved. Characteristic are preivascular and meningeal infiltration by lymphocytes accompanied by neutrophilic granulocytes and histiocytes neural neucrosis, demyelinization, glia cell infiltration, hyperaemia, hemorrhages and intranuclear inclusions.

In sheep the histological lesions (20) are in the brain stem, the medulla, the ganglia of the cranial nerves, and the spinal cord. They represent mainly neural degeneration, intranuclear inclusions preferably in glia cells, preivascular infiltration by lymphocytes and macrophages, meningeal infiltration and microglial foci.

PATHOGENESIS

Experimental infection of ruminants can be performed via different routes intradermal (i.d.), subcutanerous (s.c.) intramuscular (i.m.), intravenous (i.v.), intranasal (i.n.), oral, vaginal and rectal. From the cranial pattern of pruritus observed with most of the AD field outbreaks in cattle, one can conclude that under natural conditions the virus enters the body mainly by the respiratory route (8, 9). In 29 outbreaks of AD in cattle, involving 54 animals, virus was demonstrated in the CNS and,

additionally, in the oral, pharyngeal and nasal mucosa of 8 and in the vaginal mucosa of 3 cows. Pruritus occurred twice as frequently on the anterior part of the body as on the posterior part (4). After i.n. infection ADV was found with most of the cattle but not in calves in the nasal secretion. The titres fluctuated between $10^{1.0}$ and $10^{4.0}$ $TCID_{50}$/ml nasal swab, but in one animal a titre of $10^{5.0}$ $TCID_{50}$ was reached (6, 16, 17, 25). This amount of virus can be sufficient to infect pigs (26, 27, 28), but frequently insufficient to infect cattle, since they require between $10^{4.0}$ and $10^{4.6}$ $TCID_{50}$ (3, 16) or $10^{3.2}$ PFU (25). The cattle infective dose may be dependent on the virulence of the virus strain used. However, it must be considered that accumulation of the virus to the environment occurs and in some instances the amount of totally excreted virus may reach the cattle infective dose. Thus it is not surprising that the infection rate in cattle herds fluctuates between 3 and 60%.

The virus has a marked neurotropism in cattle, even after i.m. or i.v. infection (24). After i.n. infection the virus was isolated in some cases from the nasal and pharyngeal mucosa, tonsils, retropharyngeal lymph nodes, and thymus of some of the cattle and calves dying between 3 and 7 days post infection (DPI). The predilection sites for virus isolation were the olfactory bulb, brain stem, medulla, trigeminal ganglion and cranial nerves and cranial, thoracical less frequently lumbal spinal cord (8, 16, 17). After oral infection virus was detected in the CNS, pituitary, pharynx and submaxillary lymph nodes (12). No virus could be isolated from the sacral cord and from several other organs tested. After rectal and vaginal infection ADV was predominantly detected in the thoracic lumbal and sacral cord, caudal nerve roots, vaginal mucosa and uterus (8).

These data indicate that primary virus multiplication takes place at the site of virus entry, where the virus enters the peripheral nerves almost simultaneously and migrates centripetally to the brain and the spinal cord from where virus spread progresses cranially and caudally along the spinal cord. The spread of virus along the peripheral nerves proceeds with a

velocity of about 75 cm in less than 72 hrs. There was no evidence of virus transport in the perineural fluid and no virus was seen electron microscopically in the nerves or ganglions. However virus particles were seen in the axoplasm of the nerve fibres which seem to be the pathway of the virus (6, 55).

Nothing is known about virus spread via the hematopoetic system, but the failure to isolate virus from different organs and the results of i.v. infection (24) are not very conclusive as to whether it occurs.

Natural infection of 4 and 5-days old calves via the umbilical cord has been reported (23) and virus was isolated from brain, lumbal cord and liver.

Latent virus infection cannot be established in cattle, since the animals die within a short time. But if in exceptional cases cattle survive it cannot be completely excluded.

In sheep a similar pathogenetic mechanism is assumed to take place (21, 29) and with goats it may be the same. AD virus was excreted in the nasal discharge of infected sheep with titres up to 10^6 $TCID_{50}$/1.0 ml, however the horizontal transmission to contact lambs failed (29).

DIAGNOSTIC PROCEDURES

Clinical diagnosis of AD is easy in cattle and sheep when pruritus is present and the animals are kept in or near pig-houses. It is not necessary for the pigs to be ill (inapparent or latent infection). It is difficult to differentiate AD from real colic, lead poisoning and rabies when pruritus is absent, however, pruritus can also be a sign of ectoparasites. Licking also occurs with mineral salt deficiency.

Histological changes are very helpful for post-mortem diagnosis. The alterations are very prominent in the spinal cord, except the sacral compartment, and in ganglia. In the brain region the olfactory bulb, the adjacent cerebral cortex, the brain stem, the medulla and the trigeminal ganglion are predominantly involved.

Antigen detection by means of immunofluorescence (IF) is

apparently less sensitive than virus isolation in cell cultures. About 50% of the samples from cattle were positive in cell cultures but negative in IF. The reverse was true with 16.5% of the samples (40). The opposite was found in sheep, where the sensitivity of the tests declined in the order IF → cell culture → histological examination (37).

IMMUNITY

No ADV-specific Ab could be detected in ADV-infected cattle, that died on DPI 6 or 7 (3, 15, 16, 25), but low titres of neutralizing Ab were present in surviving cattle, persisting several months (18, 57). However, the presence of cross-reacting BHV-1 Ab must be excluded (41, 42, 43). ADV-Ab can also be developed after vaccination, but not in every case (3, 17, 25, 37, 44).

EPIZOOTIOLOGY

AD in cattle always appears to be closely connected with pigs, which are the main source of infection. Outbreaks in cattle either occur in mingled herds or in separated cattle units connected by floors or openings in the wall with or situated near the pig unit. It is assumed that man may be involved in direct and indirect virus transmission to cattle. Another way of virus transmission is by air currents produced by ventilators in the pig house over distances of 10 to 20 m (4, 30).

Experimental contact infection from calf to calf and sheep to sheep was unsuccessful, but contact infection from calf to pig was observed (6, 11, 29, 31). However, contact infection from cattle to cattle cannot be completely excluded on account of the data of virus excretion given previously. Ruminants obviously do not play an essential role in maintaining the chain of infection.

Virus transmission can also occur by means of ADV contaminated injection needles and syringes. Some AD outbreaks have been reported in cattle and sheep after the use of unsterilized injection needles and syringes, or virus contaminated saline used for rinsing the syringe. The syringes had been

previously used to vaccinate pigs with live vaccines that were insufficiently attenuated for ruminants (32, 33, 34).

Pigs are also the source of infection for sheep and goats (35, 36) and transmission may be the same as with cattle. The disease appears to be less common in small ruminants, especially in goats, because possibilities of contact between infected pigs and sheep and goat herds are rather rare. Mostly single animals are affected, when kept in pig premises.

VACCINES AND VACCINATION

Live and inactivated vaccines have been used to vaccinate cattle and sheep. The different live vaccines vary in their virulence for ruminants (see also chapter AD in pigs). The live vaccines K61 Bartha (26, 37), NIA-4 (3), MK-35 (38) and A-26 (39) appear to have no harmful effect in ruminants. However, histological changes in the CNS were detected in 3 of 22 cattle vaccinated with K61 Bartha, though it was not ascertained that these alterations were of vaccine origin (26). The MK-25 and MK-35 vaccines seem to be innocuous for ruminants (19, 38, 45), but Akkermans (46) reported residual virulence of MK-25 for cattle and sheep in the field without giving details. Data concerning virulence for ruminants of the BUK and BUK-TK vaccines are contradictory. Skoda (47) reported that BUK/387 should be avirulent for cattle, but Zuffa (48) found BUK-TK/400 to be virulent for cattle. Zuffa and Dlhy (49) considered BUK-TK/840 as avirulent for cattle but Skoda and Jamrichova (50) mention that the highly attenuated BUK/1000 vaccine was irregularly virulent for cattle and sheep. Clark et al. (51) and van Alstine et al. (34) demonstrated that the BUK virus variant being in the Norden vaccine evoked AD in lambs and sheep. The Ercegovac and the Pliva vaccine are still virulent for cattle and sheep (46). However it was shown that the route of inoculation is of influence of virulence. The Pliva vaccine was virulent for cattle when injected parenterally, but it was avirulent after i.n. application (58). No data concerning virulence for ruminants are available for the Dessau vaccine.

The protecting effect of some live vaccines was tested in cattle and sheep. After s.c. vaccination of 233 cattle herds with K61 Bartha vaccine the majority of the animals did not develop antibodies, even after 2 vaccinations. Protection was unsatisfactory, because from 35 mixed herds, in which AD in pigs occurred, 4 cattle herds also developed AD (44). In K61 Bartha i.m. vaccinated sheep, no antibodies were detected after the first vaccination and only low titres after the second one. But all the animals were protected against i.d. challenge (37). Bironf et al. (3) tested the immunogenic properties of the NIA-4 vaccine after i.n. inoculation in cattle by i.n. challenge. They could not detect antibodies and the animals were not protected. Tartarov (19, 38) vaccinated sheep with K-25 and K-35 vaccines by the s.c. route. All the animals were protected after s.c. challenge.

Inactivated vaccines have also been tested in cattle and sheep. Good protection in revaccinated animals of these species have been reported (28, 37, 44, 56). The challenge virus was administered by the i.m. (10^6 $TCID_{50}$) and the i.d. ($10^{3.8}$ $TCID_{50}$) route. In contrast no protection was found in revaccinated cattle after i.n. challenge with 10^6 to 10^9 $TCID_{50}$, despite the presence of high neutralizing antibody titres (17). This result is worse than that of Bironf et al. (3) where 50% of the revaccinated cattle were protected. However, challenge was done by them with a dose near to the threshold of infectivity ($10^{3.7}$ $TCID_{50}$). Since the surviving animals did not seroconvert even after a second challenge, the dose of challenge virus might have been too small. Protection of revaccinated cattle was obtained by van Oirschot et al. (25) after challenge with 30 cattle LD_{50} (= $10^{4.9}$ PFU), but not with higher virus doses, however, seroconversion did not occur after the first and the second challenge. He concluded that under natural conditions cattle are more likely exposed to low than to high ADV dose. That this might be true was shown by a field experiment (52) where no AD occurred in the vaccinated cattle herds, whereas in previous years cattle had died from AD without vaccination. Besides, in some of these vaccinated farms AD did occur in pigs. However, such field trials do not strictly

correspond to scientific rules, since the incidence of AD in these cattle herds without vaccination is unknown.

The contradictory results of the protection experiments both with live and inactivated vaccines may be dependent on the virus dose used for challenge, the virulence of the virus strain for cattle and the route of virus application. The results with i.n. virus application were worse than those with i.m. or i.d. application. This can be explained by the fact that after i.n. infection the virus enters the nerve endings in the mucosa and migrates on the neural pathway to the CNS. In this way it is protected against the action of antibodies, which cannot overcome the neural barrier very well (53). After i.d., s.c. or i.m. infection the virus may be neutralized at the site of infection by antibodies already present. Good protection was obtained when virus doses below 10^5 $TCID_{50}$ were used for challenge. Since the vaccinated animals which remained healthy after infection did not seroconvert the virus dose might have been too low to initiate infection. Apparently good results of vaccination of cattle in the field should not only be attributed to the vaccine but to the amount of virus present which might have been too little for infection of cattle.

CONTROL

Ruminants play a minor role in virus transmission and virus spread. Therefore it is not necessary to have special control measures for cattle apart from those for pigs. Control of AD in pigs presents the disease in cattle.

Since AD may be transmitted horizontally in cattle and from cattle to pigs slaughter of the affected cattle in a herd is recommended, however, it is not necessary to kill also the unaffected animals. Virus decontamination should be performed by disinfection of the stable.

The effect of prophylactic vaccination of cattle and other ruminants is very dubious. It has not been ascertained that it is effective.

ECONOMIC CONSIDERATIONS

When pigs and cattle are housed close together one can calculate that about one outbreak of AD in cattle herds runs in parallel to about 3 outbreaks in pig herds. However, the number of infected cattle is relatively small since mostly only a few heads of cattle are infected. In the German Federal State Lower Saxony 29.272 pigs and 561 cattle were killed or died on account of AD in 1983 and 1984. The compensation amounted to DM 4.8 million for pigs and DM 1.1 million for cattle. With regard to the species the proportion is 52:1, but with regard to compensation about 4:1. Thus AD in cattle is a great economic factor in the total cost of the disease.

FUTURE ASPECTS

AD in cattle is closely connected with AD in pigs. Therefore, an increase of AD in pigs will cause an increase of AD in cattle and small ruminants, too.

There are many open fields for research concerning pathogenesis and immunity. The role of the immune mechanism in the course of ADV infection is quite unknown. Why do cattle not produce antibodies during the first week of infection? Why do antibodies present after vaccination not protect against infection? Does ADV immediately enter the nerve endings after infection thus avoiding contact with antibodies, or is virus multiplication necessary at the site of infection? What is the role of cell-mediated immunity? Is the hematopoetic system involved in infection? Are immune cells destroyed by the ADV? Do cattle develop local immunity? Is virus latency induced in recovered cattle?

Vaccination of cattle against AD has not been solved yet. More attention should be given to the intranasal application of live vaccines to prevent field virus multiplication in the naso-pharyngeal and respiratory tract. Can the efficacy of inactivated and live vaccines be improved by new adjuvants or immunomodulators? Can efficient vaccines be constructed by genetic engineering? What viral genes are important for virulence in

cattle? Leads their deletion to efficient live vaccines?

However, these investigations may be hampered since cattle are very expensive experimental animals. Nevertheless, the economic loss caused by AD in cattle is considerable and it would be worthwhile to enhance research on AD in these animals.

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6

HERPESVIRAL DISEASES OF THE HORSE

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INTRODUCTION

Equidae are susceptible to infection by three viruses of the family Herpetoviridae. Equine herpesvirus 1 (EHV-1), an alphaherpesvirus which exists as two subtypes, is responsible for respiratory disease of young animals as well as for sporadic and epizootic abortigenic or neurologic disease. Epizootic abortion caused by EHV-1 infection can cause economically devastating losses.

Equine herpesvirus 2 (EHV-2) resembles in many of its biological properties the Cytomegaloviruses (CMV) of man and other animals. In contrast to knowledge of the disease producing capabilities of those viruses, the consequences of infection of horses by their CMV are largely unknown.

Equine herpesvirus 3 (EHV-3), a second equine alphaherpesvirus, is the cause of a benign progenital exanthematous disease which is of comparatively minor economic importance.

Dimock and Edwards (1) documented discovery of the first herpesviral disease of the horse in 1936 with their description of "Equine viral abortion". Manninger and Csontos (2) reported the occurrence of the same disease from Hungary in 1941 and recorded observation of signs of respiratory disease in mares which later aborted. This observation led to their

suggestion that "virus born(e) abortion" was a manifestation of infection of pregnant mares by the then putative equine "influenza" virus (3). Doll et al. (4,5) experimentally produced both respiratory disease and abortion by inoculating horses intranasally with virus suspensions prepared from organs of aborted fetuses. They labeled the virus Equine rhinopneumonitis virus (ERV) to distinguish it from the newly discovered Equine arteritis virus which had also been found to cause abortion.

Although the structures which later became known as typical herpetic intranuclear inclusion bodies were described by Dimock and Edwards, the virus was not recognized as a member of the Herpetoviridae until 30 years later when Plummer (6) and Darlington and James (7) compared it with Herpes simplex virus and concluded that it should be so classified.

The first example of the virus which has become known as EHV-2 was reported, in 1963 (6), to have been isolated in England from the nose of one of a group of horses with "catarrh and coughing". Similar viruses have since been isolated from the leukocytes, upper respiratory tract, bone marrow and other tissues of apparently healthy horses by a number of investigators (8,9,10,11,12,13). Such viruses are also commonly found in specimens taken from horses with a variety of diseases known to be caused by unrelated infectious agents. They are frequently encountered as contaminants in primary cell cultures derived from tissues of normal horses. The viruses are morphologically typical Herpesviruses (6) which resemble in their habit of replication and cytopathic effects upon cell cultures (13) the CMV which infect other species.

Although descriptions of the disease now known as equine coital exanthema may be found in early veterinary literature, the viral etiology of the disease was not demonstrated until 1968 when the virus was first isolated (8,14,15) and identified as a unique herpesvirus (16,17).

CLINICAL SIGNS

EHV-1 Infection: Respiratory disease caused by EHV-1 is a disease of young, immunologically inexperienced horses. The primary infection is usually contracted during their first year of life, frequently about the time of weaning. The virus infects upper respiratory mucosa causing vesiculation of the epithelium. It infects lymphoreticular tissues in which it produces focal necrosis and spreads systemically. After an incubation period of about forty hours, which may vary with the relative virulence of the infecting virus, disease presents as febrile respiratory illness in which the temperature may reach 41.5 C. and remain elevated for 8 to 10 days. The relative severity of physical signs of disease appears from observation of both naturally acquired and experimentally induced infections of young horses to be related to the infecting viral subtype, i.e. disease caused by subtype 1 (S-1) virus is generally more severe than disease caused by subtype 2 (S-2). Hyperthermia may be recorded as a biphasic curve especially in patients which experience secondary infections. Infected foals and yearlings display a serous trickling nasal efflux early in the course of disease. This becomes a clear mucoid discharge on the second or third day and commonly becomes mucopurulent on the fourth day as secondary

streptococcal infections progress. Bronchopneumonia occurs commonly especially in foals which are not protected from undue stress. The morbidity rate within herds of immunologically naive weanlings approaches 100 %. The mortality rate in the absence of uncontrolled supervening bacterial pneumonia is negligible. Neutropenia occurs during the first few days of fever, neutrophilia may occur with the development of secondary infections.

Abortigenic infection by EHV-1 is, from the economic standpoint, the more important of the three types of disease produced by this virus. Although abortigenic disease in an individual mare can conceivably arise as a result of recrudescence of infection in a latently infected subject, most such disease results from a progression of pathological events (18) originating with reinfection of the respiratory tract in an immunologically experienced mare during the terminal four months of pregnancy. Because of the ubiquitousness of infection of horses by the subtypes of this virus and because of the fleeting nature of immunity to reinfection, mares rarely reach breeding age without having been exposed to infection several times. Abortigenic disease is therefore a disease of immunologically experienced individuals. Infection of such individuals usually does not result in appearance of clinically detectable respiratory disease. The incubation period, from infection of the respiratory tract until abortion, varies from about 9 days to, in rare cases, several months (19). Ninety-five percent of abortions occur in the terminal three months of pregnancy. Abortion from naturally acquired infection has not been observed to occur in mares less than five months pregnant.

Most affected mares show few, if any, signs of impending abortion. Abortion is, except in cases complicated by fetal dystocia, a precipitous event. It may occur while the mare is standing or almost immediately after a mare lies down after a short period of apparent unease. The placenta is usually delivered with the fetus or shortly thereafter. The fetus is commonly delivered encased in the amniotic membrane and almost never displays any evidence of autolytic alteration. Mares recover from the abortion as from normal parturition. Their future reproductive capacity, in the absence of damage from dystocia or bacterial infections acquired at the time of abortion, is not compromised. The aborted fetus, its fluids and membranes represent a rich source of virus for infection of other horses but the mare's reproductive tract is cleared of virus within a day.

Neurological disease associated with infection of horses by EHV-1 may affect animals of any age, including suckling foals (20,21). It may be preceded by the occurrence of respiratory disease in young animals or abortions or as the only clinical manifestation of infection by the virus. Infection is acquired via the respiratory tract and young horses may therefore show signs of respiratory disease. The incubation period between infection and the appearance of neurological signs has been determined experimentally to be 6 to 9 days (20,22). The earliest sign of neurological disease that is usually noticed is a proprioceptive defect of the hind limbs evident by a reluctance to move or ataxia with dragging of the feet. These signs are the result of lesions in the spinal cord resulting from vasculitis. The body temperature is usually not elevated at the time of

onset of neurological disease. Lesions may occur in any part of the CNS but are, in many cases, confined to the posterior thoracic, lumbar and sacral regions of the spinal cord. Individuals may develop hind limb ataxia, regional sensory deficits, as well as tail and bladder paralysis. The development of more extensive lesions may produce quadrilateral ataxia as well as such signs as abnormal carriage of the head, nystagmus or iridocyclitis. The most severely affected animals become paralyzed and recumbent early in the course of the disease. It is typical of EHV-1 neurological disease that the course is rapidly progressive; all signs develop within a matter of a few hours and, barring complications unrelated to primary etiology, reach their ultimate degree of severity within a matter of 12 hours or less. Affected animals which do not become recumbent usually recover completely with supportive treatment. The disease may occur as a single case in a herd or may progressively involve many of its members over a period of weeks. Permanent gait abnormalities occur rarely. The mortality rate is usually low, the majority of deaths are due to respiratory and circulatory complications associated with paralytic recumbency. Some animals recumbent as a result of paralysis of the hind limbs may injure themselves as a result of violent struggling. The severity of the complicating conditions in many cases leads to a decision for euthanasia.

EHV-2 Infection: Although the incidence of infection of horses by their CMV's has been shown virologically to be very high, little evidence has been forthcoming which identifies these viruses unequivocably as the particular or initiating cause of any disease. The principle difficulty in interpreting

the observations which have been reported from both natural (6,23,24) and experimental circumstances (25,26) arises from the fact that the virus(es) can be isolated with like frequency from the respiratory tract, the leukocytes, the conjunctivae and other anatomic sites of normal horses (8,27,28). Infection by equine CMV has been held responsible for severe pneumonia of foals (23,24) complicated by bacterial infection (Corynebacterium equi, Streptococci, etc.) as well as, in two foals, for experimentally induced, asymptomatic cases of nodular pharyngeal hyperplasia which occurred three to eight months after their inoculation(25).

EHV-3 Infection: The earliest lesions of infection by this virus appear as small (2-3 mm), seldom noticed, vesicles in the skin of the vulva or the penis. The first lesion which is usually noticed is a shallow erosion with a hyperemic floor and irregular margin which, in most cases is covered by a scab. These lesions may enlarge peripherally for a few days and may coalesce to form larger erosions. The lesions are confined to the skin, they do not extend beyond the vulvovaginal or balanourethral mucocutaneous junction. The erosions often appear symmetrically on opposite vulvar surfaces and may also occur in the dependent skin. Unless antibiotic therapy is applied, secondary bacterial infections localized to the viral lesions occur routinely. Such infections in stallions may produce severe necrotizing balanitis which is medically the most serious consequence of the infection. Progenital disease occurs in maiden colts and fillies and infection by the virus can be acquired by the respiratory route. Although the disease occurs in pregnant mares, the virus has not been isolated

from a naturally infected fetus and infection appears not to constitute a threat of abortigenic disease (17).

PATHOLOGY

EHV-1 Respiratory Disease: The primary infection of the upper respiratory tract of young horses by EHV-1 produces lesions typical of herpetic disease. The virus multiplies in epithelium of the respiratory mucosa causing necrosis of cells and formation of thin walled vesicles with irregular margins and associated local inflammation. The infection spreads peripherally to regional lymph nodes where it produces necrosis and thence systemically. The consequences, if any, of systemic spread of the virus, except in the fetus and in animals which experience neurological disease, have not been described. The lesions produced by virus in the upper respiratory tract are routinely superinfected by bacteria during the first 48 hours of the infection. This infection, usually caused by Streptococcus zooepidemicus, produces abscesses of the solitary lymphoid follicles of the pharynx. The secondary infection may also produce pyogenic tonsillitis and inflammatory hypertrophy or abscessation of the retropharyngeal and intermandibular lymph nodes. The only description of the gross and microscopic pathology of uncomplicated viral bronchopneumonia produced by EHV-1 has been provided by Prickett's (29) observations in experimentally infected foals which were killed in the early stages of the infection. The viral infection produced an acute bronchopneumonia with necrosis of the bronchial epithelium, peribronchiolar and perivascular infiltration of mononuclear cells, serofibrinous exudate into the alveoli and necrosis of

the bronchial lymph nodes. Typical herpetic inclusion bodies were observed in the bronchial epithelium and in affected lymphoid tissue. This pathognomonic lesion is usually not demonstrable in naturally infected foals which succumb to viral pneumonia complicated by bacterial infection.

EHV-1 Abortion: As opposed to most fetuses aborted as a result of microbial placentitis or infection of mares by the equine arteritis virus, those infected by EHV-1 show no postmortem autolytic changes. The fetus may be aborted while still encased in the amniotic membrane. Demonstrable lesions are confined to the fetus, no gross or microscopic lesions of the viral infection have been described for the placental tissues. The majority of fetuses are viable until immediately before they are aborted; they die of suffocation. Suffocation produces agonal petechial and ecchymotic hemorrhages of the visible mucosae as well as fetal diarrhea which results in staining of the fetal footpads by meconium. The placenta may in some cases be moderately edematous but no lesions attributable to viral infection have been detected. Although some aborted fetuses attempt to breathe and succeed in partially inflating their lungs, the lungs of most remain collapsed, completely edematous and non-functional. The interlobular septa are distended and the thoracic cavity may contain from a few milliliters to a liter or more of clear, yellow colored transudate. Multiple grey foci of necrosis may be observed on the surface of the liver, the spleen is enlarged and the thymus may be grossly necrotic and therefore abnormally friable. The adrenal cortex may contain small areas of necrosis and hemorrhage. Except for a generally juandiced appearance and the presence

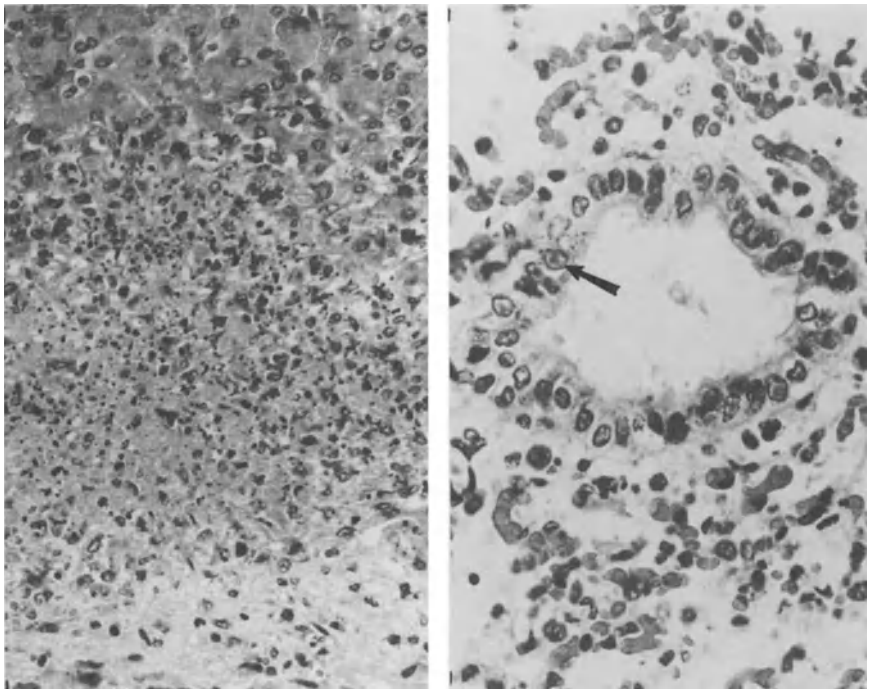
of petechial and ecchymotic hemorrhages on the serosal and mucosal surfaces of many organs, no other remarkable gross lesions are present.

Histologically, in addition to pronounced edema, the lung presents lesions of bronchopneumonia with necrosis of bronchial epithelium, sloughing of cells into the bronchial lumen, serofibrinous exudation and the pathognomonic intranuclear herpetic inclusion body which is most prominent (Fig. 1) in epithelial cells of small bronchi.

Focal necrotic areas outside of the bronchial tree may also be found in the lung parenchyma. The liver is hyperemic and may contain miliary petechial hemorrhages and areas of focal necrosis. Foci of necrosis are most commonly located in areas closely adjoining the portal triads. Inclusion bodies are present in hepatic and reticular cells at the periphery of these lesions and have also been described in biliary epithelium, vascular endothelium, in cells of the walls of arteries (30), in splenic lymphocytes and cells of the adrenal cortex (31).

