Virus Infections of Vertebrates

Volumes in the Series

VIRUS INFECTIONS OF RUMINANTS

Edited by

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Preface

The series "Virus Infection of Vertebrates" is intended to supplement the plethora of monographs devoted to individual viruses. It was the editors' aim to present a "zoocentric" approach instead of the "virocentric" course most authors follow. Information on infections of vertebrates is scattered over textbooks of veterinary medicine, where it has been selected mainly on the basis of clinical and (local) epidemiological importance. However, the concept of a series of books summarizing established knowledge on the viruses infecting animals within a taxonomic cluster (species, genus, family) was not developed with specifically the veterinary practitioner in mind; any non-veterinarian who produces, studies or employs animals for scientific purposes is a potential reader. The many authors contributing to the present volume have been advised to observe a pragmatic approach, with emphasis on essential and useful facts. Information leading to a diagnosis, to the interpretation of laboratory data, to prophylactic or other control measures was favored, whereas presentation of details on the history of a disease or molecular data was discouraged.

Consequently, this series is intended for the non-virologist as a work of reference as well as for the scientist as a first start to find out what the present state of knowledge on an infection is. The organization of the chapters is strictly hierarchical and follows virus taxonomy. Table 1 presents an overview of the virus families causing infections in ruminants. A short family description precedes the first chapter discussing an infection caused by a virus from this family. The chapters have been written either in an epic style, without subdivisions (when only few data were available) or in subdivisions dedicated to virus properties (physical and chemical characteristics, antigenic properties and cultivation), epizootiology, pathogenesis, disease signs, pathology (gross lesions and histopathology), immune reaction, laboratory diagnosis, prophylaxis and control; extensive literature references have been provided for further study. Pictograms symbolizing the subdivisions have been printed in the margins to facilitate the search. Overview chapters are included when syndromes have a multifactorial etiology (such as the respiratory and enteric infections of ruminants).

There are, of course, more viruses in cattle, sheep and goats than those discussed in this volume. I am not referring to the hitherto unknown agents but to viruses with little pathogenic significance or none at all. Viruses have also been encountered as contaminants in bovine cell culture but appear to cause no harm to the animal host. Electron microscopy of bovine tissue and fecal materials has revealed particles of virion morphology that await identification and classification (Moussa, personal communication, 1986). Infection of ruminants with arthropod-borne viruses has been evidenced by serology and, less

TABLE 1

Viral taxonomy and arrangement of subjects in the ruminants volume (numbers refer to pages)

Nucleic acid envelope	Family/ subfamily	Genus	Page
dsDNA, enveloped			
	Poxviridae/	*Orthopoxvirus*	1
	Chordopoxvirinae	*Parapoxvirus*	23
		Capripoxvirus	43
	Herpesviridae/		
	Alphaherpesvirinae	*Simplexvirus*	69
	Betaherpesvirinae		
	Gammaherpesvirinae		
dsDNA, nonenveloped			
	Adenoviridae	*Mastadenovirus*	159
	Papovaviridae	*Papillomavirus*	187
ssDNA, nonenveloped			
	Parvoviridae	*Parvovirus*	201
dsRNA, nonenveloped			
	Reoviridae	*Reovirus*	215
		Orbivirus	227
		Rotavirus	239
ssRNA, enveloped positive-sense genome			
	Togaviridae	*Pestivirus*	245
	Flaviviridae		279
	Coronaviridae		295
	Toroviridae		309
negative-sense genome			
	Paramyxoviridae	*Paramyxovirus*	317
		Morbillivirus	341
		Pneumovirus	363
	Rhabdoviridae	*Vesiculovirus*	379
		Lyssavirus	393
DNA step in replication			
	Retroviridae/		417
	Oncovirinae		
	Spumavirinae		
	Lentivirinae		431
ambisense genome			
	Bunyaviridae	*Bunyavirus*	465
		Phlebovirus	481
		Nairovirus	495
ssRNA, nonenveloped			
	Picornaviridae	*Aphthovirus*	501
		Enterovirus	513
		Rhinovirus	517
	Caliciviridae		519
	Astroviruses		523
Unclassified RNA viruses			
	Borna virus		529
Unconventional infectious agents			
	Scrapie agent		541

frequently, by virus isolation. The listing in Table 2 has been compiled from the International Catalogue of Arboviruses 1985 and gives an impression of the geographic distribution of arboviruses infecting bovines, sheep, goats and wild ruminants.

I should like to thank all contributors for their efforts, especially those who kept the deadlines. The volume editors Prof. Dr. Bror Morein and especially Prof. emerit. Dr. Zvonimir Dinter have done a fine job in having most of the manuscripts updated by the authors so that the ruminant virus infections monograph is the third to appear — after the Carnivore and Porcine volumes. A monograph on infections of avian species is presently being edited and volumes on rodents and lagomorphs, equines, and fishes are in preparation.

Marian C. Horzinek

TABLE 2

Viruses infecting ruminants as evidenced by isolation (I), serology (S) or experimental infection (E)

Virus	Family	Serogroup	Continent	Detection
Colorado tick	Orbi	Colorado	America	S
Bunyip Creek		Palyam	Australia	I, S
CSIRO Village			Australia	I, S, sentinel (bovine)
d'Aguilar			Australia	S
Marrakai			Australia	I, S
Vellore			Asia	S
EHD		EHD	America	S, E
Ibaraki			Asia	I, S
Eubenangee		Eubenangee	Australia	S
Kemerovo		Kemerovo	Europe	S
Seletar			Asia	S
Tribec			Europe	I, sentinel (goat)
Mitchell River		Warrego	Australia	S
Warrego			Australia	S
Chobar Gorge		ungrouped	Asia	S
Orungo			Africa	S
Getah	Toga	Alpha	Australia	S
Middelburg			Africa	S
Ndumu			Africa	I
Ross River			Australia	E
Sagiyama			Asia	S
Absettarov	Flavi	TBE	Europe	I, S, E
Hypr				S, E
Banzi		Uganda S	Africa	S
Kokobera		B	Australia	S
Kumlinge			Europe	S
Kunjin			Australia	S
Kyasanur Forest disease			Asia	S, E
Murray Valley encephalitis			Australia	E
Negishi			Asia	E
Omsk haemorrhagic fever			Asia	S, E
RSSE			Asia	E
Saboya			Africa	S
Stratford			Australia	S
Usutu			Africa	S
Wesselsbron			Africa	I, S

(Table continued overleaf)

TABLE 2, contd.

Viruses infecting ruminants as evidenced by isolation (I), serology (S) or experimental infection (E)

Virus	Family	Serogroup	Continent	Detection
Chandipura	Rhabdo	Vesiculo	Asia	S
Piry			America	S
Kotonkan		Lyssa	Africa	S
Adelaide River		BEF	Australia	I, S, sentinel (bovine)
Berrimah			Australia	I
Kimberley			Australia	S
Joinjakaka		ungrouped	Australia	S
Tibrogargan			Australia	S
Aino	Bunya	Simbu	Australia	S
Buttonwillow			America	S
Douglas			Australia	S
Ingwavuma			Asia	S
Peaton			Australia	I, S
Sabo			Africa	I, S
Sango			Africa	S
Shamonda			Africa	I, S
Shuni			Africa	I, S
Tinaroo			Australia	S
Inkoo		California	Europe	S
Tahyna			Europe	S
Batai		Bunyamwera	Asia, Europe	S
Bunyamwera			Africa	E, S
Cache Valley			America	S
Calovo			Europe	S (roe deer, elk), E
Germiston			Africa	S
Lokern			America	S
Maguari			America	S
Main Drain			America	S
Tlacotalpan			America	S
Pongola		Bwamba	Africa	S
Uukuniemi		Uuku	Europe	S
Congo		Nairo	Africa	I, E
Crimean hemorrh. fever			Asia, Africa Europe	S, E
Dugbe			Africa	I, S
Ganjam			Asia	I
Bhanja		Bhanja	Africa, Europe	I, S
Salehabad		Phlebo	Asia	S
Toscana			Europe	S
Zinga			Africa	S
Silverwater		Kaisodi	America	S
Trubanaman		Mapputta	Australia	S
Belmont	Bunya-like		Australia	S
Upolu			Australia	S
Thogoto	Orthomyxo		Africa	I, S
Ungrouped unclassified				
Batken			Asia	S
Ngaingan			Australia	S
Wongorr			Australia	S

BEF: bovine ephemeral fever. EHD: epizootic hemorrhagic disease of deer. RSSE: Russian spring summer encephalitis.
Source: International Catalogue of Arboviruses 1985.

Introduction

This volume is devoted to viral infections and diseases of ruminants, of which cattle and sheep deserve particular consideration. They nourish a vast majority of the human population, and their skin and wool are important to the clothing industry. In many countries great effort is therefore required to overcome contagious viral diseases such as foot-and-mouth disease (FMD) and rinderpest — epizootics that may threaten the entire national stock of cattle — and bluetongue, which is transmitted by biting flies (*Culicoides*) and is a considerable menace to rearing of sheep. Countries that are free, or mostly free, of FMD aim to eradicate infectious bovine rhinotracheitis (IBR) and bovine leukosis. Large collectives of feedlot calves are exposed to infections with viruses that cause enzootics, such as adenoviruses, parainfluenza-3 virus and bovine viral diarrhoea virus (BVDV). Transmitted from carriers, these viruses — either as solitary agents or as participants of mixed infections — initiate respiratory and/or enteric disease, often aggravated by secondary bacterial invasion. Neonatal calf diarrheas, caused by rotaviruses, toroviruses or a coronavirus, are an additional menace. The pathogenesis of mucosal disease is still an unresolved, intriguing problem. Based on development of immunotolerance towards noncytopathic biotypes of BVDV during fetal life, mucosal disease during postnatal life is caused by cytopathic biotypes, presumably mutants of the former. The economic losses by enzootics are difficult to estimate but may reach levels that make farming less profitable.

Apart from economics, there is another important aspect to viral infections of ruminants: that of comparison with human disease. Bovine leukemia virus, discovered in 1969, proved to be precedent of human T-lymphotropic leukemia and lymphoma viruses, described during the early 1980s, when the era of human retrovirology began. And maedi-visna virus of sheep, with its restricted replication in vivo, is the prototype of lentiviruses, one of which is the human immunodeficiency virus (HIV). A remarkable coincidence was the recent report that a bovine lentivirus, first described in 1972, proved to be antigenically related to HIV. Furthermore, our understanding of the etiology of Creutzfeldt-Jakob disease was inconceivable without the study of scrapie of sheep and its "unconventional virus" after successful transmission to mice and hamsters.

In the present volume, the steadily growing number of reports on development of rapid procedures had to be considered in describing viral diagnosis; however, virus isolation and identification by traditional methods is still the "gold standard", particularly for index cases of epizootics and enzootics. These methods should not preclude parallel application of rapid tests for detection of viruses via their antigens, or of antibodies to them, by various modifications of enzyme-linked immunosorbent assays (ELISA); and monoclonal antibodies, if

prepared against well-chosen epitopes, do facilitate identifiation of a virus or differentiation of antigenically closely related viruses. Subtypes of FMD virus are now identified in field samples by ELISA and no longer by the less sensitive complement-fixation test. However, the question whether or not FMD outbreaks had derived from old standard strains, present in the vaccines, could only be answered when gene sequences encoding VP1 of the isolates from outbreaks were compared to corresponding sequences of old vaccinal strains. Recently, rapid detection of IBR virus, a herpesvirus, in clinical specimens from infected cattle was achieved by dot-blot hydridization with probes selected and amplified by recombinant DNA technique.

The present volume was edited in a time of transition from identification of viruses as phenotypes (antigens) to their identification as genotypes (genomes), which also will influence future classification.

For viral proteins as "protective antigens", an appropriate way of presenting them to the body's defence system was studied, resulting in a construct called "immunostimulating complex (iscom)". An iscom prepared with a separated envelope glycoprotein of IBR virus protected cattle against disease experimentally induced by the virus and secondary infection with *Pasteurella haemolytica*. Immunizing oligopeptides from VP1 of FMD virus, even synthetic ones, are studied as constituents of future subunit vaccines, and so on. Thus, even with regard to the preparation of vaccines against viral diseases of ruminants, there are new, promising avenues of approach.

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Abbreviations

ADCC – antibody-dependent cytotoxicity
AGID – agar gel immunodiffusion
AH – arthrogryposis-hydranencephaly
BAD – Borna disease
BAV – bovine adenovirus
BD – border disease
BEF – bovine ephemeral fever
BEV – bovine enterovirus
BF (A, I, K, L, S, T) – bovine fetal (adrenal, intestine, kidney, lung, spleen, testicle) cells
BHV – bovine herpesvirus
BIV – bovine immunodeficiency virus
BLV – bovine leukemia virus
BMV – bovine mamillitis virus
BPS – bovine papular stomatitis
BPV – bovine papilloma virus
BPoV – bovine parvovirus
BRD – bovine respiratory disease
BRSFV – bovine respiratory syncytium forming virus
BTV – bluetongue virus
BVD–MD – bovine viral diarrhea–mucosal disease
BVD – bovine viral diarrhea
CAE – caprine arthritis-encephalitis
CAM – chorioallantoic membrane
C(BVD) – cytopathogenic (BVD)
CF – complement fixation
CHV – caprine herpesvirus
CIEP – counterimmunoelectrophoresis
CMI – cell-mediated immunity
CNS – central nervous system
CPE – cytopathic effect
DEA – diffuse early antigen (MCF)
DFV – deer fibroma virus
EAV – equine arteritis virus
EBL – enzootic bovine leukosis
EEPV – European elk papilloma virus
EIA – equine infectious anemia
EID50 – 50% egg infectious dose
ELISA – enzyme-linked immunosorbent assay
EM – electron microscope, electron microscopy

F – fusion protein (paramyxovirus)
FMD – foot-and-mouth disease
FTLV – feline T-lymphotropic lentivirus
GPV – goatpox virus
H – hemagglutinin (paramyxovirus)
HA – hemagglutination
HADEN – hemadsorbing enteric (virus)
HCV – hog cholera virus
H&E – hematoxylin and eosin
HI – hemagglutination inhibition
HIV – human immunodeficiency virus
HSV – herpes simplex virus
IBP – infectious balanoposthitis
IBR – infectious bovine rhinotracheitis
i.c. – intracerebral
ID – immunodiffusion
IEM – immuno electron microscopy
IF – immunofluorescence
IgG, IgM – immunoglobulin G, M
IIP – indirect immunoperoxidase
i.n. – intranasal
i.p. – intraperitoneal
IPV – infectious pustular vulvovaginitis
i.v. – intravenous
JSRV – jaagsiekte retrovirus
LD50 – 50% lethal dose
LDV – lactic dehydrogenase virus
LRS – lymphoreticular system
LSD – lumpy skin disease
MCF – malignant catarrhal fever
MD – mucosal disease
MMTV – mouse mammary tumor virus
MN – micro-neutralization
MNC – mononuclear cells
MPMV – Mason-Pfizer monkey virus
MVV – maedi-visna virus
N – neuraminidase (paramyxovirus)
NC(BVD) – noncytopathogenic (BVD)
NI – neutralization index
NSD – Nairobi sheep disease
OAV – ovine adenovirus
OPV – ovine papilloma virus
PAGE – polyacrylamide gel electrophoresis
PEA – particulate early antigen (MCF)
PFU – plaque-forming unit
p.i. – post infection
PI-3 – parainfluenza type 3
PMN – polymorphonuclear (leukocytes)
PN – plaque-neutralization
PPR – peste-des-petits-ruminants
PR – plaque reduction
PRV – pseudorabies virus
RBC – red blood cell
RIA – radioimmunoassay
RSV – respiratory syncytial virus

RVF – Rift Valley fever
SA(MCF) – sheep-associated (MCF)
S-antigen – soluble antigen (BAD)
SBL – sporadic bovine leukosis
SN – serum neutralization
SPV – sheeppox virus
STLV – simian T-lymphotropic lentivirus
SV – simian virus
TCID50 – 50% tissue culture infectious dose
VN – virus neutralization
WBV – Wesselsbron virus
WD(MCF) – wildebeest-derived (MCF)

Poxviridae

The name of this family is derived from the plural of old English "poc", meaning a vesicular skin lesion. The family contains six genera, of which members of the *Orthopoxvirus*, *Parapoxvirus* and *Capripoxvirus* genera (sub-family Chordopoxvirinae) occur in ruminants. The total number of poxviruses demonstrated in vertebrates is in excess of 30.

Poxviruses are the largest of all viruses. The particle may measure 220–450 × 140–260 nm and can thus be seen by light microscopy. The virion is brick-shaped (*Orthopoxvirus*), oblong (*Capripoxvirus*) or ovoid (*Parapoxvirus*). The surface structure of the virion is of diagnostic relevance: parapoxvirions

TABLE 3

General properties of orthopoxviruses of domestic animals*

Characteristics	Vaccinia	Cowpox	Buffalopox	Camelpox
Isolated from	man	cow, man, large felines	buffalo, man	camel
Pock on chick embryo chorioallantois	large white to grey/pale haemorrhagic ulcer	haemorrhagic	large opaque/ white/pale haemorrhagic ulcer	small opaque white
Ceiling temperature on chorioallantois of chick embryo (°C)	41.0	40.0	38.5	38.5
Rabbit skin lesions	different strains give variety of lesions	indurated with purple centre	erythematous red nodule	some strains produce transient nodule, whereas others do not produce any lesion
Pathogenicity				
Baby mice	high	high	high	low
Adult mice	+	+	−	−
Chick mortality	very high	high	medium	very low–low
Antigenic specificity to				
Vaccinia	+	+	+	+
Variola	+	?	+	+
Monkeypox	−	?	−	−
Polypeptide pattern	character of vaccinia	character of cowpox	character of buffalopox	character of camelpox
Thymidine kinase activity induced by orthopox viruses: sensitivity to inhibition by thymidine triphosphate (TTP)	resistant to inhibition by TTP	resistant to inhibition by TTP	resistant to inhibition by TTP	resistant to inhibition by TTP

*From: Pandey, R., Kaushik, A.K., Grover, Y.P., 1985. Biology of orthopoxvirus infections of domestic ruminants. Prog. Vet. Microbiol. Immun., 1: 199–228. Reprinted with permission from the publisher (Karger, Basel).
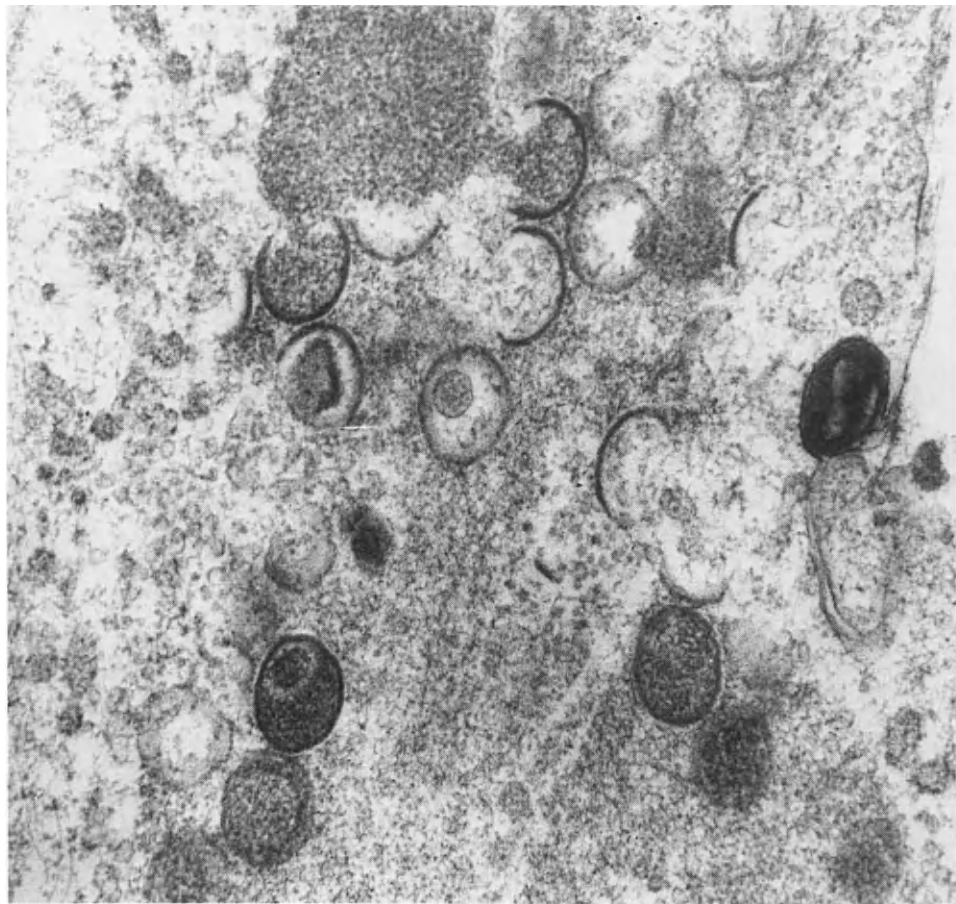
Fig. 1. Vaccinia virus viroplasm with "empty shells", immature and complete particles (× 60 000).

show a pattern of coiled parallel strands on their surface whereas members of the other two genera possess irregular filaments. Orthopoxviruses have been studied in detail. They have an external coat containing lipid and enclosing one or two lateral bodies and a core; the outer envelope is easily lost during manipulation. The biconcave core contains the nucleocapsid which, in turn, encloses a single molecule of double-stranded DNA with a mol.wt. in the range between 150 and 240 × 10^6. Virus particles are built from more than 30 structural proteins and several viral enzymes concerned with nucleic acid synthesis and processing, including a DNA-dependent transcriptase.