Focal necrosis of the lymphoreticular system including the splenic follicular tissues, thymus and various lymph nodes is found in most infected fetuses. The thymus and splenic follicles may exhibit massive necrosis. Focal necrosis also occurs in the splenic red pulp and inclusion bodies may be found in reticular cells at the periphery of necrotic areas. Lesions in peripheral lymph nodes are usually demonstrable only in nodes afferent to organs such as the spleen, lung and liver in which extensive lesions produced by the viral infection are demonstrable.

EHV-1 Neurological Disease: In contrast to herpesviral encephalomyelitides in other species,



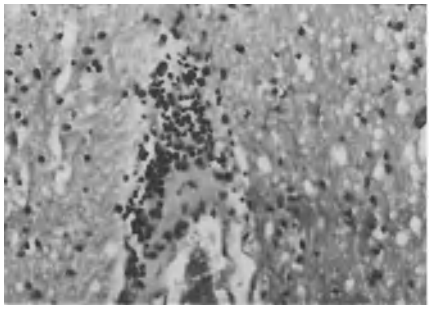
A B

Figure 1: Histologic lesions typical of EHV-1 infection in an equine fetus; (A) intra-nuclear inclusion bodies (arrow) in bronchiolar epithelium,(B) focal hepatic necrosis.

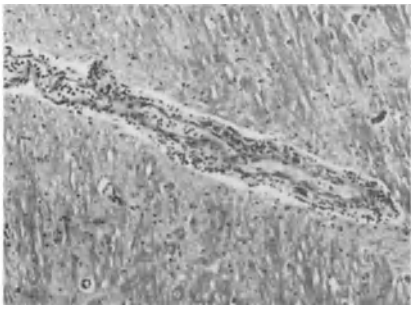
neurological disease associated with infection of horses by EHV-1 apparently does not occur as a result of a productive viral infection of the central nervous system. There is no histologic evidence of infection of neurons or other cells of the CNS and virological evidence for such infection (32,33,34) is rendered equivocal by the fact that EHV-1 viremia is demonstrable in many cases of the disease. The lesions consist of vasculitis (Fig. 2) involving both arteries and veins and ischemic damage of both grey and white matter in the brain and of the lateral and ventral white columns of the spinal cord. Vasculitis is evident by proliferation of endothelial cells, focal necrosis of the tunica media, subendothelial accumulation of plasma and erythrocytes, and perivascular cuffing of some vessels. Meningeal and penetrating vessels are most prominently involved in both the brain and cord. Perivascular cuffing of some vessels is the only lesion of the three pathologic hallmarks of encephalitis which is present (35). Marked axonal swelling without necrosis with distension of nerve sheaths is a constant lesion in the spinal cord. This lesion appears, along with the occurrence of areas of malacia to be associated with vasculitis and thrombosis of blood vessels (35,36).

As suggested by Jackson et al.(35), this pattern of lesions illustrates that EHV-1 produces a neurologic syndrome which results from pathogenetic mechanisms apparently unique among herpesviruses.

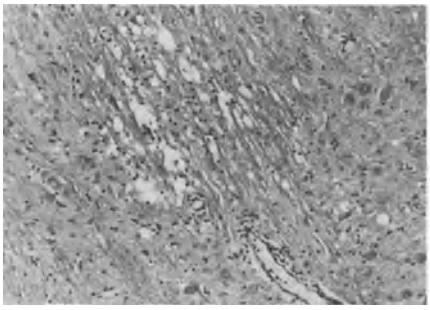
EHV-3 Infection: The gross lesions of progenital disease of mares and stallions produced by this virus are described above. Histologically, the lesions are shallow erosions extending to a depth approximately twice the thickness of the epithelium. The transition



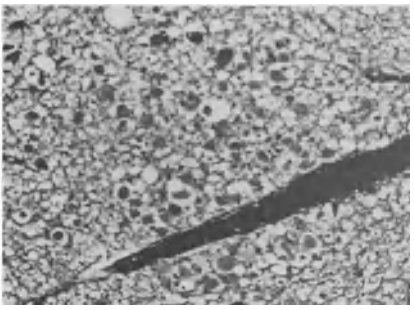
A.Cerebrum: perivascular cuff and vasculitis.



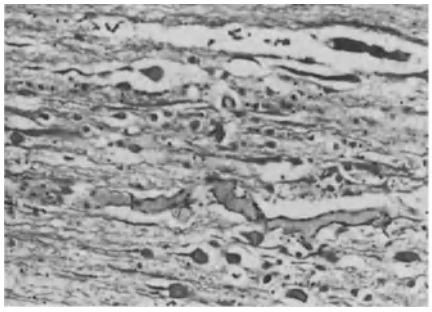
B.Midbrain; perivascular cuff.



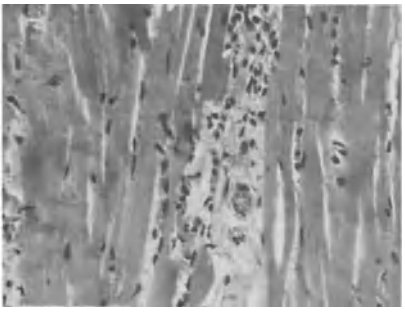
C.Midbrain: perivenous demyelination.



D.Spinal cord; perivenous demyelinization, axonal swelling.



E.Spinal cord; axonal swelling.



F.Myocardium; perivascular infiltration.

Figure 2. Histologic lesions typical of neurological disease associated with EHV-1 infection of horses.

to normal epithelium at the lateral edges of the erosion is very sharply defined. Typical herpetic inclusion bodies may be found in this area, in cells of the germinal epithelium or in remnants of cells in the necrotic areas. Although some vessels in areas adjacent to the lesions exhibit intense perivascular mononuclear cell cuffs, the vessels are apparently not otherwise affected (17). It should be noted that Burrows (41) has described isolation of an antigenically unique herpesvirus with other biological properties apparently identical to those of EHV-3 from similar lesions of the muzzle, chin and lips of a suckling donkey foal whose dam was found to have identical and extensive lesions of the vulva, teats and udder.

PATHOGENESIS

Two of the Herpesviruses which infect Equidae, EHV-1 and EHV-3, are alphaherpesviruses; they replicate and spread comparatively rapidly in cell cultures, are efficiently destructive of susceptible cells and their ability to produce latent infections in their natural host helps to insure their survival. The results of experimental studies (4,5,17), indicate that they behave similarly in the horse. Epidemic disease caused by EHV-1 occurs as a result of spread of the virus via the respiratory route and initial infection or reinfection occurs in the tissues of the nasopharynx. Horses are also susceptible to infection by EHV-3 via the respiratory route and the occurrence of progenital disease in maiden colts and fillies as well as the widespread occurrence of antibodies to this virus indicates that the disease is transmitted naturally in some cases thereby. The

incubation periods of the diseases produced by EHV-1 and 3, as estimable from inoculation of susceptible horses, are relatively short and they produce disease and lesions characteristic of inflammatory processes reacting to the cytocidal activity of virus. The primary lesion of infection of epithelium by EHV-1 and EHV-3 is formation of a vesicle with an inflammatory base. Infection of lymphoreticular and other tissues by EHV-1 produces focal necrosis. Although there is little definitive information to describe the mechanisms of response of the host to primary infection by EHV-1, the results of the investigations which have been carried out suggest that the horse responds similarly as other species which are susceptible to herpesviral infections. Disease produced in hamsters by EHV-1 strains adapted to that species is a fulminantly progressive and uniformly fatal hepatitis (37) which does not have a counterpart in the natural host species.

Abortigenic infection by EHV-1 occurs as a result of transplacental transport of virus in immunologically experienced mares. Viremia has been shown to occur in mares possessing high titers of neutralizing antibodies for the virus used to infect their nasopharynx. The virus has been shown by co-cultivation of leukocytes with susceptible cells to be associated with an as yet unidentified component of the leukocyte fraction of the blood (18). This apparently immunologically privileged cell associated viremia has been shown to persist for as long as 24 days but the question remains as to whether it is a product of multifocal infection of lymphoid tissues in regions other than the pharynx and if so, how long it can persist continuously or intermittently to threaten

infection of the susceptible fetus. Incubation periods as long as 90 days have been observed for mares inoculated by the oral or nasal routes (19). If fetuses are inoculated directly with the virus, abortion always ensues within 3 to 9 days thereafter establishing that lengthy "incubation periods" are difficult to account for by time required for the virus to produce an abortigenic infection once it initiates infection of the susceptible fetus.

Although the lesions demonstrable in aborted fetuses are qualitatively the same, they vary quantitatively to a great degree. It appears however that, no matter how severe the lesions, the disease of the fetus is not incompatible with its life in-utero during the period from infection to abortion. It has been suggested that lesions histologically demonstrable in the uterus of mares after their fetuses were infected by transplacental inoculation are interpretable as evidence that abortion is an immunologically mediated phenomenon (38). Similar lesions have been found in the uteri of pregnant mares infected by subcutaneous inoculation (35).

The essential lesion in the pathogenesis of neurological disease caused by EHV-1 infection is vasculitis and thrombosis which leads to ischemic damage to focal areas of the brain and spinal cord. Vasculitis in affected horses is not confined to the CNS. Similar lesions have been demonstrated in the endometrium, uvea, (35) nasal mucosa, lungs and at other anatomic sites (34,36) in affected animals. Although the virus is not routinely isolatable from the CNS it is commonly present in peripheral blood leukocytes during the course of CNS disease. Histological evidence (inclusion bodies) of viral

infection of endothelial cells in the equine fetus (30) as well as evidence for the presence of viral antigen in endothelial cells of vessels of the CNS of horses with EHV-1 neurological disease has been presented (39). Evidence for the presence of immune complexes presumed to be EHV-1 viral antigen-antibodies in the serum of horses inoculated with a "paretic" isolate of EHV-1 and for thrombocytopenia indicative of the early formation of thrombi in the same experimental subjects has been presented more recently (40). The description of the development of vasculitis presented by Jackson et al. (35) demonstrated that the initiation of the inflammatory alterations of vessels in the CNS is proliferation and necrosis of endothelium. The presence of viral antigen in endothelium and the pattern of progressive histological alterations of vessels which leads to functional damage to the CNS suggests that EHV-1 encephalomyelitis of the horse is the result of a generalized Arthus (Type III) immunological reaction analogous to that which is responsible for equine purpura hemorrhagica.

Equine herpesvirus 3 is commonly transmitted by coitus between animals with lesions. The virus infects and in some individual lesions destroys the stratum germinativium. Scars resulting from healing of such lesions are not uncommonly present in the skin of the vulva of mares that have recovered from the disease.

Progenital exanthematous disease may also result from infection of mares by EHV-1 virus. Such disease has been produced experimentally by intradermal inoculation (17) and the virus has been isolated from naturally occurring lesions (42,43). Most of the lesions produced by inoculation of dermis with EHV-1

are confined to the more superficial layers of epithelium and prominent intranuclear inclusion bodies are present in cells at the periphery of necrotic areas. There is no evidence to suggest that these superficial lesions are ever responsible for initiation of transcervical infection of the fetus.

LATENCY

An ability to establish latent infection is crucial to the survival of herpesviruses in the immune environment which results from the host's reaction to infection. Although comparatively little effort has been applied to investigations of latency for the equine herpesviruses, convincing evidence has accumulated which supports the concept that the equine viruses survive as a result of their sharing with other herpesviruses the ability to establish themselves latently in their natural host species.

Epizootiological observations of diseases caused by EHV-1 provides circumstantial evidence that stress resulting from such influences as transport, other infections or vaccinations, mixing of horses from different herds together at sales or race meetings increases the likelihood of the occurrence of herpesviral disease (22). Erasmus (44) reported the apparent activation of EHV-1 infection among a group of horses vaccinated for African horsesickness. Burrows and Goodridge (45) demonstrated, coincidental with such stressful influences as weaning, relocation, castration and other illness, spontaneous shedding of EHV-1 from ponies kept for 10 years in a closed herd. Although they and other investigators (46,47) including ourselves, have been unable to reactivate EHV-1 infection by administration of corticosteroids,

a practice which has been shown to accomplish this readily for some other herpesviruses (48,49,50), such reactivation has been reported (51) to have been accomplished by the administration of dexamethazone and prednisolone to ponies that had been kept for 3 months in isolation after their initial infection. From 22 attempts we (53) were able to isolate, by co-cultivation, an EHV-1 (S-2) virus from the trigeminal ganglion of one mare that had aborted an S-1 virus infected fetus a few days earlier. The possibility that the ability to establish latent infection is a characteristic of particular strains of EHV-1 has not yet been adequately explored.

The isolation of EHV-1 from circulating leukocytes must be accomplished by co-cultivation of intact leukocytes with susceptible cells. This finding, along with the observation that the number of infectious centers which can be detected in a population of co-cultivated leukocytes increases with non-specific mitogenic stimulation of the sample (52), suggests that the virus may be present in a latent state in peripheral blood mononuclear cells. Although Burrows and Goodridge were able to produce serological evidence for periodic reactivation of both EHV-1 and EHV-3 infection in their closed herd, they were unable to demonstrate infectious virus by either cultivation of tissue explants or to precipitate viral shedding by administration of corticosteroids.

Coital exanthema has been observed to be recurrent in naturally infected mares (17,45) but EHV-3 has been recovered from such mares only in the presence of lesions of the disease (45).

CHARACTERISTICS OF THE VIRUSES

Morphology: The three herpesviruses which infect horses are morphologically typical. As is the case for all known herpesviruses, their icosahedral capsids are approximately 100 nm in diameter and are structurally indistinguishable. The capsids are composed of 150 hexameric and 12 pentameric capsomeres which enclose linear double-stranded DNA. The nucleocapsid is surrounded by an amorphous tegument of as yet uncertain composition and finally by a trilaminar envelope derived from the modified inner nuclear membrane of infected cells. The loose envelope contains viral proteins responsible for infectivity, antigenicity and presumably several other viral functions which may influence virulence and immunogenicity. At the molecular level, the viruses are distinguishable by such parameters as the physical properties of their respective genomes (16,54,55,56,57,58), the immunological specificities of their envelope glycoproteins, as well as by their patterns of replication and their in-vitro cell culture host range.

Antigenic Relationships: Except for the two major subtypes of EHV-1 which share at least four antigenically related glycoproteins (53,59) and which can be shown to be related by cross neutralization tests, there appear to be no significant antigenic relationships among the equine herpesviruses. Apparent cross reactions detected by complement-fixation (CF), immunodiffusion (ID), or fluorescent antibody (FA) techniques between EHV-1 and IBR virus (60,61), EHV-1 and EHV-3 (62), EHV-1 and various other herpesviruses (61) have been described. The detectability of such reactions appears to vary with the reagents and

techniques used in various laboratories (64,65). No antigenic relationships between the equine viruses and others have been detectable by neutralization techniques.

Host Range: In addition to their antigenic differences, the two subtypes of EHV-1 differ in respect to the base sequence structure composition of their genomes (65,66,67,68) as well as in their host range in cell cultures. Thus, the range of cell cultures which are susceptible to productive infection by the S-1 viruses is very wide while that of S-2 viruses is restricted to primary cell cultures or subcultivable cell lines of equine or some of porcine origin (53, 131,) The spectrum of susceptibility of cell cultures to EHV-2 viruses has not been thoroughly explored but, like the CMV of other species it appears to be somewhat restricted. EHV-2 is known to be capable of replication in cells of equine, rabbit or feline origin (159). The EHV-3 virus is strictly limited (17) to replication in cell cultures of equine origin. The EHV-1 S-1, but apparently not S-2 virus (69,70) is adaptable to growth in golden hamsters (Cricetus auratus) (71) and capable of producing infection of the brain of young mice (69,72,73,160). Some isolates of EHV-1 (subtype unknown) have been shown to be adaptable to infect cells of the chorioallantoic membrane of chick embryos (74) and lesions have apparently been demonstrated in fetuses of guinea pigs inoculated in-utero (75,76). These latter systems appear to have limited utility for studies of the virus.

Although antigenic types or subtypes have not been defined for the EHV-2 virus, the collections of isolates which have been studied exhibit a

considerable degree of antigenic (8,13,56) and genomic variation (77,78,79). The original isolate of the EHV-2 virus has been shown to produce a clinically inapparent infection of the central nervous system of experimentally inoculated rabbits from which virus could be recovered 20 months later (80).

All isolates of EHV-3 that have been examined, except for the virus isolated from donkeys which appears in several respects to be unique (41,81) are, by evidence of cross and kinetic neutralization tests (17), antigenically identical. All isolates of the virus that have been examined have an absolute requirement for cells of equine origin to initiate a productive infection.

In recent years, viruses apparently closely related or identical to EHV-1 have been isolated from an aborted bovine fetus (68), from fetuses aborted by a captive onager (31) and zebra (107) in the United States and from captive fallow deer (83) in Alberta, Canada. The viruses isolated from the onager, deer and zebra fetuses have been typed by electrophoretic analysis of restriction enzyme fragments of their DNA's as distinct from domestic horse isolates of S-1 viruses. These data, especially those derived from the bovine and fallow deer virus, suggest that the natural host range of the EHV-1 virus may be broader than is presently appreciated.

Inactivation: Except for an investigation of the stability of EHV-1 in the environment which was done using viral suspensions prepared from infected hamsterlivers and which therefore contained much extraneous matter, there have been no reported investigations of the effect of environmental factors on the viability of the equine viruses. Because these

viruses are chemically and structurally closely similar to other herpesviruses, we can reasonably assume that transmission of the equine viruses involves direct contact with virus-containing secretions of individuals experiencing a productive viral infection. The equine viruses are inactivated by lipid solvents such as ethyl ether, and by other chemicals (sodium deoxycholate, dithiotheitol etc.) which affect the integrity of the viral envelope. Virus suspensions prepared from infected hamster livers are inactivated by heat (56C.) in 5 to 10 minutes. They remain viable when kept at 4 C. for as long as seven months in buffered saline containing 20 % serum, are most stable between pH 6.0 and 8.0, remain viable in infected hamster tissue at -20 C. for more than a year and in infected equine fetal lung kept at -40 C for at least 17 years. Virus dried as hamster liver suspensions on glass, straw or galvanized iron remained infective at room temperature (20-27C) for less than seven days and for less than 14 days on wood, paper or Manila rope.Virus dried on oily burlap cloth or on sterilized horsehair remained infectious for 35 to 42 days (84).

MOLECULAR ASPECTS OF THE VIRUSES

The Genome: Characteristic of all herpesviruses, the genetic material of the 3 types of EHVs consists of linear, double-stranded, non-segmented DNA (6,16,85). The genomic DNAs are large : 92 to 96 million for EHV-1 and EHV-3 (86,87), 126 million for EHV-2 (88). Approximately 10 % of the total content of the equine herpesvirion is DNA (85).

The extent of evolutionary distance between the DNAs of the 3 EHVs was first suggested by the wide

range of their guanosine plus cytosine (G + C) content (EHV-1: 57 moles %, EHV-2: 57 moles %, EHV-3: 66 moles %) (16,85,89) and later confirmed by experimental attempts to form stable hybrids between the 3 EHV DNAs. Estimates of DNA sequence homology among the three viral genomes obtained by the latter method of DNA hybridization have been reported as 5 to 10 % between EHV-1 and EHV-3, less than 5 % between EHV-1 and EHV-2, and 15 to 25 % between the two subtypes of EHV-1 (90,91,92). The DNAs of the two subtypes of EHV-1 and EHV-3 have been shown to be colinear with the homologous sequences dispersed throughout the genomes (92,93).

Analysis of the genomes of EHV-1 and EHV-3 by electron microscopy as well as with restriction enzymes has shown that they consist of unique (U) sequences organized in both a long (L) and short (S) section with the S section bracketed by terminal (TR) and inverted repeat (IR) sequences (94,95,96,97). When isolated from purified virions, the DNA of EHV-1 and EHV-3 is present in two possible isomeric arrangements, the prototype (P) and inverted (I) isomers, as a consequence of the inversion of the S sequences relative to those of the L segment.

The electrophoretic patterns of the DNA fragments obtained by digestion of all 3 EHV DNAs with different restriction enzymes have been identified and published (88,94,95,96,97,98). Determination of the linear order of such DNA restriction fragments has also been achieved, making available restriction enzyme cleavage maps for each of the three EHV's (95,97,98). The absence of any common restriction cleavage sites identifiable among the three gemones has added genetic support for the view, first suggested by serological,

biological and pathological data, that EHV-1, EHV-2 and EHV-3 represent three distinct and evolutionarily divergent herpesviruses of the horse.

In a search for intratypic strain variability within the genome of the EHVs, the restriction patterns of the DNAs of various field isolates have been examined in several laboratories (66,99,100,101,102). Variation in the sizes of genomic restriction fragments can be identified among different isolates of all three viruses. Some regions of the viral genome, such as the termini and repeat sequences, exhibit this type of fragment size variability more than others (100,103).

A second type of variability in the restriction patterns of different field isolates of the EHVs is due to nucleotide sequence alternation, resulting in a loss or acquisition of one or more restriction cleavage sites. The latter type of genetic variability has been used to categorize EHV isolates into distinct DNA fingerprint types (electropherotypes)(66,99,102). The several EHV electropherotypes of particular interest include: (i) EHV-1 1B which has risen, since 1981, from a position of rarity among abortigenic field isolates to one of dominance as the cause of herpesviral abortion in the central Kentucky area (104); (ii) EHV-3 large- and small-plaque (LP and sp) isolates which differ by the presence in the sp genome of an additional 5700 base pairs inserted into the S region (101,104); (iii) the division of DNA from both EHV-1 and EHV-2 isolates into two distinct electrophoretic groups with limited homology between the groups of each virus (66,92,102,103); (iv) the electrophoretically distinguishable DNA fingerprints of vaccine strains of EHV-1 attenuated by repeated

passage in cell cultures or in the Syrian hamster (66); and (v) highly variant DNA electropherotypes of EHVs isolated from animals other than the domestic horses; e.g. EHV-3 dKy from a donkey, EHV-1 1G, 1H, and 1I from an onager, zebra and fallow deer, respectively (31,106,107,108).

As is the case with other herpesviruses, expression of the viral genome during replication of the EHVs is highly regulated. Investigated most thoroughly for EHV-1, it has been demonstrated that EHV-1 transcription is divided into immediate early, early and late phases (110). Following immediate early gene expression from the viral IR DNA sequences, early and then late EHV-1 genes are sequentially expressed from the remainder of the EHV-1 genome (110).

Gene Products: From 14 to 30 new viral proteins can be identified in cells after their infection with EHV-1, EHV-2, or EHV-3 (110, 111). Both the types of EHV-1 proteins synthesized and their relative abundance vary at different times after initiation of infection (110). The virus induced proteins have been classified, on the basis of their temporal appearance during infection and the metabolic requirements for their synthesis, as either immediate early (synthesized very early during infection with no requirement for previous protein synthesis), early (synthesized only in the presence of functional immediate early viral proteins), or late (maximal synthesis occurs late during infection after initiation of viral DNA replication) (110). Selection of messenger RNA species with restriction fragments of viral DNA from defined regions of the EHV-1 genome, followed by in-vitro translation of those RNAs, has recently allowed a determination of the map positions

for many of the different EHV-1 polypeptides (112).

Extrapolation of experimental data derived from other, better characterized herpesviral systems suggests, as a generalization, that the immediate early proteins of EHV-1 are regulatory proteins, that the early proteins are enzymes involved in viral DNA synthesis, and that the late proteins are structural proteins of the progeny virions.

Several new enzyme activities, presumably viral-coded, have been identified in cells following infection with EHV-1 and EHV-3. Among these viral-induced enzymes are a deoxythymidine kinase (113,114), DNA polymerase (115), ribonucleotide reductase (116), and a virion associated protein kinase (117). In the case of EHV-1 deoxythymidine kinase and DNA polymerase, the enzymes have been purified to homogeneity, allowing precise determinations of their biochemical and enzymatic properties (118,119).

The structural proteins that comprise the virion particle have been identified for EHV-1 (121), EHV-2 (111), and EHV-3 (121). Approximately 25 bands can be detected in preparations of the purified EHV's by one-dimensional polyacrylamide gel electrophoresis. From six to nine of the structural proteins are contained within the EHV nucleocapsid; the remainder are part of the virion envelope or tegument.

When EHV-1 virions are labeled with glucosamine, eight highly-abundant and six minor species of glycoproteins can be identified (122). Three major and four minor glycoprotein species have been identified in purified virions of EHV-2 (111). Genes encoding the eight high-abundance glycoproteins of EHV-1 have been localized on the viral chromosome (123). The genes for

EHV-1 glycoproteins numbered 13 and 14 have been sequenced and shown to be the molecular homologs of gC and gB-like glycoproteins, respectively, of other alphaherpesviruses (124, 125). Monoclonal antibodies have been generated against each of the eight major glycoproteins of EHV-1 (123). The epitopes of EHV-1 glycoprotein 13 have been characterized with a panel of 42 monoclonal antibodies and shown to be predominantly subtype-specific and also to exhibit substantial intrasubtypic variability among field isolates of EHV-1 (124). Monoclonal antibodies that react with EHV-1 glycoproteins 13 and 14 neutralize viral infectivity (124). Antibodies against all the major glycoproteins, in addition to the major nucleocapsid protein, of EHV-1 have been detected in the serum of horses convalescing from EHV-1 infection (59).

DIAGNOSTIC PROCEDURES

Clinical Diagnosis: The reader is referred to pages 178 to 189 of this chapter for a description of the clinically observable signs and lesions characteristic of infection of horses by the EHV's.

Laboratory Diagnosis: Isolation of virus in susceptible cell cultures remains the only recognized method for making a definitive laboratory diagnosis of EHV infection. The specimens of choice for attempting virus isolation include: (i) lung, liver, spleen and thymus from fetuses suspected of aborting as a result of EHV-1 infection; (ii) exudate collected by swabbing the nasopharynx of horses with EHV-1 respiratory infections; (iii) blood samples collected in 3% (w/v) sodium citrate from horses thought to be affected with EHV-1 encephalomyelopathy; (iv) skin scrapings from

the edge of exanthematous lesions present on the genitalia or adjacent skin of EHV-3 infected horses; and (v) citrated blood or nasopharygeal swab samples for isolation of EHV-2.

EHV-1 subtype 1 and EHV-2 may be isolated in either rabbit kidney or equine cell lines. Only equine derived cell lines or primary equine cell cultures will be routinely successful for use in the isolation of EHV-1 S-2 or EHV-3 viruses (17,53). Positive identification of primary EHV isolates recovered in cell culture can be made by neutralization (132) or immunofluorescence (126) with reference antiserums or by determination of the viral DNA fingerprint (66,67,68). Pools of subtype-specific monoclonal antibodies have been developed for rapid differentiation of the 2 subtypes of EHV-1 (126). EHV-1 antigen may be detected in impression smears, cryosections, or sections from formalin-fixed tissues of EHV-1 aborted fetuses with the use of specific viral antibodies conjugated with either fluorescein or peroxidase.

Serological evidence of infection by the EHVs may be obtained by demonstration of a significant (i.e. 4-fold or greater) rise in virus specific antibody titer in serum samples collected during the acute and convalescent stages of infection. Neutralization (84), ELISA (59) and complement fixation (160) assays have been developed for such testing.

A diagnosis of EHV-1 abortion by serological testing performed on the aborting mare is not possible because of the often prolonged interval between the initial respiratory infection of the mare and subsequent abortion.

Only the neutralization assay is useful in attempts to serologically differentiate infection by

the two subtypes of EHV-1. If an acute phase serum sample is not available for EHV-1 serology, it is advisable to test the convalescent sample by complement fixation. The existence of an elevated complement fixation titer is suggestive of recent infection by the virus (see Immunology). It should be noted that the interpretation of anti-EHV-1 titers obtained from horses by any type of serological assay may be complicated by the occurrence of recent vaccination of the subjects tested.

IMMUNOLOGY

Infection of horses by EHV-1 results in an immune response demonstrable by development of antibodies (127), cell immune responsiveness to viral antigens (128), and development of resistance to reinfection (18,129,130). Resistance to reinfection of the respiratory tract is of short duration and immunologically experienced horses may be reinfected repeatedly. Such reinfection may be accompanied by cell-associated viremia and result in abortion or neurological disease.(18).

The existence of a state of immune resistance to infection by EHV-1 may be tested virologically by controlled intranasal challenge inoculation of horses followed by attempts to reisolate virus from the upper respiratory tract and blood. Concurrent measurements of antibody responses to such challenge inoculation contribute also to evaluation of the immune status of subject animals (18). The results of such tests illustrate that immunity to reinfection of horses by the same virus which produced a previous infection lasts for as short a period of time as 3 months.