Multiplication occurs exclusively in the cytoplasm (in special viroplasma "factories", Fig. 1) and is accompanied by the formation of inclusion bodies. Mature virus particles are released from microvilli or by cellular disruption. Genetic recombination has been reported within genera.

Some properties of poxviruses infecting ruminants are summarized in Table 3.

Chapter 1

Vaccinia Virus

A. MAYR and C.-P. CZERNY

INTRODUCTION

Vaccinia virus received its name from the Latin word "vacca" (= cow) after it was isolated from cowpox in 1796 by E. Jenner; since then, it has been cultured uninterruptedly in laboratories (in animals, chick embryos and cell cultures) to produce smallpox vaccine. It is not possible to establish whether this virus was a genuine cowpox virus or a naturally occurring vaccinia virus. The animal poxvirus used by Jenner almost 200 years ago for the first human vaccinations was probably a cowpox virus whose original strain no longer exists. Passages may have changed its original biological properties (Mayr and Danner, 1979). Due to this process, virus reservoirs may have come into existence similar to those discussed in connection with the genuine cowpox virus in carnivores and rodents (Arita and Breman, 1979; Mayr and Danner, 1979; Marennikova et al., 1984; Webster and Jefferies, 1984; Bennett et al., 1989).

It is important to note that vaccinia virus is widespread in animals and causes local affections and generalized disease (Mayr, 1976); also, it can be transmitted from affected animals to humans, where it can cause manifest infection (zoonosis). Virulent vaccinia virus strains are particularly dangerous to immunosuppressed persons where generalized vaccinia may be fatal. Vaccinia virus infections in animals must therefore be considered as dangerous (Herrlich, 1965; Hassan et al., 1970).

Vaccinia virus can be transmitted to virtually all mammals (Baxby, 1977); of the ruminants, sheep, goats, camels and buffaloes are particularly susceptible.

VIRUS PROPERTIES

Vaccinia virus is the type species of the genus *Orthopoxvirus*. Within the genus all viruses are interrelated as found by cross-protection experiments. Ectromelia virus shows the lowest degree of relationship (Müller et al., 1978; Mayr et al., 1983).

Physical and chemical characteristics

Vaccinia virus is labile to fat solvents (chloroform, ether) and at pH values below 7 and above 9. The optimum pH is around 7.6. Under natural conditions, dry scabs can remain infectious for several weeks (3–6 weeks in the tropical environment). Cell-free virus is inactivated in 1–2 h at +56°C. When lyophilized, it remains stable for unlimited periods at temperatures of +4°C and below. Suitable disinfectants are formalin (1–3%), detergents, acids and all virucidal disinfectants.

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Antigenic properties

Vaccinia virus is serologically uniform. It includes approximately 17 different antigens which can be divided into three groups: (1) heat-labile antigens (L-antigens); (2) heat-stable antigens (S-antigens); (3) nucleoprotein antigens (NP-antigens).

All three antigen complexes induce antibodies which can be detected by complement-fixation, precipitation, ELISA and RIA. The NP-antigen cross-reacts widely with other poxviruses (in some cases even with representatives of different genera).

The antigens which are responsible for the induction of protective immunity are localized exclusively in the virus coat. They give rise to virus-neutralizing and hemagglutination-inhibiting antibodies. These antibodies cross-react with other viruses within the *Orthopoxvirus* genus, which makes it possible to differentiate between the vaccinia virus cluster and the viruses belonging to other genera of the Poxviridae family.

Cultivation

Small experimental animals (baby mice, rabbits, guinea pigs, rats, chick embryos) and a large number of cell cultures are suitable for propagation of vaccinia virus. Isolation, using the chorioallantoic membrane of embryonated eggs, is the preferred method for diagnosis, since the nature of the primary foci permits differentiation from other orthopoxviruses. The broad cell spectrum (virtually all cell cultures from mammals and birds are susceptible) is of additional assistance in the diagnostic work. Replication is usually accompanied by a CPE, leading to cytolysis after 3–5 days. The virus harvests contain up to $10^{7.0}$–$10^{8.5}$ $TCID_{50}$/ml (Mayr, 1966; Mayr et al., 1972; Mayr et al., 1983).

Vaccinia virus was initially propagated in calves (dermovaccine). For vaccine production, it was also propagated in donkeys, sheep, goats, rabbits and subsequently in chick embryo cell cultures. The attenuated vaccinia virus strains have been obtained by repeated passage in cell culture. They are considerably less virulent for humans and animals, and their ability to induce protective immunity against smallpox remains unchanged. The prototype for these attenuated vaccine strains is the MVA strain (Mayr et al., 1978).

Mutations leading to reduced virulence are frequently associated with deletions in the DNA molecule. The genome of the MVA strain, for instance, is 9% shorter than that of the Elstree WHO reference strain (Mayr et al., 1978; Altenburger et al., 1989). "White cowpox" variants also occur as a result of a deficit in terminal sequences of the red cowpox virus genome, affecting approximately 16–18 × 10^6 daltons, or 11.4% of the genome (Archard and Mackett, 1979).

The most important biological characteristics which can change spontaneously affect the virulence, host range, behaviour in the host, thermostability, and the formation of hemagglutinin. Vaccinia virus mutants that do not form a hemagglutinin have repeatedly been isolated from infected animals.

Mutants possessing particular markers can be obtained as a result of treatment with chemicals. More than 50 mutants of this type have been described.

EPIZOOTIOLOGY

During the era of smallpox vaccination in humans, vaccinia infections in ruminants, as in other animal species, originated from farm children after primary vaccination. The local pustules become dry and fall off as scabs; in

them, vaccinia virus survives for a long time. It finds its way into dust and spreads in the form of airborne particles. However, it is primarily transmitted from humans to animals by direct contact and to a certain extent also by biting flies and other arthropods.

Small lesions on the skin and mucous membranes promote transmission. In the population, an infected animal with manifest disease may spread the virus, which thereby adapts to the species through animal-to-animal passages. The spread amongst animals is via the oral and aerogenic routes and by arthropods, resulting in an increase in virulence. However, foci of vaccinia virus in ruminants remain always locally restricted. The virus does not spread in an epidemic manner, since transmission only takes place by direct contact. The termination of worldwide smallpox vaccination may eventually lead to the disappearance of vaccinia virus infections in animals. Alternatively, a new situation may arise with vaccinia virus adapted to animal species that hitherto did not support its growth. However, also in these instances direct contact will probably remain the most important means of transmission. This applies both to transmission between animals, and between animals and man.

PATHOGENESIS AND PATHOLOGY

Vaccinia virus infections in ruminants can develop as local affections, generalized disease, and as latent infections. Localized affections are most common. Lesions develop on restricted areas of the mucous membranes, skin or on other organs. A generalized disease develops in immunosuppressed animals and in those with impaired nonspecific infection defense mechanisms. It arises as a result of a massive propagation of the virus in the primary target organs and progresses through a viremia, resulting in lesion on the skin, the mucous membrane and in inner organs. Latent vaccinia infections are rare. The virus occasionally persists in lymphoreticular tissue without being secreted.

The typical vaccinia pustules which develop on the skin and the mucous membranes pass from efflorescences (stadium maculosum) into papules (stadium papulosum), small blisters (stadium vesiculosum) and subsequently into pustules when necrosis of the blisters' central region (pox navel) occurs. The pustules dry up and fall off as scabs after several days.

In the inner organs, a localized proliferation initially occurs at the point where the virus is multiplying, followed by a ballooning degeneration and subsequent lysis of the affected cells.

Histologically, intracytoplasmic A- and B-type inclusion bodies (Guarnieri's bodies) can be detected. Both types of inclusion bodies constitute the viroplasm zones where virus replication takes place. The acidophilic A-type inclusion bodies are viroplasm zones in an advanced stage of virus development, in which mature particles dominate. The basophilic B-type inclusion bodies are zones in which early virus development takes place.

DISEASE SIGNS

The disease manifests itself as local affections in most instances. Typical vaccinia pustules with a wide central navel (pox navel) form on the mucous membranes, the skin of the muzzle, the udder and, rarely, in the genital tract. The incubation period is 2–3 days. After the eruption phase it takes about 5 days for complete development of the pustules. If there is no secondary bac-

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terial infection, the pustules dry up within days and fall off after one week. The animal's general state of health is not affected.

The generalized form of the infection begins with general signs of disease, e.g. anorexia, restlessness and salivation. In contrast to genuine cowpox, high fever also occurs. The incubation period is between 3 and 7 days. When the fever has subsided, pox pustules (secondary and tertiary pustules) gradually appear over the whole body. The areas most susceptible to infection are the mucous membranes of the muzzle (ulcerative stomatitis) and the genitals, and the hair-free areas of the scrotum and udder. Complications are acute ulceration and mastitis. In the absence of complications, the generalized form in cows progresses rapidly and the symptoms vanish. Infections with genuine cowpox virus take a more protracted course.

Vaccinia virus has an affinity to fetal organs; therefore, abortion and fetal malformation must be expected to occur in susceptible animals. In addition, complications such as those following pox immunization in humans can occur, e.g. the development of opportunistic infections (Keane et al., 1983). Newborn and young calves are most at risk, as vaccinia infection easily becomes generalized. In the past, young animals and dairy cows were often afflicted by outright enzootics. Animal caretakers and their families frequently became infected (Hassan et al., 1970).

The prognosis is good in most cases. The disease is lethal in only 1–2%; however, in calves the mortality can rise to 20–30% when generalized complicated forms of the disease develop.
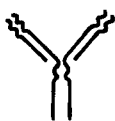
IMMUNE REACTION

Animals acquire a long-lasting immunity after recovery. This pertains both to the localized disease and to systemic disease of a cyclic pattern.

Protection is based on cellular and humoral immunity. Before the development of detectable immunity (between 3 and 8 days p.i.), a nonspecific resistance against various infections develops (as early as 8–10 h p.i.).

Cellular immunity begins 3–5 days p.i. It can be detected by delayed hypersensitivity reactions in the skin using an intracutaneous inoculation test; reactivity disappears after 2–3 months.

Specific serum antibodies appear 6–8 days after infection. They are detected in complements-fixation, precipitation, HI and VN tests. The hemagglutination-inhibiting and neutralizing antibodies are directed against surface proteins and are considered to prevent the development of systemic disease. They last for over a year, whereas the antibodies directed against other virus proteins disappear after 2–3 months.

The humoral immunity is transferred from the mother to the newborn animals via the colostrum.

LABORATORY DIAGNOSIS

The preferred method is EM detection of the typical vaccinia virus particles in fresh samples of skin and mucous membrane pustules (Fig. 2). In case of doubt it is necessary to propagate the virus on the CAM of embryonated eggs which have been pre-incubated for 10 days (appearance of primary pocks) or in cell cultures (type of cytopathic effect, immunofluorescence). Difficulties arise in distinguishing vaccinia virus from cowpox virus and also from other related poxviruses in ruminants.

A simple and rapid method of distinction is cutaneous inoculation (feather
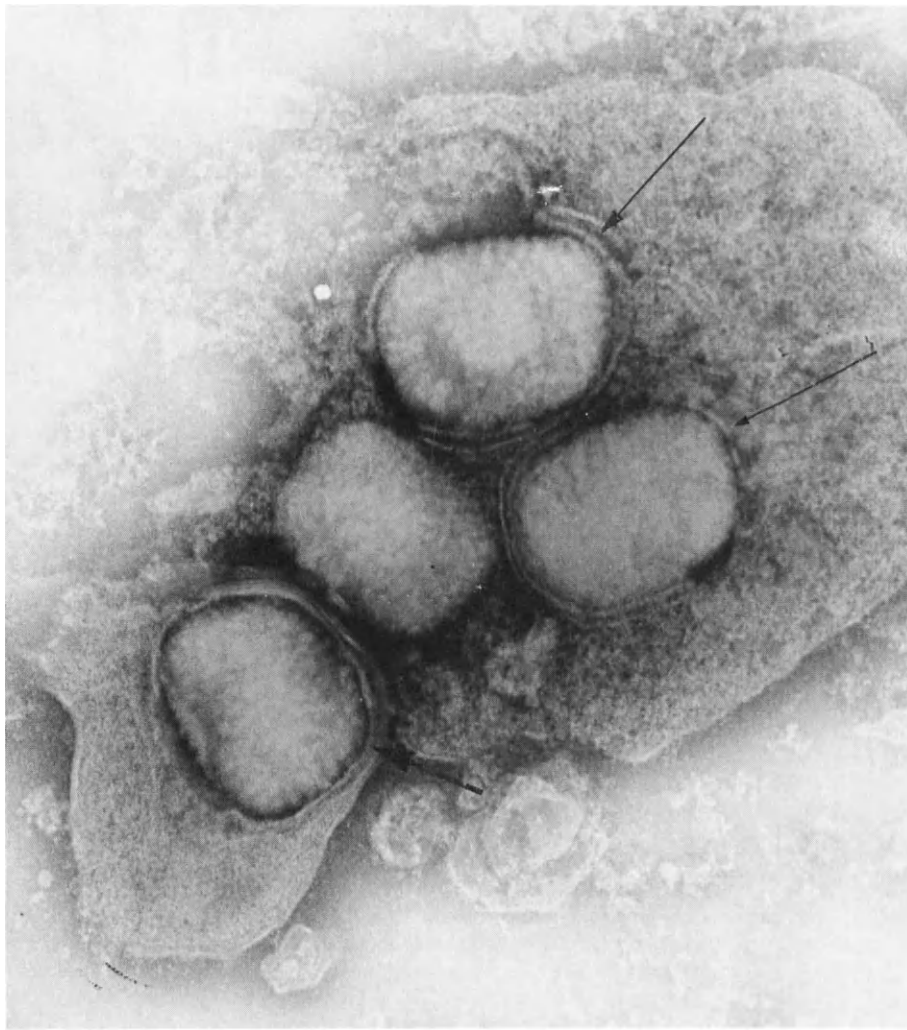
Fig. 2. Vaccinia virus: complete particle with additional membranes originating from the cell membrane.

follicle method) of chickens. Vaccinia virus strains cause typical localized pocks at the site of inoculation whereas cowpox virus strains lead to no reaction whatsoever (Mayr, 1966; Mayr et al., 1972). New methods of differentiating between both viruses are hybridization and restriction enzyme analysis of the DNA. It is possible to distinguish the virions from parapoxviruses by electron microscopy, by pock morphology on the CAM, in laboratory animals and cell cultures, and by immunofluorescence and other serological methods.

Indirect diagnosis via antibody detection is possible in individual cases by examining paired sera (HI, neutralization). For diagnosis in livestock, it is sufficient to confirm the presence of antibodies in a few animals.

Clinical methods are not sufficient for an etiological diagnosis of the disease. Cowpox, milker's nodes, parapox diseases (stomatitis papulosa, ecthyma), stomatitis vesicularis, sheep- and goatpox, bluetongue, panaritium, mastitis and occasionally foot-and-mouth disease and mucosal disease have to be excluded.

PROPHYLAXIS AND CONTROL

There is no specific therapy. Symptomatic treatment is intended to prevent secondary bacterial infections. Young and pregnant animals can be protected

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against infection by the administration of immune serum. Immune serum has no therapeutic effect.

Vaccination with live virus vaccines is the preferred method of prophylaxis. Only attenuated vaccinia virus strains may be used as vaccines; virulent viruses, e.g. the Elstree strain, present a risk. The attenuated vaccinia virus strain MVA has proved to be harmless and has good immunogenic properties; a dose should contain at least $10^{7.0}$ $TCID_{50}$ of vaccinia virus. Application is by intracutaneous injection, scarification or subcutaneous injection. Vaccines of this kind are also suitable for use in healthy animals in infected herds as an emergency measure (Mayr, 1976; Mayr et al., 1978).

REFERENCES

Altenburger, W., Süter, C.-P. and Altenburger, J., 1989. Partial deletion of the human host range gene in the attenuated vaccinia virus MVA. Arch. Virol., 105: 15–27.

Archard, L.C. and Mackett, M., 1979. Restriction endonuclease analysis of red cowpox virus and its white pock variant. J. Gen. Virol., 45: 51–61.

Arita, I. and Breman, J.G., 1979. Evaluation of smallpox vaccination policy. Bull. World Health Org., 57: 1–9.

Baxby, D., 1977. Poxvirus hosts and reservoirs. Brief review. Arch. Virol., 55: 169–179.

Bennett, M., Gaskell, R.M., Gaskell, C.J., Baxby, D. and Kelly, D.F., 1989. Studies on poxvirus infection in cats. Arch. Virol., 104: 19–33.

Hassan, A. Abdel Wahab, M.F., Farid, Z., Imam, Z.E., Kent, D.C. and Labib, A., 1970. An outbreak of vaccinia in thirteen Egyptian slaughterhouse labourers in Cairo. J. Trop. Med. Hyg., 73: 51–53.