Although attempts have been made to correlate SN antibody titers and immunity to disease (18,131,132), the consequences of reinfection of pregnant mares or of other horses which have had multiple infection experiences remain largely unpredictable. The interpretation of the significance of serum neutralizing antibody titers in mature horses used as experimental subjects has been somewhat confounded by the inability of the testing methods that were available to discriminate between immune responses resulting from infection by the individual subtype viruses.

The primary virus neutralizing antibody (SN) response to infection acquired by intranasal administration of virus is detectable eight to nine days later. Anamnestic responses are detectable in five days and their kinetics are typical (127) The kinetics of the antibody response as measured by complement fixation (CF) and enzyme linked immunosorbent assay (ELISA) (59) techniques are similar. Complement fixation antibody titers reach their height about three weeks after infection and may no longer be detectable after 60 days. The SN antibody responses are more persistent; most last for more than a year (131). It is highly unusual to find any horse more than a year old in a population in which either of the EHV-1 viral subtypes are endemic to be negative to the SN test (127)

In addition to the development of specificially reactive immunoglobulins which are detectable in both serum and nasal secretions (127,133,135); the occurrence of cell immune responses to EHV-1 infection can be demonstrated by viral antigen induced

transformation of peripheral blood lymphocytes (PBL) (128,135), development of delayed hypersensitivity (134), and immune lymphocyte cytotoxicity for virus infected cells (136). Additionally, evidence has been presented to illustrate that mononuclear phagocytes from horses susceptible to infection become activated as a result of infection and that cultures of macrophages prepared from the same horses during convalescence become resistant to infection (138). Unfortunately, except for results available from virological and serological monitoring of challenge inoculation, the presently available bits and pieces of information descriptive of increments of the immune response to EHV-1 do not provide information sufficient to construct a reasonable definition of an immune horse.

It seems clear that the immunological responses which are critical to the development of serviceable immunity to infection or disease are conditioned specifically by the antigenicity of the envelope glycoproteins of the virus (137). On a basis of results of immunoblotting assays using both convalescent serum from a horse infected with the same virus from which the proteins were prepared as well as polyclonal antiserum produced in rabbits, four of the six major envelope glycoprotein antigens of the EHV-1 viruses have been shown to be held by the subtypes in common and two shown to be subtype specific (59). The results of experiments conducted by immunization of hamsters with both subtype viruses and challenge with hamster adapted S-1, as well as the results of cross-protection tests in horses infected by one subtype and later challenged with the second establish that the serologically detectable antigenic

relationships between the subtypes are immunologically significant (53).

Little is known about the immune response of horses to EHV-2 other than that they develop SN antibodies as a result of their infection by antigenically diverse strains of this virus and that apparently persistent infection without recognized signs of disease exists in a very high percentage of horses (8). The prophylactic use of hyperimmune serum in an effort to prevent respiratory disease of foals apparently associated with their infection by a strain of EHV-1 has been explored and reported in one case to have been successful (139).

The kinetics of the antibody response to infection of horses with EHV-3 as measured by CF and SN tests appear similar to those provoked by EHV-1 infection (17).

EPIZOOTIOLOGY

EHV-1: The three equine herpesviruses have been described from most countries in which horses are economically important enough for the application of modern laboratory diagnostic methods to their diseases. The viruses are apparently worldwide in distribution and infect both domestic and feral species of Equidae kept in zoological parks. Until the discovery of the existence of EHV-1 as two subtypes and the association of subtype genomic and antigenic differences with characteristics of virulence, it was assumed, on the basis of subtype non-specific serological tests, that populations of horses in all countries were infected with the same virus. The first indication of the existence of antigenic differences among isolates of EHV-1 obtained from aborted fetuses

in different countries came from Japan in 1959 (140). The results of a worldwide serological survey using one of the Japanese strains of virus, now known to be S-2, and an S-1 strain isolated in Kentucky suggested that horses in some countries were infected predominately by one viral subtype (141). The occurrence of epizootic abortigenic disease in Japan in 1969 (142) and Australia in 1977 (143) appears to have been a consequence of introduction of the more virulent S-1 virus into populations in which the S-2 virus, which is an infrequent cause of abortigenic disease, was the sole or predominant EHV-1 subtype.

Under natural conditions the epizootiology of EHV-1 disease is influenced predominantly by the frequency of occurrence of latent infections, the occurrence of those environmental circumstances (shipment, crowding, weaning, other disease etc.) which produce stress and thereby influence activation of latent herpesviral infections, and the immunological status of the population at risk to infection.

The introduction of either subtype virus into a population of immunologically naive foals produces epizootic infections which become clinically evident as respiratory disease. Although S-1 viruses produce more severe disease than S-2, the latter appears to spread as efficiently as the more virulent subtype.

The occurrence of epizootic respiratory disease caused by S-1 virus among weanling foals amplifies the amount of virus in the environment and, if an infected group of foals is in contact with pregnant mares, increases the risk of abortigenic infection (144). Although such an epizootiological scenario is obviously one which produces great risk to pregnant

broodmares, more recently acquired data (53) suggests that most outbreaks of EHV-1 infection among foals, as evident in the central Kentucky area, are caused by the less likely to be abortigenic S-2 virus. The reciprocal immunogenicity of the subtype viruses may, if frequent exposure of pregnant mares to S-2 virus occurs, positively influence herd immunity to the S-1 subtype and therefore modulate favorably the epizootiology of the more economically important forms of disease caused by EHV-1 (53).

Although immunity to infection by either subtype of EHV-1 may result from repeated infection by the other (53), it appears that the influence of naturally acquired immunity in a given population depends, so far as prevention of abortigenic or encephalomyelitic disease is concerned, upon the frequency of exposure of candidate animals to either of the subtype viruses and the time which has elapsed since their last exposure. Accordingly, horses which have had repeated infection experiences with EHV-1 viruses, as their last experience becomes more remote become at increasing risk to abortion or neurological disease when exposed to S-1 virus.

Although the origin of virus which produces individual cases or epizootic abortigenic or neurological disease is almost never established, circumstantial evidence suggests that it is frequently by introduction of new members to a herd from sales yards, racing stables, other farms or even another group of animals kept on the same farm. Any management situation which results in crowding or which necessitates reestablishment of the "pecking order" among a group of horses appears capable of producing a degree of stress which results in the activation of

herpesviral disease in an individual animal and its subsequent spread to cohorts (145). The frequency of occurrence of respiratory disease caused by EHV-1 S-2 virus among groups of foals at the time they are weaned, which coincides closely with their loss of maternally acquired immunity, suggests that latent infections by this virus are established in foals early in life. Subtype 1 viruses are the principle cause of abortigenic infection and apparently the sole cause of neurological disease. Genetic variability among isolates of the S-1 virus as determined by restriction endonuclease electrophoretic analysis is relatively slight, while that of S-2 is extensive (66). Of 282 isolates of S-1 virus obtained from aborted fetuses during a 24 year period in our laboratory, more than 90% were found to represent two DNA electropherotypes. One, labeled 1P, was found responsible for 86% of the total number of abortions during a 20 year period. The second, designated 1B, was not detected among the viruses isolated prior to 1972 but was found to have caused, during 1980 and the three subsequent years, 62% of abortion epizootics and more than 50% of all abortigenic infections (104). The influence of intrasubtypic genetic variation detected by this method upon the epizootiology of EHV-1 disease has not been elucidated but, as has been pointed out by Allen et al. (66), if the concept outlined by Buchman et al. for Herpes simplex virus (HSV) (146), i.e. that the coexistence of a large number of genetically diverse herpesvirus strains depends upon latency is correct; then the greater genetic heterogeneity and absence of a dominant strain among field isolates of EHV-1 S-2 would indicate that these viruses would be more likely to establish latent

infections than would the more genetically stable S-1 genotype. It follows that the S-2 genotype would be the virus more likely to be first encountered by foals and the virus to which horses of all ages would be exposed and re-exposed most frequently. The resolution of the questions of whether and how the intrasubtypic genetic differences as well as the antigenic differences detectable by monoclonal antibodies (126) affect epizootiological patterns of infection and disease caused by these viruses must however await the results of further studies.

EHV-2: Both serological (147,148) and virological evidence (8,149) suggests that persistent infection by EHV-2 viruses among horses without definable disease is extremely common. The incidence of such infection as defined by virological studies in a sample of normal horses in the United States was found to be 88.7% (149); while that in a group of 19 horses admitted for a variety of surgical and orthopedic conditions to a veterinary hospital in England was 89% (27).

The EHV-2 virus has not been isolated from either normal equine fetuses or from fetuses aborted as a result of other causes. Unlike EHV-1, although EHV-2 is cultivable from viremic horses only by co-cultivation of leukocytes with susceptible cells, it apparently does not cross the placenta. Transplacental inoculation of a pony fetus has been shown to result in persistent productive viral infection without apparent pathological effect (26). The virus is commonly present on the mucosa of the nasopharynx and has been isolated as well from the conjunctivae and the vaginal mucosa of normal horses. Newborn foals have not been found to be infected but

foals have been shown to become infected when less than 30 days of age during which period they maintain high titers of antibodies acquired from the colostrum. The virus appears to be maintained by a very high incidence of activated latent or persistent infections among horses and infection to be acquired via the respiratory tract by foals at an early age (24,102,150).

EHV-3: Equine herpesvirus 3 infection has been shown experimentally to be transmittable via the respiratory route. Progenital disease caused by this virus has also been observed to occur in purportedly maiden colts and fillies which suggests that such disease may be a consequence of infection via means other than coitus. However, transmissions of the virus which result in progenital exanthematous disease most commonly result from coitus with a partner bearing recognizable lesions (17).

VACCINES AND VACCINATION

The vaccines currently available for use as aids in the prevention of herpesviral diseases of the horse are designed to provide immunity to EHV-1 S-1. The strategies for employment of the vaccines are based on the concept that naturally acquired immunity to respiratory disease caused by EHV-1 is a product of the immunogenic conditioning of repeated infection experience (130); that immunity to reinfection is short lived (18), and that protection against abortigenic infection is required from the onset of the sixth month of gestation until term (144). Two vaccines are licensed and are in widespread use in the United States; one is a live virus product attenuated empirically by multiple passages in swine cell

cultures followed by passage in equine cells (151). It was originally recommended as a preventive for abortigenic infection by EHV-1 as well as respiratory disease in performance horses. Apparently because of repeated failure of this vaccine to satisfy the claims made for its efficacy for prevention of abortigenic disease (152,153), its label claims are now limited to use for respiratory disease. The vaccine is, nevertheless, still employed in brood mares by some veterinarians. The second vaccine licensed for use in the United States is a formalin inactivated, adjuvanted formulation containing, like the attenuated live vaccine, a strain of EHV-1 S-1 virus (154,155). This vaccine was subjected to testing for efficacy by controlled vaccination and challenge of pregnant broodmares twice (154) prior to licensing and general distribution. Its performance in field use was closely monitored for a period of four years prior and subsequent to its licensing during which it was employed in approximately 65 percent of the pregnant broodmare population of central Kentucky. The vaccine was injected during the fifth, seventh and ninth months of pregnancy in all pregnant brood mares on each farm. Recommendations for the use of that protocol were based on responses of pregnant mares immunologically primed, as most mares of breeding age are, by previous naturally acquired infections to injection of the vaccine as that could be estimated in-vitro by measurement of both humoral and cell-mediated immune responses (128,156). The mean incidence of EHV-1 abortigenic infection in central Kentucky during a six year period for which records are complete (1980-1985) since the inactivated vaccine was made available to veterinarians declined

from 7.4 /1000 pregnant Thoroughbred mares (range 4.0 - 17.0/1000) to 2.3/1000 (range 1.0 - 3.1/1000). This decrease in incidence of the disease occurred during a period when the pregnant broodmare population at apparent risk increased more than 40%. Although the overall incidence of the disease decreased, both single and multiple abortions continued to occur in herds vaccinated with either the inactivated or the modified live virus vaccines during this period. Because of this some practicing veterinarians devised their own particular protocols for vaccination of pregnant mares. Among the vaccination schemes employed have been: administration of both vaccines at various intervals, use of the inactivated vaccine at intervals of two months throughout the year, and injection of pregnant mares as well as all other horses on individual farms with the modified live vaccine every month. Although the incidence of the disease remains depressed, this chaotic application of vaccines renders extremely difficult any attempt to continue to monitor the efficacy of vaccines except in individual herds for which reliable vaccination records are available.

Neither the live nor inactivated vaccines pose appreciable danger of untoward reaction if properly employed. The virus used in the live vaccine, which is genotypically unique, was isolated from three aborted fetuses on one farm during the first year of its use but has not been detected to be a cause of abortigenic infection in any other herd in the same area since (53). The inactivated vaccine may produce both immediate and delayed hypersensitivity reactions if horses other than immunologically naive foals are

injected at less than the 60 day intervals recommended (8). Systemic hypersensitivity reactions resulting from the injection of either vaccine have not been observed when recommended vaccination procedures were followed.

No vaccines specifically designed to protect horses against infection or reinfection by S-2 virus are available. Both the vaccines in use are recommended for prevention of EHV-1 respiratory disease on the basis that the S-1 and S-2 viruses are reciprocally immunogenic as a result of repeated infection experience by either virus (53). A combined EHV-1 S-1 and bivalent (H3,H7) equine influenza virus vaccine has recently been introduced in the United States and a multivalent vaccine employing inactivated EHV-1 S-1, H3 and H7 influenza viruses and three subtypes of Reovirus is manufactured in Germany and recommended for prevention of equine viral respiratory diseases (157). No data allowing an objective evaluation of the efficacy of these latter vaccines has yet been published.

No vaccines intended for immunization of horses against EHV-2 and -3 have to our knowledge been manufactured. One, (139) reportedly successful, attempt to provide passive immunity by administration of antiserum to foals threatened by EHV-2 infection has been made.

CONTROL

The results of investigations of circumstances under which multiple abortigenic or neurologic disease caused by EHV-1 S-1 virus have occurred indicate that management practices may substantially influence the likelihood of occurrence of such disease (145). Proper

management practices including provision of adequate space for individual animals in both housing and pasture, application of quarantine measures prior to the addition of new members to herds, avoidance of stress by the use of gentle methods for weaning and provision of well ventilated, clean quarters, whether or not vaccination is practiced, need to be given careful attention if one is to avoid the economic penalties of diseases caused by EHV-1.

Abortigenic disease among Thoroughbred and other breeds subjected to a controlled breeding season occurs very commonly when pregnant mares purchased at public sales late in the year are added to herds. Aborted fetuses infected by EHV-1 are the richest possible source of virulent virus for other mares. If a mare aborts such a fetus in a paddock or field, all of the mares in the group usually become exposed to virus in a short period of time. Pregnant mares purchased at salesor brought from another farm should not share paddocks with the longer term resident pregnant mares. If the premises afford an adequate facility such newly purchased mares should be kept from contact with resident pregnant mares until after they foal. If the facility is not adequate for long term quarantine, newly introduced mares should be kept away for no less a period than three weeks which is about twice as long as immunologically experienced mares susceptible to infection shed virus from the nasopharynx after deliberate intranasal inoculation (18). If a single foaling barn unit is used to foal all mares on a farm, pregnant mares should be sorted into foaling groups as soon as possible after they are confirmed to be in-foal. If this procedure is not followed, the necessary reorganization of the mares

into such groups late in the season presents the same hazards as the introduction of strange mares from outside the farm.

The reproductive tract of mares which abort EHV-1 infected fetuses is free of the virus within a matter of a day after abortion occurs. They may be moved and added to barren mare groups whenever convenient and unless complications such as bacterial infection or injury resulting from dystocia occur, may be bred as normal mares. Unless submitted to a diagnostic laboratory, infected fetuses along with the placenta, bedding and feed should be disposed of by burning.

Vaccination of pregnant mares using the inactivated viral vaccine should be practiced as an aid in control of the abortigenic disease. Mares should be vaccinated either individually during the fifth, seventh and ninth months of gestation or herd vaccination should be practiced every two months beginning when the first mare in the group reaches the fifth month of pregnancy.

There is little basis for advice on the routine application of presently available vaccines for prevention of neurological disease. When such disease is recognized in a stable or herd of unvaccinated horses, it may be recommended that all unaffected mature animals in the group be given a single injection of the inactivated vaccine and that younger animals be given two injections of the vaccine, the second being given six weeks after the first.

It has been determined serologically (53,144) that about 85% of weanling foals in central Kentucky contract respiratory infection by EHV-1 during their first year of life. It is now known that most of these infections are caused by S-2 virus for which no

specific vaccine is available. Vaccination of foals with the inactivated vaccine which contains related S-1 virus requires a series of at least three injections. The first two injections should be administered at about the time of weaning, the third about a month prior to the sale of yearlings or the beginning of their preparation for training. Because of a dearth of data descriptive of the role of EHV-1 viruses in the etiology of respiratory disease of racehorses and of data allowing evaluation of the efficacy of various vaccines for prevention of such disease, it is not possible to recommend a protocol for vaccination of racing or other performance animals. Considering however that such animals may become at-risk to neurological disease and that females may enter breeding herds later in life, periodic single injections of EHV-1 vaccine may be found advisable by veterinarians.

Although there is evidence of widespread latent and persistent infection of horses by EHV-2 the incompleteness of our knowledge of its clinical importance, immunology or epizootiology provides no basis for promulgation of methods for control.

Control of the spread of disease caused by EHV-3 may be obtained by prevention of sexual contact. There is no specific treatment or method for prophylaxis for this disease available.

FUTURE ASPECTS

While no basis exists for estimation of the overall economic loss caused by herpesviral infections of the horse, losses produced by epizootic abortigenic or neurologic disease are well established to be economically costly to individual breeders worldwide.

There is an obvious need for improvement of presently available vaccinal approaches to control of these diseases. The development of such improved methods for control requires a much better understanding of both the virus and the reaction of the host to infection. Our knowledge of the immunology of infection of horses by EHV-1 is descriptive predominately of the clinically qualitative aspects of the immune responses of horses to infection by the virus. The existence of immunity protective against infection by the virus has been repeatedly demonstrated by challenge by the natural route of infection with virulent virus but little analytically useful information to afford an opportunity to dissect the immune response is available. Necessary advances in our understanding of the functional anatomy of the genome of EHV-1 are beginning to be made (124, 125). These may be expected to provide clues about the role played by various viral proteins in pathogenesis, virulence and the immune response to infection. Development of an understanding of the function of individual viral proteins should allow exploitation of purified immunogenic viral proteins as vaccines while eliminating others that may have undesirable effects upon the development of immunity.

Among the several important questions that remain to be answered before the epizootiology of infection by any of the equine herpesviruses can be more productively understood is that of how and where latent infection becomes established and of such factors as the possible effect of latent infection by one herpesvirus upon the ability of another to establish itself in a like situation (158). In regard to development of better methods for control of the

abortigenic and neurological diseases caused by EHV-1 S-1 it would be most interesting to learn what the relative distribution of the carrier state by each of the subtype viruses is in various populations and to explore the effect that a predominance of one or the other type may have on the incidence of disease. The development of subgenomic DNA probes specific for the viruses and their application in routinely applicable hybridization techniques can be expected to contribute much information critical to the solution of such problems.

Among the potentially more critical questions that need to be asked about pathogenesis are those which deal with the nature of the relationships between EHV-1 and -2 and leukocytes and the mechanisms by which the virus is transported to or prevented from reaching the susceptible fetus.

The Australian experience (159) illustrates, as was suggested by earlier serological studies (141) that the epizootiological pattern of disease caused by EHV-1 may be influenced by the occurrence in a given population of only the S-2 virus and that such populations may be at particular risk to the more serious forms of disease produced by the S-1 variety. The application of contemporary methods for the identification of the EHV-1 subtypes is capable of producing important information relative to prognostication of the seriousness of a potential epizootic or to control of the introduction of virulent viruses into susceptible populations.

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7

AUJESZKY'S DISEASE (PSEUDORABIES) IN PIGS

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INTRODUCTION

Aujeszky's disease (AD) is a contagious, epidemic disease which is characterized by enzephalomyelitis, frequently accompanied by inflammation of the upper respiratory tract and the lungs. The causative agent of the disease is a herpesvirus.

AD affects a large number of mammalian species. The animals usually die after infection, with the only exception of adult pigs which survive. Therefore, the pig is of particular significance for maintaining the chain of infection.

The history of AD began in 1813 when a disease in cattle was described in the USA, characterized by heavy itching. Therefore, the disease was called "mad itch". In 1849 a similar disease occurred in Switzerland and was mistaken for rabies because of the similarity of the symptoms in cattle and dogs. In 1902 the Hungarian veterinary surgeon Aujeszky (1) succeeded in distinguishing the disease from rabies by its behaviour in experimentally infected rabbits. He demonstrated that the disease is not caused by bacteria and called it "pseudorabies". In 1910 Schmiedhofer (2) confirmed the viral nature of the agent by filtration experiments. Shope (3) reported in 1931 that mad itch and pseudorabies are caused by the same virus. In the USA, the disease has been termed "pseudorabies", whereas in Europe it was called "Aujeszky's disease" instead of "mad itch" or "infectious bulbar paralysis", respectively. In 1934 Sabin (4) and Sabin and Wright (5) reported that AD virus (ADV) was immunologically

related to herpes simplex virus and herpes B virus. The virus was classified into the herpesvirus group.

From 1902 to 1930, only single outbreaks of AD were reported predominantly in cattle and dogs in Hungary, Rumania, France, Russia, Brazil and the USA. The origin of infection was unknown. AD in pigs was first described in 1920, and the first enzootic in pigs appeared in The Netherlands in 1931. In the following years, sporadic outbreaks occurred in several European countries, North Africa, Turkey and the USA (for review see 6).

This epidemiological pattern changed in the fifties and sixties. AD became enzootic now in the big piggeries and fattening farms in Eastern and South-eastern Europe and in the Middle West of the USA. In parallel to intensive pig-keeping, AD has spread further to certain regions in Central and Western Europe and in South-east Asia. Thus, AD has become a worldwide disease of great economic importance. It causes heavy economic loss and mostly withstands the efforts of the veterinary services to combat it.

CLINICAL SYMPTOMS

The clinical picture of AD in pigs considerably varies according to the age of the animal. The younger the animals, the more serious the symptoms and the higher the mortality. The incubation period ranges from 1 to 11 days, mostly being 3 to 6 days. The mortality rate is up to 100% in piglets less than 2 wk of age, about 50% in 3-wk-old piglets and decreases to less than 5% in mature pigs. However, not only age but also other factors influence the course of the disease, e.g. amount and virulence of the virus, individual condition of the animal and stress situations (7). Accordingly, mortality rates can augment at any age.

In piglets less than 3 wk old, sudden death can occur with few if any clinical signs, especially in baby pigs. But more often death is preceded by fever, lethargy, loss of appetite, weakness, lack of coordination and convulsions. Vomiting and diarrhea can be present. Pigs less than 2 wk old usually die. Intrauterinely infected suckling piglets die within 2 days after birth,

occasionally showing violent shaking and shivering (shaker pig syndrome). Piglets infected immediately after birth show clinical signs within the first 2 days and usually die before they are 5 days old.

In older pigs, the symptoms start with fever followed by loss of appetite, listlessness, loss of voice, somnolence, vomiting, tremor and, in some animals, with lack of coordination and weakness of the hindquarters. Involvement of the respiratory tract is indicated by dyspnoea, sneezing, coughing and nasal discharge. Death is usually preceded by convulsions. Recovered pigs show significant loss in weight.

The intensity of the clinical signs decreases with rising age. Therefore, the disease is usually not severe in adult pigs. Fever is always present, and nasal discharge, coughing, loss of voice and somnolence frequently occur, but typical nervous symptoms are only observed occasionally. Usually, no marked pruritus develops in pigs of every age, but aggressiveness may occur.

In addition to other symptoms, infection of boars results in scrotal swelling due to subcutaneous oedema, in testicular degeneration and in poor semen quality about 10-14 days post infection for a period of 1-2 wk (8, 9, 10).

ADV infection of sows in the early stage of pregnancy is followed by death and resorption of their fetuses. Infection in mid pregnancy causes abortion of mumified fetuses, and in the late stage of pregnancy abortion, stillbirth or birth of weak piglets, which die within a few days, occur.

PATHOLOGY

No macroscopic lesions typical for AD are found in pigs that succumbed to the infection. The following pathological alterations can be observed: oedema and haemorrhages in the retropharyngeal and mandibular lymph nodes and in the lungs, interstitial pneumonia which is caused by secondary infections with bacteria (11), degenerate foci in the myocardium, pleuritis and peritonitis with exsudate, haemorrhages under the endocardium, severe

tonsillitis with ulcera and diphtheroid layers which spreads to the epiglottis, congestion in different organs, especially of the brain and the spinal cord, and necrotic foci in the spleen, the liver and the suprarenal gland. Frequently, a hypoplasia of the thymus occurs (12).

Histological changes indicating AD in pigs are only found in the CNS, mainly restricted to the brain, where a non-suppurative meningoencephalitis with relatively mild myelitis develops (13). The predominant sites are the cerebral and cerabellar cortices, whereas lesions in the brainstem are less often noted. The lesions are characterized by diffuse and focal microglial infiltrations occasionally combined with necrosis of neurons and perivascular and meningeal infiltrations by lymphocytes, neutrophilic granulocytes and macrophages. Sometimes, intranuclear inclusions of type A are found. Corresponding lesions in the spinal cord are frequently mild, and they decrease from the cranial to the caudal part.

PATHOGENESIS

Route of infection

Pigs are mainly infected by aspirating virus aerosols or by sniffing sick animals. Oral infection takes place by consuming virus-contaminated food or milk. The virus can be transmitted by mating or by artificial insemination or intrauterinely to the fetus or embryo.

Susceptibility to infection is dependent on several factors (14, 15, 16, 17, 18): degree of virulence of the virus strain, amount of infectious virus, route of infection, animal species, age of the animal, individual conditions of the animals and stress situations. For example, for oral infection larger quantities of virus are necessary than for nasal infection, and piglets need less virus than adult pigs. For intranasal infection, piglets require between 10^1 and 10^3 $TCID_{50}$, young pigs about 10^4 $TCID_{50}$ and grown-up pigs about 10^4 to 10^5 $TCID_{50}$ (14, 16, 20). For infection of vaccinated pigs, 100-to-1000-fold higher virus doses are necessary than for unvaccinated pigs (19). Thus, ADV is not

very contagious, and the percentage of animals infected in a herd fluctuates between 50% and 100%.

Virus multiplication

Primary virus multiplication in pigs takes place in the nasopharyngeal region and in the respiratory tract. From here the virus invades the CNS by the neural pathway (17, 18, 19, 21, 22, 23, 24). It can be assumed that the virus also spreads centrifugally from the CNS via the nerves to other parts of the body. Besides, the virus is apparently disseminated throughout the body to certain organs and tissues by peripheral blood lymphocytes (PBL). Multiplication of ADV occurs in bone marrow cells, thymus cells and PBL (12, 15, 23, 24, 25, 26). The susceptibility to ADV of various PBL populations was high in T lymphoblasts and adherent monocytes, medium in resting lymphocytes and low in B lymphocytes and granulocytes (27). This suggests a possible mechanism by which ADV could have an immunosuppressive effect as well as a pathway of dissemination of ADV. The frequency of ADV-replicating cells in the hemopoietic system decreased with increasing cell differentiation in parallel to the age of the animals, and it is tempting to speculate that this phenomenon is connected with age resistance of pigs against ADV infection (12).

The intensity of virus multiplication varies in different parts of the body. The largest amounts of virus are detected at the sites of primary virus multiplication, especially in the tonsils and pharyngeal lymph nodes. Smaller amounts of virus are present in the lungs, and fairly small amounts of virus are found in the CNS and in other organs. High virus multiplication in organs is correlated with long virus isolation periods. Virus persists in pharyngeal lymph nodes and in tonsils for up to 35 days, in the lungs for up to 14 days, in the CNS for up to 10 days and in other organs for up to 7 days (23).

Virus excretion

Nasal viral excretion occurs for 8 to 17 days with maximum titres of between $10^{5.8}$ and $10^{8.3}$ $TCID_{50}$ per swab (22, 28, 29, Wittmann, unpubl.). From oropharyngeal swabs, it can be isolated for 18 to 25 days with titres up to 10^{6} $TCID_{50}$ (30). Virus is

found in vaginal secretions and foreskin secretions (ejaculate) for up to 12 days (15, 20, 31, 32). The virus multiplies in the serosa, plexus pampiniformis, ductus deferens and tunica vaginalis of the testicles and is also isolated from the scrotal fluid (33). Virus is excreted in the milk for 2 to 3 days (34) and occasionally in the urine, but has not been isolated from the faeces (20, 34), though it was found in rectal swabs up to 10 days (15). Virus excretion always starts before the onset of clinical symptoms.

LATENT INFECTION

ADV can persist in infected pigs recovered from the disease in a latent state (18, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44) independent on the degree of immunity of the animal. ADV infection of pigs with colostral antibodies (45) or of pigs vaccinated with inactivated or live vaccines can also result in latent infection, however, latency is frequently reduced compared to non-vaccinated animals (42, 45, 46, 47, 48, 49). Reactivation of the latent virus is followed by virus excretion to a reduced rate.

Latent infection can be present in cells of the tonsils, thymus, lymph nodes, lungs, trigeminal ganglion, brain, spinal cord, internal ear and in cells of the hemopoietic system (macrophages, lymphocytes) predominantly in the bone marrow (40, 41, 50; Ohlinger et al., unpubl.).

To detect latent virus in tissues or cells, special biological techniques are required. The most sensitive method to recover ADV is the enzymatic or physical dispersion of tissues followed by cultivation of the tissue fragments or by co-cultivation of fragments or cells on indicator cell monolayer for prolonged time (18, 37, 41, 42). In contrast to HSV, it is generally more difficult to rescue latent ADV. The reactivation rate from neural tiusses can vary between 50% to 75% (43, 51, 52). For such variation genotypic differences between the virus strains used for infection might be responsible (53). Additionally, successful virus recovery decreases in later times after infection (42). By the described isolation methods virus was detected in

non-immunosuppressed vaccinated animals up to 6.5 mo and in immunosuppressed vaccinated pigs up to 18 mo. Virus reactivation failed at 22.5 mo. In non-vaccinated pigs virus reactivation succeeded at least up to 16 mo, but latency may last even longer (42).

As shown later, molecular DNA hybridization techniques are more sensitive than cell culture methods to detect latency (see paragraph "Molecular aspects of latency").

Certain stimuli can reactivate latent virus, resulting in virus excretion, a fact of great epizootic importance. Experimental treatment of the animals with immunosuppressants induces reactivation from latency (e.g. injection of 1250 mg or 1875 mg of prednisolon on 4 consecutive days in adult pigs) (42, 45) or dexamethasone (54). However, this treatment does not simulate natural conditions, since the immune mechanisms are drastically impaired, as will be described later. Better simulation of the natural stress is accomplished by intravaginal application of prostaglandine E2 (3 mg in pills, every 24 hr, for 4 days; Ohlinger, unpubl.). Virus reactivation can also be evoked by fluctuations in environmental temperature (from $18^{o}C/19^{o}C$ to $22^{o}C/23^{o}C$ and vice versa) for several days (Ohlinger, unpubl.).

Virus can be excreted after reactivation but clinical symptoms are rarely observed, however, pathological alterations in brain and lymph nodes may occur (55). Virus can be isolated from nasal swabs with titres ranging between null and $10^{3.5}$ $TCID_{50}$, and the maximal excretion time lasted from day 4 to day 19 after immunosuppression (42). The amount of excreted virus is reduced in comparison to non-vaccinated pigs (42, 45, 49) but can be sufficient to infect non-immune and a part of the vaccinated sentinel pigs (42; Ohlinger et al., unpubl.). The failure of virus isolation from nasal swabs does not reflect the natural conditions, as sentinel vaccinated and unvaccinated pigs were infected by contact with immunosuppressed latently infected pigs although no virus was isolated from the nasal swabs of the latter (Ohlinger et al., unpubl.). This signifies that virus excretion may be intermittent and that excreted virus gradually accumulates

to infectious levels in the environment. Virus reactivation is usually accompanied by an increase of antibody titres (42, 45, 49, 54).

Since most of the data on reactivation have been obtained from experimentally infected pigs, the question of the real frequency and significance of ADV reactivated from naturally infected animals in the field remains open. Natural reactivation has been seldomly reported. Only one case is described concerning a farrowing sow (38), and there are hints that reactivation occurs after transport of pigs (56) and under extreme climate conditions (57).

CHARACTERISTICS OF THE VIRUS

Taxonomic status

The AD virus belongs to the herpesviridae family, genus/subfamily alphaherpesviruses. Only a single serological type is known (58).

Morphology

ADV is cubically shaped with an overall diameter of 150-180 nm. The inner part of the virus is formed by the core, 75 nm in diameter, containing the linear double-stranded DNA and bound protein (59, 60). The core is surrounded by a protein shell, the capsid, with a diameter of 105 nm. It is composed of 162 protein subunits (capsomeres) arranged in icosapentahedron symmetry, which are 12-13 nm in length, 9-10 nm in width and which possess a central hole of 4 nm. Core and capsid form the nucleocapsid, which is coated by an irregularly shaped envelope, predominantly containing glycoproteins and lipoproteins. The envelope is essential for virus adsorption to the host cell and responsible for the immunogenicity of the virus.

Antigenic relationships

Antigenic relationship exists between ADV and other herpesviruses (61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71). The one-way relationship between ADV and IBR virus in the neutralization test is of practical interest, since ADV is neutralized by IBR antibodies but usually not vice versa (61, 69,

72). That means that ADV neutralization by cattle serum can be caused by IBR antibodies. On the other hand, it is rather unlikely that positive ADV neutralization in pig serum is established by antibodies against IBR virus or herpes simplex virus (73, 74, 75).

Physicochemical properties

ADV has a density of 1.278 in CsCl (76).

The virus is inactivated at 60°C within 30-60 min, at 70°C within 10-15 min, at 80°C within 3 min and at 100°C within 1 min (77). It stays alive at 25°C about 6 wk, at 15°C about 9 wk, at 4°C about 20 wk and at -40°C for years. However, the virus is relatively unstable at -18°C to -25°C, where inactivation occurs within 12 wk (78, 79).

At pH values between 5.0 and 12.0 the virus is stable, and even at pH values of 2.0 and 13.5 it takes 2 to 4 hr until the virus is completely inactivated (80). The inactivation time is significantly reduced by combining low or high pH levels with elevated temperatures (78).

ADV is sensitive to chloroform (81) and ether (76). Some virus strains show trypsin sensitivity (82). Several chemicals induce inactivation of the virus (83, 84): phenolics (orthophenyl phenol compounds) completely destroy the virus at room temperature within 5 min. Under the same conditions, inactivation of 90% of the virus is achieved by 70% ethanol, iodines, quaternary ammonium compounds, chlorhexidine diacetate and 5% sodium hydroxide, but 1% NaOH does not inactivate the virus within 6 hr (84). 70% of the virus is inactivated by 2% glutaraldehyde and 5.25% sodium hypochlorite within 5 min. Treatment with 3% or 1% chloramine inactivates ADV within 10 min or 30 min, respectively; 4% formaldehyde reduces virus infectivity by 60% within 5 min, but inactivation is complete after 3 hr. Binary ethyleneimine (0.001 mol) inactivates the virus at 37°C within 6 hr. It is frequently used as inactivant for vaccine production (85). Butylated hydroxytoluene (0.35 mM) inactivates the virus at 37°C in 1 hr (86). Detergents like Nonidet P-40 (0.5%, 4°C, 60 min) and Triton X-100 (0.5%, 45°C, 10 min, pH 8.5) destroy purified concentrated virus.

Gamma irradiation results in inactivation of the virus (87, 88). Direct UV radiation (15 W, 30-inch distance) reduces the infectivity of ADV by 4 log10 at pH 8.6 in 20 min and at pH 5.3 within 40 to 60 min, respectively. At pH 7.0 the virus shows intermediate sensitivity (78). The indirect atmospheric effect evoked by the UV light did not affect the virus at all.

The stability of ADV during freeze-drying and subsequent storage depends on the media used. Media containing 5% glutamate with 5% sucrose or culture media with 2% serum yield the highest degree of protection (89).

Disinfection

For disinfection of ADV-contaminated premises, the virus-inactivating chemicals cited above can be used. In practice, the mainly used disinfectants contain phenolics or formaldehyde, but calcium hydroxide or thick milk of lime are also applied. The efficiency of disinfection depends as well on the resistance of the virus to the chemicals and to the environmental conditions such as temperature and protective organic material. Besides one has to consider the corrosive effect of certain compounds on metal and clothing. The efficiency of compounds splitting off chlorine is strongly reduced in the presence of organic matter. Therefore, a careful cleaning of the objects' surface must precede disinfection. The same applies when using formaldehyde (90), which has a low penetrating power. Disinfection of large volumes of slurry is a well-known problem: thick milk of lime (40 kg/m^3), freshly hydrated lime (calcium hydroxide, 20 kg/m^3), formalin containing at least 35% formaldehyde (6 kg/m^3), sodium hydroxide (8 kg/m^3) and peracetic acid (40 kg/m^3) are recommended (91). The use of the latter is limited since strong foam formation occurs in the mixture. All these compounds inactivate $10^{6.0}$ $TCID_{50}$/ml of ADV in slurry at temperatures of 4°C and 23°C within 4 days. Chlorinated lime (calcium hypochlorite, 15 kg/m^3) is not suitable.

Resistance to environmental conditions

Resistance of ADV is very high under complex natural environmental conditions, because adverse and favourable factors for virus survival are involved. Therefore, the given data must be

only taken as clues to the result.

The virus is not killed during the maturation of pig meat at 4°C (92). It is, however, inactivated in the meat at -18°C within 35 to 40 days (15, 93) and also after heat treatment at temperatures of at least 80°C in every part of the meat, sausages or boiled ham (94). In urine, the virus survives for 3 wk in summer and for 8-15 wk in winter (95), in slurry for about 1 mo in summer and for 2 mo in winter (34, 96), but a much shorter inactivation time of 4 days is reported by Šmid et al. (97) at 15°C and pH 6.5. On the other hand, virus was found by Strauch (pers. comm.) in slurry experimentally contaminated with $10^{6.5}$ $TCID_{50}$/ml at 4°C up to 27 wk and at 23°C up to 15 wk, respectively. Regarding these results, it must be considered that the virus content of slurry under field conditions is much lower than that used in experiments. In biothermically treated slurry, the virus is inactivated within 5 days in summer and within 12 days in winter (98) and in aerated slurry (pH 9.6, temperature up to 44°C) within 8 to 21 days (99). The survival time of the virus in cleaning water at 9°C is 20 to 50 days (100). In soil the virus is found for 5 to 6 wk (34). In hay and straw the virus can survive 15 days in summer and 40 days in winter, whereas virus dried on sacks and wood survives about 10 days in summer and 15 days in winter (101). No data are available on untreated waste food. In waste food, fermented by Lactobacillus acidophilus, the virus was inactivated at 20°C and 30°C within 24 hr, but it survived for at least 48 hr at 10°C and for 96 hr at 5°C (89). Heating of waste food to 70°C or to 80°C inactivated the virus within 10 min or 5 min, respectively (103).

Biological properties

Host range: ADV has a very broad host range. Natural infection of domestic animal occurs in pigs, cattle, sheep, goats, dogs and cats. Fur animals like minks, polar foxes and silver foxes are susceptible, too. Amongst wild life, AD has been reported in hares, wild rabbits, foxes, badgers, polecats, martens, wild pigs, ferrets, deer and stags, porcupines, hedgehogs, coatls, skunks, racoons, polar bears, jackals,

leopards, otters, rats and mice. The list of susceptible wild animals may be considerably longer.

Natural infection of horses, chickens, turkeys, geese, ducks and pigeons is not reported, but experimental infection of these animals is possible if large virus doses are parenterally injected. In this relation, a publication is of interest reporting that after vaccination of a batch of 49,000 1-day-old chickens against Marek's disease, 10,000 of them died of AD 2 or 3 days later (104). The authors concluded that the vaccine had been contaminated with ADV. Experimental infection of European starlings failed (105). Rhesus monkeys, macaques and Grivet monkeys but not baboons and chimpanzees can be experimentally infected. Man is considered to be insusceptible to ADV, since ADV infection has neither been proved virologically nor serologically in suspected cases (106, 107, 108). A recent report on seropositive evidence of human infection 5 to 15 mo after the onset of suspected clinical illness (109) is not convincing, since cross-reaction with herpes simplex virus has obviously not been excluded.

Regarding laboratory animals, the rabbit is the most sensitive one (16, 110, 111). Most virus strains evoke pruritus, however, exceptions exist (112, 113, 114). Suckling mice up to 1 wk old are highly susceptible, but sensitivity decreases with rising age (111, 115). The sensitivity of rats is about the same as of adult mice (16). Guinea pigs are susceptible to intranasal and parenteral infection (116). The use of minks and ferrets is described (111). Day-old chicks (117) were found to be highly susceptible. ADV has been propagated in embryonate hen's eggs (118). However, eggs are no sensitive indicators of infection, since adaptation of the virus is necessary.

Laboratory animals are most efficiently infected by the intracerebral pathway, but intramuscular or subcutaneous applications also give satisfactory results. Intranasal rather than subcutaneous infection of rats turns out to be more sensitive (119), but the contrary is valid for mice (16).

Differences in virulence of field virus strains: The present

epizootic behaviour of AD indicates that alterations in ADV virulence may have occurred in the past. Many of the early reports deal with AD in cattle, which apparently were the main host of ADV. In pigs, only sporadic cases comprising CNS affection and death of suckling piglets were described. Nowadays pigs are the main host, and adult pigs are attacked more and more; besides CNS symptoms, respiratory illness is one of the new features. It seems that ADV has acquired higher degrees of pig virulence. Apparently, multiple virus passages in the dense pig population have favoured this change. However, increasing incidence of AD in cattle is observed again in the last years, and links to infected pigs could not be established in every case.

There are some indications that field virus strains of different virulence do exist. In Northern Ireland, four virus strains have been isolated which differ in virulence (120). The NIA-1 strain is neurotropic and kills 5-20% of 7-wk-old pigs. Strain NIA-2 evokes a similar mortality but differs from NIA-1 by causing severe respiratory illness. Strain NIA-3 causes 80-100% mortality in 7-wk-old pigs and up to 20% mortality in 14-to-20-wk-old pigs. Strain NIA-4 was isolated accidentally from the lymph node of a cow which suffered from bovine malignant catarrhal fever. This virus is apathogenic for pigs of all age as well as for cattle and sheep and is now used as live vaccine. Strain NIA-6 was recently isolated from pigs (121). It causes no disease in 4-wk-old piglets, however, it kills 2-wk-old piglets. Škoda et al. (122) isolated an avirulent ADV strain (SUCH-1) from pigs, and Christov et al. (113) isolated a virus strain (Kostinbrode-1) from the brain of a dead calf which only evoked pruritus in 1-mo-old calves and no symptoms in calves older than 6 mo.

Virus propagation in cell cultures: More than half a century ago, Traub (123) succeeded to cultivate ADV in Maitland-type tissue explants of rabbit and guinea pig testis as well as of chick embryos. Practical use of cell cultures for ADV growth started in 1952 when the monolayer technique was developed. Nowadays, cell cultures have nearly completely replaced the use of laboratory animals in AD research.

ADV multiplies in a large variety of cell cultures of different origin. Most frequently used are (a) the porcine cell lines PK-15, SK and SK-6 and primary porcine kidney cells; (b) the bovine cell line MDBK and primary calf testis and kidney cells; (c) primary lamb kidney cells; (d) the rabbit cell lines RK-13 and NRK and primary rabbit kidney cells; (e) different clones of BHK-21 cell lines of hamster origin and (f) the Vero cell line of simian origin. Furthermore, the virus multiplies in chick embryo fibroblast cells, primary chicken kidney cells, dog kidney cells, the canine cell line MDCK, primary cat kidney cells, the feline cell line CRFK, primary ferret kidney cells and the human cell lines HeLa and HEP-2.

Some kinds of cells show a different sensitivity to ADV than others. Virus titres were equal in chick embryo cells and in pig kidney cells (124, 125) as well as in rabbit kidney and pig kidney cells (110). Burrows (126) found rabbit, pig, dog, sheep and ferret kidney cells to be very sensitive, and McFerran et al. (127) reported that pig kidney and PK-15 cells are most sensitive but Vero, lamb kidney and calf kidney cells are also very susceptible. In a comparative study (128), some differences in virus growth were found between cell lines. The titres obtained were $10^{6.7}$ $TCID_{50}/0.1$ in Vero cells, $10^{5.5}$ in SK cells, $10^{6.5}$ in MDBK cells, $10^{7.5}$ in two PK-15 cell lines from different laboratories, $10^{4.9}$ in a BHK-21 cell line and $10^{7.3}$ in the BHK-21 clone Tübingen (CT). The latter shows that different cell clones of the same cell line can have different susceptibility, and thus results of different laboratories working with the same cell line can differ.

ADV forms plaques in susceptible cell monolayers (129, 130). The plaque size depends on the virus strains and cell types used. Therefore, plaque size is applied to distinguish virus strains, especially attenuated strains. This aspect will be discussed later.

Virus replication in cell cultures induces two types of CPE (76): syncytial formation and rounding of cells. Both types result in cell lysis. Syncytia are mainly formed by highly virulent virus

strains, whereas cell rounding is found with virus strains of lower virulence (131). This difference is especially marked in primary pig kidney cells, whereas mixed CPE types appear in other cell cultures with the prevalence of one of the two CPE types.

Single Feulgen-positive intranuclear inclusion bodies are found in infected cells (132). The inclusions shrink later on and a surrounding halo appears, representing typical Cowdry type A inclusions. These inclusions become Feulgen-negative and eosinophilic in a later stage.

MOLECULAR ASPECTS OF THE VIRUS

The genome

The genome of ADV represents a linear, double-stranded DNA with a size of about 145 kilobase pairs (kbp) and a relatively high G+C content (73-74 mole%). The viral DNA is composed of a long (L) and a short (S) component. The S region consists of a unique short (U_S) sequence comprising about 9.2 kbp, which is embraced by inverted repeated sequences of about 15.4 kbp leaving the remainder of the molecule as a long unique (U_L) sequence (for more details see 133). The terminal repeat (TR) sequence is present in an inverted form (the internal repeat; IR) at the other end of U_S; the terminal sequence of TR seems at least not in all molecules to be part of IR (134). This genomic composition is classified as a class II (135) or type D (136) herpesviral DNA molecule. Since the U_S inverts itself relative to the U_L, two isomeric forms of ADV DNA are present in equimolar amounts in the virions which are both infectious (133). Recently, however, it was reported (137) that the ADV genome can also exist as a class III (type E) molecule like HSV. The analysis of several independently derived vaccine strains (all BUK-derivatives; Tatarov strain) reveals that 4 isomeric structures do occur, because of the presence of an invertible U_L (138). This striking change in genomic structure is explained by the translocation of a DNA piece originating from the left end of U_L to the boundary between L and S region. Whereas a minority of ADV isolates in nature display such an invertible U_L, passage of strains of field isolates in

chicken cells, but not in rabbit or pig cells, rapidly leads to the accumulation of class III genomes (137). The translocation is accompanied by the deletion of DNA sequences in the right end of U_L (in BamHI fragment 8'). The size both of the translocated and the deleted sequences varies between some hundred to some thousand base pairs during earlier passages, but become similar in size at later passages. Obviously, ADV with invertible U_L possesses a selective growth advantage in chicken cells which are distinct to swine.

Within the nucleus of the infected cell the ADV genome replicates via circular and concatemeric ("head-to-tail") DNA molecules in the mode of a rolling circle (for review: 59, 60, 133, 139). Although circle formation does occur, prerequisites for circularization as e.g. homology between the DNA ends or the presence of protein(s) bound to the ends of the genome are not detectable (133). Recently, Harper et al. (140) demonstrated that the concatemeric DNA molecules are cleaved to mature genomic DNA resulting in a 2-base (GG) 3'overhang at the S terminus, whereas the L terminus remains blunt-ended. During replication the mature genomic ends become joined by blunt-end ligation after repair of the 2-base gap at the S terminus. For the cleavage of the replicating concatemeric DNA molecules to unit-size molecules, it has been found that recognition signals are present at both ends (141). The BamHI fragments 14' and 13 are both necessary for efficient cleavage-encapsidation of the viral genome. In addition to an origin of replication located in the inverted repeat sequences, two other origins of replication have been identified in the U_L, one at the end of the U_L (in BamHI fragment 14') and another in the middle of the U_L (in the BamHI fragment 15).

Proteins

Because of its complexity the ADV genome possesses a potential coding capacity of about 100 polypeptides. By Northern blot analysis more than 70 abundant viral RNA species can be found (133). During the infectious cycle, the synthesis and processing of cellular polypeptides is gradually inhibited and numerous virus-encoded and virus-induced proteins can be detected.

Depending on the resolution of the gel systems used, between 20 (142), 27 (143) or 34 viral genomes (144) have been described after one-dimensional gel electrophoresis, and in two-dimensional gels at least 40 different unique ADV protein spots can be found (133, 145). Several viral polypeptides are phosphorylated both by preexisting cellular protein kinase(s) without the requirement of the expression of the viral genome (146, 147) and apparently also by virus-coded (148) or virus-induced protein kinase (147).

As with all herpesviruses, gene expression of ADV is regulated in a cascade-like fashion and can be divided into five classes (133, 149):

Immediate-early (IE) or alpha-genes are expressed at very early times after infection without viral DNA synthesis. ADV is coding for only one IE protein of 180 kilodalton (kd) apparent molecular weight; the IE gene is located in the inverted repeat sequences of the genome (133, 149, 150, 151). S1 analysis and DNA sequencing identified a single IE mRNA of 5.1 kb in size. The IE protein represents a nonstructural, regulatory polypeptide, which inhibits the cellular protein synthesis (150) and controls the transcription of the consecutively expressed early genes of ADV (133). Sequences flanking the 5′ end of the IE gene contain a strong promoter and probably also an enhancer (152) for activating the transcription of other viral and eucaryotic gene promoters (153, 154, 155, 156, 157, 158). The IE protein seems to be necessary for the formation but not for the maintenance of a stable transcription complex (159), which then remains active for hours also in the absence of a functional IE protein (160). This transcription complex is presumed to stimulate transcription by class II (160) and class III RNA polymerase (157). The ability of ADV IE protein to activate transcription does not seem to be correlated with its ability to bind to single-stranded DNA (161). Finally, the IE protein is essential to mediate efficient recombination between parental viral genomes preceding viral DNA replication (133). The IE protein synthesis in the infected cell is controlled both by self-regulation at the level of transcription and by regulation of mRNA stability (for review:

133).

Early or beta-genes of ADV (class II) are expressed prior and during viral DNA synthesis (1-4 hr after infection [p.i.]), thereafter their synthesis declines (133). At 2 hr p.i. approximately 30 virus-specific early RNAs can be detected which are all found polysome-associated (149). Probably the virus-encoded enzymes (e.g. DNA polymerase, thymidine-kinase) belong to these class II ADV proteins. Another early viral protein, the major DNA-binding protein (DBP; 136 kd) reaches its maximal rate of synthesis before the onset of viral DNA synthesis (162). This protein seems to be responsible for retaining the viral DNA in the nuclear matrix of the infected cell (163) and is required for initiation and later rounds of DNA replication (133). The gene encoding the DBP is located in the left part of U_L (0.14-0.18 map units), the specific mRNA is 4.3 kb in size (149).

The synthesis of early-late proteins of ADV (class III) also starts at early times after infection (1.5 hr p.i.), but they are produced at maximal rates between 4 to 9 hr p.i. after viral DNA synthesis has been initiated (133, 149). The major capsid protein (MCP; 142 kd apparent molecular weight) is one representative of this protein class. The MCP gene resides in the BamHI fragment 4, transcribing a MCP-specific mRNA of 4.4 kb in size (149). In addition, three further polysome-associated RNAs (1.5 kb, 2.1 kb, 5.5 kb) do hybridize with the BamHI fragment 4, and after immunoprecipitation of hybrid-selected in vitro translation products with anti-capsid protein antiserum, a 62 kd and a 32 kd protein are detected together with the MCP (158). Thus, this DNA fragment codes for four early-late genes involved in capsid assembly, which are also suggested to play an important role in virulence of ADV (158; see also below).

After the onset of DNA replication, the late or gamma-genes of ADV (class IV proteins) become expressed, being not detectable before 2.5 to 3 hr p.i. Some of the glycoproteins of the ADV envelope belong to this class, as well as the structural 10 kd and the non-structural 15 kd protein (133). These two DNA-binding proteins associated with concatemeric ADV DNA are suspected in

anchoring the viral DNA to the nuclear matrix during DNA replication (163).

Finally, ADV-specific proteins not synthesized in uninfected cells are detectable in unvarying amounts throughout the infectious cycle and are designated as class V proteins (133).

Glycoproteins

During the last years, several groups focused their interest on the investigation of the viral glycoproteins. Thus, since 1984 considerable progress has been made in the identification, characterization, gene mapping and sequencing. To date, five glycoproteins localized in the viral envelope as well as in the membrane of the infected cell are known: gI, gII, gIII, gp63 and gp50. In addition, one glycoprotein, the gX, is released into the medium of virus-infected cells. Althqugh the actual functions of each of these glycoproteins are just beginning to be elucidated, the results obtained so far already opened new, promising possibilities for the control of AD. In particular, the role of glycoproteins in the control of virulence and release of ADV from infected cells is of importance from a pratical point of view. The availability of defined genetically engineered ADV mutants will render it possible to reveal functions of the individual glycoproteins not only in vitro but also in vivo. In the following sections, the known properties of the single glycoproteins are separately described. A physical map of the ADV genome including the map locations of the described proteins and glycoproteins is depicted in Table 1.

Glycoprotein gI: The glycoprotein gI of ADV has achieved a great deal of attention during the last years. The apparent molecular weight of this glycoprotein ranges between 120 kd and 130 kd (145, 164), and its structural gene has been mapped into the right part of the U_S region of the viral genome (165). Earlier studies have already shown that most of the attenuated ADV strains used for vaccination display a similar DNA deletion of approximately 4 kbp in size in the U_S part of their genomes (138, 166, 167, 168). This deletion comprises the gI-gene, and it could be demonstrated (169) that in those avirulent strains neither gI-

Table 1. Properties of the ADV glycoproteins

Designation	gI	gp63	gp50	gIII	gII	gX
Structural	+	+	+	+	+	-
Mol. wt.[a)] - mature	130 kd	63 kd	50-60 kd	ca. 90 kd	155 kd[b)] IIa: 120 kd[c)] IIb: 67 kd IIc: 58 kd	95-99 kd
- precursor	2 pgI 78-83 kd	pgp63 36 kd	pgp50 44 kd	pgIII 60 kd	pgII 110 kd	pgX 65 kd
Complexed[d)]	gp63 115 kd gIV/gV	gI			gIIa gIIb gIIc	
ORF (AA)[e)]	1731 (577)	1050 (350)	1206 (402)	1437 (479)	2976 (913)	1494 (498)
mRNA	2.8 kb (1.7 kb)	2.4 kb colinear mRNA		1.55 kb	3.0 kb	1.6 kb
Gene location	U_S	U_S	U_S	U_1	U_1	U_S
MCA:[f)]						
- C′	-		+	+	+	-
+ C′	+		+	+	+	-
protection	+		+	+	+	-

Table 1. Continued

Designation	gI	gp63	gp50	gIII	gII	gX
Functions	Virulence Release	Release	Virulence Release Adsorption Thermostability		Penetration ?	
In vivo neutralization	?	?	+	+	+?	-
Essential[g)]	-	-	+	-	+	-
Homology:[h)]	HSV gE VZV gpI	HSV gI VZV gpIV	HSV gD	HSV gC VZV gpV	HSV gB VZV gpII BHV-1 g130 EBV gp110 CMV 145 kd	HSV gG ?
- map location	colinear	colinear	colinear	inverted	inverted	
- DNA	+	-	-	-	+	
- cys-alignment	+/-	+/-	+	+	+	

Legend to Table 1:

a) Apparent molecular weight (mol. wt.) from SDS-PAGE.

b) Under non-reducing conditions.

c) Under reducing conditions.

d) Association with the indicated viral proteins in the virion; gII represents a complex of gIIa-c linked by disulfide bonds.

e) ORF = open reading frame indicated in nucleotides;
AA = amino acids as deduced from DNA sequence.

f) MCA = monoclonal antibodies; in vitro neutralizing activity in the absence (- C′) and in the presence (+ C′) of complement; protection of mice passively immunized with the MCAs.

g) Essential for in vitro replication.

h) Homology to glycoproteins of other herpesviruses; the indications refer to genomic map location and to the extent of DNA homology and of alignment of cysteine residues; HSV = herpes simplex virus, VZV = varicella zoster virus, BHV-1 = bovine herpes virus type 1, EBV = Epstein-Barr virus, CMV = cytomegalovirus.