Herrlich, A., 1965. Die Pockenschutzimpfung. In: A. Herrlich (Editor), Handbuch der Schutzimpfungen. Springer-Verlag, Berlin, Heidelberg, New York, pp. 60–312.

Marennikova, S.S., Shelukhina, E.M. and Efremova, E.V., 1984. New outlook on the biology of cowpox virus. Acta Virol., 28: 437–444.

Mayr, A., 1966. Eine einfache und schnelle Methode zur Differenzierung zwischen Vaccine- und Kuhpockenvirus. Zentralbl. Bakteriol. I Orig., 199: 144–151.

Mayr, A., 1976. TC-Marker des attenuierten Vaccinia-Impfstammes "MVA" in menschlichen Zellkulturen. Zugleich ein Beitrag zur Schutzimpfung gegen Orthopox-Krankheiten beim Tier. Zentralbl. Veterinärmed. B, 23: 417–430.

Mayr, A. and Danner, K., 1979. Aufhebung der Pflichtimpfung gegen Pocken. Münchner Med. Wochenschr., 50: 1675–1678.

Mayr, A., Mahnel, H. and Munz, E., 1972. Systematisierung und Differenzierung der Pockenviren. Zentralbl. Veterinärmed. B, 19: 69–88.

Mayr, A., Stickl, H., Müller, H.K., Danner, K. and Singer, H., 1978. Der Pockenimpfstamm MVA: Marker, genetische Struktur, Erfahrungen mit der parenteralen Schutzimpfung und Verhalten im abwehrgeschwächten Organismus. Zentralbl. Bakeriol. Hyg. I Abt. Orig. B, 167: 375–390.

Mayr, A., Eissner, G. and Mayr-Bibrack, B., 1983. Vaccinia Virus. In: Handbuch der Schutzimpfungen in der Tiermedizin. Paul Parey, Berlin, Hamburg, pp. 739–740.

Müller, H.K., Wittek, R., Schaffner, W., Schümperli, A., Menna, A. and Wyler, R., 1978. Comparison of five poxvirus genomes by analysis with restriction endonucleases Hind. III, Bam I and EcoRI, J. Gen. Virol., 38: 135–147.

Webster, J. and Jefferies, J.R., 1984. Cowpox in cats. Vet. Rec., 11: 151.

Chapter 2

Cowpox Virus

A. MAYR and C.-P. CZERNY

INTRODUCTION

The term "cowpox" is somewhat indiscriminately applied to all diseases in cows where a local or generalized pox exanthema of the mucous membranes and skin occurs, especially on the udder (udder pox). Etiologically these diseases are not uniform; the agents involved are parapoxviruses (stomatitis papulosa, milker's node, ecthyma), vaccinia virus and the genuine cowpox virus.

Only the disease caused by cowpox virus should be designated as cowpox. The disease pictures include on the one hand pustulous local lesions restricted to particular areas of the animal's body, e.g. the muzzle, udder, teats or scrotum, and on the other hand systemic disease of a cyclic nature, with pocks covering the whole body.

The cowpox disease is as old as variola, but it was recognized only fairly late. The origin and first outbreaks of cowpox are unknown. It is assumed that the bovine epidemics of ancient times described as "smallpox", were, in fact, outbreaks of cowpox. When Edward Jenner applied a suspension from the pustulous infected skin of a cow to a person and proved that this treatment protected against smallpox (1796), he showed that a pox disease occurring in cows is related to a similar disease in man. It will remain unknown whether the orthopoxvirus experimentally transmitted from cowpox at that time was the genuine cowpox virus as it has been isolated in recent decades, or whether it more closely resembled vaccinia virus.

Cowpox had a purely economic significance during the period in which smallpox vaccination took place. It occurred sporadically, only occasionally reaching epidemic proportions. Since the termination of worldwide compulsory vaccination against smallpox, however, cowpox has become important again, in view of the danger of its transmission to humans as a zoonosis (Mayr and Danner, 1979).
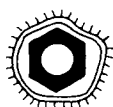
VIRUS PROPERTIES

Physical and chemical characteristics

The tenacity of the cowpox virus resembles that of vaccinia virus. This also applies to the use of disinfectants.

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Antigenic properties

Cowpox virus belongs to the Poxviridae family, Chordopoxvirinae subfamily and to the *Orthopoxvirus* genus. It resembles vaccinia virus in size, shape (Fig. 2), composition, chemicophysical properties and autonomous multiplication in "viroplasm zones". Immunologically, cowpox virus is a single serotype. It cross-reacts with the variola and vaccinia viruses as well as with the other orthopoxviruses. It is possible to differentiate cowpox virus from other orthopoxviruses by the character of primary foci on the CAM of chick embryos, the host spectrum and its behaviour in various cell cultures. More recently, serological tests with the aid of monoclonal antibodies are used, as well as DNA-analysis by restriction enzymes and hybridization. However, differentiation tests of this kind are only applicable to field strains. The differences become slight in laboratory strains which have been passaged and attenuated in cell cultures or which have become adapted by passage through other animal species; this applies in particular to the differentiation between the vaccinia and cowpox viruses. Common methods of differentiating between orthopoxviruses of animal origin are listed in Table 3.

Cowpox virus is closely related to the poxviruses of elephants, buffalos and other zoo animals but differs relatively much from camelpox virus.

Cultivation

For cultivation of cowpox virus the CAM of 10–12-day-old chick embryos and a large number of cell cultures are suitable (Mahnel, 1974). The preferred methods are inoculation of the CAM and propagation in secondary cell cultures of fetal bovine lung and kidney. Hemorrhagic foci with a central necrosis between 1.5 and 2 mm in diameter arise on the CAM after 2–3 days. The proliferative phase of focus formation is highly accentuated, lasting somewhat longer than in vaccinia. Correspondingly, the central lysis occurs slightly later. The mesoderm of the CAM is enlarged by infiltrating cells. Stasis and thrombosis in the terminal blood vessels and considerable damage to the vessel walls accompanied by diapedesis are characteristic of the primary cowpox foci. After 60–72 h, the infection becomes generalized throughout the CAM along the blood vessels. The generalization includes the embryo and its internal organs. During this stage the embryo dies. After generalization, titers between $10^{7.5}$ and $10^{8.5}$ EID_{50}/ml can be found in the CAM.

Repeated passages of cowpox virus can lead to the appearance of "white mutants" which resemble vaccinia foci on the CAM (Herrlich et al., 1967). Such mutants can occur in virtually all orthopoxviruses; they have been described for simian and rabbit poxviruses. Mutants are always the result of terminal deletions in the viral DNA (Archard et al., 1984).

Virus replication in cell culture is accompanied by a CPE. It begins with rounding of the cells and results in lysis, cell fusion and formation of syncytia. Optimum virus harvests contain $10^{7.0}$–$10^{8.5}$ $TCID_{50}$/ml.

Of the common laboratory animals, rabbits (cutaneous route, mildly affected, only partly hemorrhagic), guinea pigs (cutaneous infection), rats (cutaneous infection), and mice (intracerebral, parenteral infection) are susceptible to cowpox virus. In newborn animals, parenteral infection can lead to generalization, with fatal consequences.

Cowpox virus can be distinguished from the camelpox and vaccinia viruses by inoculation of chickens and rabbits. Details are given in Table 3.

EPIZOOTIOLOGY

Cowpox is transmitted both directly and indirectly. The most likely means of transmission is direct contact. The virus passes into the environment by way of pustules from the skin and mucous membranes which have dried up and peeled off, ocular secretion and secretions of the upper respiratory and digestive tract. It enters the body principally through broken skin, but can also be contracted from dust containing the virus via the mucous membranes of the respiratory and digestive tract. Occasionally, a large number of animals may contract the infection while grazing. Cowpox can often also be transmitted indirectly, e.g. during milking (via the milk pail, humans or animals; Ghosh et al., 1977). In addition, different invertebrates can spread cowpox virus in a purely mechanical way. Intrauterine transmission can occur in the course of generalized disease.

A large number of domestic animals and animals kept in zoos are susceptible to cowpox virus. Among the ruminants, sheep, goats, camels and buffalos in particular contract cowpox. Cowpox virus is also transmitted to humans, where it may cause local affections in the form of pocks on the hands, arms and face after direct contact with pustule material, e.g. during milking. Systemic disease with fever, lymphangitis, lymphadenitis, conjunctivitis and disseminated pox exanthema may also develop. Fatal cases of meningo-encephalitis have been reported (Herrlich et al., 1967). In recent years, the disease has occurred in humans with no contact with cattle. It is therefore suspected that reservoirs of cowpox virus exist in other animal species. The presence of a natural reservoir in rodents is discussed (Marennikova et al., 1984).

Cowpox can cause epidemics with a periodic occurrence. Epidemics of this kind are known from Germany (intervals of 10 years), England, France, Poland and the USSR. In the 1950s, epizootic cowpox in cattle occurred in Holland, Great Britain, France and Brazil (Baxby, 1977). Then, between 1962 and 1972, only five cases were reported in these countries. Consequently, the epidemiology of cowpox has not yet been explained (Marennikova et al., 1984). In many cases the sources of infection cannot be traced. The possibility that animal species other than ruminants may serve as reservoir hosts became a topic of discussion at the very beginning of research. In 1962, Dixon described the periodic outbreaks as "mysterious". After 1970, cowpox virus infections attracted more attention. Five okapis contracted the disease at a zoo in Rotterdam. Cheetahs in a British zoo, and elephants belonging to travelling circuses in Germany, Austria, Poland and England were found infected; the virus was also isolated from kangaroos, rhinoceroses and dolphins (for references, see Pandey et al., 1985).

A new epidemiological aspect emerged when cowpox infections were observed in various carnivorous species (Felidae); in some cases in a generalized form, they were found in cats, leopards, lions, ocelots, cheetahs, jaguars, and the like (Webster and Jefferies, 1984; Bennett et al., 1989; Mahnel et al., 1989).

In the search for a virus reservoir, infections in laboratory rats were discovered. The virus which was first isolated from the lungs of diseased rats was given the name "pneumotropic virus of white rats" (Krikun, 1974), but was later characterized as cowpox virus. Further investigations showed that infections with viruses similar to cowpox virus also occur in wild rats (*Rattus norvegicus*). They take an inapparent course but may lead to systemic diseases (pneumonia, exanthema).

The virus is excreted with urine and feces. Serological tests (hemagglutination-inhibition and neutralization) confirmed the presence of cowpox infection in various rodent species. It is possible to differentiate cowpox from ectromelia (mousepox), which is caused by the only rodent poxvirus known.

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Fig. 3. Cowpox lesions on teats.

Marennikova et al. (1984) concluded that various species of wild rodents are a hidden natural reservoir for cowpox virus, maintaining the chain of infection. The final hosts are ungulates, carnivores and humans.

PATHOGENESIS AND PATHOLOGY

The pathogenesis of cowpox resembles that of vaccinia virus infections. Besides local affections with pustule formation on the muzzle, udder and

scrotum, generalized systemic disease occurs, which often takes a more severe course. Systemic forms of infection have a cyclical pattern, affecting the skin and mucous membranes, internal organs and the central nervous system. They are the result of virus replication in organs of primary affinity (bone marrow, liver) following viremia. Latent infections are conceivable, but serological tests have not yet provided reliable proof. Interpretation of the test results is difficult due to the immunological cross-reactions with vaccinia virus.

In pathological–anatomical terms, cowpox is a typical pox disease with pustulation of the skin and mucous membranes, and disseminated foci in the internal organs (pin-sized, whitish), in particular in the lungs, liver, kidneys and lymph nodes. In severe cases, hemorrhagic diathesis occurs, leading to petechiae and extensive ecchymosis. Hemorrhagic exudates are found in the pleural cavity and the pericardium. Hemorrhages occur above all in the liver, spleen, renal capsule and central nervous system.

Histologically, cytoplasmic inclusion bodies (acidophilic) are present in the infected tissue. In contrast to vaccinia inclusion bodies, they are large, compact and less granular, and occur only in small numbers. Inclusions produced by cowpox virus in cell cultures have been analyzed by Patel et al. (1986).

DISEASE SIGNS

The incubation period generally lasts for 3–7 days; it is normally shorter for vaccinia than for cowpox virus infections. The disease begins with moderate fever which remains unnoticed in many cases. The experimental infection of bovines with vaccinia virus results in a fever higher than 40.5°C as early as 3 days after inoculation, lasting for about 10 days. In contrast, after cowpox virus infection, the body temperature does not rise above 39.5°C before the 4th day p.i. The temperature curve reaches its peak on the 8th day. Shortly after or at the same time as the fever rises, pocks appear on the udder, teats and testes, and only very rarely on other parts of the body. Infection of the udder is mostly confined to the teats (Fig. 3). The pustules reach maturity after 8–11 days, then dry up into a scab. Infection with cowpox virus is frequently accompanied by hemorrhagic symptoms resulting in scabs with a darker color than those occurring after vaccinia virus infection.

Localized pox lesions usually take a benign course unless the tops of broken pustules become infected by bacteria and abscesses or ulcers form. The generalized infection, however, leads to severe disease characterized by generalized manifestations on the skin, mucous membrane and internal organs (Fig. 4). The pocks are often hemorrhagic.

High fever and heart and circulatory complications are the dominant symptoms. In the advanced stage of the disease, central nervous system disorders become apparent. Abortions occur in pregnant animals. As a result of secondary bacterial infections, acute inflammation of the udder and ulceration of the affected areas of skin and mucous membranes occur.

The prognosis is favourable for the localized form of the disease. In systemic disease, when hemorrhagic symptoms are involved, mortality can reach 50%. Young animals are more seriously affected than older ones.
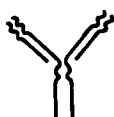
IMMUNE REACTION

When animals have recovered, they possess a protective immunity lasting for several years. The immunity is more pronounced and lasts longer than after vaccinia virus infection.

Fig. 4. Generalized cowpox.

Protective immunity is determined by humoral and cellular factors. Cellular immunity measured by delayed hypersensitivity disappears faster than the humoral immunity. In general, cellular immunity can be detected for up to a year after exposure. The protective humoral immunity is considered to be based on antibodies detectable by HI and VN tests. These antibodies appear to prevent a generalized, systemic disease and last for between 2 and 3 years. Newborn animals receive maternal antibodies via the colostrum.

LABORATORY DIAGNOSIS

The diagnosis of cowpox resembles that of vaccinia virus infection. Differentiation between the cowpox and vaccinia viruses is difficult. The methods for differential diagnosis are listed in Table 3.

In clinical terms, only a tentative diagnosis is possible. Infections involving vaccinia virus and parapoxvirus must be excluded by virological and serological methods. The following diseases are important for differential diagnosis: stomatitis vesicularis, bluetongue, panaritium and mastitis, and occasionally foot-and-mouth disease and bovine diarrhea/mucosal disease.
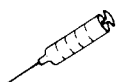
PROPHYLAXIS AND CONTROL

There is no specific therapy for cowpox. The localized skin and mucous membrane inflammations are treated symptomatically (iodine- and halogen-based ointments and acidic solutions, sprays or embrocations). Secondary bacterial infections are treated with antibiotics. In most cases it is too late for

serum therapy, which does not prevent the local affections from developing into a generalized systemic disease.

The preferred method of prophylaxis makes use of live virus vaccines for healthy animals after an outbreak has started in a herd or a zoo (emergency vaccination). Only attenuated vaccine strains should be used whose virulence for cows has been reduced. Attenuated vaccinia strains used as vaccines have proved innocuous; the MVA vaccinia strain is recommended (Mayr, 1976; Mayr et al., 1978). It became attenuated through numerous cell culture passages and has been tested over a period of several years. For primary vaccination, two parenteral injections at an interval of 3–4 weeks are recommended. In case of emergency one injection is sufficient. Re-vaccination after 2–3 years should be sufficient. The MVA vaccines should contain at least $10^{7.0}$ $TCID_{50}$ per dose to ensure a high degree of efficacy.

REFERENCES

Archard, L.C., Mackett, M., Barnes, D.E. and Dumbell, K.R., 1984. The genome structure of cowpox virus white pock variants. J. Gen. Virol., 65: 875–886.

Baxby, D., 1977. Poxvirus hosts and reservoirs. Arch. Virol., 55: 169–179.

Bennett, M., Gaskell, R.M., Gaskell, C.J., Baxby, D. and Kelly, D.F., 1989. Studies on poxvirus infection in cats. Arch. Virol., 104: 19–33.

Dixon, C.W., 1962. Smallpox. Churchill, London, 512 pp.

Ghosh, T.K., Arora, R.R., Sehgal, C.L., Ray, S.N. and Wattal, B.L., 1977. An investigation of buffalo-pox outbreak in animals and human beings in Dhulia District (Maharashtra State). 2. Epidemiological studies. J. Communicable Dis., 9: 93–101.

Herrlich, A., Mayr, A. and Munz, E., 1967. Die Pocken. G. Thieme Verlag, Stuttgart, 2nd edn., 295 pp.

Krikun, V.A., 1974. Isolation of pneumotropic virus from white rats during outbreak of a disease of unknown etiology in an animal farm. Conference "Laboratornye Zhivornye v meditsinskikh Issledovaniyakh", Academy of Medical Sciences, Moscow, Abstracts, pp. 96–97 (in Russian).

Mahnel, H., 1974. Laboratordifferenzierungen der Orthopockviren. Zentralbl. Veterinärmed., 21: 242–258.

Mahnel, H., Czerny, C.-P. and Mayr, A., 1989. Nachweis und Identifizierung von Pockenvirus bei Hauskatzen. J. Vet. Med. B., 36: 231–236.

Marennikova, S.S., Shelukhina, E.M. and Efremova, E.V., 1984. New outlook on the biology of cowpox virus. Acta Virol., 28: 437–444.

Mayr, A., 1976. TC-Marker des attenuierten Vaccinia-Impfstammes "MVA" in menschlichen Zellkulturen. Zentralbl. Veterinärmed. B, 23: 417–430.

Mayr, A. and Danner, K., 1979. Aufhebung der Pflichtimpfung gegen Pocken. Münchn. Med. Wochenschr., 50: 1675–1678.

Mayr, A., Stickl, H., Müller, H.K., Danner, K. and Singer, H., 1978. Der Pockenimpfstamm MVA: Marker, genetische Struktur, Erfahrungen mit der parenteralen Schutzimpfung und Verhalten im abwehrgeschwächten Organismus. Zentralbl. Bakteriol. Hyg. I Abt. Orig., 167: 375–390.

Pandey, R., Kaushik, A.K., Grover, Y.P., 1985. Biology of orthopoxvirus infections of domestic ruminants. Prog. Vet. Microbiol. Immunol., 1: 199–228.

Patel, D.D., Pickup, D.J. and Joklik, W.K., 1986. Isolation of cowpox virus A-type inclusions and characterization of their major protein component. Virology, 149: 174–189.

Webster, J. and Jefferies, J.R., 1984. Cowpox in cats. Vet. Rec., 114: 151.

Chapter 3

Buffalopox Virus

A. MAYR and C.-P. CZERNY

Clinically, buffalopox resemble vaccinia virus infections and cowpox. They mostly take the form of local pox exanthema (pustulation with central necrosis) on the muzzle, udder, teats or scrotum. In individual cases, buffalopox can also develop as a severe systemic disease of a cyclical pattern.

Buffalopox was first described in India (Ramkrishnan and Anathapadmanabham, 1957; Maqusood, 1958; Singh and Singh, 1967). It was contemporary with smallpox epidemics and the vaccinia virus vaccination programs. Vaccinia virus vaccines were initially produced in buffaloes (dermo-vaccine) in India (Hobday et al., 1961). Buffaloes cannot be infected with variola virus —only man and apes are susceptible. Cases of buffalopox have meanwhile also been reported from other countries (Mathew, 1967; Tantawi, 1974; Tantawi et al., 1977). A connection with smallpox immunization of humans has been excluded, but infection with cowpox virus is discussed (Tantawi et al., 1979). There is a possibility that the vaccinia and cowpox viruses have adapted to the buffalo via several passages through highly susceptible animals.

As far as its epidemiology and potential virus reservoirs are concerned, the same considerations as for cowpox apply. The epidemiology of buffalopox should be reconsidered some 10–20 years after the termination of smallpox immunization in humans.

Buffalopox virus is currently classified as a separate species of the *Orthopoxvirus* genus (Baxby and Hill, 1971; Mayr et al., 1972). Its size, shape, structure (Bloch and Lal, 1975), physico-chemical properties and replication in "viroplasm zones" resemble those of the cowpox and vaccinia viruses. It is immunologically uniform and cross-reacts both with vaccinia and cowpox, as well as with other orthopoxviruses. It can be distinguished from cowpox and vaccinia viruses by double ID, CF, immunoelectrophoresis and neutralization tests. It is serologically more closely related to the cowpox and vaccinia viruses than to the other orthopoxviruses (Gispen, 1955; Rondle and Dumbell, 1962). However, its biological property differs slightly from those of the cowpox and vaccinia viruses (Mathew, 1970; Baxby and Hill, 1971; Dogra et al., 1978).

Buffalopox virus replicates in a wide range of cell cultures and in the chick embryo. Cell cultures of bovine origin and of chick embryos are most frequently used for virus propagation that is accompanied by a cytopathic effect.

As regards the pathogenesis, pathology and histology (formation of cytoplasmic inclusion bodies) buffalopox resembles vaccinia and cowpox.

After recovery the animal is protected by both cell- and antibody-mediated immunity. Neutralizing, hemagglutination-inhibiting and precipitating antibodies may be detected. Following experimental infection, neutralizing and precipitating antibodies appear after approximately 12 days. The maternal antibodies are transferred to the newborn animals via the colostrum.

Although buffalopox does not occur very frequently, the disease is economically important in countries where buffalo are reared. It may be transmitted to humans who are in close contact with affected buffaloes (Ghosh et al., 1977).

Prophylactic control is possible with a live virus vaccine based on an attenuated vaccinia vaccine strain. The program resembles that of cowpox prophylaxis. Such vaccines can also be used to protect animals in an infected herd.

REFERENCES

Baxby, D. and Hill, B.J., 1971. Characteristics of a new poxvirus isolated from Indian buffaloes. Arch. Ges. Virusforsch., 35: 70-79.

Bloch, B. and Lal, S.M., 1975. A study of the ultrastructure of the buffalo pox virus. Acta Pathol. Microbiol. Scand., 83B: 191-200.

Dogra, S.C., Sharma, V.K. and Pandey, R., 1978. Susceptibility of infant white mice to buffalo-pox virus. Vet. Rec., 102: 382-383.

Gispen, R., 1955. Analysis of poxvirus antigens by means of double-diffusion. A method for direct serological differentiation of cowpox. J. Immunol., 74: 134-141.

Ghosh, T.K., Arora, R.R., Sehgal, C.L., Ray, S.N. and Wattal, B.L., 1977. An investigation of buffalopox outbreak in animals and human beings in Dhulia district (Maharashtra State). 2. Epidemiological studies. J. Communicable Dis., 9: 93-101.

Hobday, R.L., Rao, A.R., Kempe, C.H. and Downie, A.W., 1961. Comparison of dried vaccine with fresh Indian buffalo-calf lymph in revaccination against smallpox. Bull. World Health Org., 25: 69-71.

Maqusood, M., 1958. Generalisierte Büffelpocken. Vet. Rec., 70: 321-322.

Mathew, T., 1967. Virus study of pox disease among buffalo. Indian J. Pathol. Bacteriol., 10: 101-102.

Mathew, G., 1970. Comparative studies on buffalopox, vaccinia and cowpox in hamster kidney cell cultures. Acta Virol., 14: 513.

Mayr, A., Mahnel, H. and Munz, E., 1972. Systematisierung und Differenzierung der Pockenviren. Zentralbl. Veterinärmed. B, 19: 69-88.

Ramkrishnan, M. and Ananthapadmanabham, K., 1957. An experimental study on the buffalo pox virus. Indian Vet. J., 34: 23-30.

Rondle, C.J.M. and Dumbell, K.R., 1962. Antigens of cowpox virus. J. Hyg. (Lond.), 60: 41-44.

Singh, I.P. and Singh, S.B., 1967. Isolation and characterization of the aetiological agent of buffalo pox. J. Res. Ludhiana, 4: 440-448.

Tantawi, H.H., 1974. Study of poxvirus isolated from buffaloes in Egypt and India. Veterinarija, Moscow, 8: 119-121.

Tantawi, H.H., Fayed, A.A., Shalaby, M.A. and Skalinsky, E.I., 1977. Isolation, cultivation and characterization of poxviruses from Egyptian water buffaloes. J. Egypt. Vet. Med. Assoc., 37: 15-23.

Tantawi, H.H., Fayed, A.A. and El Sherif, M.T., 1979. Experimental inoculation of buffaloes with cowpox, buffalopox and vaccinia viruses. Bull. Anim. Health Prod. Afr., 27: 101-104.

Chapter 4

Camelpox Virus

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INTRODUCTION

Camelpox is a systemic disease of a cyclic pattern. A typical pox exanthema appears over the entire body, and on the head in particular. The first symptom is usually swelling of the entire face. Pustules then form on the lips, nostrils and ears, resulting in severe secondary bacterial infections of the eyes and ears, which may cause blindness. The disease occurs enzootically and causes high mortality among young animals.

Camelpox has been known since the Middle Ages and is caused by a poxvirus with a relatively restricted host spectrum (Tantawi, 1974). It occurs in the Bactrian camel (*Camelus bactrianus* L.) and in the dromedary (*Camelus dromedarius* L.). Pox diseases are unknown in the South American tylopods (llama and related species). Camelpox is widespread in Asia and Africa, especially Algeria, the Sahara, Egypt, Sudan, Kenya and Somalia, the Asiatic part of the Soviet Union and India (Baxby, 1972).
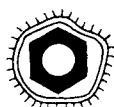
VIRUS PROPERTIES

Camelpox virus belongs to the Poxviridae family (Chordopoxvirinae subfamily) and is classified under the *Orthopoxvirus* genus (Mahnel, 1974; Kriz, 1982; Mayr et al., 1983). In terms of size, shape, structure, physico-chemical properties and autonomous replication in "viroplasm zones", it resembles vaccinia virus. It is immunologically uniform and closely related to other members of the *Orthopoxvirus* genus. There is no relationship with *Parapoxvirus*, the etiological agent of contagious dermatitis (Asduk) (Roslyakov, 1972; Dashtseren et al., 1984).

It is possible to differentiate within the orthopoxviruses by the nature of primary foci on the CAM of embryonated eggs, the host spectrum (apes, mice) and its behaviour in various cell cultures. Other methods for differentiation are immunological techniques where monoclonal antibodies are useful, e.g. HI, neutralization or ELISA tests, or by nucleic acid analysis using restriction enzymes or DNA-hybridization. Small proliferative focuses occur on the CAM, the morphology of which depends on the incubation temperature, without the tendency to generalize or to kill the embryo. Virus replication causes complete CPE in a wide range of cell cultures. However, virus strains from the USSR, Iran and Egypt behave differently as far as temperature sensitivity and hemagglutinin formation are concerned. When injected intracutaneously into rabbits, sheep and chickens, not all virus strains cause infection. Apes appear to be more susceptible. Upon intracerebral and intraperitoneal inoculation into

young mice, all strains were found to be only mildly virulent (Mahnel and Bartenbach, 1973; Marennikova et al., 1974; Davies et al., 1975; Tantawi et al., 1978). Histologically, cytoplasmic eosinophilic inclusion bodies can be detected in the infected cells (Tantawi and Sokar, 1976).

EPIZOOTIOLOGY

Camelpox can be transmitted both directly and indirectly. Once it has established itself in a herd, it is transmitted by contact. The virus contaminates the environment when pustules on the skin and mucous membranes become dry and fall off; it is frequently shed with secretions from the eyes, the upper respiratory tract and the digestive tract. Feed, grazing and contaminated objects play an important role in spreading the infection. Virus in dry scabs or crusts remains infectious for $4\frac{1}{2}$ months or longer. Camelpox spreads rapidly in camel populations, affecting young animals in particular. Epidemics occur every 3–5 years, mostly in the warm, humid seasons.

Only few cases of transmission to humans are known, and such reports are for the most part not very reliable. However, Kriz (1982) reports a WHO investigation of 280 persons in Somalia, 33% of whom had been vaccinated against smallpox, and all of whom were in constant contact with severely diseased camels. Of this group, three unvaccinated people fell ill with exanthema, and in one case a pox infection was suspected on the basis of a positive HI test against orthopoxviruses. Although transmission to humans may be rare, camelpox must be considered a zoonosis which may cause illness, particularly in immunosuppressed persons.

DISEASE SIGNS

The incubation period in the adult camel is usually 6–7 days (range 3–15 days). Newborn foals of non-immune camels fall ill between 3 and 5 days after being exposed to the virus at birth.

Pustule formation starts 1–3 days after the prodromal symptoms (fever, poor general state of health, salivation). In mild cases the pustules are restricted to the lips, nose, eyelids and the respective mucous membranes. In severe cases with fever, the pustules spread over the whole body, being manifested on the udder, genitals and the anal region. An edema frequently appears on the head and limbs. Animals may recover after 1–2 months. Pregnant female camels mostly suffer abortions.

Secondary infections result in suppuration beneath the pock scabs, abscesses in the subcutis, muscles, lymph nodes and lungs, and general pyemic symptoms. In such cases the animals suffer exhaustion, loss of appetite, diarrhea, and rapid loss of weight. They die quickly or after a few months as a result of total exhaustion. In general, young animals are most severely affected. The mortality rate in infected herds ranges from 25 to 100% for young animals, and from 5 to 25% for older animals. Mortality is higher in males than in female animals; it has reached 8.8% in male animals and 4.4% in female animals (Ramyar and Hessami, 1972; Kriz, 1982).
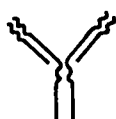
IMMUNE REACTION

Animals which have recovered from local affections or systemic disease have protective immunity for several years. In its development and duration,

it resembles the immunity gained after infection with vaccinia virus, but it is more and lasts longer.

Protective immunity is humoral and cell-mediated. Cellular immunity disappears more rapidly than the humoral immunity. The former can generally be detected for up to a year by delayed hypersensitivity reactions. The humoral immune response is detected by HI and VN tests. These antibodies are considered to prevent generalization of the virus and the development of a cyclic disease. It lasts for 2-3 years. Maternal immunity is transferred to newborn animals via the colostrum.

LABORATORY DIAGNOSIS

The methods for diagnosis of camelpox resemble those used for vaccinia and cowpox virus infections. The preferred method is EM detection of the typical orthopox virus particles in the pustules. The virus can be isolated in 10-12-day-old chick embryos (inoculation on the CAM) or in cell cultures. A difficulty is the differentiation of viruses within the *Orthopoxvirus* species, and in particular between the cowpox, variola and vaccinia viruses. Camelpox virus can be easily distinguished from parapox virus by electron microscopy, by cultivation and by serological methods.

PROPHYLAXIS AND CONTROL

Some camel-owning tribes still use the empirical but outdated and dangerous method of "variolation", i.e. inoculation with virulent field virus. They collect dried-up scabs from diseased camels and keep them until the following year. Before the onset of the humid season, milk containing ground scabs is inoculated into the lips of the animals. The rest of the herd thereby becomes infected by a natural process, and infection with virulent virus is constantly maintained.

Live vaccines from attenuated vaccinia strains are harmless and effective. The attenuated MVA vaccinia strain is recommended for this purpose. For primary vaccination, it is advisable to administer two parenteral doses at an interval of 3-4 weeks. One dose is sufficient for vaccination of healthy animals in an infected herd (emergency vaccination). Revaccination at intervals of 2-3 years is sufficient (Mayr, 1976; Mayr et al., 1978).

REFERENCES

Baxby, D., 1972. Smallpox-like viruses from camels in Iran. Lancet, 2: 1063-1065.

Baxby, D. and Hill, B.J., 1971. Characteristics of a new poxvirus isolated from Indian buffaloes. Arch. Ges. Virusforsch., 35: 70-79.

Dashtseren, T.S., Solovyev, B.V., Varejka, Khokhoo, 1984. Camel contagious ecthyma (pustular dermatitis). Acta Virol., 28: 122-127.

Davies, F.G., Mungai, H.N. and Shaw, T., 1975. Characteristics of a Kenyan camelpox virus. J. Hyg., 75: 381-385.

Kriz, B., 1982. A study of camelpox in Somalia. J. Comp. Pathol., 92: 1-8.

Mahnel, H. and Bartenbach, G., 1973. Systematization the camelpox virus (CPV). Zentralbl. Veterinärmed. B, 20: 572-576.

Mahnel, H., 1974. Laboratordifferenzierungen der Orthopockviren. Zentralbl. Veterinärmed. B., 21: 242-258.

Marennikova, S.S., Shenkman, L.S., Shelukhina, E.M. and Maltseva, N.N., 1974. Isolation of camelpox virus and investigation of its properties. Acta Virol., 18: 423-428.

Mayr, A., 1976. TC-Marker des attenuierten Vaccinia-Impfstammes "MVA" in menschlichen

Zellkulturen. Zentralbl. Veterinärmed., 19: 69–88.

Mayr, A., Stickl, H., Müller, H.K., Danner, K. and Singer, H., 1978. Der Pockenimpfstamm MVA: Marker, genetische Struktur, Erfahrungen mit der parenteralen Schutzimpfung und Verhalten im abwehrgeschwächten Organismus. Zentralbl. Bakteriol. Hyg. I Abt. Orig., 167: 375–390.

Mayr, A., Eissner, G. and Mayr-Bibrack, B., 1983. Handbuch der Schutzimpfungen in der Tier-medizin. Verlag Paul Parey, Berlin, Hamburg, pp. 742–743.

Ramyar, H. and Hessami, M., 1972. Isolation, cultivation and characterization of camelpox virus. Zentralbl. Veterinärmed. B, 19: 182–189.

Roslyakov, A.A., 1972. Comparative ultrastructure of the viruses of camelpox, a pox-like disease of camels ("Auzyk") and contagious ecthyma of sheep. Vopr. Virusol., 17: 26–30 (in Russian).

Tantawi, H.H., 1974. Comparative studies on camelpox, sheeppox and vaccinia viruses. Acta Virol., 18: 347–351.

Tantawi, H.H. and Sokar, M.S., 1976. Histopathological study of tissue reactions in chorioallantoic membrane and cell-culture infected with camelpox and buffalopox viruses. Indian J. Anim. Sci., 45: 76–80.

Tantawi, H.H., El-Dahaby, H. and Fahmy, L.S., 1978. Comparative studies on poxvirus strains isolated from camels. Acta Virol., 22: 451–457.

Chapter 5

Bovine Papular Stomatitis Virus

A. MAYR and M. BÜTTNER

INTRODUCTION

Bovine papular stomatitis (BPS) is a mild generalized viral disease. It frequently becomes apparent after activation of latent virus, which is widespread in cattle. It manifests itself typically on the skin and mucous membranes, with papulation occurring particularly around the mouth and occasionally on the udder. The papules are not, as has often erroneously been supposed, a local affection, but rather the result of a viremic phase with secondary viral localization in the skin, mucous membranes and internal organs. The disease may resemble local affections as seen in hyperkeratosis (X disease, chloronaphthalene poisoning and other intoxications; Schmidt, 1967). The terms "stomatitis pseudoaphtosa" or "oral papillomatosis" are misleading, since they suggest an other etiology.

Bovine papular stomatitis is occasionally transmitted to humans, causing mainly local skin affections (Nagington et al., 1965; Priesslinger, 1982).
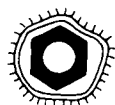
VIRUS PROPERTIES

Bovine papular stomatitis virus (BPSV) belongs to the family Poxviridae (subfamily Chordopoxvirinae) and is classified in the genus *Parapoxvirus*. It is morphologically similar to ovine orf virus and to the parapoxvirus causing the milker's nodes or udder pox. These three viruses are antigenically closely related (Mayr et al., 1972). A comparative study between neutralization kinetic tests and restriction enzyme analysis was carried out by Wittek et al. (1980) on various isolates of the three viruses. Serological cross-reactions were demonstrated between all strains tested. Antibodies to the outer coat structures were detected by Rosenbusch and Reed (1983) in low cell culture passage virus using immunoelectron microscopy. Antibodies to antigens in the coat permit the serological distinction between the three parapoxviruses. Restriction enzyme analysis of the DNAs of several isolates revealed a broad heterogeneity within orf virus and the milker's node parapoxviruses. Different strains of BPSV showed less heterogeneity. Presently, only the DNA hybridization technique allows a differentiation between the three viruses (Wittek et al., 1980; Gassman et al., 1985; Rafii and Burger, 1985).

The chemical and physical properties of BPSV resemble those of orf virus. The BPSV can be propagated in cell cultures; primary and secondary cultures of fetal lung, testes and kidney from cattle, sheep and man are particularly suitable (Nagington et al., 1965). A CPE with cell fusion, giant cell formation and granular lysis usually accompanies the replication, and cytoplasmic in-

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clusion bodies are formed. On the other hand, propagation in chick embryo fibroblast cultures or in the CAM of embryonated eggs fails, this in contrast to the bovine orthopoxviruses and to the capripoxvirus that causes lumpy skin disease. Small laboratory animals (including baby mice) are not susceptible to the BPSV.

EPIZOOTIOLOGY

At birth, BPSV is transmitted to the offspring of latently infected mothers; later in life it is transmitted by contact with latently infected or diseased animals (e.g. when penned together for fattening), and by virus intake via food and water. The virus is excreted from the upper digestive and respiratory tracts (Moreno-Lopez and Lif, 1979). Latent infections are frequent in cattle. It can be assumed that the virus occurs worldwide (Aguilar-Setién et al., 1980).

Although BPS occurs also in wild ruminants, the etiology is not clear in many cases since, for example, ovine orf virus can also be involved (Kummeneje, 1979). BPSV does not appear to be harmful to pigs and horses, and reports of infection in carnivores, particularly in dogs, are infrequent and contradictory (Liebermann, 1967).