More details are given in the text.

specific mRNA is transcribed nor the gI is expressed. Thus, these data as well as marker rescue and marker transfer experiments (168, 170, 171) suggested the influence of gI in the control of ADV virulence. Further work proved that gI, which is non-essential for virus replication in vitro and in vivo, controls the virulent character of ADV strains. However, it must be emphasized that the virulence of ADV is controlled multigenically. The expression of gI or the presence of an intact U_S region as well as the expression of the viral thymidine kinase (tk) is apparantly not sufficient for the expression of high levels of virulence (170, 172, 173, 174). From recent studies it became clear that in addition to genes located in the U_S region of the ADV genome, in particular that coding for gI, functions involved in the nucleocapsid assembly mapping in the U_L (BamHI fragment 4) may also play a role in virulence (174). However, restoration of the U_S and of the BamHI fragment 4 in strain Bartha revealed that yet another function is required for the expression of wild-type virus virulence (174). In mice, Kost et al. (175) observed that intracerebrally inoculated tk-negative vaccinia virus recombinants expressing gI exhibited increased virulence over tk-negative wild-type vaccinia virus. This increased pathogenicity seemed to be potentiated when gI was expressed in concert with gp50 and gp63 (175). However, the vaccinia-gI-recombinants were not lethal to mice after intraperitoneal infection.

A correlation between virulence and both virus release from certain infected cells and the ability of replication in chicken brain has been postulated (172, 174). It could be demonstrated (176) that gI affects the release of ADV from certain cells, but that this effect depends on the genetic background of the virus. The virus release was impaired after the deletion of the gI gene from a rescued Bartha virus (strain Bartha to which an intact U_S had been restored), but not after removing gI from wild-type ADV. In addition, the gI inactivation rendered the rescued Bartha avirulent, but not wild-type virus (173). One further interesting finding concerning the deletion in U_S and the gI expression has been reported recently (137, 177). Most of the attenuated vaccine

strains have in common to be propagated in chicken cells and/or in embryonated eggs. Those attenuated strains exhibit a similar, but not completely identical DNA deletion in the U_S; the sequences bordering the deleted part are different in several attenuated vaccines (137, 177, 178). Comparative analysis between attenuated ADV variants, variants with a restored U_S and wild-type virus clearly demonstrate a selective enrichment of gI-negative ADV (according to the deleted U_S) (177). In contrast, passage in rabbit or pig kidney cells does not select for gI-deleted viruses. In addition, after mixed infection of chicken cells with gI-negative variants and wild-type virus also the deleted variants are enriched, but not after passage in other cell species (177). Thus, it is concluded that the expression of gI (and possibly in addition that of gp63) may be deleterious for the growth of ADV in chicken cells which are distinct to swine, the natural host. However, despite this growth advantage the Bartha strain is not able to replicate in day-old chicken brain (174).

DNA sequencing of the gI-coding region revealed an open reading frame encoding 577 amino acids with 6 cysteine residues and 5 potential N-glycosylation sites (179). A specific mRNA of about 3.8 kb in size is detectable in Northern blots from 2 hr p.i. onward increasing with later times p.i. (133, 165). By S1 analysis the size of the gI-mRNA was determined to be 2.8 kb and is suggested to represent a colinear transcript for gI (1.7 kb) and for a putative 36 kd protein (175). After in vitro translation and radioimmunoprecipitation with different monoclonal antibodies against gI, two non-glycosylated precursor polypeptides (pgI) are translated in equimolar amounts (165). The synthesis of two pgI (between 78 kd and 83 kd in size) is consistently found in more than 100 ADV isolates (Mettenleiter, unpubl.), except for one single case displaying a truncated pgI and gI (180) as described below. Interestingly, there exists a virus strain-specific size pattern of the two pgI independent of the host cells used for infection (165). The individual pgI pattern in distinct virus strains and isolates (e.g. for isolates from Northern Ireland) is stable for at least 20 yr. The occurrence of two pgI cannot be

explained by a mere heterogeneity in the virus population, since single plaque isolates of a given ADV strain also reveal the existence of both pgI (165). The reason and possible importance of this finding is unclear, and it is also unknown whether one or both of the two pgI will be processed to mature gI. In pulse-chase experiments, the earliest detectable form represents a 86 kd polypeptide, which is rapidly processed to the mature gI involving the Golgi apparatus (165). In the virion envelope, the gI can be found complexed together with a 115 kd protein and two other glycoproteins designated gIV and gV (145).

The expression of gI can exhibit a high degree of variability in different ADV strains, both quantitatively and qualitatively. Mettenleiter et al. (180) demonstrated that this variable gI expression depends to a large extent on the virus strain or even on the particular plaque isolate. Thus, one can distinguish between (i) stable expression of high amounts of gI (e.g. in strain Phylaxia), (ii) variable amounts of gI expressed ranging from high quantity to undetectable amounts of gI (e.g. in plaque isolates of strain Ka), (iii) synthesis of a truncated, probably non-glycosylated gI (designated as gI*) which was detected in one exceptional field isolate or after in vitro passage of strain NIA-5, and (iv) complete lack of gI due to the large DNA deletion, as already discussed. This finding explains that Hampl et al. (145) designated this glycoprotein as a minor one in the strain Ka, whereas Lukàcs et al. (164) found gI to represent a major glycoprotein in strain Phylaxia. Finally, the gI of the vaccine strain Tatarov appears to be altered in at least one epitope. Ben-Porat et al. (181) reported that one of different gI-specific monoclonal antibodies does not react with this virus strain.

Although some variability of gI expression can occur, none of numerous field strains of ADV tested have been found negative for gI (172, 181, 182; Mettenleiter and Rziha, unpubl.). Such a conservation of gI among ADV is also indicated by the restriction enzyme analysis of the genome of several hundreds of ADV field isolates and strains which all did not exhibit detectable deletion of the gI gene. Thus, the missing gI expression represents a

testable marker for gI-negative ADV vaccines. Using gI-specific monoclonal antibodies a serological differentiation between vaccinated and wild-type-infected pigs has been achieved by a competitive enzyme immunoassay (182). Recently a more simple, but specific and sensitive blocking ELISA has been developed to detect serum antibodies against gI in infected animals (183). Now it seems very attractive to use gI-deleted vaccine strains and a gI-specific antibody assay for a combined eradication programme of AD. However, the infected cell lysate used as antigen in those tests must always be ascertained for sufficient gI synthesis. Of course, the available, well-characterized vaccine strains constructed by the removal of viral gene(s) have great advantage over the use of attenuated ADV strains with unknown mutations. But although the gI plays an important role in the control of ADV virulence, one must bear in mind that it represents only one among other viral (and cellular?) genes involved in the virus-host relationship. For instance, restoration of the Bartha strain by wild-type gIII-gene and wild-type capsid genes (BamHI fragment 4) rendered a virulent ADV, although gI-negative (184). Furthermore, the actual function of gI during infection of the natural host remains to be elucidated.

Existing monoclonal antibodies against gI are strictly dependent on complement in in vitro neutralization of ADV (164). Some of these monoclonal antibodies have the ability to protect passively immunized mice against lethal virus challenge (Rziha et al., in prep.). Humoral antibodies specific for gI can be certainly detected in swine either during the acute or the latent state of infection (182), but the experiments reported by Ben-Porat et al. (181) indicate that in swine gI may not elucidate neutralizing antibodies. Some of the anti-gI monoclonal antibodies tested are not active in complement-mediated cell lysis and in virus-specific cell-mediated cytotoxicity (Ben-Porat, pers. commun.; Ohlinger and Rziha, unpubl.). Thus, what kind of influence the gI might exert on the host's immune response remains to be clarified.

In comparison to other herpesviruses, the gI of ADV and both

the gE of HSV-1 and the gpI of varicella zoster virus show colinear gene location and share a region of extensive amino acid homology with good alignment of some but not all cysteine residues (179). It is tempting to make functional deductions from the partially conserved gene organization between HSV-gE and ADV-gI, but no information exists whether ADV encodes e.g. for a receptor to bind to the Fc-component of immunoglobulin.

Glycoprotein gp63: In the U_S region of the ADV genome another glycoprotein could be identified using a lambda-gt11 expression library of ADV DNA fragments (179). This glycoprotein has been designated gp63 and shows an apparent mol. wt. of 63 kd. The structural gene is adjacent to that of gp50, the DNA sequence reveals an open reading frame coding for 350 amino acids. It starts with three consecutive ATG codons 20 nucleotides downstream of the stop codon of gp50, without the presence of typical motifs known for the regulation of transcription (e.g. polyadenylation signal, CAT or TATA box). Downstream of the termination codon of gp63 the sequence reveals consensus sequences of polyadenylation. Thus, it is likely to assume that the transcripts of gp50 and gp63 have a coterminal 3'end (175, 179). The mentioned DNA deletion in U_S of various vaccine strains removes about 75% of the gp63 coding region in the Bartha strain, but in the Norden strain (178) and variants of it (177) no coding sequences of gp63 are deleted. Although the complete coding region of gp63 seems to be present, no authentic gp63 is synthesized in the Norden strain; instead of it a 36 kd form of gp63 is detected in the infected cells and a 44 kd form is secreted into the medium (178). Probably, the 36 kd protein represents a precursor molecule of the truncated gp63 (44 kd) in strain Norden. It remains to clarify, whether in this virus strain e.g. a point mutation might be responsible for the loss or the cleavage of the transmembrane region of gp63 leading to excretion from the infected cell.

It has been found (179) that the amino acid sequence of gp63 has one region of extensive homology with HSV-1 gI (corresponding to US7) and with varicella zoster virus gpIV, and some but not all

cysteine residues can be aligned. The gp63 is dispensible for replication of ADV in cells. It has been suggested that the gp63 might be involved in the control of virus release from certain infected cells (178), since this function is absent in the Bartha strain but not in the Norden strain (172). Recent studies (173, 176, 177) do not exclude such a function, but they rather indicate that gp63 in concert with gI - and another additional function - plays a significant role in virus release. These studies also show that gp63 seems not to be involved in controlling ADV virulence, but that in concert with gI it can be deleterious for virus growth in chicken cells.

Glycoprotein gp50: The glycoprotein gp50, also designated gVI (181), was first mapped into the U_S part of the viral genome by Wathen and Wathen (185). The authors selected a mutant resistant against in vitro neutralization with gp50-specific monoclonal antibodies and determined the genomic location by marker rescue experiments. After DNA sequencing of the corresponding region (162, 175), an open reading frame coding for a peptide of 402 amino acids (44.5 kd) was found. The identity of that open reading frame with gp50 could be proven after expression in a mammalian vector (162). The mature gp50, 50 kd (185) to 60 kd (162) in size, most likely contains O-linked carbohydrate (162), although direct biochemical evidence is lacking. The absence of amino acid consensus sequences typical for N-linked glycosylation sites and experiments with tunicamycin, monensin and endo-ß-N-acetylglucosaminidase H (162) strongly indicate the lack of N-linked carbohydrates in gp50. Immediately downstream of the open reading frame no polyadenylation signal is present in the DNA sequence of the gp50 gene (162, 179). Similar to HSV gD the gp50 transcript appears to be coterminal together with that of the adjacent gene coding for gp63 (162, 175, 179). Indeed, a 2.4 kb mRNA specific for gp50 and gp63 could be identified (175). This might indicate an evolutionary relationship to HSV gD together with the finding that a conserved, internal amino acid region exists between HSV and ADV, although most of the remaining amino acid sequences are not homologous (162). In the homologous part of

gD and gp50, all but one cysteine residues can be aligned which might be suggestive for possible conserved disulfide linkages important for the structure and function of these glycoproteins (162). A possible relationship of HSV gD and ADV gp50 has been already suggested by Wathen and Wathen (185).

They also demonstrated that the gp50 might represent one of the important immunogens of ADV. Polyvalent immune serum is highly reactive with gp50, and monoclonal antibodies against gp50 effectively neutralize ADV in vitro with and without the aid of complement (185, 186, 187). Passive immunization with gp50-specific MCA can protect both mice (186, 187, 188) and swine (188) against lethal challenge with ADV. The protective activity of those monoclonal antibodies recognizing different epitopes of gp50 was correlated neither with their dependence of complement for in vitro neutralization (186) nor with their in vitro neutralizing activity at all (188). Interestingly, the data of Marchioli et al. (188) showed that the results of mice protection experiments cannot always be extrapolated to the natural host, swine, with respect to the protective activity of individual monoclonal antibodies. The application of gp50 alone, as a vaccinia recombinant virus or expressed in mammalian cells (189), protected mice and swine against lethal ADV challenge and induced virus-neutralizing antibodies. To protect the animals very low amounts of the expressed gp50 appeared to be sufficient. Similar results are found with vaccinia recombinants expressing both gp50 and gp63 (175). These data together with the described conserved amino acid region and comparable gene organization of ADV and HSV might indicate that gp50 represents an equivalent to gD. However, a functional relationship to HSV gD with regard to a possible importance of the glycoprotein in membrane fusion and virus adsorption to the cell remains to be shown. The significance of the gp50 glycosylation pattern unusual in herpesviruses is obscure, too.

Glycoprotein gX: This glycoprotein is synthesized in high amounts in infected cells and is excreted into the supernatant of infected cell cultures. The structural gene encoding gX was mapped

in the U_S part of the viral genome, and an unspliced mRNA of 1.6 kp in size was found to be specifically transcribed (190). The DNA sequence revealed an open reading frame of 1494 nucleotides corresponding to a polypeptide of 498 amino acids and a calculated mol. wt. of 53.7 kd (190). The apparent mol. wt. of the primary translational product (pgX) is about 65 kd (191), that of the mature gX is between 95 and 99 kd (190, 192). The pgX is processed to a high mannose-type 90 kd polypeptide and to a 115 kd intracellular form, probably after the addition of O-linked oligosaccharides, which is further cleaved proteolytically into the 99 kd mature gX (192). Presumably this secreted end product results from the removal of the transmembrane and cytoplasmic domain. The gX has been found to be sulfated to a higher extent than other glycoproteins of ADV. The sulfate moiety is not linked to carbohydrates (192, 193), possibly to tyrosine residues on the protein backbone as hypothesized for secreted proteins (192). The processing of gX seems to be a consequence of the protein sequence itself, since the same processing events can be found during expression of the gX-gene in eucaryotic cells. The mature gX also contains O-linked oligosaccharides (192). It is unlikely that gX represents a structural glycoprotein of ADV. It seems not to be incorporated into the virion (133, 191) as also indicated by our results of Western blot experiments (Rziha, unpubl.); neither with polyclonal anti-gX serum nor with specific monoclonal antibody a specifically reacting polypeptide was detected in purified virions.

Obviously, the gX is similarly expressed in various field isolates and strains of ADV (178; Rziha, unpubl.), although a slightly larger pgX (69 kd in size) is synthesized in the vaccine strain Norden (Mettenleiter, unpubl.). The reported lack of gX production in cells infected with the vaccine strains Bartha and NIA-4 (194) is in contrast to the results of Thomsen et al. (195). These conflicting data might not be unimportant, in particular with regard to a possible introduction of a gX-deleted ADV vaccine in combination with a gX-specific ELISA for the serological distinction between vaccinated and infected pigs (195, 196). Using

anti-gX-specific polyclonal and monoclonal antibodies, synthesis of gX was demonstrable in cells infected with the vaccine strains Bartha, NIA-4, Tatarov MK-35, Dessau and Norden provided that a sufficiently high-titre virus preparation was used for infection (Rziha, unpubl.).

The function of gX is completely unknown. It does not seem to be involved in virus neutralization and in virulence. No in vitro neutralization of ADV has been achieved with gX-specific monoclonal antibodies or polyclonal antiserum (188, 196) or with swine sera displaying high titres of anti-gX antibodies (195). The application of genetically engineered gX subunit vaccines did not protect animals against lethal challenge with wild-type ADV (195). Finally, gX is non-essential for in vitro growth of ADV, and a gX-deletion mutant has been constructed for possible use as a live vaccine (195, 196). However, since in nature no gX-negative ADV seems to arise, one can assume that gX should provide some function to ADV. No significant homology between gX and any sequenced HSV protein has been found so far.

Glycoprotein gIII: Another major glycoprotein of ADV represents the gIII (145, 164). Depending on the gel systems used, the apparent molecular weight of this glycoprotein ranges between 82 kd (197), 90 kd (164), 92 kd (198) or 98 kd (145). The gIII exists as a monomer not complexed with other viral proteins (145). After in vitro translation a primary translational product of about 60 kd in size can be found. The coding region is localized in the middle of the U_L part of the viral genome (197, 199, 200), and the corresponding DNA sequence reveals an open reading frame of 1437 bp encoding for a peptide of 479 amino acids (50.86 kd) with 8 potential N-linked glycosylation sites (198). An unspliced mRNA of 1.55 kb in size is synthesized with increasing amounts from 4 hr p.i. on or earlier. The gIII is non-essential for in vitro growth of ADV (198).

Different ADV mutants deleted of gIII have been constructed, which synthesize either a truncated gIII or no more gIII (201, 202). Those mutants are in vitro as infectious as their parental strain and the virions produced are indistinguishable from wild-

type virions by electron microscopy (201). Earlier results (145) indicate a role of gIII in virus adsorption, but in concert with other viral functions as suggested from studies with gIII mutants (201). Ryan et al. (202) concluded a role of gIII in virus release, since they had found some effect of the gIII mutation leading to a delayed and decreased release of virions into the medium of the infected cell cultures. Furthermore, mar-mutants (mutants resistant against neutralization with individual monoclonal antibodies) selected by anti-gIII monoclonal antibodies display an altered plaque morphology showing syncytial formation (197). Very recently, Schreurs et al. (203) clearly demonstrated the role of gIII in stable virus adsorption (as one of several glycoproteins or as part of a protein complex) using constructed gIII-deletion mutants and mar-mutants. From these studies it became evident that the adsorption process of ADV can be divided into (i) a gIII-mediated rapid adsorption and (ii) an alternative slower adsorption mode which is used by the gIII mutants.

Furthermore, it has been shown that gIII is a multifunctional glycoprotein (203). Except for its role in virus adsorption, gIII also affects thermostability of ADV and virus release. Virus release is markedly affected in conjunction with gI and constitutes a gIII function separate from that in virus adsorption (203). Similar studies (184) revealed a synergistic function of gIII in conjunction with gI or gp63 on ADV virulence. Because of its importance in mediating attachment of ADV to host cells, it does not surprise that gIII also influences the virulent or pathogenic character of virus strains. However, as discussed for gI, neither gIII or gI or gp63 alone are sufficient to confer complete virulence on certain strains (e.g. Bartha), but the genetic background of a given ADV strain is also very important. At least one of the capsid protein genes appears to play a crucial role in determining virulence (174, 184). Using the constructed gIII mutant viruses, more detailed analysis of the processing and of the functional domains of this glycoprotein could be made (202). The primary precursor polypeptide is glycosylated to a 74 kd form (pre-Golgi localized; not found in mature virions) after

the addition of N-linked sugars and subsequently converted to the mature 92 kd form of gIII in the Golgi apparatus. These studies have also shown that the signal sequence of gIII contains sufficient information for the export and release of the glycoprotein, and that the carboxy-terminus of gIII is responsible for its anchoring in the cell membrane and in the virion envelope. It has been also shown that a gIII missing e.g. the middle third of its protein is not excluded from the virion envelope and is also expressed on the surface of the infected cell (202).

The suggestion that the ADV gIII might represent a functional equivalent of the HSV gC (197) has to be proven yet. Comparison of the amino acid sequence of both glycoproteins reveals approximately 20% homology (198), but serological cross-reaction with other herpesviral glycoproteins has been not observed so far. Monoclonal antibodies against gIII neutralize ADV in vitro, not only in the presence but also in the absence of complement (145, 187, 197). In addition, some of these monoclonal antibodies have been shown to protect passively immunized mice and swine against lethal virus challenge (188).

Ben-Porat et al. (181) conclude from their studies that a major part of swine-neutralizing serum antibodies are directed against gIII. However, these authors also demonstrate that a considerable antigenic drift of gIII can occur. Virus isolates obtained from the same geographic area antigenically appear very similar (181, 204), but ADV isolates from different regions can exhibit antigenic variations, especially with regard to their sensitivity to anti-gIII monoclonal antibodies (181). Furthermore, Ben-Porat et al. (181) have shown that the vaccine strains Bartha K, Norden (BVK) and Tatarov (MK-35) were not neutralized with different monoclonal antibodies recognizing different epitopes of gIII. In general, compensatory alterations in other glycoproteins of those gIII mutants could not be detected (181, 202), except of the Bartha K strain (181). From the results of virus neutralization with anti-gIII antibodies without the aid of complement and of the role of gIII in virus attachment, it appears that the gIII of ADV and the gC of HSV-1 functionally differ

albeit the observed sequence homology.

Glycoprotein gII: One of the major glycoproteins of ADV, the gII, represents a complex of glycoproteins linked by disulfide bonds (145, 164). In the presence of 2-mercaptoethanol or dithiothreitol this complex reveals three structurally unrelated glycoproteins sharing homologous sequences (145, 146). They were designated gIIa, gIIb and gIIc showing apparent molecular weights of 120-125 kd (a), 74-67 kd (b) and 58 kd (c), respectively (145, 146). Under non-reducing conditions gIIa-gIIc remain linked, co-sediment in sucrose gradient or co-chromatographe in sephacryl gels (145) and display a single band of approximately 155 kd in size in PAGE (164). One primary unglycosylated precursor molecule (pgII; 110 kd in size) has been determined (205) and has been corroborated by sequencing the gII gene (206, Simon and Rziha, unpubl.). The pgII is quickly glycosylated to a 115-120 kd form (164; Schreurs, unpubl.) which is cleaved at the same time into gIIb and gIIc. Lukàcs et al. (164) hypothesize that gIIb and gIIc are linked via disulfide bonds after the cleavage of a common glycosylated precursor. A potential cleavage site deduced from the amino acid sequence has been described recently (206). The structural gene of gII has been mapped into the left part of the U_L region (205) and is expressed early-late during the infectious cycle (133, 149). About 8 hr p.i., most abundant transcription of gII mRNA can be found (206; Rziha and Mettenleiter, unpubl.) representing a 3.0-kb RNA, formerly identified as a 3.5 kb RNA (205). The gene comprises 2976 nucleotides in the Becker strain of ADV (206) or 2880 nucleotides in the Phylaxia strain (Simon and Rziha, unpubl.). It is notable that the 913 amino acids of the gII include an unusually long signal peptide of approximately 54 amino acids (206). Comparison of the gII-sequence of these two strains does not reveal striking differences except of some single exchanges of bases which are located in the third codon position (Simon and Rziha, unpubl.). Immediately downstream of the 3'end, a repetitive sequence of 15 bases occurs in varying copy numbers (between 3 and 50) differing both in different ADV strains and in individual plaque isolates of a given strain (Simon et al.,

submitted). The importance of this extragenic direct repeat unit is obscure.

The gII complex appears to represent a stably expressed antigen and might play an important role in the infectivity of ADV and in the immune response of the infected host. Up to now, no gII-negative ADV has been found. All ADV strains and isolates tested so far synthesize the gII in comparable quantity and quality (181; Mettenleiter, unpubl.). Merely the vaccine strain Bartha has been demonstrated to be neutralized more effectively in vitro with anti-gII antibodies than other virus strains (181). Ben-Porat et al. (181) suggest that the obvious overproduction of gII in Bartha might compensate for both the missing gI-expression and the underproduction of gIII.

Detailed functional analysis of gII is missing so far. A possible important role of this major viral glycoprotein in ADV neutralization can be suggested (Schreurs et al., unpubl.). A panel of monoclonal antibodies directed against at least 5 different epitopes of gII all neutralize ADV in vitro with the aid of complement. Three groups of monoclonal antibodies displayed also neutralizing activity in the absence of complement. Only those monoclonal antibodies with complement-independent neutralizing activity protected mice after passive immunization (Schreurs et al., unpubl.), and some of them were also highly reactive in antibody-dependent cell-mediated cytotoxity (Ohlinger and Rziha, unpubl.). These protective monoclonal antibodies recognize at least two different antigenic determinants of gII. However, the results of mouse protection experiments cannot be easily conferred to swine, the natural host. Although preliminar results from our own experiments indicate that some of the monoclonal antibodies protective in mice can also protect swine from the disease to some extent, Marchioli et al. (188) have shown the opposite for single anti-gII as well as for anti-gp50 monoclonal antibodies.

Further expectations on the function of gII must remain speculative to date, although it seems justified to assume that ADV might use the gII for similar functions as HSV uses its gB.

This implication can be made from data showing extensive structural homologies between both glycoproteins (206). The DNA sequences of gII and gB display 62% homology, and more than 50% of the amino acid sequences have been found to be identical in both genes (206; Simon and Rziha, unpubl.). All cysteine residues (but one located in the signal peptide) of gII can be aligned with those of gB, and 4 of the 6 potential N-linked glycosylation sites are conserved (206). This might indicate a common tertiary structure of both glycoproteins. Monospecific antibody against gII also immunoprecipitates gB. Furthermore, some relationship between gII and a similar glycoprotein complex of bovine herpesvirus type 1 (207) showing also some homology to HSV gB is indicated by DNA homology of their coding regions. A very similar degree of homology is demonstrable between the ADV gII and the gpII of varicella zoster virus by comparing both sequences.

Molecular aspects of latency

Investigations of the molecular basis of ADV latency in swine shall help to elucidate the mechanisms involved. In addition, the detection of virus-specific nucleic acids can provide more accurate data on the presence of latent ADV in the different organs of swine, even when infectious virus cannot be rescued. Thus, the viral genome (or at least part of it) is detected by molecular hybridization in latently infected tissues (25, 40, 41, 50, 208, 209, 210, 211, 212).

The availability of highly specific ADV DNA probes makes the use of molecular hybridization attractive for a routine detection of latent ADV. Although hybridization also with non-radioactive biotine probes can be excellently used for tissue cells of acutely infected swine (25, 211, 212; Rziha, unpubl.), its application in routine diagnosis of latent ADV remains to be further improved with respect to reliability (i.e. specificity and sensitivity) and simplification.

Studying a greater number of pigs over a period of 13 mo. p.i., the presence of viral DNA is demonstrable in at least 30% of cases negative for virus rescue (41). These results show that also neural tissues other than trigeminal ganglia can regularly harbour

latent ADV, in particular the olfactory bulb, medulla, brain stem and spinal cord. Furthermore, by in situ hybridization ADV DNA is found in neuronal cells of the inner ear of acutely and latently infected animals (213, 214). In addition to the known neural latency of ADV, some reports indicate the persistence of the virus also in extraneural tissues. From tonsils and nasal mucosa infectious virus is rescueable in about 20% of latently infected pigs (43, 52), approximately at the same rate the viral genome is demonstrable in tonsils (208; Rziha et al., in preparation), and viral DNA can be detected in liver and spleen (209). Interestingly, ADV-DNA is also present in white blood cells and thymus cells and to a surprisingly high percentage of about 50% in the bone marrow cells of latently infected swine (Rziha et al., in preparation). These data are in accordance with results (23, 44) showing the possible hematogenous and lymphoid spread of ADV. More recently, the susceptiblity for ADV of mononuclear cells and T cells of swine has been demonstrated (27; Ohlinger et al., unpubl.) confirming this pathway for viremic spread of the virus. It remains to determine whether and to what extent productive, abortive or latent infection takes place in the different blood components. Thus, more thorough investigations have to substantiate these findings to reveal the acutal function of extraneural tissues in the establishment, maintenance and/or reactivation of latent ADV.