People in close contact with diseased animals may become infected; local papular and pustular lesions develop on the hands, arms and legs. The lesions heal either spontaneously or during symptomatic treatment (Nasemann and Sauerbrey, 1981). Such affections in man may be caused by any of the three viruses of the *Parapoxvirus* genus; recently, the incidence seems to have increased (Schnurrenberger et al., 1980). When cases occur it is often difficult to establish the actual cause of the disease. Most of the cases in man, however, can be attributed to orf virus and BPSV; the latter can also manifest itself on the udders, predominantly of older animals (PHLS Communicable Disease Surveillance Centre, 1982). The expression "milker's node" (see chapter 6) has established itself in human medicine as the designation of a zoonosis, regardless which one of the three relevant virus species has caused the infection.

PATHOGENESIS AND PATHOLOGY

Bovine papular stomatitis is a cyclic, generalized disease. The pathogenetic chain of events is similar to that of the ovine ecthyma contagiosum. During generalized disease, the symptoms may disappear at any stage with ensuing subclinical and latent infections accompanied by virus excretion. Clinically inapparent infections predominate in obviously healthy animals and can be activated by various stress factors.

More recently, infections in immunosuppressive stress situations (e.g. crowding, misuse of medication) have led—particularly in fattening herds—to activation of latent infections with widespread outbreaks (Snider et al., 1982) and severe cases of the disease (Bohac and Yates, 1980).

When the latent infection is activated, the disease manifests itself on the mucous membranes of the muzzle (Fig. 5), on the udder (of older animals), the palatal mucous membrane, the esophagus and rumen. The infected areas become inflamed and develop into erosions and papulae at a later stage; these are characterized by concentric zones of inflammation (Figs. 6 and 7). Virus multiplication occurs in the spinous layer of the epidermis and leads to ballooning degeneration and vacuolization of infected cells, as well as to basophilic and eosinophilic cytoplasmic inclusion bodies (Pospischil and Bachmann, 1980). A small number of lesions on mucous membranes of the head and occasionally on

Fig. 5. First clinical signs of BPS: erythematous circular changes on the skin and mucous membranes around the mouth (arrow).

the udder are pathognostic for classical BPS; despite the viremia these animals' general state of health is hardly affected (fever is rare and food uptake is normal).

DISEASE SIGNS

The period of incubation is between 2 and 5 days. The course of the disease is generally mild. Initial erythematous changes on the mucous membranes of the muzzle develop into erosive and proliferative stomatitis. There is no formation of blisters. The primary lesions are often followed 2–3 weeks later by secondary papulation, which causes the animal severe irritation. The papulae may turn ulcerous in rare progressive cases, and the general state of health is then disturbed. The prodromal symptoms are increased salivation and reduced food intake. During generalization the infection also manifests itself on the udder as papulation and subsequent ulceration (Figs. 8 and 9). Such manifestations can occur enzootically and are referred to by Nagington et al. (1965) as "benign or chronic", or as "acutely progressive". The duration of the disease varies greatly; depending upon the nature of its progression and secondary infections, it can last for several months. In the case of chronic progression, repeated phases of sickness every 2–3 weeks are typical. A simultaneous infection with adeno-, mucosal disease- and IBR-IPV viruses may occur and often initiates the outbreak of BPS. Secondary bacterial infections, especially with pasteurellae may complicate its course.
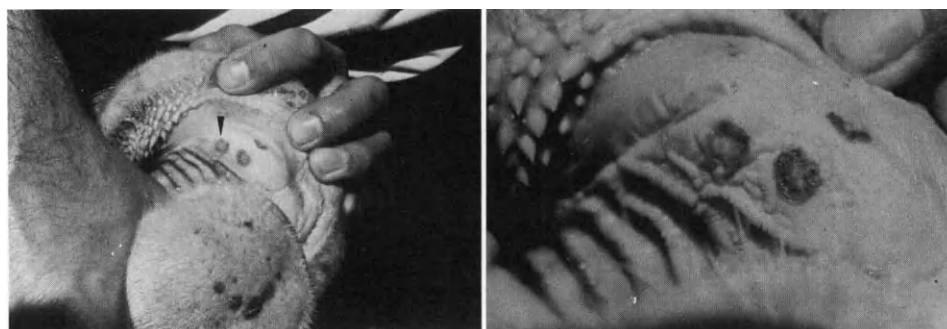
Fig. 6 (left). Typical cockade-like appearance of BPS erosions in the form of concentric ring zones around a central papula (arrow) on the gum of a calf.

Fig. 7 (right). Enlarged view of Fig. 6.

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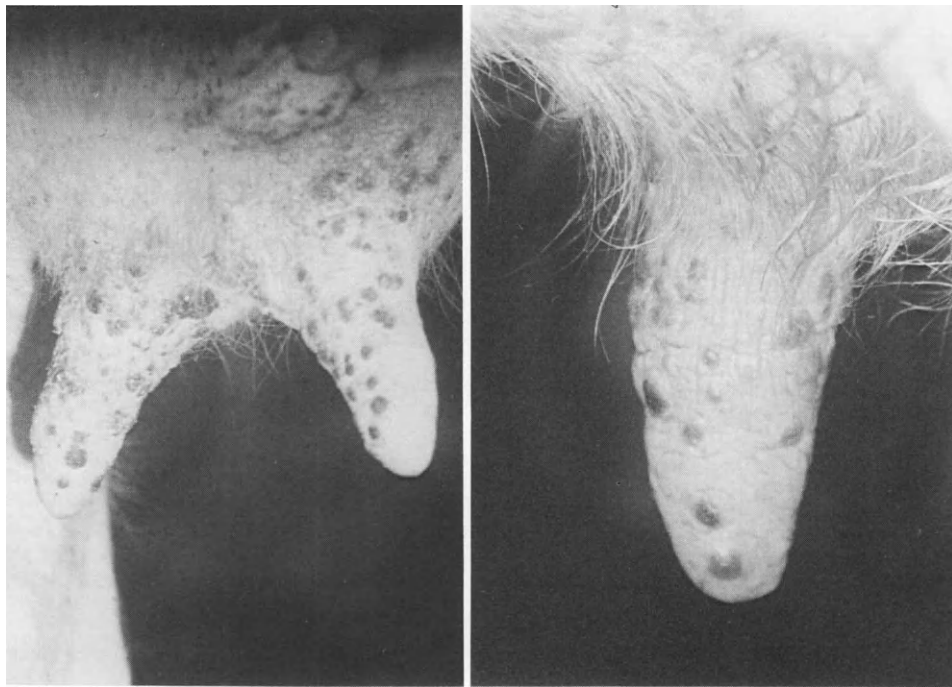
Fig. 8 (left). BPS manifestation on the udder. Papulation with subsequent ulceration.

Fig. 9 (right). As Fig. 8, enlarged view.

The economic significance of bovine papular stomatitis is greater for fattening than for breeding cattle. Besides causing acute sickness in calves soon after they are stalled up for fattening, it causes diminished food uptake and supports multiple infections such as "crowding disease".
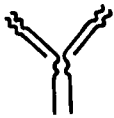
IMMUNE REACTION

Most animals develop antibodies that can be detected by the agar gel precipitation test, by IF or by the ELISA technique (Aguilar-Setién et al., 1981). The complement-dependent cytolytic activity of serum antibodies against ^{51}Cr-labelled virus-infected cells can also be tested; sera reacted with higher titers against homologous than against heterologous strains (Rosenbusch and Reed, 1983). Virus-neutralizing antibodies are extremely rare. On the other hand, a powerful cell-mediated response can be diagnosed by the delayed hypersensitivity skin reaction.

Newborn animals acquire antibodies from the mother but are not protected.

In general, protective immunity to BPS is very unstable. It develops slowly, is primarily cell-mediated and is short-lived. If animals possess neutralizing antibodies, they are protected against generalized disease. Neutralizing antibodies are only detectable for a period of several months. The parapoxviruses are reported to induce nonspecific defense mechanisms also in heterologous hosts (Mayr, 1981).

LABORATORY DIAGNOSIS

The fastest and most reliable diagnosis is obtained by EM detection of the characteristic parapox virions in fresh samples of typical skin and mucous

membrane lesions. In doubtful cases, virus is propagated in suitable cell cultures where viral antigen is detected by IF. For differential diagnosis, material may be inoculated onto the CAM of 10–12-day-old chick embryos or onto chick embryo fibroblast cultures in which orthopoxviruses would replicate in contrast to parapoxvirus.

In individual cases a serological diagnosis should be made on paired sera; only a positive result is conclusive.

For herd diagnosis, detection of serum antibody in a small number of animals is sufficient to diagnose virus presence. The intracutaneous hypersensitivity test using purified BPSV antigen also demonstrates the presence of the virus.

The most important diagnostic distinction has to be made between BPS and mucosal disease (BVD-MD). Both diseases are similar in the primary stage. They may occur together and influence each other synergistically (Ernst and Butler, 1983). Besides BVD-MD, we must also take into account infectious bovine rhinotracheitis, malignant catarrhal fever and progressive exanthematic diseases (vesicular complex), especially foot-and-mouth disease (Griesemer and Cole, 1960).

PROPHYLAXIS AND CONTROL

Until now, no specific preventive measures against BPS have been taken. The importance of such measures has been realized only in the last few years, when reports have accumulated on the role of BPSV in calf diseases (fattening farms) and on enzootics among breeding animals. The infection's ubiquitous occurrence and its zoonotic character are factors which underline the importance of control.

The close immunological relationship of BPSV to the ovine parapoxvirus (orf) prompted us to test the attenuated orf virus strain D 1701 as a vaccine. This strain proved innocuous for cattle when given subcutaneously or intramuscularly, and the inoculated virus was not excreted. The protection awarded was almost equally good against experimental and natural infection. The attenuated strain D1701 enhanced the immune response against BVD-MD virus when present in a combined vaccine, whereas its efficacy against BPS remained unaffected (Mayr et al., 1980; Mayr, 1981; Büttner, 1985; Chen et al., 1985).

REFERENCES

Aguilar-Setién, A., Correa-Giron, P., Hermandez-Baumgarten, E., Cruz-Gomez, A. and Hernandez-Jauregui, P., 1980. Bovine stomatitis, first report of the disease in Mexico. Cornell Vet., 70: 10–18.

Aguilar-Setién, A., Pastoret, P.-P., Thiry, E., Pivont, P., Gregoire, R., Antoine, H., Michaux, C. and Lekeux, P., 1981. Enquête sérologique sur la stomatite papuleuse bovine. Ann. Med. Vet., 125: 111–116.

Bohac, H.G. and Yates, W.D.G., 1980. Concurrent bovine virus diarrhea and bovine papular stomatitis infection in a calf. Can. Vet. J., 21: 310–313.

Büttner, M., 1985. Safety and efficacy of a combined Parapox/BVD vaccine. Develop. Biol. Stand., 65: 221–226.

Chen, K.S., Johnson, D.W. and Muscoplat, C.C., 1985. Adjuvant enhancement of humoral immune response to chemically inactivated bovine viral diarrhea virus. Can. J. Comp. Med., 49: 91–94.

Ernst, P.B. and Butler, D.G., 1983. A bovine virus diarrhea calfhood vaccination trial in a persistently infected herd: effects on titers, health and growth. Can. J. Comp. Med., 47: 118–123.

Gassmann, U., Wyler, R. and Wittek, R., 1985. Analysis of *Parapoxvirus* genomes. Arch. Virol., 83: 17–31.

Griesemer, R.A. and Cole, C.R., 1960. Bovine papular stomatitis. J. Am. Vet. Med. Assoc., 137: 404–409.

Kummeneje, K., 1979. Contagious ecthyma (orf) in reindeer (*Rangifer tarandus*). Vet. Rec., 105: 60–61.

Liebermann, H., 1967. Untersuchungen über die Stomatitis papulosa unter Berücksichtigung der Differentialdiagnose. Arch. Exp. Vet. Med., 21: 1319–1336.

Mayr, A., 1981. Induction of paramunity. In: P.A. Bachmann (Editor), Biological Products for Viral Diseases. Taylor and Francis, London, pp. 201–227.

Mayr, A., Mahnel, H. and Munz, E., 1972. Systematisierung und Differenzierung der Pockenviren. Zentralbl. Veterinärmed. B, 19: 69–88.

Mayr, A., Baljer, G. and Büttner, M., 1980. Aktive Schutzimpfung mit heterologen Impfstoffen. Tierärztl. Umschau, 35: 560–576.

Moreno-Lopez, J. and Lif, I., 1979. A parapoxvirus isolated from nasal secretion of a calf with respiratory disease. Vet. Microbiol., 4: 85–88.

Naginton, J., Tee, G.H. and Smith, J.S., 1965. Milker's nodule virus infections in Dorset and their similarity to orf. Nature, 30: 505–507.

Nasemann, Th. and Sauerbrey, W., 1981. Textbook of Skin Disease and Veneral Infections. Springer Verlag, Berlin, 475 pp.

PHLS Communicable Disease Surveillance Centre and the Communicable Disease Unit, 1982. Orf paravaccinia infections, British Isles: 1975–81. Brit. Med. J., 284: 1958.

Pospischil, A. and Bachmann, P.A., 1980. Nuclear change in cells infected with parapoxviruses stomatitis papulosa and orf: an in vivo and in vitro ultrastructural study. J. Gen. Virol., 47: 113–121.

Priesslinger, G., 1982. Occurrence of so-called Pseudopox in a veterinary practice and its transmission to humans. Prakt. Tierarzt., 1: 34–38.

Rafii, F. and Burger, D., 1985. Comparison of contagious ecthyma virus genomes by restriction endonucleases. Arch. Virol., 84: 283–289.

Rosenbusch, R.F. and Reed, D.E., 1983. Reaction of convalescent bovine antisera with strain-specific antigens of parapoxviruses. Am. J. Vet. Res., 44: 875–878.

Schmidt, D., 1967. Stomatitis papulosa of cattle. In: H. Röhrer (Editor), Handbook of Viral Diseases of Animals. G. Fischer Verlag, Jena, Band II, 1, p. 661.

Schnurrenberger, P.R., Swango, L.J., Bowman, G.M. and Luttgen, P.O., 1980. Bovine papular stomatitis incidence in veterinary students. Can. J. Comp. Med., 44: 239–243.

Snider, T.G., McConell, S. and Pierce, K.R., 1982. Increased incidence of bovine papular stomatitis in neonatal calves. Arch. Virol., 71: 251–258.

Wittek, R., Herlyn, M., Schümperli, D., Bachmann, P.A., Mayr, A. and Wyler, R., 1980. Genetic and antigenic heterogeneity of different parapoxvirus strains. Intervirology, 13: 33–41.

Chapter 6

Milker's Node Virus

A. MAYR and M. BÜTTNER

INTRODUCTION

Milker's node is a virus-induced local skin infection in humans, characterized by the appearance of nodules between the size of a pea and a cherry pit. It is transmitted to humans by close contact with sheep, goats and cows in particular, where skin lesions of the animals promote transmission of the infection. In rare cases and under immunosuppressive conditions, a generalizing systemic disease can develop (Leavell et al., 1968). Milker's node is a zoonosis; other names for the disease are pseudo cowpox, spurious cowpox, milker's nodule and farmyard pox. All these designations are ill-chosen but are still in use, irrespective of proposals to change the terminology (Shelley and Shelley, 1983).
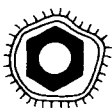
VIRUS PROPERTIES

The etiological agent is a virus of the family Poxviridae, subfamily Chordopoxvirinae of the genus *Parapoxvirus*. This genus includes orf virus in sheep and goats, and BPSV in cows. All three viruses can be transmitted to humans and may lead to the development of "milker's nodes".

These viruses are immunologically closely related and cannot be distinguished in cross-neutralization tests. Distinction was achieved however, by DNA restriction analysis and hybridization (Gassmann et al., 1985). Using the same methods, differences were found also within groups of strains belonging to the ovine and bovine parapoxviruses. It is therefore a matter of discussion whether the three viruses should be regarded as variants of one virus which has adapted to different hosts. In morphological and physico-chemical terms, the three viruses are identical. Parapoxviruses can easily be distinguished from other poxvirus genera by EM. There are a few cross-reacting antigens with orthopoxviruses in agar gel precipitation, but there is no protective cross-immunity between the genera (Renshaw and Dodd, 1978; Subba Rao et al., 1984).

Primary and secondary cultures of fetal lungs, kidneys, and skin from cows, sheep and humans are suitable for propagation of milker's node parapoxvirus. A CPE characterized by cell fusion and the formation of giant cells leads to lysis. Cytoplasmic inclusion bodies are formed in the infected cells.

EPIZOOTIOLOGY

Humans acquire the disease by direct contact with lesions from infected animals. When milking by hand was common, lesions on the udders of cows

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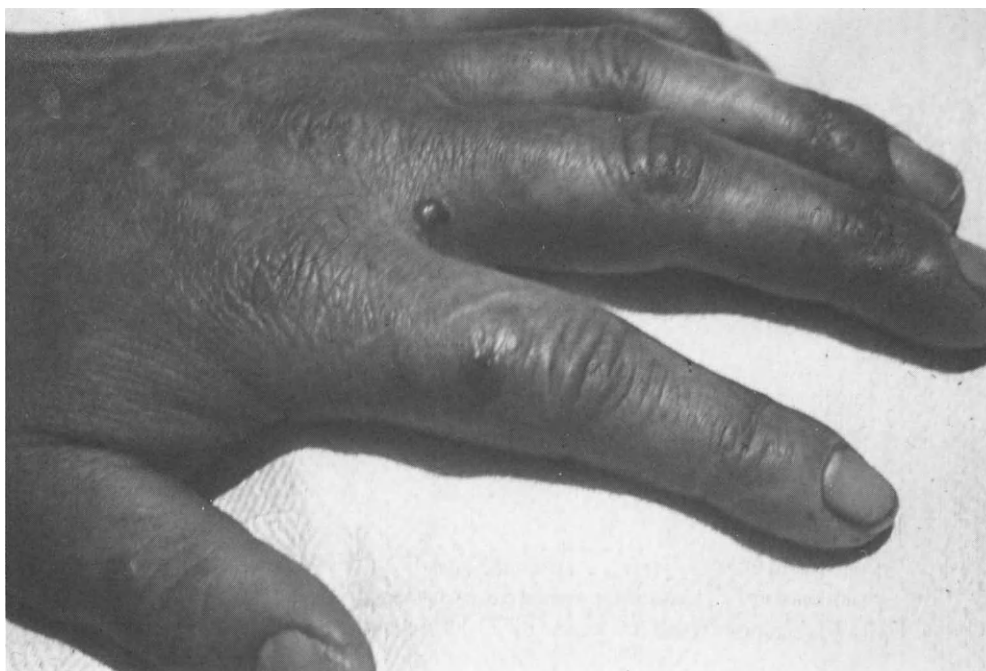
Fig. 10. Milker's nodes on the hand of a man.

frequently transmitted the infection (Fig. 10). Milkers and livestock farmers are most prone to infection with parapoxviruses. However, abattoir workers and veterinary surgeons can also contract the disease, and under exceptional circumstances, housewives preparing contaminated meat became infected (Günes et al., 1982; Brueschke, 1983). Parapoxviruses can be transmitted by inanimate carriers such as apparatuses and abattoir products. In New Zealand abattoir workers handling virus-contaminated wool were found to be at great risk of infection (Robinson and Petersen, 1983).

According to an epidemiological study carried out in Great Britain (1975–1981), most human infections arose from contact with infected sheep (PHLS Communicable Disease Surveillance Centre, 1982; Moore et al., 1983; Shelley and Shelley, 1983).

DISEASE SIGNS

Milker's node is characterized by rather coarse nodes of a roughly spherical shape, up to the size of a cherry pit, of a reddish-blue color, which occur individually or in clusters, primarily on the fingers, ball of the thumb, back of the hand and forearm, rarely in the face (Fig. 11). The nodules have a narrow, reddish limbus and are somewhat elevated in the center (Nasemann and Sauerbrey, 1981; Brueschke, 1983). As the infection progresses, a central navel forms which develops into a brownish-black crust during the healing stage. The nodes are not particularly painful. In contrast to cowpox, the general state of health of the patient is not affected. Secondary infections with bacteria and fungi may complicate the course of the disease (Hartman et al., 1985).

Fig. 11. Milker's nodes in the face of a woman.

PATHOLOGY

The following lesions are typical of coarse milker's nodes (Brueschke, 1983):
— hyperkeratosis and parakeratosis with lamellar layers but with intact epidermis;
— localized reticuloform degeneration of the stratum spinosum, transition to small intra-epidermal blisters, no pustulation;
— strong epithelial ridges spreading into the cutis, more frequent necrosis of the tips of the epithelial ridges;
— cell infiltration, principally of polynuclear leukocytes;
— edema of the cutis with enlarged vessels;
— Feulgen-positive cytoplasmic inclusion bodies in the rete Maplpighi.
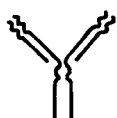
IMMUNE REACTION

A brief period of cellular immunity occurs in all cases and can be detected by delayed hypersensitivity (intracutaneous) tests with parapoxvirus antigen. Antibodies may sometimes be detected—the most reliable method is ELISA (Hartmann et al., 1985). Antibodies are less frequently detected by VN; they are of the IgG and IgM class. When they are present, immunity is strong, it lasts for a longer period of time and it indicates that a generalized infection has taken place.

LABORATORY DIAGNOSIS

Clinically, only a tentative diagnosis is possible, which can be supported by histology. The virus can be propagated in cell culture from biopsy specimens

(Hessami et al., 1979). A suspected case of parapox virus infection can rapidly and reliably be identified by EM.

For a differential diagnosis in man, infections with orthopoxviruses, in particular vaccinia, cowpox and molluscum contagiosum, and further tuberculosis cutis verrucosa, chancrous pyoderma and warts have to be excluded.

PROPHYLAXIS AND CONTROL

Localized milker's nodes are usually treated symptomatically by applying protective bandages with desiccating ointments, powders and local antibiotics to prevent secondary infections. The best prophylactic measure is vaccination with attenuated orf virus of cell culture origin. Sheep, goats and cows should be vaccinated against ecthyma contagiosum and stomatitis papulosa to control virus spread among the animals as well as transmission to humans (Mayr et al., 1981). It is advisable to apply emergency vaccination to healthy animals in an infected herd.

REFERENCES

Brueschke, G., 1983. Handbook on Internal Diseases, Vol. 5, Infectious Diseases. G. Fischer Verlag, Jena, 900 p.

Gassmann, U., Wyler, R. and Wittek, R., 1985. Analysis of parapoxvirus genomes. Arch. Virol., 87: 17–31.

Günes, A.T., Gezen D., Kapdagh, H. and Marschall, H.J., 1982. Ecthyma contagiosum epidemics in Turkey. Hautarzt, 33: 384–387.

Hartmann, A.A., Büttner, M., Stanka, F. and Elsner, P., 1985. Sero- und Immuno-diagnostik bei Parapoxvirus-Infektionen des Menschen. Hautarzt, 36: 663–669.

Hessami, M., Keney, D.A., Pearson, L.D. and Storz, J., 1979. Isolation of parapoxviruses from man and animals: cultivation and cellular changes in bovine fetal spleen cells. Comp. Immunol. Microbiol. Infect. Dis., 2: 1–7.

Leavell, U.W., McNamara, M.J., Muelling, R., Talbert, W.M., Rucker, R.C. and Dalton, A.J., 1968. Orf: Report of 19 human cases. J. Am. Med. Assoc., 204(8): 109–116.

Mayr, A., Herlyn, M., Mahnel, H., Danko, A., Zach, A. and Bostedt, H., 1981. Bekämpfung des Ecthyma contagiosum (Pustulardermatitis) der Schafe mit einem neuen Parenteral–Zellkultur–Lebendimpfstoff. Zentralbl. Veterinärmed. B, 28: 535–552.

Moore, D.M., MacKenzie, W.F., Doepel, F. and Hansen, T.N., 1983. Contagious ecthyma in lambs and laboratory personnel. Lab. Anim. Sci., 33: 473–475.

Nasemann, Th. and Sauerbrey, W., 1981. Textbook of Skin Diseases and Veneral Infections. Springer Verlag, Berlin, 4th edn., 475 pp.

PHLS Communicable Disease Surveillance Centre and Communicable Disease Unit, 1982. Orf paravaccinia infections, British Isles: 1975–1981. Brit. Med. J., 284: 1958.

Renshaw, H.W. and Dodd, A.G., 1978. Serologic and cross-immunity studies with contagious ecthyma and goat pox virus isolates from the western United States. Arch. Virol., 56: 201–210.

Robinson, A.J. and Petersen, G.V., 1983. Orf virus infection of workers in the meat industry. N.Z. Med. J., 96: 81–85.

Shelly, W.B. and Shelley, E.D., 1983. Farmyard pox: parapox virus infection in man. Br. J. Dermatol., 108: 725–727.

Subba Rao, M.V., Malik, B.S. and Sharma, S.N., 1984. Antigenic relationships among sheep pox, goat pox and contagious pustular dermatitis viruses. Acta Virol., 28: 380–387.

Chapter 7

Ecthyma (Orf) Virus

A. MAYR and M. BÜTTNER

INTRODUCTION

Ecthyma contagiosum is a generalized viral disease incurred by sheep, goats and chamois that normally takes a cyclic course; local lesions occur occasionally. The infection frequently leads to clinically inapparent forms which can be activated by stress, immunosuppression and other noxae.

Ecthyma is transmitted to humans (zoonosis), where it mostly results in local affections.

There are more than 80 synonyms for ecthyma contagiosum, an indication of the multifarious clinical picture of this disease. The designations most commonly used are: contagious ecthyma (CE), contagious pustular dermatitis, ecthyma contagiosum and orf.

The word "ecthyma" means skin purulence with ulcer formation; the pathogen is known as orf virus, and the disease also often bears this name, which probably originated in Scottish or English. It may have derived from the old Icelandic word "hurf" meaning a scab or crust (Robinson and Balassu, 1981).
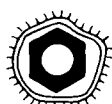
VIRUS PROPERTIES

Physical and chemical characteristics

Orf virus is a member of the family Poxviridae, subfamily Chordopoxvirinae, genus *Parapoxvirus*. Infection confers cross-immunity to the other parapoxviruses, the agents causing BPS and milker's node.

Parapoxviruses differ from orthopoxviruses structurally in terms of:
—the size, shape and virion axial ratio;
—the arrangement of the filaments on the viral membrane;
—the dimensions of lateral bodies and the internal substructure.

The parapoxvirion is somewhat smaller than, for example, vaccinia virus, and the shape is an elongated oval. The dimensions are 250 nm for the longitudinal axis and 160 nm for the shorter axis (ratio about 1.5; Fig. 12). The most important difference is found in the arrangement of the filaments in the outer membrane. The tubular subunits are, unlike those of other poxviruses, arranged regularly like a single coiled-up rope—the "clew" morphology (Fig. 13). The strand is 8–9 nm thick and has a total length of about 8–10 μm. Lateral body, surface protein and internal body are smaller (Nagington and Horne, 1962). Also, parapoxviruses replicate in their own "factories" in the cytoplasm (viroplasm) where virions may be found in several development stages.

Orf virus is extremely stable. It can be kept for many years in liquid medium

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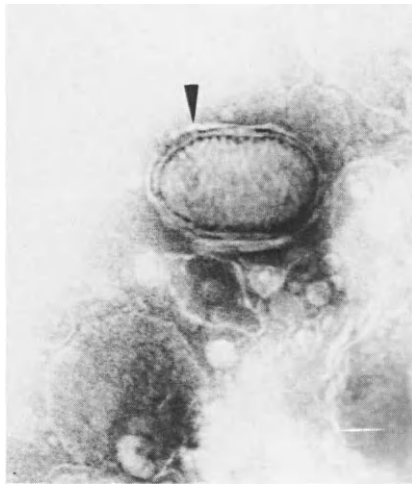
Fig. 12. Electron microscopy of orf virions with typical "clew" morphology and envelopes (arrow) surrounding the virus particles (× 60 000).

at temperatures below −70°C, or after drying or lyophilization at +4°C. Infectivity is maintained at room temperature in thick, dry scabs for up to 10 years, in scabs after grinding for 14 months, in 5% glycerin for 3–8 months and in putrid material for at least 17 days. Pasture remains infectious for many months. In summer, the virus loses its infectivity on the ground only after 50–60
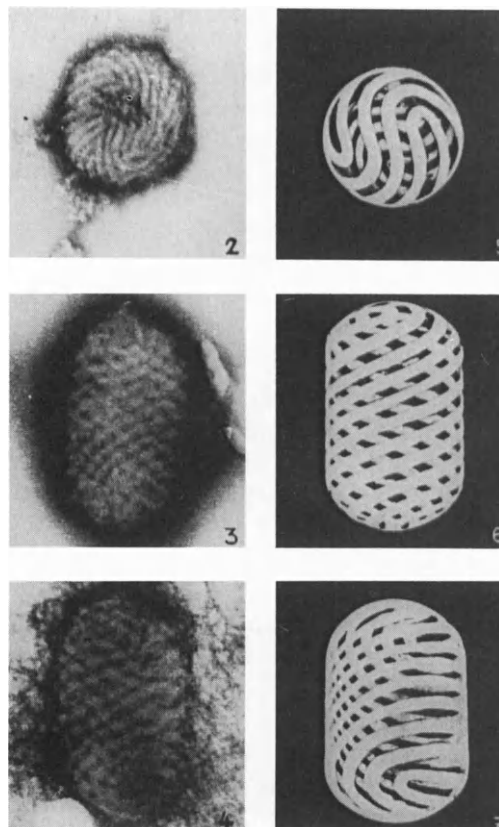
Fig. 13. Orf virus particles (negative staining) and schematic representation of structure: (2) and (5), particle seen on end with threads or tubules curving round the end; particle (250 × 160 nm) of symmetrical shape; (3) and (6), series of figure-of-eight loops, each circuit having one crossover; (4) and (7), spiral arrangement of tubules (from Nagington, 1962).

days; it can withstand the winter months. Contaminated animals remain infectious for several weeks. The virus survives heating to 56°C for several hours. Suitable disinfectants are a 5% creolin solution, chloramine, chloride of lime, formalin, detergents and commercially available virucide disinfectants (Mayr et al., 1983).

Antigenic properties

All orf strains isolated so far from sheep, goats and humans are immunologically uniform. Serologically (CF, precipitation, neutralization tests), there are slight differences between strains. Restriction analysis revealed genetic heterogeneity among orf virus isolates (Grassmann et al., 1985). The existence and nature of phenotypic differences as well as possible pathobiological consequences are still uncertain, although recent experiments support this (Hussain and Burger, 1989).

Cultivation

Cell cultures that are particularly suitable for growth of parapoxviruses are derived from fetal kidneys, lungs and from the skin of sheep, cattle and humans. Adaptation of orf virus to growth in monkey kidney cell lines is possible and has been demonstrated by Hussain and Burger (1989) for three field isolates in Vero cells and in our laboratory for an attenuated vaccine strain in MA cells. Replication of orf virus produces a CPE either immediately or after two–three blind passages; cell fusion, formation of giant cells and subsequent granular breakdown are observed.

EPIZOOTIOLOGY

Orf is highly contagious among sheep and goats. Natural transmission is by contact, aerogenic, or by virus remaining infectious in lost scabs in the ground during the winter. The virus is indirectly transmitted by way of carcasses or processed meat from latently infected animals or those slaughtered in the viremic phase. Carcasses may also contaminate the environment. In addition, dried virus-containing material can spread to the environment by way of feed, wool, fleece, etc. The virus can be transmitted to lambs by infected ewes at birth or later. Orf can spread at an explosive rate in a susceptible population, especially if the sheep are kept in stables and fattened early after weaning, as is currently practiced.

Morbidity can reach 100% (Gardiner et al., 1967), but mortality is usually not more than 1%. Changes in animal husbandry (early weaning, crowding, etc.) weaken the animals' defenses and increase virulence as a result of rapid passages of orf virus through host animals. This has given rise to more severe forms of ecthyma, with mortality reaching 20–50% among suckling lambs and causing severe financial losses (Valder et al., 1979). The persistance of the virus has led to worldwide spread by way of trade in animals and products derived from sheep (Obi and Gibbs, 1978).

Latently infected sheep, goats and chamois are regarded as virus reservoirs for other animals (Robinson and Balassu, 1981). In Norway contagious ecthyma was diagnosed by EM in musk ox (Kummeneje and Krogsrud, 1978; Mathiesen et al., 1985) and reindeer (Kummeneje and Krogsrud, 1979). In musk ox the clinical picture was characterized by cauliflower-like papillomas mainly on the lips, muzzle and nostrils. The disease caused high mortality in calves and adult males while adult females were more resistant. In reindeer the clinical picture

was mild and characterized by similar papillomas (1–2 cm in diameter) on the oral mucocutaneous junction. The lesions disappeared within 3–4 weeks. Other animals species which can contract orf are bighorn sheep, chamois, thar ibex, camels and alpaca.

There is one report of an outbreak of orf in dogs (Wilkinson et al., 1970). It was suspected that the dogs became infected after feeding off unskinned sheep carcasses. The situation is still uncertain with regard to cattle. When kept together with infected sheep, cattle normally do not become ill. However, several authors have described experimental infections by way of the mucous membranes of the muzzle (Aynaud, 1923; Bennet et al., 1944; Huck, 1966; Liebermann, 1967). Of the small laboratory animals, apparently only the rabbit can be infected, but not reproducibly and only with extremely large amounts of virus (Abdussalam, 1957; Darbyshire, 1961). Horses and pigs are resistant to orf, but monkeys can probably acquire the disease.

Ecthyma is a zoonosis. It normally induces proliferative local lesions on the skin and mucous membranes (Liess, 1962; Falk, 1978). The virus can become generalized in immunosuppressed animals and humans. Human cases are attributable to contact with sheep, but also reindeer and musk-ox were reported as source of infection (Falk, 1978). They are usually found in members of particularly exposed professions such as shepherds, farmers, sheep-shearers, taxidermists, butchers and veterinary physicians, but housewives have also become infected after handling mutton or lamb (PHLS Communicable Disease Surveillance Center and Communicable Diseases Unit, 1982).

PATHOGENESIS AND PATHOLOGY

Ecthyma is a generalized viral disease which runs a cyclic course. Its preferred portal of entry are the skin and mucous membranes. After virus multiplication at these sites a primary viremia develops, during which organs of primary affinity, e.g. the lympho-reticular tissue, bone marrow, and liver become infected. Whether the virus becomes generalized in a second viremic phase depends on its replication in these organs. Preferred targets of symptom manifestation are the head, extremities, udder, genitals, lungs and liver. The proliferative, typical changes in the skin and mucous membranes are the result of viremia and not attributable to primary virus settlement. The cause of severe, sometimes fatal disease is virus replication in the internal organs, which takes place simultaneously with growth in the skin and mucous membranes. The chain of pathogenetic events leading to generalized disease can be interrupted in any phase. This results in subclinical and latent forms of the infection with virus excretion but without symptoms. Inapparent infections can be activated by stress, leading to immunosuppression. Secondary infections with certain viruses (e.g. adenoviruses and BVD-MD/border disease viruses) and bacteria promote clinical manifestation.

Histologically, virus multiplication leads through a proliferation phase to ballooning degeneration with pustule formation and subsequent cell lysis (McKeever et al., 1988). Cytoplasmic inclusion bodies occur in the affected cells, a visible sign of virus multiplication. However, also nuclear changes visible as fine intranuclear filaments occur approximately 36 h p.i. (Pospischil and Bachmann, 1980; Fig. 14).

DISEASE SIGNS

Ecthyma appears clinically with different symptomatologies, namely as: the labial form; the podal form; the genital form; the malignant form.
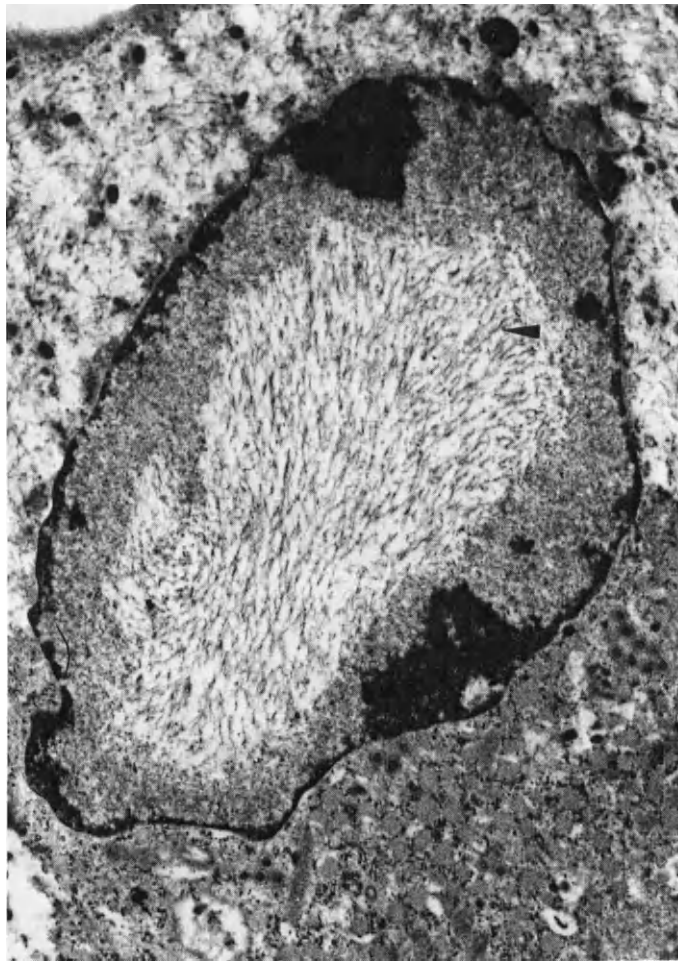
Fig. 14. Formation of intranuclear filaments (arrow) during infection of bovine embryonic lung cells with orf virus 36 h p.i. (from Pospischil and Bachmann, 1980).