In situ hybridization reveals that ADV DNA persists in less than 1% of the latently infected neurons (41, 210), both of the peripheral and the central nervous system (41). The copy number of the viral genome (0.3 - 0.05) per cell as well as the spectrum of neural sites harbouring ADV DNA is independent from the time of established latency after actue infection (41). The detectable, DNA-positive cell nuclei contain at least 30 copies of the viral genome (41), which can also be observed as discrete foci in the neuron after in situ hybridization (210). In some cases, unusually large amounts of viral DNA are present without any indication for a productive virus multiplication (41, 209). It remains speculative, whether in those cases a high amount of virus

replicated at the periphery leads to a consequent accumulation of latent virus in ganglia, or whether some kind of abortive (non-lytic) infection can lead to increased levels of viral DNA.

In contrast to HSV, the physical state of the latent ADV genome predominantly represents linear, non-integrated molecules (41). On the other hand, similarities between ADV and HSV or bovine herpes virus 1 are indicated by the transcriptional activity of the latent genome. In situ hybridization with probes specific for the immediate early (IE) gene region shows the presence of viral RNA in the nuclei of the latently infected neurons (210). Our preliminary results with strand-specific probes, however, are conflicting: (i) in neural tissues of indivdual pigs, RNA is found transcribed in the same orientation as the IE-specific mRNA, whereas in a few other cases antisense RNA is detected with probes spanning the IE gene region (as reported for HSV-1 and BHV-1); (ii) up to now ADV-specific transcripts cannot be regularly demonstrated in neural tissues of latently infected pigs (Rziha, unpubl.). Thus, a possible functional role of viral RNA for the establishment and/or maintenance of the latent state has to be determined by more thorough investigation.

DIAGNOSTIC PROCEDURES

Clinical diagnosis

Clinical diagnosis of AD in individual pigs is difficult, but AD can be suspected if whole herd symptoms are considered: numerous deaths of suckling piglets during the first 3 wk of life, nasal discharge, coughing, dullness, somnolence and nervous disorders with older pigs, high frequency of abortions and stillbirths. It is characteristic that morbidity and mortality decrease with rising age of the pigs. Furthermore, AD-suggestive signs in cattle, dogs and cats on the farm or discovery of dead dogs and cats are further hints at AD. Post-mortem does not reveal any pathological alterations typical for AD.

Concerning differential diagnosis AD resembles transmissible gastroenteritis or Coli enterotoxicosis in new-born piglets when

diarrhea is present. Respiratory signs can be caused by bacteria, especially pasteurella, and by swine influenza virus. In the latter case pigs of all ages become severely ill but do not die. Hog cholera infection can produce nervous signs, but when no gross pathological changes typical for hog cholera are present, differentiation from AD is difficult. Neural disturbances evoked by Teschen disease are not accompanied by infection of the respiratory tract as in AD. NaCl poisoning causes excitement, whereas arsanilic acid and mercurial poisoning results in lethargy of the animals. However, in contrast to AP, poisoning occurs suddenly without fever. Stillbirth and abortion can also be evoked by parvovirus infection. In any case, laboratory diagnosis will be necessary to confirm AD.

Histological diagnosis

Histological changes in pigs indicative of AD have been described in a previous section of this review (see Pathology). It can be difficult to differentiate the neural changes in AD from those in Teschen disease and classical swine fever (CSF) (13). Teschen disease lesions are prominent in the basal layer of the cerebellum and in the lumbar region of the spinal cord and the dorsal root ganglia, while in AD lesions in the caudal spinal cord are less marked and usually no reactions take place in the dorsal root ganglia. In CSF the brain stem is most severely involved, while in contrast to AD cerebrum and cerebellum are relatively less affected. Besides, the perivascular infiltrates in hog cholera are associated with marked endothelial damage. No inclusions are observed in Teschen disease and CSF.

Isolation of virus and detection of virus antigen and DNA

Tonsils, brain (especially olfactory bulb, quadrigeminal plate, hypocampus, pons, cerebellum, medulla oblongata), cervical and lumbal part of the spinal cord, lungs and spleen (especially in suckling piglets) are the most appropriate tissues for detecting virus in pigs. The distribution of the virus in the tissues, especially in the nervous tissues, can vary. Thus, several parts of the organ should be examined or mixed tissue homogenates prepared.

For virus isolation, tissue homogenates are inoculated into cell cultures and into small laboratory animals. Rabbits are the most susceptible ones, followed by mice and rats (16, 110, 215), but nowadays the use of cell cultures is favoured. As previously mentioned, the virus multiplies in a great number of cell cultures of different kind.

Immunofluorescence (IF) is the predominant technique to detect ADV antigen in tissue sections (35, 216, 217) or in impression smears (218, 219). IF usually is detected in the cytoplasm and only exceptionally in the nucleus. Pig serum is the most suitable serum for conjugate preparation (220). Comparing the sensitivity of IF and virus isolation in cell cultures (CC), Neumann and Bechmann (217) detected 14% of the samples to be positive in CC but negative in IF, and Hirchert (221) found 15.6% of the samples to be only positive in CC and 21.3% only positive in IF. A rather similar distribution was also described by Akkermans et al. (215). If virus isolation in cell culture is negative in contrast to IF, the presence of antibodies which has neutralized the virus or virus inactivation in aged samples may be responsible. In the opposite case virus concentration may be too small to be detected in IF.

Another tool to detect ADV antigen is the immuno-peroxydase technique (IPT) (222) which is applied like IF, but positive reactions involve both nucleus and cytoplasm. IPT in paraffin-embedded sections is, however, more complicated and time-consuming than IF. The IP-labeling of impression smears, described by Allan et al. (223), may be of more practical interest. In brain smears the IPT was as sensitive as IF and more sensitive than virus isolation. A rapid and specific method to detect ADV antigen in cell cultures is indirect IPT (224, 225, 226). For the detection of viral DNA in samples and in tissue DNA hybridization techniques are used (25, 40, 41, 50, 208, 209, 210, 211, 212). They are dealt with in the paragraph "Molecular aspects of latency".

Detection of antibody

Serum-neutralization test (SNT): The SNT in cell cultures is widely used for detecting ADV antibodies (227, 228, 229, 230,

231, 232) and is usually performed as microtest in plastic plates. The sensitivity of the test depends on several factors: type of the cell culture, macro- or microtest, number of cells used, amount of virus used, preincubation period of the virus/serum mixture, addition of complement, time of reading the test, type of diluter, quality of the plates, etc. (233). The most drastic influence on the sensitivity of the SNT is, however, evoked by the mode of incubation of the virus/serum mixture. Neutralization can be significantly enhanced by prolonging the incubation at 37°C to 24 hrs (228, 233).

Further enhancement of neutralization can be achieved by adding guinea-pig or rabbit complement (5% final dilution) to the virus/serum mixture during the incubation period. This is important for detection of virus-specific IgM antibodies in 'early' sera from 3 to 7 days after infection or vaccination (228, 234), but enhancement of neutralization also occurs in 'late' sera, because some IgG subclasses are complement-dependent.

SN-titres of 1:4 are considered as positive. Titres below are doubtful and afford testing of a second serum sample. Since ADV shares common antigenic components with other herpesviruses, serological cross-reactions take place (see paragraph "Antigenic relationship").

Enzyme-linked immunosorbent assay (ELISA): Antibody detection by ELISA (227, 235, 236, 237, 238, 239) is easy and cheap to perform, yields results within a few hours, is highly sensitive and well correlated with SNT. The ELISA can be readily automated, it is not disturbed by cytolytic properties of the serum and independent of a continuous supply of cell cultures. Every laboratory can employ the ELISA without great effort, since several test kits are commercially available; however, some of them do not detect IgM antibodies. Because of these advantages, ELISA is more and more replacing SNT. The ELISA detects a broader spectrum of antibodies than the SNT, including those which are not involved in neutralization. Therefore ELISA titres are usually higher than SN titres. The ELISA can be also used to detect antibodies in the exudates of organs, like liver, kidney and

muscles (240). Paper discs sucked full with serum (241) or blood (242) can also be used in ELISA. These methods facilitate the bleeding of the animals, since only small amounts of blood are needed.

The ELISA is superior to SNT techniques of low sensitivity if weakly positive sera are to be detected, but this advantage makes no more difference, when the sensitivity of the SNT is optimal. Goyal et al. (243) compared ELISA, SNT and RIDEA and they prefer the use of the SNT because of possible false positive reactions with ELISA and RIDEA. Sensitivity may be different between various commercial ELISA kits and between different kit batches (232).

Immunodiffusion test (IDT): Detection of ADV antibodies can be achieved either by double or radial ID (28, 227, 230, 244). The test is dependent both upon the method used for antigen preparation and the specificity of the antiserum (245). The IDT is less sensitive than the SNT when the SN titres are below 1:16. A very bad correlation between the two tests was found with pigs possessing maternally derived antibodies, even at SN titres above 1:16. The low sensitivity of the IDT may be the reason why antibodies cannot be detected before DPI 10 and only irregularly before DPI 14, although IgM as well as IgG and IgA antibodies react in the test. On account of its low sensitivity, the IDT is advantageous to screen pig herds, but not to test individual animals.

Radial immunodiffusion enzyme assay (RIDEA): In order to improve the sensitivity of the IDT the RIDEA was developed which combines the principle of radial immunodiffusion with ELISA (246). In a field trial (247), RIDEA and SNT were equally sensitive in detecting antibodies resulting from infection with a field strain of the virus. Sensitivity of RIDEA was, however, slightly reduced by using sera from vaccinated sows and significantly lower in detecting maternally transmitted antibodies. In all instances, RIDEA was as sensitive as SNT at SN titres of $\geq$ 1:16.

Complement fixation test (CFT): The CFT can be used to detect ADV antibodies in pig serum (228, 248, 249). There is a good conformity between CF and SN titres. However, CFT is less used

since most of the pig sera show haemolytic activity in the presence of complement up to dilutions of 1:32.

Radioimmunoassay (RIA): The RIA is a highly sensitive method for detecting ADV antibody (250, 251, 252). It is, however, restricted to laboratories with special equipment, since the use of radioactive iodine is necessary. Therefore, the test has gained no practical importance and no advantages over the ELISA.

Countercurrent immunoelectrophoresis (CIE): CIE for antibody detection (227) is of no practical interest, because its sensitivity lies between those of the IDT and the SNT.

Indirect hemagglutination test (IHA): An IHA test was developed by Haffer et al. (253). Positive reactions were observed on DPI 5.

Detection of immune response by the skin test: Cutaneously delayed hypersensitivity to ADV antigens can be used to detect ADV-infected pig herds (230, 248, 254, 255, 256). The test is much less sensitive than the SNT. Thus, only ADV-infected herds with infection rates of at least 46.7% can be identified by testing adult pigs. In young animals, particularly suckling and weaning pigs, the test is unsatisfactory. The overall correlation between skin test and SNT fluctuates between 10.5% and 80%, but it is not correlated with the SN titres (254). On the other hand, pigs vaccinated with inactivated ADV vaccines showed specific skin reactions while the SN titres were below 1:4 (257, 258). These divergences are not surprising since the skin test detects cell-mediated immunity. Therefore, pigs with maternal antibody do not react in the skin test. On the other hand, the skin test may be positive early after infection when antibodies are not yet detectable. Serological surveys by the SNT are not impaired by the skin test, since the antigens used in the latter do not evoke seroconversion.

IMMUNITY

Antibody production

Neutralizing antibodies (Ab) can be first detected in the serum of some animals between day post infection (DPI) 4 and DPI

6, when guinea pig or rabbit complement is added to the serum-neutralization test (SNT) (24, 128, 234, 259). Without complement positive results are obtained from DPI 9 or DPI 10 onward, however, great individual variations appear during the early phase of Ab production (22, 24, 227, 228, 260, 261, 262, 263). Maximum Ab levels are reached between DPI 14 and DPI 21. They fluctuate between 1:8 (261) and 1:512 (233), depending on the sensitivity of the SNT technique used and on individual variations of the animals. However, the majority of the titres are above 1:32.

The fact that complement is needed to detect neutralizing Ab in the early phase of infection indicates that IgM and complement-requiring IgG subclasses predominate. IgM Ab peak between DPI 7 and DPI 11 and then decline. They are no longer detectable at DPI 14 or DPI 25. IgG Ab rise from DPI 3 or 4 onward and reach their peak around DPI 14 (227, 253, 260). No data are available of the humoral profile on ADV-neutralizing IgA Ab.

With regard to the persistence of neutralizing Ab after infection, Gutekunst (264) has reported ND_{50} values between 1:64 and 1:512 after 7 mo, and Papp-Vid and Dulac (262) have found titres around 1:100 after 7 to 10 mo. Wittmann et al. (42) detected titres between 1:169 and 1:294 after 9.5 mo and titres between 1:11 and 1:18 after 16 mo. Furthermore they demonstrated titres between 1:128 and 1:512 in vaccinated infected pigs after 22.5 mo.

The long persistence of neutralizing Ab indicates that several antigenic stimuli must have occurred. Latent infection may be responsible for this phenomenon if reinfection can be excluded. During the latent phase several virus reactivation processes may take place, evoking booster effects on Ab production (42, 45, 54), which continuously maintain Ab levels. Since reactivation is accompanied by virus excretion immune contact animals can be infected and boostered. This explains field observations of Skoda et al. (266) who demonstrated high Ab titres in sows in breeding farms up to 4.5 yr after ceasing of clinical AD.

ELISA Ab are first detected between DPI 5 and 6. They are of IgM and IgG type. IgM peak on DPI 9 and 10 and then decrease, IgG

peak between DPI 10 and 15 (260). Other authors found the first positive reaction on DPI 7 (227) or DPI 10 (238). These discrepancies may be due to the conjugated serum, since Martin et al. used anti-IgM and anti-IgG whereas Banks et al. and Moennig et al. only used anti-IgG. Maximum titres were obtained from DPI 10 to DPI 24 (22) or on DPI 42 (238), respectively; they fluctuated between 1:530 and 1:1250 and higher. A comparative ELISA study concerning Ig classes was performed by Rodák et al. (241). The first anti-ADV IgG were detected in serum on DPI 5, and they persisted at least until DPI 18. IgG was first detected on DPI 7 and peaked on DPI 14. IgA appeared on DPI 10 and rose at least until DPI 18. A similar Ab response was found in oropharyngeal secretions, but the IgA titres were higher than in the serum on DPI 10 and DPI 14. Pigs vaccinated with an inactivated vaccine showed a similar IgM and IgG response on a lower level, however, no IgG could be detected.

Nothing is known about the long-term persistence of ELISA Ab. On account of comparative studies with field sera (237, 238) which show very similar results in ELISA and SNT, one can conclude that persistence of ELISA Ab may be similar to that of neutralizing Ab.

Complement-fixing Ab are not detected before DPI 7 (259) or DPI 14 (248). They peak between DPI 12 and DPI 28 with maximum titres between 1:64 and 1:256, and disappear 5 to 8 wk after infection (265).

Precipitating Ab are not detected before DPI 7, giving weakly positive reactions only, but strongly positive reactions appear from DPI 14 onward (28, 227, 230, 261).

Antibodies which are demonstrated by indirect hemagglutination appear on DPI 5 (253).

Complement-facilitated lysis of ADV-infected target cells by Ab appears in some cases on DPI 9 and 10 and is constantly present from DPI 15 onward (260).

Maternally derived antibodies

Maternally derived Ab are transferred from ADV-infected sows to the offspring by colostrum. The titres of neutralizing Ab in the colostrum are usually higher than in the serum of the sow

(263, 266, 267). Neutralizing Ab in the milk of sows are predominantly of the IgG class and only small amounts are of the IgG class; no ADV specific activity was found in the IgA class (267).

The half-life of Ab in piglets is estimated between 7.4 and 10.8 days (266, 263, 268). The level of neutralizing maternally derived Ab in the serum of piglets depends on the amount of Ab ingested with the colostrum. The ND_{50} values gradually decline in the piglets. Depending on this, colostral neutralizing Ab can be detected in the serum of piglets at minimum for 5 wk and at maximum up to 17 wk (266, 267, 268, 269, 270, 271). With the ELISA maternally derived Ab were detectable until 12 wk after birth (272).

Other immune factors like immune cells or immune mediators might be transferred with the colostrum, since ADV-specific lymphocyte stimulation was detected in a few piglets of vaccinated sows, which had never had contact with ADV (266, 273).

Correlation between antibody and protection

The serum titres of ADV-specific neutralizing Ab are one of the main parameters of measuring protection against ADV. In general there is a correlation between Ab titres and protection, however, individual exceptions exist (274, 275). Thus, some suckling piglets and vaccinated pigs were not protected against ADV infection despite the presence of Ab, but on the other hand passively or actively immunized pigs were protected despite undetectable or very low Ab levels (30, 47, 257, 266, 274, 275, 276, 277, 278, 279, 280).

The reason for these discrepancies may be that ADV locally spreads directly from cell to cell, is disseminated throughout the body in lymphocytes and macrophages and migrates on the neural pathways. Therefore, the virus is not accessible to Ab because it is not extracellular or is protected from Ab by the nerve/blood barrier. In these cases, the virus is more likely controlled by cellular defense mechanisms. Furthermore, serum Ab do not reflect local production of interferon and antibodies or local cellular defense mechanisms at the site of virus entry, like local

production of interferon and antibodies or local cellular defense processes. However, the relations between local immunity and protection yet are quite unclear.

The close correlation between SNT and ELISA indicates that neutralizing Ab are also involved in the ELISA, however, the ELISA also detects other functional Ab types. Therefore, the results of ELISA may be less indicative of protection than those of the SNT. On the other hand, the ELISA may recognize non-neutralizing opsonizing Ab which act in virus clearance and thus indirectly in immunity.

Since Ab production starts between DPI 3 and 6, it cannot evoke any antiviral effects in early primary infection. Therefore Ab may primarily act in recovery. Ab evoke a protective effect at reinfection (281) and at infection of vaccinated or passively immune animals. In these cases higher doses of virus are needed for infection, the intensity of virus multiplication and virus dissemination is reduced and severe clinical symptoms do not occur (19, 23). However, the establishment of latent infection is not prevented in each case.

Complement-mediated lysis of virus-infected cells by Ab acts in parallel or even later than neutralization of free virus (260, 277). Therefore this mechanism may be important for recovery but not for the early defense.

Cell-mediated immunity (CMI)

CMI can be demonstrated in pigs after ADV infection and after vaccination. However, the correlation between in vitro parameters of CMI and in vivo events is quite unclear. In comparison to humoral immunity, much greater temporal, individual and litter-dependent variations occur in CMI (282). Such variations are not unusual and can be evoked by genetic, age-dependent and seasonal factors (283, 284, 285, 286, 287, 288). Thus it is rather difficult to interpret the results of CMI tests on an individual base. Besides, it must be considered that many of the investigations were done with peripheral blood leukocytes (PBL), but the main processes may take place locally in the tissues and be not reflected in the peripheral blood.

Some parameters of CMI have been investigated: inhibition and enhancement of macrophage migration (289), inhibition of leukocyte migration (264), lymphocyte stimulation (233, 249, 288), spontaneous cell-mediated cytotoxicity (233, 273, 290) and antibody-dependent cell-mediated cytotoxicity (260, 273, 274, 277, 291, 292). Nothing is known about the cytotoxic activity of T lymphocytes in ADV infection, because it is difficult to establish a well-working isogenic test system.

Inhibition of macrophage and leukocyte migration (MMI, LMI): With the indirect MMI test (289), it could be demonstrated that lymphocytes from ADV infected pigs specifically altered the mobility of normal guinea pig macrophages in the presence of ADV-soluble antigen. The first lymphocyte reactions on macrophages occurred with blood lymphocytes and thymus lymphocytes on DPI 4. The number of positive reactions increased between day 7 and 35, the latest day tested. The lymphocyte action could be demonstrated nearly equally well with lymphocytes from retropharyngeal and inguinal lymph nodes, and with lymphocytes from spleen, blood, thymus and bone marrow. Besides macrophage migration inhibition, migration enhancement occurred.

The results of the MMI test were compared with those of lymphocyte stimulation (266), and it was found that both tests measure different parameters of lymphocyte activity, but give in principle similar results concerning the appearance of both activities. Rather similar results have been obtained by Gutekunst (264) with the LMI test, however, enhancement of migration was not found here.

Lymphocyte stimulation (LYST): ADV-specific LYST in infected pigs occurs in single cases on DPI 4 but regularly between DPI 7 and 14 (233, 249) and lasts at least 16 mo (42, 288). Thus LYST runs rather parallel to antibody formation. LYST was mostly marked with lymphocytes from lymph nodes, predominantly those draining the site of infection, and from spleen, whereas blood and thymus lymphocytes reacted less frequently; bone marrow lymphocytes showed no response (249, 288). Wittmann and Ohlinger (282, 288), detected LYST with spleen cells of piglets from immune sows,

indicating an ADV-specific sensitization of the lymphocytes via colostrum or a maternal transfer of sensitized lymphocytes.

Spontaneous cell-mediated cytotoxicity (SCC): Macrophages, neutrophilic granulocytes and lymphocytes (natural killer (NK) cells) are involved in SCC. In pigs at least two subpopulations of NK cells exist (290), and enhanced NK activity of blood lymphocytes was evoked on ADV-infected IBRS-2 targets. No significant variation of NK cytotoxicity was found in 3 pigs during the first 3 wk post infection, but in one pig a marked decline of NK activity occurred 2 days before death (290). By using blood lymphocytes as effector cells and Vero cells as targets a high percentage of the pigs was found to react with SCC against ADV-infected and non-infected targets before infection (233, 282, 290, 291). In the first week post infection, SCC against non-infected targets remained more or less at the preinfectious level, however SCC against ADV-infected targets was distinctly reduced during the first days of infection, particularly with effector cells from severely diseased animals. When non-infected YAC cells were used instead of Vero cell targets, a significant reduction of NK activity was demonstrated in the first week after infection (Ohlinger, unpubl.).

After immunosuppression of latently infected pigs by prednisolon a temporary significant decrease of SCC occurred (42). Recent experiments (Ohlinger, unpubl.) have shown that this phenomenon was not due to the reactivation process itself but to the drug, since reactivation evoked by thermostress or prostaglandin did not alter the NK activity.

SCC against non-infected and ADV-infected Vero cells was also present in piglets either from immune or non-immune sows from birth onward. Liver and spleen cells were the most active, whereas peripheral blood lymphocytes and cells from lymph nodes reacted only in a few cases. There was no difference whether the targets were ADV-infected or not (273).

From all these data one may assume that NK cells participate in the very early defense mechanisms against ADV infection.

Antibody-dependent cell-mediated cytotoxicity (ADCC): ADCC is

evoked by a variety of cells. Macrophages proved to be most efficient against ADV-infected cells, followed by neutrophils and lymphocytes (K cells) (292). The appearance of ADCC coincides with the production and presence of neutralizing antibodies, and the correlation is significant (274). Accordingly, ADCC is not detected before DPI 9 in ADV-infected pigs and it lasts as long as neutralizing antibodies are present (233, 260, 277). Therefore, ADCC is apparently important for recovery, but besides a correlation was found between ADCC titres in the serum of vaccinated pigs and the degree of protection against ADV infection (274, 277). Peripheral blood leukocytes are the most active cells, followed by liver and spleen cells (42, 273). Reactivation of latent virus does not enhance ADCC, which usually is on a high level even 16 mo post infection (42). ADCC is also present in piglets with colostrally derived antibodies (273, 274), however the reactivity of these antibodies in ADCC is apparently less than that of actively produced antibodies (273, 293).

CMI in vaccinated pigs: Some parameters of CMI were also investigated after vaccination of pigs. ADV-specific leukocyte migration inhibition (LMI) was detected 1 wk after the application of an inactivated vaccine (earlier times apparently not tested). LMI increased until wk 5, remained on the elevated level until wk 8 and 9 and then slightly decreased until wk 14. After challenge infection at this time, LMI increased (28).

ADV-specific LYST of PBL appeared after the first week of vaccination with an inactivated vaccine. It could be demonstrated until 3 wk, but the number of reacting animals decreased. No LYST could be found after 4.5 and 6 wk. A second vaccination led to a temporary increase of LYST which was especially marked after 1 wk. However, the number of reacting animals was reduced after 2 or 3.5 wk. A high percentage of the animals reacted in LYST against non-viral vaccine constituents which may have masked ADV-specific LYST (233, 282). LYST in vaccinated virus-challenged pigs was detected for at least 22.5 mo (42) and immunosuppression resulted in loss of LYST activity.

By using a live vaccine, van Oirschot (288) found a much

higher LYST response which lasted at least up to 35 days after revaccination, indicating that live vaccines evoke better LYST than inactivated vaccines. This was confirmed by Alva-Valdes et al. (294) who could not detect LYST after the application of an inactivated vaccine from 1 to 14 mo post vaccination, but they found LYST after vaccination with a live vaccine after 3 mo. After challenge, the intensity of LYST was higher in animals vaccinated with a live vaccine than in those vaccinated with an inactivated vaccine.

SCC against ADV-infected and non-infected target cells was significantly influenced by the application of inactivated vaccines. After revaccination, however, NK activity against non-infected targets temporarily increased during the first week after vaccination and that against ADV-infected targets during the second week. After challenge infection, a temporary reduction of SCC against ADV-infected and non-infected targets was detected on DPI 2, but thereafter an increase set in until DPI 7, which was followed by a decrease to preinfectious levels (42, 233, 282).

ADV-specific ADCC after vaccination was detected in parallel to IgG antibody production, however, no evident correlation to the titres of neutralizing serum antibodies (nAb) existed in the early stage of vaccination, where only about 44% of the animals responded in ADCC despite the presence of nAb in all the animals (233, 274, 292). After revaccination ADCC was regularly found (233, 282). Maternal nAb markedly interfered with ADCC response after challenge infection (274). No difference in ADCC response could be detected in pigs vaccinated with an inactivated or a live vaccine (277).

Interferon (IFN): Porcine IFN-alpha evokes a strong antiviral effect against ADV (295). Traces of IFN could be detected in serum 6 hrs after intranasal infection with ADV in some cases, and it was regularly present after 24 hrs. IFN disappeared between DPI 6 and DPI 11, depending on individual variations (23, 233, 296). However, no correlation could be found between IFN levels and the degree of clinical symptoms, except in one case. But one has to consider that serum IFN does not really reflect local IFN

production in the tissues or in the mucosa lining the nasopharynx region and the respiratory tract.

EPIZOOTIOLOGY

Geographic distribution and incidence of the disease in infected countries

AD exists in most parts of the world (297). The affected countries are listed in Table 2. Countries not listed need not be free of AD in any case, since single outbreaks may remain undetected or no data are available. Finland (298), Switzerland (299) and the German Democratic Republic (GDR) (300) have been approved to be free of AD by serological surveys. In Switzerland only 5 AD foci were reported in 1977 (301) and AD was eradicated in 1983 (299). In the GDR eradication was finished in 1985 (300), but in the meantime two new outbreaks have occurred (Epizootiological information of O.I.E.). The only countries where AD has no chance are the Islamic ones because pigs play no role there and thus the basis of the disease is absent.