Fig. 15. "Scabby mouth": labial form of contagious ecthyma in sheep. Blisters and yellowish pustules form on the upper and lower lip.
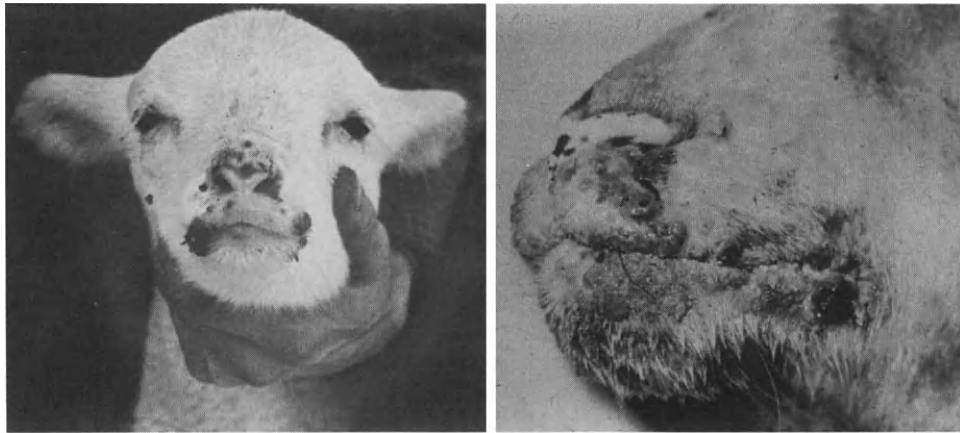
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Fig. 16. Malignant form of ecthyma contagiosum in sheep: severe cauliflower-like tumors on the lips and mucous membranes of the mouth. Glossitis phlegmatosa et ulcerosa complicated by secondary bacterial infections.

The incubation period is 3–8 days.

In the *labial form* (scabby mouth), blisters and yellowish pustules, which may reach approximately pea size, are formed on the lips. The lesions extend to the corners of the mouth and up to the nose, ears and eyelids, changing gradually into brownish-black, hard scabs and reaching walnut size (Fig. 15). The scabs can be removed, sometimes with difficulty, revealing easily bleeding granulated tissue. The formation of pus and abscesses in older scabs is caused by secondary infections with bacteria (*Spherophorus necrophorum*, staphylococci) and insect larvae. The mild labial form generally heals in 3 weeks. Pustules sometimes occur on the udders of ewes shortly before the lambing period. Mastitis can also develop, with complications caused by secondary bacterial infection.

The *podal form* (scabby foot) occurs either at the same time as the labial form or independently. In the latter case, changes develop at the coronar edges of the hooves, at the pasterns and in the hoof gaps. The ends of the digits are painful, leading to lameness and the refusal to stand. Secondary bacterial infection may cause whitlows and pododermatitis.

The *genital form* is less common. Typical pustule and crust formation occurs on the udder in particular, developing into mastitis. Furthermore, skin changes occur on the inner leg, the labia and the prepuce. Abortion sometimes takes place without skin or mucous membrane changes.

In the *malignant forms*, the skin and mucous membrane changes appear together with virus manifestation in the internal organs. Secondary bacterial infections complicate the course of the disease. The result is a breakdown of the general well-being, leading to prostration. The general symptoms occurring in parallel with the skin and mucous membrane disease are high temperature, general debility, edema of the head, swelling of regional lymph nodes, pneumonia and gastroenteritis. If no complications occur, the disease lasts for 2–4 weeks.

Malignant forms affect predominantly lambs, but also older sheep. Lambs may show cauliflower-shaped tumors on the mucous membranes of the mouth, particularly around the incisors, and glossitis phlegmonosa et ulcerosa (scabby mouth) extending to the pharynx, the esophagus and as far as to the entry of the rumen (Figs. 16 and 17); these manifestations lead to unwillingness to suck, inanition and frequent deaths. If the herd has become infected for the first time, severe forms may also be observed in older sheep. In such cases foot-and-mouth disease has to be excluded. Blisters varying from millet-seed to lentil size may
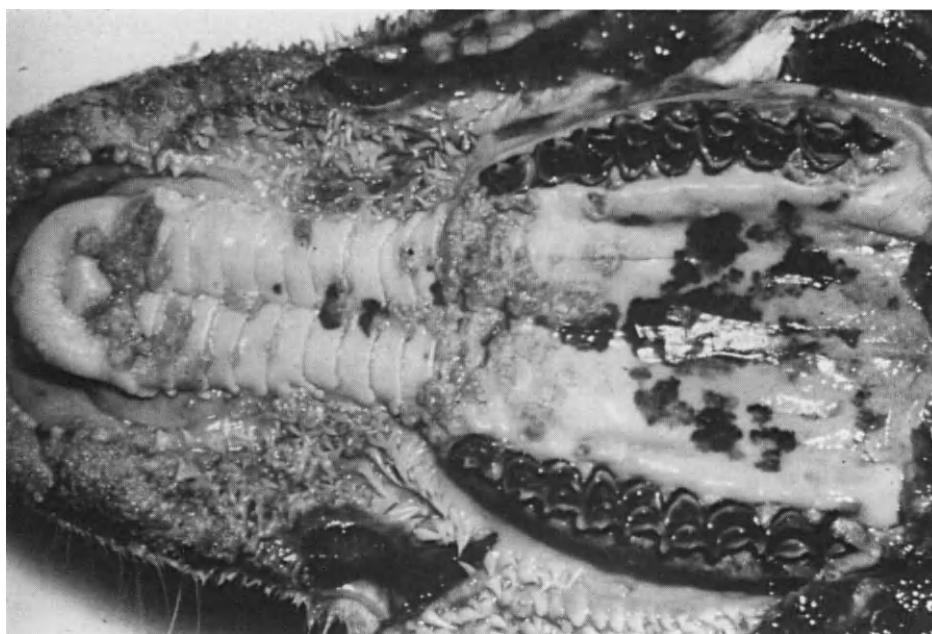
Fig. 17. Gum of a sheep with a malignant form of ecthyma contagiosum: glossitis that can extend to the pharynx, the esophagus and as far as the entry to the rumen.

occur, in particular on the mucous membrane of the mouth and tongue, later modifying into tumor-like growth with necrosis. About 8 weeks later, most animals become lame, and cases of hoof horn shedding are frequently observed. The old hoof horn is rejected only after new horn has been formed.
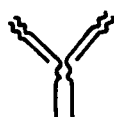
IMMUNE REACTION

Animals which went either through the disease or the inapparent infection are protected against reinfection. However, the immunity is labile, especially under stress. A distinction must be made between systemic and local ecthyma immunity in the skin and mucous membranes. Systemic immunity is both cellular (e.g. measured by delayed hypersensitivity) and humoral (circulating antibodies). The skin and mucous membrane immunity is carried by cytotoxic T-lymphocytes and secreted IgA-antibodies.

Before specific immunity develops, non-specific short-term defense is activated, sustaining the disease or promoting latent forms of it. It includes viral interference, induction of interferon and other mediators, and increased macrophage and natural killer-cell activity.

Cellular immunity dependent on T-cells develops about 5–7 days after infection. Secretion of local antibodies occurs after 7–9 days and appearance of serum antibodies at about 15–20 days p.i. Depending on the nature and course of the infection and the age of the animal, the production of serum antibodies may not take place. However, the animals continue to be protected against reinfection.

Often serum antibodies cannot be demonstrated until after reinfection, particularly in lambs. In adult sheep, on the other hand, antibodies are found quite frequently, probably because they have been infected repeatedly in their lives.

Virus neutralizing antibodies are usually produced only to low titers (Fredrichs, 1980). There is neither cross-neutralization nor cross-immunity to chal-

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lenge with sheeppox virus (Renshaw and Dodd, 1978; Dubey and Sawhney, 1979).

Antibodies are transferred to newborn animals from the mother and provide them with a low and inadequate level of immunity (Poulain et al., 1972; Le Jan et al., 1978; Buddle and Pulford, 1984).

In the absence of a booster infection the humoral antibodies disappear after about 5 months (first the precipitation and complement-fixation reactions become negative and then the neutralizing antibodies disappear). Cellular immunity does not last for more than 8 months.

LABORATORY DIAGNOSIS

The most rapid and reliable diagnosis is obtained by EM, when the typical parapoxvirus particles are found in freshly obtained specimens from sheep which exhibit the characteristic skin and mucous membrane alterations. In case of doubt, the virus should be isolated in suitable cell cultures and viral antigen demonstrated by IF. In order to distinguish orf virus from orthopox-viruses, the material under investigation can be used in parallel to inoculate both the CAM of 10–12-day-old chicken embryos and chicken embryo fibroblast cultures; orf virus would not grow in these tissues.

In individual cases, paired sera can be tested for an indirect diagnosis by demonstrating the presence of antibodies (neutralization test, ELISA, agar gel precipitation, etc.). Complement-mediated antibody reactions against virus-infected cells can be detected by a chromium-51 release assay in the form of antibody-dependent cytotoxicity (ADCC) (DeMartini et al., 1978). Individual serological diagnosis is needed especially when humans have contracted the disease.

Serological methods are useful for diagnosing field outbreaks after vaccination or an enzootic infection. In both cases, demonstration of antibodies in only a few animals will suffice. More recently we have developed monoclonal antibodies which give the possibility of a sensitive and reliable detection of parapox-viruses in ELISA, immunoperoxidase staining and immunoblotting. Sheep pox, foot rot, blue-tongue, whitlows, mastitis and foot-and-mouth disease should be excluded.

PROPHYLAXIS AND CONTROL

Inactivated virus has not proven successful as a vaccine against orf. Vaccination of ewes does not give rise to protection of their offspring, but it does prevent the spread of virus in a herd.

Live vaccines administered cutaneously have been used for many years, but not very successfully (Robinson and Balassu, 1981). In some cases poorly defined virus isolates from sheep were used, which resulted in vaccines containing, for example, too low virus concentrations per dose or even highly virulent virus (for details see Buddle et al., 1984). Such preparations have been superseded by high-titered cell culture vaccines based on attenuated, genetically stable, avirulent, noncontagious vaccine strains that can be applied parenterally. Mayr et al. (1981) developed a vaccine of this type, based on a highly attenuated (135 passages), plaque purified orf virus strain (D1701); it is applied subcutaneously and is innocuous. After inoculation, virus-neutralizing antibodies were found in the majority of sheep. A booster effect was also observed. Since vaccination of gestating ewes does not provide sufficient protection to the offspring, the lambs have also to be vaccinated (Buddle and Pulford, 1984).

The vaccine has proved successful under extreme field conditions as an "emergency" vaccine for lambs (Mayr et al., 1983). Parenteral live vaccines induce both cellular and humoral systemic immunity. With the subcutaneous method of administration the risk of postvaccinal disease is avoided and so is the excretion of vaccine virus; this has frequently occurred after scarification of the skin in the groin area as practiced previously.

Based on experience to date, the following vaccination programs can be recommended for the new parenteral (subcutaneous) cell culture live vaccines (Mayr et al., 1983).

Vaccination in noninfected herds: All animals older than 3 months are vaccinated once, regardless of the stage of gestation; lambs less than 3 months of age are vaccinated twice at an interval of 4–6 weeks; depending upon the degree of exposure, the inoculations are repeated at 6–12-month intervals.

Vaccination in infected herds (emergency vaccination): Like in noninfected herds, all animals without symptoms are vaccinated; newborn lambs are inoculated when they are 1–2 days old; they are revaccinated after 10–14 days and after 3 months.

For all vaccination programmes it is important that all healthy animals are included.

REFERENCES

Abdussalam, M., 1957. Contagious pustular dermatitis. III. Experimental infection. J. Comp. Pathol., 67: 305–319.

Aynaud, M., 1923. La stomatite pustuleuse contagieuse des ovins (chancre du mouton). Ann. Inst. Pasteur, 37: 498–527.

Bennet, S.C.J., Horgan, E.S. and Hasseb, M.A., 1944. The pox disease of sheep and goats. J. Comp. Pathol., 54: 131–160.

Buddle, B.M. and Pulford, H.D., 1984. Effect of passively-acquired antibodies and vaccination on the immune response to contagious ecthyma virus. Vet. Microbiol., 9: 515–522.

Buddle, B.M., Dellers, R.W. and Schurig, G.G., 1984. Contagious ecthyma virus vaccination failures. Am. J. Vet. Res., 45: 263–266.

Darbyshire, J.H., 1961. A fatal ulcerative mucosal condition of sheep associated with the virus of contagious pustular dermatitis. Br. Vet. J., 117: 97–105.

DeMartini, J.C., Pearson, L.D. and Fiscus, S.A., 1978. Chromium-51-release assay of antibody and complement-mediated cytotoxicity for contagious ecthyma virus-infected cells. Am. J. Vet. Res., 39: 1922–1926.

Dubey, S.C. and Sawhney, A.N., 1979. Serological relationship between viruses of goat-pox and contagious pustular dermatitis. Ind. J. Animal Sci., 49: 135–139.

Falk, E.S., 1978. Parapoxvirus infections of reindeer and musk ox associated with unusual human infections. Br. J. Dermatol., 99: 647.

Gardiner, M.R., Craig, J. and Nairn, M.E., 1967. An unusual outbreak of contagious ecthyma (scabby mouth) in sheep. Aust. Vet. J., 43: 163–165.

Gassmann, U., Wyler, R. and Wittek, R., 1985. Analysis of parapoxvirus genomes. Arch. Virol., 83: 17–31.

Huck, R.A., 1966. A paravaccinia virus isolated from cows' teats. Vet. Rec., 78: 503–505.

Hussain, K.A. and Burger, D., 1989. In vivo and in vitro characteristics of contagious ecthyma virus isolates: host response mechanism. Vet. Microbiol., 19: 23–26.

Kummeneje, K. and Krogsrud, J., 1978. Contagious ecthyma (orf) in the musk ox (*Ovibos moschatus*). Acta Vet. Scand., 19: 461–462.

Kummeneje, K. and Krogsrud, J., 1979. Contagious ecthyma (orf) in reindeer (*Rangifer tarandus*). Vet. Rec., 105: 60–61.

Le Jan, C., L'Haridon, R., Madelaine, M.F., Cornu, C. and Asso, J., 1978. Transfer of antibodies against the CPD virus through colostrum and milk. Ann. Rech. Vet., 9: 343–346.

Liebermann, H., 1967. Das Infektionsspektrum des Virus der Stomatitis papulosa. Arch. Exp. Vet. Med., 21: 1337–1352.

Liess, B., 1962. Beobachtungen und Untersuchungen über den Lippengrind (Ecthyma contagiosum) der Schafe als Zooanthroponose. Zentralbl. Bakteriol. Hyg. Orig. I, 185–189.

McKeever, D.J., McEwan Jenkinson, D., Hutchinson, G. and Reid, H.W., 1988. Studies of the pathogenesis of orf virus infection in sheep. J. Comp. Pathol., 99: 317–328.

Mathiesen, S.D. Jorgensen, T., Traavik, T. and Blix, A.S., 1985. On contagious ecthyma and its treatment in muskoxen (*Ovibos moschatus*). Acta Vet. Scand., 26: 120–126.

Mayr, A., Herlyn, M., Mahnel, H., Danko, A., Zach, A. and Bostedt, H., 1981. Bekämpfung des Ecthyma contagiosum (Pustulardermatitis) der Schafe mit einem neuen Parenteral-Zellkultur-Lebendimpfstoff. Zentralbl. Veterinärmed. B, 28: 535–552.

Mayr, A., Eißner, G. und Mayr-Bibrack, B., 1983. Handbuch der Schutzimpfungen in der Tiermedizin. Verlag Paul Parey, Berlin, Hamburg, p. 1100.

Naginton, H. and Horne, R.W., 1962. Morphological studies of orf and vaccinia viruses. Virology, 16: 248–260.

Obi, T.U. and Gibbs, E.P.J., 1978. Orf in sheep and goats in Nigeria. Trop. Anim. Health Prod., 10: 233–235.

PHLS Communicable Disease Surveillance Centre and Communicable Disease Unit, 1982. Orf paravaccinia infections, British Isles: 1975–1981. Br. Med. J., 284: 1958.

Pospischil, A. and Bachmann, P.A., 1980. Nuclear changes in cells infected with Parapoxviruses stomatitis papulosa and Orf: an in vivo and in vitro ultrastructural study. J. Gen. Virol., 47: 113–121.

Poulain, J., Gourreau, J.-M. and Dautigny, A., 1972. Ecthyma contagieux du mouton: anticorps sériques neutralisants. Ann. Rech. Vet., 3: 571–579.

Renshaw, H.W. and Dodd, A.G., 1978. Serologic and cross-immunity studies with contagious ecthyma and goat pox virus isolates from the western United States. Arch. Virol., 56: 201–210.

Robinson, A.H. and Balassu, T.C., 1981. Contagious pustular dermatitis (Orf). Vet. Bull., 51: 771–782.

Valder, W.-A., Straub, O.C., Thiel, W., Wachendörfer, G. and Zettl, K., 1979. Ecthyma contagiosum des Schafes—Wandel des klinischen Bildes. Tierärztl. Umschau, 34: 828–836.

Wilkinson, G.T., Prydie, J. and Scarnell, J., 1970. Possible "Orf" (contagious pustular dermatitis, contagious ecthyma of sheep) infection in the dog. Vet. Rec., 87: 766–767.

Chapter 8

Sheeppox Virus

M. MERZA and E.Z. MUSHI

INTRODUCTION

Sheeppox is a contagious disease of sheep caused by sheeppox virus (SPV). It is considered one of the most dangerous pox virus infections among animals. The disease is characterized by a rise of body temperature and skin lesions in the areas denied of wool; the disease may also affect the mucous membranes of the respiratory and gastrointestinal tracts. Sheep are the natural host of the virus; affection is determined by age and breed (Jubb and Kennedy, 1970; Buxton and Fraser, 1977).

VIRUS PROPERTIES

The virus has been classified by the International Committee of Taxonomy of Viruses as belonging to the subfamily Chordopoxvirinae and the genus *Capripoxvirus* (Andrews et al., 1978), which includes goatpox virus and lumpy skin disease virus. Viruses of the *Capripoxvirus* genus are found not to agglutinate red blood cells (Andrews et al., 1978). The chemical and physical properties of SPV resemble those of orf virus, but the morphology is different. Abdussalam (1957) was first to identify the virus by EM; it is brick-shaped, with dimensions of 90–140 by 150–250 nm; it has an outer membrane surrounding an electron-dense nucleoid which contains the genomic DNA. The lateral bodies of this virus are larger than those of other members of the Poxviridae family (Cohen et al., 1971).

In negatively stained EM preparations two forms of this virus were revealed: the beaded "mulberry" form (M-form) and the capsular form (C-form; Al-Bana, 1978). A similar morphology characterizes the vaccinia virus (Westwood et al., 1964).

Most of the strains isolated in different parts of the world infect only sheep under natural conditions and not cattle or goats (Murty and Singh, 1971; Khan 1973). One strain was isolated in Kenya which caused disease under natural conditions in both sheep and goats. After experimental infection this strain showed the same pathogenicity for sheep and goats; it was therefore called sheep- and goatpox virus (Davis, 1976). In its pathogenicity the Kenyan strain is different from strains isolated in the Middle East and India. Al-Bana (1978) observed mild lesions in goats inoculated intradermally with the Held strain of SPV, while no clinical signs were seen following intravenous inoculation. The goats that developed lesions resisted the challenge with goatpox virus (GPV) and so did the goats given the SPV intravenously.

There appears to be a considerable difference in the susceptibility of dif-

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ferent breeds of sheep to different strains of SPV (Arik and Kurtul, 1974; Davis and Otema, 1981).