Data concerning the incidence of AD are available with some countries. In Belgium and France the number of outbreaks was below 20 per year until 1971. Thereafter, a moderate annual increase set in until 1973 and 1974, which was followed by a steep increase, reaching 200 outbreaks in France (pig population 11 million) and 300 outbreaks in Belgium (pig population 5 million) in 1977 (302). In France the disease had spread to 5 Departments by 1972 and to 53 by 1983 (303). A rather similar outbreak profile was shown in The Netherlands and the USA, though the number of outbreaks in these countries was higher (302). While it was below 100 until 1971, the number rose to 2000 in The Netherlands (pig population 8 million) and to 1300 in the USA (pig population 72 million in 1977). At present 6.4 million of the 80 million pigs are considered to be infected in the USA. An opposite course of AD appeared in Denmark (302). A peak of 180 outbreaks was reached from 1969 to 1971, but it gradually declined to 100 outbreaks in 1977. In the Federal Republic of Germany (pig population 22 million) AD was no problem until 1976 (between 12 and 24 outbreaks

Table 2. Control of AD in different countries

Country	N	Qf	Qi	S	Sp	I	Ca	Cc	V	T	AD present
Namibia	+					+					-
Reunion	+	+				+					-
South Africa	+		+	+					p		-
Seychelles						+					-
Swaziland		+									-
Argentina	+	+	+					+			+
Bermuda	+										-
Bahamas	+	+									-
Brazil	+	+	+						+		+
Canada	+										-
Chile		+									-
Columbia											+
Cuba	+			+					+		+
Ecuador		+									-
Falkland		+									-
Guatemala	+		+						p		+
Haiti		+									-
Jamaica						+					-
Mexico	+	+	+	+	+		+		+		+
Nicaragua	+										-
Panama											?
Peru						+					-
USA	+						+		+		+
Venezuela	+	+	+						+		+

Table 2. Continued

Country	N	Qf	Qi	S	Sp	I	Ca	Cc	V	T	AD present
Afghanistan											+
Cyprus	+										-
Hong Kong									+		+
Israel		+									-
Iran	+	+									-
Japan	+	+									+
Korea D.R.P.									+		+
Korea Republic		+							p	+	-
Laos											+
Malaysia (Peninsula)									+		+
Philippines		+	+							+	+
Singapore									+		+
Syria						+					-
Thailand									+		+
Vietnam				+							+
Albania										+	+
Austria	+	+	+		+			+	p		+
Belgium									+		+
Bulgaria	+			+			+		+		+
Czechoslovakia	+			+					+		+
Denmark	+	+	+		+		+		p		+
Fed. Rep. Germany	+		+		+		+		+*		+
France	+		+				+	+	+	+	+
German Dem. Rep.	+			+						+	-
Greece		+	+						+		+
Hungary	+		+					+	+	+	+
Ireland									+		+

Table 2. Continued

Country	N	Qf	Qi	S	Sp	I	Ca	Cc	V	T	AD present
Italy	+								+!		+
Luxembourg	+		+		+		+		p	+	+ dog
Malta	+	+				+					-
Netherlands									+		+
Norway	+			+		+					-
Poland			+						+		+
Portugal	+	+	+				+		+	+	+
Romania	+		+	+				+	+	+	+
Spain	+		+				+				+
Sweden	+						+				+
Switzerland				+							-
United Kingdom	+	+		+				+	p	+	?
Northern Ireland			+						+		+
Yugoslavia	+	+	+	+		+	+		+	+	+
Australia	+	+									-
Fiji						+					-
New Caledonia						+				+	-
New Zealand	+					+					+
Papua New Guinea						+					-
Samoa						+					+
Salomon Island						+					-
Tonga	+										+
Vanuata						+					-
USSR	+		+	+		+		+	+	+	+

Explanation to Table 2:

N Notifiable

Qf Quarantine or other precautions at frontier

Qi Quarantine of infected zones or herds and movement control inside the country

S Stamping out

Sp Partial stamping out

I Prohibition of import from infected countries

Ca Control programme for only some areas of the country or certain breedings

Cc Control programme for the whole country

V Vaccination

* Live vaccines only for non-breeding pigs

! Live vaccines prohibited

? Suspected but not confined

per year). Thereafter, a gradual increase occurred until 1978 (175 outbreaks) which converted to a steep rise from 1979 onward reaching a peak of 1,968 outbreaks in 1987 despite of legislative measures and increasing vaccination. Despite duty of notification a considerable number of non-notified outbreaks may exist. In the United Kingdom a gradual increase of AD occurred in 1971 (5 outbreaks) reaching 60 outbreaks in 1982, when an eradication programme has been started (302).

AD is endemic in areas with dense pig population and intensive, specialized farming management which involves a lot of animal movement between the farms. AD occurs most frequently in farms which buy pigs from different sources, thus fattening farms are predominantly affected. AD is not endemic in districts where small farms with own pig breeding predominate or which buy the pigs from well-known farms of their surroundings. If single outbreaks occur there, they are usually caused by pigs imported from infected areas. In the FRG, 78% of the outbreaks occur in the

northern part of the country where 64% of the fattening pigs and 75% of the breeding pigs are produced.

The epidemiological course of AD shows seasonal cycles. During the warm season the number of outbreaks drops while in the cold season a peak is reached. The reasons may be that the survival conditions for the virus are better in winter than in summer, and the climate stress by temperature fluctuations, leading to reactivation of latent virus, may be more marked in winter than in summer.

Transmission of the virus

ADV-infected pigs are the main source of virus spread. Other species are less important since they usually die and virus spread is interrupted. AD is predominantly transmitted by trade with ADV-infected unvaccinated or vaccinated pigs being either in the incubation period, in a subclinical or in a latent phase of infection. In latter case, the stress of transportation may evoke reactivation followed by virus excretion.

Genital virus transmission can be induced by artificial insemination with ADV-contaminated semen or when sows are serviced in ADV-infected boar sations (31, 32, 215). ADV can be transmitted by embryo transfer, since it is adsorbed to the zona pellucida, but it does not penetrate into the embryo (304, 305, 306). The virus is not removed by several washings, but trypsin treatment removes most of it. This may be the reason why virus transmission was not found in hundreds of embryo transfers from seropositive pigs (307). Furthermore, the amount of virus used for experimental infection of embryos was important. Bolin et al. (305) induced seroconversion in the recipients, when embryos exposed to 10^8 $TCID_{50}$ of ADV were washed and transferred, but they failed to do the same with embryos exposed to 10^4 $TCID_{50}$.

ADV can be transmitted by contaminated implements, vehicles, food and by man, but the likelihood of these routes must not be overestimated. The same applies with regard to virus transmission by cats, dogs and rats, since virus excretion by these animals is very low (308, 309). Experimentally, no contact transmission was found between sick cats and pigs (309). The role of the racoon as

virus reservoir is controversial. Wright and Thawley (310, 311) are of the opinion that the racoon may serve as a short-term reservoir for ADV, but it is unlikely to act as a long-term carrier of the virus. Platt et al. (312) believe that the racoon may be a potential reservoir of the virus. Experimentally, the virus could not be transmitted between racoons but it was transmitted between racoon and pig by contact (313). Insects are apparently not important in virus spread. However, house flies might play a role in mechanical transmission of ADV within a herd, but less likely between herds (419). Virus transmission to dogs, cats, racoons and other carnivores as well as rats occurs via ADV-infected meat, offal or carcasses. Pigs can be infected orally by ADV-contaminated garbage, and nursing piglets can acquire ADV from the milk of the infected sow or they become infected in the uterus.

Air-borne transmission of ADV is possible. An infected pig excretes up to $10^{5.8}$ $TCID_{50}$ of ADV into the air during 24 hr (15), and ADV could be isolated from air samples of ADV-infected farms (314). Further experiments showed that the air stream of a ventilator transports the virus to premises 10 to 20 m apart (15, 315). Air-borne transmission may occur even over larger distances ranging from 500 m up to 2000 m (315; Bitsch, pers. comm.). Discussion on air-borne virus transmission across 10 km (Taylor, pers. comm.) or 80 km (Andersen, pers. comm.) are merely speculative. Air-borne virus may originate also from aerosols when ADV-contaminated liquid manure is sprayed on land (314).

VACCINES AND VACCINATION

Live virus vaccines

Numerous variants of ADV with reduced virulence are used as live vaccines (review articles 316, 317). Either apthogenic field virus isolates (natural variants) or virus attenuated by serial passages in cell cultures (biologically induced variants), or virus modified by chemicals (chemically induced mutants). The most frequently used live vaccines are:

Natural variants: The K 61 Bartha strain, isolated from a

pig, attenuated by passages in pig kidney cell cultures (318, 319) and adapted to chick embryo fibroblast (CEF) cultures.

The NIA-4 strain from Northern Ireland was isolated from the lymph node of a cow (120) and adapted to a pig testis cell line (NL-ST-1).

Biologically induced variants: Serial passages of ADV in the CAM of fertilized hen's eggs and in CEF cultures led to the Bukarest variants which served as origins for further variants (320, 321, 322). Two independent groups made further passages in CEF cultures resulting in the BUK-TK variants (268, 323, 324) and the BUK variants (325, 326). The number behind the letters indicates the number of passages in CEF cultures, e.g. TK/900 or BUK/624. There is no indication that the BUK-TK and BUK variants differ fundamentally from each other.

The strain Ercegovac (327) has been attenuated by passages in the CAM and in rats and was adapted to CEF cultures.

The B-Kal 68 strain was attenuated in CEF cultures (328).

Toma et al. (329, 330) isolated an attenuated variant (Alfort 26, A-26) at low temperature (26°C) after passages in a pig kidney cell line.

The attenuated strain "Tornau" (no details known) is used in the "Dessau" vaccine (331, 332).

Chemically induced mutants: Tatarov (333) produced a mutant (MK-25) in CEF cultures in the presence of 5-iodo-deoxy-uridine which is thymidinekinase-negative. A further mutant (MK-35) was induced by 5-bromo-deoxyuridine (334).

To differentiate attenuated from virulent virus strains, markers of the attenuated strains were investigated. Several parameters have been tested: temperature resistance, trypsin resistance, plaque size, type of CPE, thymidine-kinase activity, virus multiplication at supraoptimal temperature and virulence in mice, rats, rabbits and chickens (111, 279, 335, 336, 337, 338).

Trypsin and thermoresistance (336) and thymidine-kinase activity (279) are not specific markers of attenuation. Plaque size does also not allow conclusions to attenuation, though most of the attenuated strains tested produce small plaques in CEF

monolayers, but large plaque producers have as well been found (338). Attenuated strains preferably evoke a CPE characterized by cell rounding in porcine kidney cells, whereas virulent strains cause syncytial formation (338). In other cell cultures, both CPE types appear in parallel, one or the other predominating. Both plaque size and type of CPE were dependent on the type of cell culture used. Virus multiplication in cell cultures at supraoptimal temperature (40°C) did also not give unequivocal results (338).

The degree of virulence of attenuated strains in mice, rabbits and chickens allows conclusions to the degree of attenuation. All of the attenuated strains tested (K 61 Bartha, BUK, BUK-TK, NIA-4, A-26, MK-25, MK-35) showed reduced virulence for these animals (111, 279, 336, 337, 339). However, this only indicates attenuation of the virus, and no conclusion can be drawn with regard to virulence for other species.

Therefore physicochemical and biological markers only characterize a certain attenuated virus strain, but they are not decisive for and not linked with the degree of attenuation. It is more promising to identify attenuated strains on molecular basis. It was found that the attenuated virus strains Bartha K 61, BUK TK/900 (but not BUK TK 650) and NIA-4 carry deletions in the U_S region of the genome where the gene for gI has been mapped (138, 165, 169, 340, 341, 342).

Furthermore, the Bartha K 61 strain has an additional deletion in the U_S region of the genome, coding for gp63, which is not present in the BUK strain (178). The thymidine-kinase-negative live vaccines MK-25 and MK-35 are partially gI-deleted. An ELISA has been developed which enables to distinguish pigs vaccinated with the gI-negative mutants from pigs either infected with wild-type virus or vaccinated with gI-positive mutants or gI-positive inactivated vaccines (182) which evoke a gI-antibody production.

By means of virus DNA analysis of certain live vaccines, the vaccines Ercegovac, Ay-Vak and K 61 Bartha, in contrast to MK-25, were demonstrated not to be genetically uniform and to contain

several genetic variants (342, 343). In addition, it was found that a single passage of the virus in pigs was sufficient to cause changes in the DNA. Changes in the virus genome of attenuated BUK virus (Norden vaccine) also occur after passages of the virus in CEF, but not in RK or PK cells (137). CEF passages of the virus result in deletions in the gI region of the genome (177). Thus, continuous variations can take place during virus passage in animals and cell cultures. Therefore, the appearance of virulent mutants cannot be excluded, as some reports indicate: Gielkens et al. (342) isolated the vaccine strains Ercegovac and MK-25 from an ill pig, and Sabo et al. (344) succeeded to isolate an attenuated virus from five cattle that had died of AD. This attenuated virus was not different from the live vaccine BUK TK/900 which ought to be avirulent for cattle.

Virulence of ADV for animals is reduced during the attenuation process in serial passages in the order pig -> cattle -> sheep -> dog. Immunogenicity for the preceding species decreases in the same order. Therefore, Zuffa (323) recommends to produce a proper vaccine for each species. Avirulence can also be presented when small vaccine doses are used. Vitin et al. (345) found that 5×10^7 $TCID_{50}$ of a BUK vaccine were lethal for newborn piglets, whereas 5×10^6 $TCID_{50}$ did not cause death but the virus could be isolated from all organs.

The pathogenic porperties of the most important live vaccines are summarized in Table 3. All the attenuated strains are largely avirulent for weaning and older pigs, though a part of them still evoke elevated temperatures and short-lasting slight anorexia. But some of the vaccines are still virulent for suckling piglets, especially after intracerebral application. Great differences of virulence appear in cattle and sheep. The innocuity tests are usually performed in small groups of animals, but this must not reflect the situation in large numbers. Skoda and Jamrichová (366) have reported that the highly attenuated vaccine BUK/1000 showed irregular virulence when tested in 1000 cattle and sheep. The degree of virulence is also dependent on the route of inoculation (367). All the vaccines still have considerable virulence for

dogs. Even the "avirulent" K 61 Bartha strain evoked severe clinical symptoms in dogs with a lethality of 40% (347, 350). Since the majority of vaccines has not been tested in dogs it has to be assumed that they are still virulent for this species until the contrary will be proved.

All vaccine strains multiply locally at the site of application and in the regional lymph nodes. After intranasal application the virus can be demonstrated in the tonsils, the nasal and oral mucosa and in the respiratory tract. Most of the strains tested evoke various degrees of generalized infection.

Little is known about intrauterine transmission of attenuated virus to the fetus. After injection into 88-day-old fetus the highly attenuated strain BUK 624 was lethal to the fetus. The strain BUK TK/900 multiplied in the fetus and caused contact infection of the other fetus without killing. Antibody production in the fetus was observed with TK/900 but not with BUK 624 (360). No virus could be isolated from new-born piglets of sows vaccinated with BUK TK/200 (323) or BUK TK/650 at day of gestation 14 to 65 (protocol of the vaccine producer). However, BUK 628 was isolated from the fetus after killing of the vaccinated sow 72 hr after vaccination (361), but no harmful effects were observed in new-born piglets of sows vaccinated with BUK vaccines (268, 355).

Since attenuated virus can be detected in the oronasal mucosa and the tonsils, virus excretion is very likely, but the amount of virus shedded seems to be too low for contact infection. However, these experiments were performed with a small number of animals. Therefore, it cannot be completely excluded that after mass vaccination contact infection might occur in a few cases.

The ability of vaccine virus to convert into latency is quite obscure, though van Oirschot and Gielkens (47) could not detect K 61 Bartha virus after immunosuppression of vaccinated pigs.

A series of reports deals with immunity and protection after the application of live vaccines (K 61 Bartha: 318, 319, 346, 351, 368; BUK: 18, 294, 313, 322, 323, 325, 326, 355, 358, 359, 369; NIA-4: 369; MK-25, MK-35: 333, 334, 364; A-26: 271, 329, 330; Dessau: 332).

Table 3. Virulence of attenuated ADV strains

Vaccine strains	Virulence for Suckl. pig-lets	Older pigs	Cattle	Sheep	Dogs	Virus multiplication Local	Gener-alized	Fetus	Virus excre-tion	Contact infec-tion	Latency
Bartha K 61	-	-	-	-	+	+	-		-/+[7]	-	-
NIA-4	-	-	-	-		+	+[6]		-/+[7]	-	
BUK 387/0[2]	+	-				+	+		+	-	
/250	+	-	+	+	-?[5]	+	+		+	-	
/437	-	-	+	+		+			+	-	
/628	(+)[3]	-				+	+	+[12]		-	
/1000		-	(+)[8]	(+)[8]		+		+[12]			
BUK-TK/200	-	-	+	+		+	+	-	+	+	
/300		-	-	+		+					
/400		-	+	+		+				-	
/650	-	-	+	+		+	+[11]	-	-[9]	-	-
/840		-	-			+					
/900		-	(+)[8]			+		-			
Ercegovac	+	-	+	+	+	+			+	+	
B-Kal 68		-	+[10]	+	+	+			+	-	
MK-25		-	-	-	+	+	+		+	-	
MK-35		-	-	-		+	+			-	
A-26	-[4]	-		-	+	+	-			-	
Dessau		-				+				-	

Explanation to Table 3:

[1] 10 days old

[2] Last figure: number of passages in cell cultures

[3] After i.c. application

[4] Avirulent after i.c. application in 2-day-old piglets

[5] Only 2 animals used

[6] Occasionally in the liver

[7] Excretion after i.n. application only

[8] Occasionally virulent

[9] I.n. application not tested

[10] After i.n. application avirulent, after parenteral application virulent (Oirschot 1987 Vet.Rec.)

[11] Only in tonsils

[12] Virus multiplication after experimental infection of the fetus

Literature cited:
Bartha K 61: 47, 319, 346, 347, 348, 349, 350, 351, 352, 353. NIA-4: 120, 280, 354. BVK: 122, 268, 275, 294, 308, 319, 320, 321, 322, 323, 324, 325, 345, 355, 356, 357, 358, 359, 360, 361, 362, 363. Ercegovac: 327, 364. B-Kal 68: 275, 328, 364. MK-25/MK-35: 275, 333, 334, 353, 364, 365. A-27: 329, 330. Dessau: 331, 332.

Summarizing these results, neutralizing antibodies can be detected 2 wk after the application of live vaccines, but rather low serum-neutralization (SN) titres are induced. A booster effect occurs after a second vaccination 3 or 4 wk later, but nevertheless the SN titres are lower than after the application of inactivated vaccines (268, 273). Although intranasal application of live vaccines evokes lower SN titres than parenteral application, protection against nasal ADV infection is better (367, 368). Intranasal vaccination may induce local immunity in the oronasal mucosa and the respiratory tract. Several investigations reveal that SN titres are not regularly correlated with protection.

Vaccination with attenuated virus does not prevent field

virus infection and local field virus multiplication which can be followed by generalization. Field virus is excreted for some days until 2 wk, and the amount of virus shedded is high enough to infect other animals. Field virus latency is not prevented by vaccination (46, 47). Clinical symptoms of various severity can occur after field virus infection in vaccinated animals, mostly slight to moderate fever and slight anorexia, but the resulting loss of weight is much lower than in unvaccinated pigs. After infection a strong secondary antibody response is developed.

According to the results of Tatarov (334) immunity may start early after infection. He found protection on day 4 after vaccination. However, it is not certain if this is true for all the live vaccines. One can presume, however, that interferon production or genuine virus interference by the vaccine virus induces local resistance within a few hours. Long-term immunity after a single vaccination may last 4 to 6 mo, depending on the vaccine used and the genetic and individual conditions of the animal (372). There are great differences in the immunogenic and protective properties of the vaccines. In general, the immunogenic properties decline with the degree of attenuation. Accordingly, two vaccinations are recommended e.g. for BUK TK/900 and Bartha K 61. The percentage of protected animals fluctuates between 94% and 10% at the end of the fattening period (275, 328, 373). On the other hand, it was found that a potent inactivated vaccine evokes protection in about 67% of the pigs vaccinated once at this time (275).

Maternally derived protection lasted in pigs from BUK-vaccinated sows from at least 36 days (326) until 85 days (268). On the other hand, Vannier (271) could not detect maternal antibodies in piglets from A-26-vaccinated sows after 5 wk, whereas in pigs from sows vaccinated with an inactivated vaccine, maternal antibodies were detected up to 17 wk. The superiority of an inactivated vaccine to a live vaccine (K 61 Bartha) to evoke maternal protection in piglets was also reported by Andries et al. (276). Thus, in general live vaccines apparently induce weaker maternal immunity than inactivated vaccines, but differences exist

between different live vaccines.

However, this phenomenon can be of advantage, since the blockade of maternal antibodies on active immunity (345, 369, 370, 373, 374, 375, 376) is easier to overcome by vaccination. This effect is particularly prominent when live vaccines are inoculated intranasally (367, 370, 371). Nevertheless, the interfering effect of maternal immunity was still marked very often in 4- to 6-wk-old piglets and even demonstrable in 12-wk-old piglets (369, 370).

In summary, the advantages of live vaccines to inactivated vaccines are: lower costs, early protection, apparently better protection after a single vaccination, lower interference with maternal immunity, and no local adjuvant reactions at the site of inoculation. The disadvantages are: genetic heterogenicity and instability, iatrogenic transmission of the vaccine virus to other susceptible species, e.g. cattle, sheep and dogs (362, 377, 378), and weak maternal immunity. The evaluation of advantages and disadvantages depends on the point of view. If economic interests dominate the advantages prevail, if epidemiological aspects predominate the disadvantages must be considered.

It should be mentioned that a live vaccine (BUK-628) was also effective when mixed with an FMD and a swine fever vaccine (379).

Inactivated virus vaccines

Efficient, inactivated vaccines were not available until 1973 (for review see 85). At this time Škoda and Wittmann (85) developed a potent vaccine basing on ethyleneimine-inactivated, BHK-cell-adapted virus and DEAD dextran as an adjuvant.

After vaccination of pigs from 2 wk of age onward, neutralizing antibodies (Ab) were detected from day 7 up to 12 wk. Revaccination evoked a considerable booster effect when done 3 to 4 wk after the first vaccination. The Ab persisted until 18 to 20 wk after revaccination. After intracerebral challenge infection, 65% of the animals vaccinated once were protected 85 to 108 days post vaccination and 61% 137 to 217 days post revaccination (85, 266, 278, 380, 381). Vaccination did not prevent virus multiplication, virus distribution across the body and virus excretion after challenge, but clinical symptoms were strongly

reduced or missed (24). Vaccination during the incubation period of infection did not enhance the clinical course of AD (234). Neutralizing Ab were transmitted from vaccinated sows to their offspring (266). They interfered with active Ab production after the first vaccination of these piglets, but no interference occurred when the piglets were vaccinated at the age of 4 wk and revaccinated 2 wk later. However, partial interference was demonstrated when 2- to 3-wk-old vaccinated pigs were revaccinated (234). A disadvantage of the vaccine was the appearance of hypersensitivity of immediate type after vaccination and revaccination in some of the pigs, apparently evoked by anti-dextran Ab which had been present in pigs before vaccination (234).

Another effective inactivated vaccine was developed by a French research group in 1975 (383, 384, 385). They used IBRS-2 cell-adapted virus, inactivated by glutaric aldehyde supplemented by an oil adjuvant. This vaccine was by far superior to a vaccine which contained $Al(OH)_3$ and saponin as an adjuvant. Neutralizing Ab were detected in vaccinated pigs on day 15 post vaccination and titres increased until day 30. The vaccine evoked good protection in pigs after 4 wk. Ab were colostrally transmitted from vaccinated sows to their piglets which were protected against challenge at 3 wk of age. Bommeli (30) found good protection in pigs 3 wk post vaccination, but the challenge virus was excreted and isolated from the tonsils up to 35 days and detected by immunofluorescence for 51 days. Allergic reactions were detected by means of the skin test (385).

Since the IBRS-2 cells used for virus production are persistently infected with classical swine fever (CSF) virus, it was suspected that vaccinated animals may develop Ab against this virus and disturbances of serological surveys on CSF may occur. This could be confirmed by Jensen (386) but not by Reuss et al. (387).

Gutekunst (257) developed a vaccine which consisted of PK-15 cell adapted virus, inactivated by acetylethyleneimine and conjugated with lauric acid. This lipid-conjugated vaccine did not

evoke neutralizing Ab, but precipitating Ab were detectable on day 14 and the animals showed positive skin test reaction against AD virus. After challenge infection mild respiratory symptoms developed. By combining this vaccine with an Al(OH)-adjuvanted one, neutralizing Ab appeared at wk 2 and they persisted up to 14 wk. Challenge resulted in mild resporatory tract distress (28).

In recent years work was done on subunit vaccines. The virions were split by detergents (Nonidet P-40, Triton-X-100) and the resulting glycoprotein suspension was partly purified and concentrated and its immunizing capacity demonstrated in mice (388). Mixed with Freund's incomplete adjuvant the vaccine was tested in pigs (29). Neutralizing Ab appeared after 2 wk and they were boostered after revaccination. After challenge (4 wk post revaccination) the pigs did not shed virus, but since only 3 pigs were used, this finding is not convincing. Platt (389) developed a lectin-agarose base subunit vaccine, supplemented with incomplete Freund's adjuvant. The vaccine evoked low titres of neutralizing Ab after the first, but high titres after the second dose. The vaccinated pigs showed high protection after challenge at day 21 to 25, but they excreted virus.

Several inactivated vaccines have been developed on commercial base. They contain cell-culture-adapted virus inactivated by ethyleneimine or glutaraldehyde and complemented in most cases with oil adjuvants, in some cases with aluminum oxide.

From a series of publications (32, 42, 233, 266, 275, 276, 277, 278, 294, 317, 351, 352, 359, 390, 391, 392) the following conclusions can be drawn:

Pigs of all age can be vaccinated, however the immune response in piglets and young pigs is lower than in older pigs. Therefore, two vaccinations are recommended, 3 to 6 wk apart, to evoke optimal immunity. With breeding pigs and boars vaccination must be repeated every 6 mo or 4 to 6 wk before farrowing. From the practical and economic point of view, fattening pigs are usually vaccinated once when 8 to 10 wk old, accepting non-optimal degrees of immunity. Immunity after primovaccination develops within 2 wk, and after revaccination it lasts up to 5 to 6 mo.

This time is shorter after one vaccination only. The duration of immunity is also dependent on the quality of the vaccine and the individual reactivity of the animal. De Leeuw and van Oirschot (275) have reported that 67% of the pigs vaccinated once were protected at the end of the fattening period. Lenihan and O'Connor (392) examined five commercial, inactivated vaccines in pigs vaccinated at 2 to 4 mo of age and challenged 6 to 7 wk afterwards. The survival rates were 100%, 94%, 94%, 88% and 53% per vaccine. After the first vaccination, up to 10% of the vaccinated animals insufficiently respond to vaccination. In comparison to oil vaccines, vaccines containing aluminum adjuvant show a very low neutralizing Ab response after the first vaccination, revaccination improves the response. Indeed, Ab titres and protection are not correlated evidently (30, 47, 257, 266, 274, 275, 276, 277, 278, 279, 280), but field observations indicate that aluminum vaccines are less efficient than oil vaccines. All the published results indicate that the vast majority of animals with neutralizing Ab titres above 1:10 are protected, and Ab titres are the most reliable in vitro parameter of protection.

Vaccination of piglets and young pigs from vaccinated sows is unsatisfactory, since maternally derived Ab interfere with active immunit (266, 271, 282, 352, 369, 370, 375, 393, 394, 395). Maternally derived Ab could be demonstrated by the ELISA up to 12 wk (272) and by the neutralizing test up to 17 wk (271), however the protection level of Ab vanishes much earlier, namely after 4 to 10 wk, depending on the amount of Ab transferred. But the Ab blockade can be partially overcome by vaccination when the Ab titre has dropped to a low level, or by revaccination (266, 288). The first vaccination sensitizes the immune system and a secondary response develops after revaccination. Therefore, most of the vaccine producers recommend vaccination of pigs from vaccinated sows when 6 to 10 wk old and revaccination 4 to 6 wk later. According to our results (266, 282) passively immune piglets can be vaccinated when 3 to 4 week old and revaccinated 3 wk later.

Vaccinated pigs with optimum immunity are protected against

ADV infection only if they contact lower quantities of the virus, e.g. 10^4 $TCID_{50}$ intranasally. With larger quantities, virus multiplication takes place at the site of infection and the virus can be spread throughout the body to different organs, however, virus multiplication there is less than in non-vaccinated animals (19, 23, 351, 396). Slight clinical symptoms can appear and virus is shedded in nasal and pharyngeal fluids, though lower intensity of virus excretion ($10^{2.8}$ to $10^{4.3}$ $TCID_{50}$) and shorter duration (4 to 7 days) than with non-vaccinated animals can be observed (23, 264, 351). Nevertheless, the amount of totally shedded virus is high enough to infect non-vaccinated and vaccinated animals by contact (unpubl. results). Virus infection of the fetus (97, 397) and virus excretion in the semen (32) is apparently prevented by vaccination.

Virus infection of vaccinated animals can pass over to latency and the virus can be reactivated and excreted (42, 47). The same is true when passively immune piglets are infected (47, 398). Although the virus content in nasal swabs is low ($10^{0.1}$ to $10^{2.5}$ $TCID_{40}$) after revaccination, and no virus shedding is detected with some of the animals at all, the amount of totally excreted virus is sufficient to infect non-vaccinated and vaccinated pigs by contact (Wittmann, unpubl.).

Marked local adjuvant-evoked reaction can appear after the application of oil vaccines. Lesions, abscesses and long-lasting adjuvant residues are found at the site of vaccine application (muscles of the neck). These parts must be removed at meat inspection. Therefore, the use of oil vaccines for fattening pigs has strongly declined in the FRG. Another type of vaccine reactions is the appearance of hypersensitivity, mostly of the immediate type. This depends largely on the vaccine used and differs from one vaccine to another. According to field observations, vaccination should temporarily impair the fertility of boars, although Weitze and Gaus (399) could not ascertain this experimentally.

Simultaneous application of inactivated AD vaccine and live swine fever vaccine and erysipelas vaccine is effective (400,

401). The same is true for bivalent vaccine against ADV and porcine parvovirus (402).

Regulations concerning vaccines

In some countries, legislative measures for vaccination exist. Vaccination is generally forbidden in South Africa, Denmark, the United Kingdom except Northern Ireland, Luxembourg and in some States of the USA. The use of live vaccines is not allowed in Italy and in some States of the USA. In the Federal Republic of Germany the use of live vaccines is only allowed for non-breeding pigs.

Different regulations for vaccine testing exist in different countries. The European Pharmacopoeia Commission is preparing standard procedures for vaccine testing which cover the following points:

Live vaccine: The seedlot virus should be tested for the absence of contaminating agents (bacteria, mycoplasm) and extraneous viruses. It should be innocuous for piglets and pregnant sows, causing no abnormalities in the fetuses and the new-born piglets. Irreversiblility of attenuation should be ascertained by 6 consecutive passages in piglets. Identification of the seedlot should be tested with monospecific antiserum. Each vaccine batch should be tested for sterility, virus identity, extraneous viruses and innocuity in piglets. The virus titre should be ascertained. Potency should be tested in piglets by PD_{50}.

It must be mentioned that these are very preliminary proposals, especially for inactivated vaccines, and will be extended and more specified in the future.

GENETICALLY ENGINEERED VACCINES

The finding that the glycoprotein gI of ADV is both important in the determination of virulence and dispensable for virus replication (for details see paragraph "Molecular aspects") may be viewed as a milestone in the development of vaccines against AD. Since recent years, different wild-type and already existing attenuated ADV strains have been genetically engineered with the

aim to obtain safer, well-defined and efficient live vaccines.

Deletion mutants are constructed by the removal of the gI gene (or large parts of it). For this strategy of creating vaccine strains, the knowledge of the dispensability of other viral glycoproteins for ADV replication (gp63, gIII and gX; paragraph "Molecular aspects") opened further possibilites. Except for the presumed genetic stability (because of deletions), one major aspect of those vaccines is the possible serological differentiation between wild-type-infected and vaccinated animals. This is accomplished by the absence of expression of distinct glycoproteins in the vaccine strains, and the animals can be easily tested for the presence or absence of the specific antibodies. Such a serological survey opens new ways to combined vacciniation-eradication programmes, provided that after natural field infection detectable levels of antibodies against the corresponding glycoprotein are regularly present. Although this appears true for gI, gIII and gX, very thorough and extended investigations with field sera must be provided, because of the possible variable expression of glycoproteins at least in some ADV strains. In this context, the monoclonal antibodies used in those assays must be assured to be generally applicable for recognizing the corresponding glycoprotein of different strains and field isolates.

High expectations, particularly in Europe, are set on the use of gI- or gI- and gp63-deleted constructs for the use as live vaccines. In addition, a combined deletion/mutation in the viral thymidine kinase (tk) gene shall yield more improved avirulent strains, because of its suspected role in neural virulence and latency. Existing data show that the different gI-negative vaccine strains constructed are avirulent for pigs and protect against the disease as good as or even better than the existing conventional live vaccines. Slight variations between the different vaccines exist with regard to the amount and the duration of vaccine virus excreted after inoculation. However, contact infection has never been observed. Differences are found in the pathogenicity for different other species. Similar properties concerning safety and

immunogenicity display constructed mutants, where the gene coding for glycoprotein gX or for gIII has been deleted. Table 4 summarizes some characteristics of deleted vaccines against ADV, which are up to now registered and commercially available.

It seems that the constructed vaccine viruses tested (NOBI-PORVAC, TOLVID) cannot or only at a reduced rate persist in neural tissues of pigs. This is indicated by the absence of or very low amounts of viral DNA detectable in prednisolone-treated animals and the failure or rescueing latent vaccine virus (Rziha et al., unpubl.). Another important question is whether vaccination might prevent latency of wild-type ADV after superinfection. Some results indicate that vaccination can reduce the level of excretion of reactivated challenge virus (e.g. after TOLVID vaccination; L.E. Post and R. Wardley, pers. comm.). However, it remains to prove whether vaccinated pigs prevent wild-type ADV from becoming latent or whether the immunological status of the vaccinated pigs is able to prevent reactivation. It is also unknown whether the potential of vaccine virus to produce latency might be important to inhibit the establishment of challenge virus latency.

Although gI-deleted ADV vaccines look very attractive, in the present state of art it seems difficult to finally decide what kind of construct might be the most suitable one for future vaccination programmes. Finally, possible recombinational events between different mutant viruses and field strains are discussed. The frequency and consequence of those events in the field is not really known, but it has been reported that vaccine strains with different deletions can produce virulent recombinant ADV (403) in co-infected cell cultures. This points to a potential danger when using different vaccine strains in the same geographical area, since virulent recombinants could be produced in animals vaccinated with two different live virus vaccines.

Except for the efforts in constructing live vaccines, new subunit vaccines are also produced by expressing viral glycoprotein(s) in bacteria and eucaryotic cells. In this regard, the gp50 and gIII might be promising candidates (see paragraph

Table 4. Genetically engineered vaccines

NAME	Suvaxyn	Nobi-Porvac	Tolvid	Omnivac I	Omnimark	PRV-Marker
Manufacturer	Duphar	Intervet	Upjohn	Fermenta	Fermenta	Syntrov.
Parental strain	NIA-3	NIA-3	HR	BUK	BUK	IOWA
gI	-	-	+	+	+	+
gp63	+	+	+	+	+	+
gIII	+	+	+	+	-	+
gX	+	+	-	+	+	-
tk	-	-*	-	-	-	-
Virulence						
Mice	-	-	-	-	-	-
Chicks i.c.		-				
Rabbits i.m.		(+)	+	(+)	(+)	(+)
Calves i.m.		-	+	-		-
i.n.		-	-	-		
Sheep i.m.		-	+	-		-
i.n.			(+)			
Cats i.m.		-	+			
i.n.			-			
Dogs i.m.		+	+			
i.n.			+			
oral		-	-			

Legend to Table 4:

* tk-negative after chemical mutagenesis.

Virulence: i.c.: intracerebral; i.m.: intramuscular; i.n.: intranasal.
(+) reduced virulence as compared to wild-type virus.
For mice the data refer to various application routes as intraperitoneal, subcutaneous or intramuscular.

Where no indications are made, no data are available to the author.

"Molecular aspects"), however, more detailed information on the actual role of the ADV glycoproteins in the immune response has to be provided.

CONTROL AND ERADICATION

An essential precondition for the control of AD is the duty of notification. Possible measures on the infected farms are: removal of pigs only for slaughter; killing of afflicted pigs or of all pigs of the herd; vaccination of the herd; thermotreatment of meat and offal; removal of dead animals, aborted fetuses, stillborn piglets and afterbirths; non-use of semen for artificial insemination; disinfestation of rats; decontamination of dung, liquid manure and waste material; disinfection of implements, vehicles etc. and of thoroughfares, including entrance and exit of the pig-shed. Unauthorized persons, cats and dogs should be kept away from the pig-shed. Authorized persons have to disinfect and decontaminate their hands, clothing and shoes when leaving the pig-shed. The regulations for infected farms can also be applied to pig markets, pig exhibitions and pig transports, when an AD outbreak occurs there or when other animals than pigs on a farm die of AD.

If all the pigs of a herd are removed, the ban is lifted after disinfection of the premise. However, in many cases the infected herd is vaccinated and not depopulated on account of economic reasons. Usually, such a herd remains latently infected, but the restrictions must be cancelled to avoid overpopulation. Depending on the maximal period of virus excretion and incubation

time, 28 to 35 days should elapse between the last clinical case of AD in a vaccinated herd and lifting of the ban. Nevertheless, the following restrictions should be further imposed: continuation of vaccination until final sanitation and removal of pigs only for slaughter or for transfer into vaccinated herds.

Prophylactic vaccination is widely used for protecting non-infected pig herds in endemic areas. Vaccination should cover the whole infected area, as unvaccinated herds present favour virus spread. Introduced virus circulates within the vaccinated herd, since vaccination does not prevent subclinical and latent ADV infection (35, 247, 311, 404, 405). Therefore, each vaccinated pig should be marked to call a buyer's attention to the possible risk of introducing AD.

Besides, one has to keep in mind that not all pigs of a vaccinated herd are protected. Some vaccinated animals are unable to develop efficient immunity either on account of the individual conditions or for interference of maternally derived antibodies (Ab) with vaccination. Young pigs with high levels of maternal Ab are not vaccinated at all. When maternal immunity decreases they are susceptible to ADV.

Since vaccination only reduces clinical disease and therefore economic losses but does not prevent virus spread, AD cannot be eradicated only by vaccination in general. Furthermore, serological surveys are complicated by the impossibility to distinguish between vaccinated and infected animals, unless live vaccines or inactivated vaccines with stable genetic virus markers (e.g. gI^-, gX^-, $gIII^-$) are used. These vaccines do not induce Ab formation in vaccinated animals against the missing proteins. However, it must be guaranteed that the ELISA kits do not give false positive results with sera of repeatedly vaccinated animals on account of steric hindrance of anti-gI Ab attachment by the surplus of non-anti-gI Ab (van Oirschot, pers. comm.).

The final aim of the control of AD is its eradication. From the foregoing comments it is evident that eradication of AD can be best performed by slaughtering all seropositive animals and by strict control of pig movement. Such an eradication programme is

very expensive. Therefore, several sanitation programmes have been developed (270, 331, 405, 406, 407, 408, 409) basing on vaccination of the infected herd and rearing an unvaccinated seronegative offspring. However, these programmes are expensive and time-consuming. They take up to 3 yr, provided that good isolation facilities are present and any new introduction of ADV can be prevented. In fattening herds, properly controlled vaccination together with a culling programme may result in the eradication of the disease (410).

The eradication of AD in the German Democratic Republic (pig population 12 million) has been reported by Kretzschmar (300). Serological surveys of the pig population have been done since 1965. The seropositive herds were banned indefinitely and the pig trade was strictly controlled. Since 1970, all the seropositive herds were continuously vaccinated with live vaccine. These herds were considered to be ADV-infected. The non-vaccinated offspring was reared separately as far as possible. The procedure was very successful in small herds, somewhat less successful in herds up to 400 sows or 6,000 fattening pigs and unsuccessful in herds up to 5,000 sows or 25,000 fattening pigs. Therefore, in 1982 it was decided to slaughter all the seropositive animals on a farm, including the vaccinated ones, within a few months and replace them by seronegative pigs. About 250,000 pigs were slaughtered and replaced within in following 3 yr. The eradication programme was completed in autumn 1985, but in the meantime several new outbreaks had been recorded which were eradicated immediately.

The availability of vaccine strains with certain gene deletions may improve and facilitate eradication programmes. Since field virus strains and deleted virus strains evoke a different antibody pattern, it is possible to discriminate between vaccinated and latently infected pigs. The latter can be removed from the herd as soon as possible to avoid further virus spread. Vaccination of all the herds including the offspring and serological examination should be maintained until all the infected pigs are removed. An advantage of this procedure is that the offspring does not have to be separated.

The Netherlands intend to perform an eradication programme from 1989 to 1994. All the pigs shall be vaccinated with gI-deleted vaccines, breeding pigs for 3 times a yr, fattening pigs for 2 times per fattening period. Besides, all the breeding animals will be tested serologically in a corresponding ELISA, and random serum samples from slaughtered pigs will be examined. All latently infected (gI-positive) breeding animals will be removed as soon as possible. In fattening herds, intensive vaccination for years is expected to lead to disappearance of ADV infection, since virus shedding is significantly reduced and the animals are continuously slaughtered (van der Valk, pers. comm).

Which measures should be taken to prevent the introduction of ADV in an AD-free country? First of all, the trade in pigs must be carefully controlled. According to the O.I.E. International Zoo-Sanitary Code (article 3.4.3.1.), veterinary administration of importing countries should require for breeding pigs from non-vaccinated herds the presentation of an international zoo-sanitary certificate attesting that the pigs for export (a) come from a herd in which no clinical sign of AD has been observed for the past 12 mo; (b) were kept in isolation on the farm of origin during the 30 days before being moved to a quarantine station and subjected there to a sero-neutralization test or an ELISA giving negative results, and were clinically healthy; and (c) were kept in a quarantine station for 30 days before export and during this period, but not less than 21 days after the test required by (b), were subjected to a sero-neutralization test or an ELISA giving negative results. With pig products, the presentation of an international sanitary certificate is necessary attesting that the whole consignment of meat comes from animals slaughtered in an officially approved abattoir and found to be healthy both before and after slaughtering. However, this only excludes pigs with clinical AD but not inapparently infected animals. Since the presence of ADV in meat - but not in offal, bone marrow etc. - is apparently rather rare (15, 17, 21, 23), this proposal may be sufficient, especially when meat has been frozen at a temperature of about -18^{o}C for at least 40 days (15, 93). It is unknown,

however, whether this freezing period is sufficient to inactivate virus in bone marrow. One has further to keep in mind that ADV may be present in fresh rawmeat products like sausages (94).

A summary of the control measures in different countries is given in Table 2. The data are taken from the Animal Health Yearbook (297).

ECONOMIC CONSIDERATIONS

AD causes great economic losses. A French study (411) was conducted on 2 non-vaccinated farms which were quarantined after ADV infection. Each farm had 80 sows and produced piglets and fattening pigs. After infection, no killing of the infected animals and no vaccination was performed. The loss was estimated by the difference between the economic situation prior to and after infection. The minimum loss was calculated at FF 167,750, though only 3 sows and 5 fattening pigs died and 5 abortions occurred. The costs of AD per sow amounted to FF 1,000. The indirect costs from disturbance of the management (extended fattening period, small litters, return to heat etc.) were not considered. Thus, the real costs have been considerably higher.

In the UK, an economic study was performed in an infected 550-sow herd. The annual costs of AD per sow were estimated at £ 153 (412), and a similar study in a Danish sow herd resulted in a loss of $ 145 per sow/yr (413).

The collective losses in 16 farms in Iowa were estimated at $ 462,587 during 3 yr. Pilot studies conducted in the USA showed that total costs per infected herd were $ 2,441 in Wisconsin, $ 3,939 in Iowa and $ 12,622 in Pennsylvania (415). Total losses in the USA resulting from AD mortality and abortions were estimated at between $ 21.4 million and $ 25.6 million in 1977 (416).

In The Netherlands the costs of AD per year amount to HFL 35.0 million for vaccination. Export losses are estimated at HFL 30.0 million. The eradication programme in progress will cost about HFL 77.5 million for vaccination and HFL 11.0 million for serological surveys per year. That means total costs of about HFL 365 million

until 1994 (van der Valk, pers. comm.).

In the Federal Republic of Germany, DM 61.0 million were paid in compensation for killed animals from 1980 to 1982. North-Rhine Westphalia, a state of the FRG, having had 624 outbreaks in 1987, paid compensation of DM 1.6 million for cattle (no compensation for pigs), DM 13.5 million for vaccination and DM 0.17 million for diagnosis. The estimated economic losses amounted to DM 9.5 million. Total costs DM 24.8 million. In Lower Saxony 874 outbreaks were recorded in 1987. DM 3.4 million for compensation of pigs and DM 1.5 million for cattle were paid: vaccination afforded DM 7.3 million and diagnosis DM 0.76 million. The total costs including econimic losses amounted to DM 16.7 million.

The running eradication programme in the UK cost more than £ 30.0 million from 1983 to 1987 (417), though only 46 clinical outbreaks of AD had occurred in 1982 (418), but 400,000 seropositive pigs had to be slaughtered.

FUTURE ASPECTS

The presently used live vaccines will be gradually replaced by genetically constructed ones in the future. They will be identifiable either by the insertion of identification markers in their genome or by gene deletions. In any way, they must allow the serological differentiation between vaccinated and latently infected pigs, however, further improvement of the corresponding ELISAs is necessary to avoid false positive results. Furthermore, the filter disc ELISA will become most important for mass screening of sera, since the bleeding of pigs is much facilitated.

The use of genetically identifiable virus strains in live and inactivated vaccines, which allow identification of vaccinated animals, will enhance the eradication of AD in heavily infected countries by intensive vaccination of pigs in parallel to culling of infected animals. The high costs may, however, be a limiting factor in some countries. Increasing import restrictions for pigs will nevertheless compel exporting countries to get free of AD, since the economic loss will exceed the expenses for eradication. If the combined eradication programme works, AD may disappear in

Europe within the next 10 yr.

By this development, basic research on molecular biology and gene technology especially with regard to virulence genes, immunogenic glycoproteins (subunit vaccines) and virus recombinant vaccines will be further intensified. At present, it is unlikely that vaccinia virus recombinants with inserted ADV genes will by admitted for use in the field. Therefore, search for other virus vectors is urgent, e.g. porcine cytomegalovirus. On the other hand, highly attenuated ADV may act as vector for other porcine virus recombinant vaccines, however, their use is limited by AD eradication.

The release of genetically modified viruses in the environment is a general problem. ADV will be a good model for evaluation of any possible risks.

Special attention should be paid to the role of the hemopoietic system in pathogenesis and latency of AD and to the molecular events concerned with these processes. Lack of knowledge also exists in cell-mediated immunity, especially T-cell cytotoxicity, and in local antibody and interferon production. These will be further fields of virologists and immunologists. National and international co-operation will be needed for harmonization of serological tests and for eradication programmes. The same is true with regard to potency testing of vaccines in order to get comparable results.

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8

PORCINE CYTOMEGALOVIRUS (PCMV)

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INTRODUCTION

Cytomegalovirus infections are very common in many species (1,2,3,4,5). They induce host specific cytomegalic intranuclear inclusions in various organs. PCMV was first identified as inclusion body rhinitis (IBR) of pigs in Great Britain by Done and coworkers in 1955 (6). Obviously PCMV is inapparently present in most pig herds and causes generally mild disease in young pigs, only. Therefore, the disease is of low economical importance. A generalized fatal disease frequently occurs in gnotobiotic piglets (7,8), that could also be infected congenitally (9).

CLINICAL SYMPTOMS

If the disease appears at all, the piglets of about 2 weeks of age are predominantly affected. The animals show dyspnoea, sneezing, snuffling and anorexia. The discharge from the nostrils changes from glairy to purulent. Sometimes the animals establish paresis followed by death within 7 days. Respiratory embarrassment lasting for one month or more, impaired appetite and a marked check to growth are signs of a subacute course of infection. The average death rate is about 10%, but mortality can rise to 50%. Morbidity is virtually 100% in affected litters. In some gnotobiotic pigs death is sudden without previous clinical symptoms. If PCMV infection results in fatal generalized disease, the virus shows predilection for reticulo-endothelial cells (1,7). Glomerulonephritis is also detected in some cases (10). Nearly no clinical symptoms are observed in pigs inoculated later than 2 weeks of age. In sows, which are infected during pregnancy,

afebrile anorexia and lethargy may occur for a few days between 2 to 4 weeks post infection (p.i.), but no significant fluctuations in body temperature are recorded in sows, which are infected during pregnancy (7). PCMV is isolated between week 3 and 5 from nasal swabs and at week 5 p.i. from cervical swabs (11). Infection of pregnant sows results in increased numbers of mumified fetuses.

PATHOLOGY

The main gross lesions are found in the lungs with purple areas in the ventral parts of the apical cardiac and diaphragmatic lobes. Hemorrhages are found in the alveolar wall and the interlobular septae with scattered macrophage exudation. Intranuclear inclusion bodies are difficult to find in sections of the lung. Sometimes excessive pericardial exsudation is found. Numerous petechiae are detected in the slightly enlarged kidneys. The cortical capillaries are more affected than the medullary ones, and petechiae can also be detected in pigs without clinical signs. Histopathological lesions further occur in the sinusoidal cells of the liver and adrenal glands. The lymph nodes are enlarged, edematous and petechial (7,12). PCMV infection results in an exudative inflammation with basophilic intranuclear inclusion bodies, which are associated with a mild infiltration of lymphocytes and plasma cells and occasional neutrophils.

In contrast to infections with Bordetella nasal lesions are confined to the lamina propria (8). PCMV induces basophilic, intranuclear inclusions in the mucus glands and the duct epithelium of the nasal mucosa.

Some animals develop a multifocal nonpurulent encephalitis with meningeal infiltration and perivascular accumulation of lymphocytes and proliferating glial cells. These lesions are most commonly located in the choroid plexus or the vessels outside the cerebral cortex.

Extramedullary hematopoiesis is frequently encountered, as denoted by the presence of megakaryocytic giant cells and nucleated erythrocytes.

PATHOGENESIS

In utero infection is possible (9,13). In contrast to the fetuses, no PCMV is detected in the placental tissue (11). Some of the reared piglets die during the first week without showing macroscopic lesions of diagnostic value.

After experimental intranasal infection with PCMV and an incubation period of 10 to 20 days (14), gnotobiotic pigs develop viremia for about 3 days. During the acute stage of infection PCMV exceeds $10^{6.9}$ $TCID_{50}$/g of nasal mucosa and $10^{2.5}$ $TCID_{50}$/g of kidney (7). PCMV is usually shedded for 1 to 3 weeks, but excretion up to 10 weeks is possible (15).

Fatal infection with high mortality is observed under 2 weeks of age. The virus generalizes and both reticulo-endothelial cells and epithelial cells are infected. This fatal infection must be differentiated from asymptomatic, generalized disease affecting various epithelia with decreased sensitivity of the reticulo-endothelial cell system (7).

LATENCY

Latent infections with PCMV are described by several authors (8,16). Latent virus is reactivated by application of prednisolone, dexamethasone or corticosteroids. Mild to severe lesions are detected within 1 week. The distribution of lesions after reactivation is similar to that in experimental and natural infection.

It has to be presumed, that latent PCMV infection can be reactivated by stress during parturation, growding, transport, superinfections, e.g.

CHARACTERISTICS OF THE VIRUS

Pig cytomegalovirus belongs to the Betaherpesvirinae. It is a possible member of the murine cytomegalovirus group, and it is classified as suid herpesvirus 2 (17). Mature virus particles consist of a central core (30-70 nm in diameter), a nucleocapsid of 162 capsomers arranged as an icosahedron (90-120 nm in diameter) and an envelope (170-200 nm in diameter) with small

projections of about 10 nm in size (12,18,19,20).

There are a lot of PCMV-strains and isolates. Most of the experiments are performed with following strains: ADRI-1 (21), B4 (22), B6 (23), CID (24) und J1 (12). Different serotypes of PCMV and antigenic relationship to other herpesviruses are not known.

PCMV can be purified by continuous density gradient centrifugation on 10-70% (wt/vol) sucrose or on 10-50% (wt/vol) cesium chloride. Three bands are separated. Unenveloped infective particles peak at a density of 1.315, enveloped infective particles at 1.275 and together with numerous cell debris at 1.225 g/ml (12). Other physico-chemical properties as well as survival in natural environments are not well examined.

Desinfection should be performed as for other herpesviruses.

The host range of PCMV is restricted to pigs. PCMV is normally propagated in (gnotobiotic) piglets early after birth (12). Pigs are experimentally infected with PCMV infected cells or with tissue culture fluid.

PCMV replicates in pig lung macrophages (PLM), which are washed out from gnotobiotic pig lungs. Macrophages from PCMV immune pigs show very slow susceptibility to PCMV (23).

Swine testicle cells (ST) are also susceptible to PCMV, but the virus can not be passaged. Infected ST cells show only a few refractive swelling cells and small syncytia. (12). Strain specific differences in virus replication and virulence are not detected.

MOLECULAR ASPECTS OF THE VIRUS

The DNAs of Cytomegalovirus group viruses have MW of 130-150 x $10^{6.0}$. Nothing is known on molecular biology of PCMV.

DIAGNOSTIC PROCEDURES

Clinical symptoms can only be observed in piglets. Normally, sneezing is noticed in a few pigs of the herd. Infrequent coughing tends to replace the sneezing at about three months of age. Sometimes conjunctival exudate give rise to a ring of black discoloration around the eyes of affected pigs. Mucopurulent or

haemorrhagic nasal exudate is detected after secondary infections (15). Typical signs of atrophic rhinitis with atrophy of the turbinate bones are shown after superinfection with Bordetella bronchiseptica, Pasteurella multocida, e.g. (8,10,25).

Infected sows produce smaller litters with significant increase of mumified fetuses. Cytomegaly and intranuclear inclusions are detected in liver and lungs of the dead fetuses (11).

Histopathological examination shows intranuclear inclusion bodies (several small granules of 2 um in diameter or reticulate masses up to 23 um x 45 um) in infected cells which are detected by May-Gr nwald-Giemsa or acridin-orange (26). In perinatally infected piglets, inclusion bodies are detected in the lungs, liver, spleen, kidney and adrenal glands of nearly all infected individuals. The turbinates, tonsils, lymph nodes, thymus, thyroid, bone marrow and CNS are less affected.

Virus can be isolated from turbinate mucosa, lung macrophages, kidney and sometimes salivary gland (7). Swine testicle cell cultures can harbour the virus (16). At an age of 5-8 weeks most of the infected pigs excrete virus, and nearly 75% of the nasal swabs are PCMV positive. PLM are the most susceptible cells for virus isolation. For PCMV detection the probe is passaged 4 times in PLM. Finally the cells are examined by indirect immunofluorescence (IIF) or they are stained with Giemsa for the detection of intranuclear inclusion bodies (23).

PCMV antibodies are detected with the IIF-antibody test using cultures of infected swine testicle cells (16), PLM (27) or pig fallopian tube cells (24). Perinuclear immunofluorescence is shown with PCMV-positive sera. This test is much more sensitive than the neutralisation test (15). An ELISA test basing on antigen prepared in pig fallopian tube cells results in higher titres with 25% of the sera, if compared with the IIF-test, but both tests are statistically different (24).

Normally no PCMV antibodies are induced by intra-uterine infection (15) and therefore are not detectable in neonatal sera. Postnatal infections result in IIF-titres up to 1:1024, whereas

neutralizing PCMV-antibodies only reach ND_{50}-titres of 1:16. Gnotobiotic pigs can develop unspecific IIF-titres up to 1:8.

SMEDI and Pseudorabies virus infection are to be considered for differential diagnosis.

IMMUNOLOGY

The absence of PCMV antibodies of IgG or IgM class at birth suggests that either the piglets are immuno-tolerant or the degree of antigenic stimulation was inadequate to produce antibody at term (11). Evidence for the regular transfer of colostral antibody was obtained (15) and colostral antibodies give some protection to newborn piglets that are infected transplacentally with PCMV. Colostral PCMV antibodies decrease during the first 2 months of life. Piglets with congenital or neonatal infection show virus excretion without seroconversion. All the individual PCMV IIF-antibody titres decrease, but only few piglets have PCMV IIF-antibody titres of less than 1:4 after infection. Normally PCMV antibodies appear during the 3rd month and reach maximum titres at 5 to 6 months (15). IIF-antibodies are detected within 5 weeks after infection.

EPIZOOTIOLOGY

PCMV is prevalent among pig herds in European countries (6,15,28,29,30,31,32,33,34,35,36), Japan (37), Australia (38), New Zealand (39) the U.S.A. (40,41) and Canada (42). Normally 95-100% of the pig herds show PCMV antibody titres.

The major route of infection is the transmission through nasal secretions. Small amounts of virus are also found in oropharyngeal secretions and urine (15). Infection by transplacental or cervical transmission is possible. Therefore infection is spread by grouping of piglets, when they were weaned or transferred to fattening pens (11).

CONTROL

For PCMV is a porcine herpes virus, it has to be considered, that protection against clinical symptomes and financial losses

must not be correlated with protection against viral infection. There is no effective treatment and in most herds none is warranted. Antibiotics are used to reduce secondary bacterial infections, and vaccines against Bordetella bronchiseptica minimize the appearance of rhinitis atrophicans. Severe challenge to very young piglets is avoided by management procedures (43), such as minimizing stress factors and limiting the grouping of animals from different original herds.

FUTURE ASPECTS

No vaccine against PCMV is available, since PCMV replicates very slowly and the virus is not propagated to titres which are necessary to produce vaccines with good antigenicity. Genetically engineered vaccines may overcome this difficulties in future.

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