Experimental intradermal inoculation of cattle with SPV resulted in a local reaction similar to the nodules produced by lumpy skin disease virus, and the inoculated animals also resisted the challenge with this virus (Capstic et al., 1959; Capstic and Coakley, 1961). Man appears to be insusceptible to SPV.

SPV is generally propagated in cell cultures from kidney or testicles of lambs. Subba Rao and Malik (1978) were able to adapt the Rainpet strain of SPV to growth in the egg CAM.

EPIZOOTIOLOGY

Sheeppox is prevalent in Africa, Asia, the Middle East and some Mediterranean countries. The main routes of infection are by direct contact with materials contaminated with SPV, and by the aerogenic route: inhalation of contaminated water droplets, dust or dry skin scabs (Hutyra et al., 1949; Murty and Singh, 1971). There are some reports indicating that arthropods may transmit the disease (Buxton and Fraser, 1977). Virus may be transmitted through wounds or scratches on the skin (Jensen, 1974). Experimentally it is possible to transmit the disease by inoculation of virus intradermally, subcutaneously, intraperitoneally or intravenously (Plowright et al., 1959). In the early days, Hutyra et al. (1949) infected the animals by intratracheal inoculation, which resulted in inflammation of the mucous membranes and spread of the disease.

PATHOGENESIS

Following intradermal inoculation, SPV replicates locally in the tissue (Plowright et al., 1959). Peak virus titer in the skin was attained on day 7 p.i., when pox lesions were detected. The virus spreads to the regional lymph nodes before invading the blood stream to produce primary viremia after 3–4 days (Singh et al., 1979). Internal organs such as spleen, liver and lungs are affected after viremia; lung lesions, however, may also develop directly from inhaled virus. The peak virus titers in skin nodules persist from day 7 to 14 and decline thereafter, concomitantly with the development of serum antibodies. Excretion of SPV from sheep is not documented; most likely skin scabs are the main source for virus shedding, but also ocular and nasal secretions might be infectious.

DISEASE SIGNS

The incubation period is 4–8 days after natural infection (own observations; Newson, 1965). The incubation period following experimental infection is reported to be 2–3 days.

The disease is characterized by a rise in body temperature up to 42°C, lacrimation, salivation, serous nasal discharge, swelling of the eye lids, hard respiration and congestion of mucous membranes. Two to three days later macules appear on the skin in areas denied of wool such as the lips, around the eyes, nostril, udder, genital organs, under the tail and between the legs. After 1–2 days the macules develop into red papules with a diameter up to 1 cm, which later develop into vesicles; thereafter necrotization of the skin and formation of scabs occur. Healing may take 5–6 weeks. The scabs fall off, leaving scars in their places.

Some authors reported that the stage of vesicle formation is absent and the papules develop directly into scabs (Plowright et al., 1959; Krishnan, 1968; Vegad and Sharma, 1970). Two forms of the disease are recognized. The malignant form causes high mortality in lambs (50–80%). In this form the lesions are found all over the skin denied of wool and in the mucous membranes of mouth, respiratory and gastrointestinal tracts (Murty and Singh, 1971). The mild form usually causes low mortality (2–5%) among adult animals, and the lesions appear in certain areas of the skin such as under the tail and between the legs (Buxton and Fraser, 1977). Secondary complications may occur due to bacterial infection, which often causes septicemia, tendovaginitis, orchitis and peripheral paresis (Scott, 1960).

PATHOLOGY

SPV causes generalized skin eruptions. Following an incubation period of about one week, hyperemic macules are observed on the skin which later, in a day or two, develop into papules and umbilicated vesicles followed by scab formation. Iwanoff (1941) described four forms of papules:

— Verrucoid papules, which are wart-like and are characterized by hyperplasia and parakeratosis of the epidermis; they are known as "wart pox".

— Urticaria-like papules, which are characterized by an intensive serous and cellular infiltration of the chorium, associated with thickening of the epidermis.

— Pelliculated papules, which are covered by a membranous necrotic epithelium that, when stripped off, leaves a raw ulcerating surface.

— Vesiculated papules, which are formed when the necrotic membrane of the pelliculated papules are raised from the chorium and filled with serous fluid to form a vesicle.

The main histopathological changes in the skin involve the subepithelial layers, dermis and subcutis. Borel (1903) described sheeppox cells or "cellules claveleuses", which are characterized by large oval or irregular nuclei with enlarged nucleoli. These cells arise from histiocytes (Jubb and Kennedy, 1970).

Lesions consisting of vesicles and ulcers and mucous membranes of the respiratory and alimentary tracts and small lymphoma-like nodules in the kidneys and lungs have been described (Hutyra et al., 1949; Scott, 1960; Jubb and Kennedy, 1970).

Regression of the lesions and repair with incoming fibroblasts is observed from the 16th day after the onset of disease (Ramachandran, 1967). The primary virus effect on the bone marrow appears to be hyperplasia followed by regressive changes (Vegad and Sharma, 1972).

LABORATORY DIAGNOSIS

Skin nodules should be preserved in buffered glycerol and transported on ice. Electron microscopy is advantageously performed on negatively stained samples that reveal poxvirus particles. Inoculation of lamb testicle cell cultures grown on cover slips with lesion material allows the demonstration of viral antigens by IF after 48 h. An AGID technique using convalescent serum or hyperimmune rabbit serum can be used to differentiate SPV from GPV infection.

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PROPHYLAXIS AND CONTROL

Many attempts were made towards the production of vaccines against sheep-pox; they can be classified as follows:

Inactivated virus vaccines

Many efforts have been made to inactivate SPV; e.g. by treatment with formaldehyde, butanol, boric acid, phenol and UV light (Angeloff, 1940; Uppal, 1963; Pandey et al., 1969). These treatments proved to be unsuccessful due to their denaturing effects and to the loss of immunogenicity of the preparations (Jayaraman, 1967).

Goyal and Singh (1975) used chemicals which would not affect the antigenicity of the virus, such as N-acetyl ethyleneimine, hydroxylamine or formalin in low concentrations. The resulting vaccines were safe and induced protection against challenge with high doses of virulent virus.

Sheeppox antigen in combined vaccines

SPV can be advantageously mixed with other antigens to form combined vaccines, e.g. with antrax antigen (Linkhachev et al., 1967); the preparation was shown to protect against both pathogens. Safarov and Kadymov (1970) composed a vaccine with SPV and clostridial antigens which proved effective.

Chick embryo adapted live vaccines

Yuan et al. (1957) adapted the virus to the CAM of chicken embryos and used it as a vaccine after 90 passages. Linkhachev et al. (1961) further studied the Chinese strain of SPV, which was found to be avirulent and to induce a high level of protective immunity. Borisovich (1962) and Borisovich et al. (1966) confirmed that the Chinese strain (K-strain) grown on the CAM is highly immunogenic.

Cell culture attenuated vaccines

Serial passages in tissue culture resulted in the loss of virulence while immunogenicity of the virus was retained. Large yields of virus to a low cost could be attained, and the vaccine produced in this way did not spread from the vaccinated animals. After inoculation into the original host a local reaction with rise in body temperature was observed, and a high level of immunity was induced, protecting the animal against natural or experimental infection (Ramyar and Hessami, 1968; Martin et al., 1973; Penkova et al., 1974).

The Rumanian strain of SPV (RM/65), isolated in Yougoslavia, was attenuated by 30 passages in cultures of lamb kidney cells (Ramyar, 1965; Ramyar and Hessami, 1968). This vaccine was shown to give protective immunity lasting for up to 2 years (Ramyar et al., 1976). El-Zein (1968, 1969) managed to attenuate this strain by 25 passages in lamb testicle cells and used it as a vaccine which is called sheeppox virus/Rumanian-Fannar (SPV/RF).

Precausta et al. (1979) developed a vaccine by passing the Rumanian strain 30 times in cultures of lamb kidney cells. Given subcutaneously, it protected animals for 12 months. The freeze-dried vaccine is stable for at least 2 years at 6°C.

Recently, local isolates of SPV have been attenuated. They often seem to suit the local conditions better and induce protection in a larger proportion of vaccinated animals than foreign strains.

For prophylaxis and control, the live vaccines attenuated by several passages (20–30) in tissue culture should be used instead of inactivated vaccines: they are safe and induce protective immunity. These attenuated vaccines can safely be given to lambs over 2 months of age; annual boosters are required (Precausta et al., 1979).

The vaccine is administered intradermally. At the site of inoculation a local reaction should develop. All vaccinated animals should be checked after a week for a positive reaction, and if there is no take they should be revaccinated.

Suitable disinfectants are formalin (1–3%) strong acids, caustic soda and virucidal preparations.

REFERENCES

Abdussalam, M., 1957. Elementary bodies of sheep pox. Am. J. Vet. Res., 18: 614.

Al-Bana, A.S., 1978. Purification, Physical and Chemical Analysis of Sheep Pox Virus and the Response of Sheep and Goats to Infection with Sheep and Goat Pox Viruses. Ph.D. Thesis, Cornell University, Ithaca, NY.

Andrews, C.H., Pereira, H.G. and Wildy, P., 1978. Viruses of Vertebrates. Ballier, Tindall, London, 4th edn., p. 371.

Angeloff, S., 1940. Sheep pox in Bulgaria. Abstr. Vet. Bull., 12: 584.

Arik, F. and Kurtul, Y., 1974. Culture of sheep pox virus on the chorioallantoic membrane of the chick embryo. Pendik Vet. Kontrol. Ara. Enst. Derg., 7: 20.

Borel, A., 1903. Epithelioses infectieuses et epitheliomas. Cited in: Plowright, W. and Ferris, E.D., 1958. Br. J. Exp. Pathol., 396: 424.

Borisovich, Y.F., 1962. Immunological properties of dried chick embryo virus vaccine against sheep pox. Abs. Vet. Bull., 33: 438.

Borisovich, Y.F., Koval, G.L., Bogmelt, L.G. and Palgov, A.A., 1966. Chick embryo sheep pox vaccine. Abs. Vet. Bull., 37: 296.

Buxton, A. and Fraser, G., 1977. Animal Microbiology. Blackwell Scientific Publications, London, Vol. 2, 473 pp.

Capstick, P.B. and Coakley, W., 1961. Protection of cattle against lumpy skin disease. Trials with a vaccine against Neethling type infection. Res. Vet. Sci., 2: 362–368.

Capstick, P.B., Prydie, J., Coakley, W. and Burdin, M.L., 1959. Protection of cattle against the Neethling type virus of lumpy skin disease. Vet. Rec., 71: 422.

Cohen, J., Berehi, A., Ribero, M. and Vincent, M., 1971. Étude en microscopie électronique de la morphogénèse du virus de la clavelée (variole ovine) en culture de tissu. Ann. Inst. Pasteur, 121: 569.

Davies, F.G., 1976. Characteristics of a virus causing a pox disease in sheep and goats in Kenya, with observations on the epidemiology and control. J. Hyg. Camb., 76: 163.

Davies, F.G. and Otema, C., 1981. Relationship of capripox viruses in Kenya with two Middle Eastern strains and some orthopox viruses. Res. Vet. Sci., 31: 253–255.

El-Zein, A.Z., 1968. Annual Report. Cited in: Penkova, V.M., Jassin, F.A., Thompson, J.R. and Al-Doori, T.M., 1974. Bull. Intr. Epiz., 81: 32.

El-Zein, A.Z., 1969. Annual Report. Cited in: Penkova, V.M., Jassim, F.A., Thompson, J.R. and Al-Doori, T.M., 1974. Bull. Intr. Epiz., 81: 32.

Goyal, S.M. and Singh, I.P., 1975. Inactivation of sheep pox virus by formaldehyde, hydroxylamine and N-acetyl ethyleneimine. Indian J. Anim. Sci., 45: 748.

Hutyra, F., Marek, J. and Manninger, R., 1949. In: J.R. Greig (Editor), Special Pathology and Therapeutics of Diseases of Domestic Animals. Alexander Eger, Chicago, IL, p. 353.

Iwanoff, X., 1941. Beiträge zum pathologisch-anatomischen Bilde der Schafpocken mit besonderer Berücksichtigung der sogenannten atypischen Schafpocken. Jahresber. Veterinärmed., 68: 214.

Jayaraman, M.S., 1967. Latest positions in respect of immunization against sheep pox and goat pox. Proc. 12th Conference on Animal Diseases, Poona.

Jensen, R., 1974. Sheep pox. In: Diseases of Sheep. Lea and Febiger, Philadelphia, PA, p. 281.

Jubb, K.V. and Kennedy, P.C., 1970. Pathology of Domestic Animals. Academic Press, New York, 2nd edn., 600 pp.

Khan, M.H., 1973. Sheep and goat pox, its prevalence and field control in Pakistan. In: M.M. Lowrence (Editor), Cento Semin. Control. Erad. Virol. Dis. Center, Ankara, Turkey, Reg p. 132.

Krishnan, R., 1968. Pathogenesis of sheep pox. Indian Vet. J., 45: 297.

Linkhachev, N.V., Skalinskii, E.I., Borisovich, Y.F. and Islenteva, K.M., 1961. Properties of dried live embryo vaccine prepared from sheep pox virus of low virulence. Tr. Nauchno Kontrol. Inst. Vet. Preparatov, 9: 17.

Martin, W.B., Ergin, N. and Koyln, A., 1973. Tests in sheep of attenuated sheep pox vaccines. Res. Vet. Sci., 14: 53.

Murty, D.K. and Singh, P.P., 1971. Epidemiological studies on outbreaks of sheep pox in mixed flock in Uttar pradesh. Indian J. Anim. Sci., 41: 1072.

Newson, I.E., 1965. Sheep pox. In: H. Marsh (Editor), Sheep Diseases. Williams and Wilkins, Baltimore, MD, p. 116.

Pandey, A.K., Malik, B.S. and Bansal, M.P., 1969. Studies on sheep pox virus II—Immune and antibody response with cell culture adapted virus. Indian Vet. J., 46: 1017.

Penkova, V.M., Jassim, F.A., Thompson, J.R. and Al-Doori, T.M., 1974. The propagation of an attenuated sheep pox virus and its use as a vaccine. Bull. Intr. Epiz. Bio., 81: 32.

Plowright, W., Macleod, W.G. and Ferris, R.D., 1959. The pathogenesis of sheep pox in the skin of sheep. J. Comp. Pathol., 69: 400.

Precausta, P., Kato, F. and Vellut, G., 1979. A new freeze-dried living vaccine against sheep pox. Comp. Immunol. Microbiol. Infect. Dis., 1: 305.

Ramachandran, S., 1967. Observation on histopathology of lung lesions in experimental pox infection of sheep and goats. Ceylon Vet. J., 15: 78.

Ramyar, H., 1965. Studies on the immunogenic properties of tissue culture sheep pox virus. Zentralbl. Veterinärmed., 123: 537.

Ramyar, H. and Baharsefat, M., 1969. A new approach to active immunization of sheep by combined sheep pox and anthrax vaccine. Zentralbl. Veterinärmed., 163: 585.

Ramyar, H. and Hessami, M., 1968. Development of an attenuated live virus vaccine against sheep pox. Arch. Inst. Razi., 20: 77.

Ramyar, H., Hessami, M. and Ghaboussi, B., 1976. Observation on the use of live modified tissue culture vaccine against sheep pox. Arch. Inst. Razi., 28: 11.

Safarov, Y.B. and Kadymov, R.A., 1970. Simultaneous vaccination of sheep against pox and clostridial infection. Abstr. Vet. Bull., 41: 636.

Scott, G.R., 1960. Sheep pox. In: W. Dalling (Editor), International Encyclopedia of Veterinary Medicine. Green and Sons, Edinburgh, p. 2411.

Singh, I.P., Pandey, R. and Strivastova, R.N., 1979. Sheep pox: A review. Vet. Bull., 49: 145.

Subba Rao, M.V. and Malik, B.S., 1978. Cultivation of sheep pox virus on CAM of chick embryo. Vet. Rec., 102: 264.

Uppal, P.K., 1963. Studies on sheep pox virus. MSc thesis, Agara University. Cited in: Sharma and Danda, 1971. Indian J. Anim. Sci., 41: 267.

Vegad, J.L. and Sharma, G.L., 1970. Pathogenesis of sheep pox (Variola ovina) in the skin of experimentally infected sheep. Microscopic observation. JNKVV. Res. J., 4: 32.

Vegad, J.L. and Sharma, G.L., 1972. Pathogenesis of sheep pox (Variola ovina) in the lymphatic, reticulo-endothelial and haematopoietic systems of experimentally infected sheep. JNKVV. Res. J., 6: 21.

Westwood, J.C.N., Harris, W.J., Zwartouw, H.T., Titmuss, D.H.J. and Appleyard, G., 1964. Studies on the structure of vaccinia virus. J. Gen. Microbiol., 34: 67.

Yuan, C.I., Led, P.C. and Chang, Y.S., 1957. Research on low virulence virus vaccine of sheep pox virus on the developing chick embryo. Abstr. Vet. Bull., 29: 1054.

Chapter 9

Goatpox Virus

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Goatpox is caused by goatpox virus (GPV) which is closely related to sheep-pox virus; clinically it resembles sheeppox but the disease in goats takes a milder course.

GPV is morphologically and structurally identical to SPV (Skalinski and Borisovich, 1975); it is also sensitive to ether and chloroform (Pandey and Singh, 1970). GPV is classified by the International Committee of Taxonomy of viruses as a member of the genus *(Capripoxvirus)*.

Under natural conditions GPV infects goats; there are also reports of the disease affecting mixed sheep and goat herds in Gahna and Yemen.

Bennett et al. (1944) were able to transmit Palestinian GPV to sheep, monkeys, rabbits and calves. Using a Swedish strain, Bakos and Brag (1957) also showed such a wide host range. In Sudan, Mohamed et al. (1982) have described the transmission of GPV to sheep; others, however, have failed (Hutyra et al., 1949; Andrews and Pereira, 1972). Al-Bana (1978) observed mild lesions in sheep inoculated intradermally with the Held strain of GPV. No reaction was seen following intravenous injection. The sheep that had reacted with lesions after intradermal injection, but also those that had been given the virus intravenously, resisted challenge with virulent SPV. Bennett et al. (1944) and Bakos and Brag (1957) are the only authors who report experimental infections of laboratory animals (rabbits) with GPV.

The Sersenk strain of GPV grew very well on the CAM of chick embryos (Tantawi and Falluji, 1979). An Iranian and likewise an Egyptian strain grew poorly, while a Russian strain did not grow at all. The Swedish strain of GPV caused pox-like eruptions on the hands of people in contact with infected goats (Bakos and Brag, 1957). Animal attendants were reported to be infected with an Iranian strain of GPV, and the virus isolated from the person could experimentally be transmitted back to goats (Sawhney et al., 1972).

The first to describe the disease was Hansen in Norway 1879 (cited in Andrews et al., 1978). Goatpox is now prevalent in North Africa, the Middle East, India, Australia, America and some Scandinavian countries (Andrews et al., 1978).

Under natural conditions the disease is transmitted within the flock by direct contact. Experimental infection of goats by the inoculation of virus intradermally, subcutaneously, intravenously, or intraperitoneally caused the appearance of skin lesions all over the body (Rafyi and Ramyar, 1959).

The clinical signs resemble those of sheeppox (Hutyra et al., 1949; Jubb and Kennedy, 1970). The disease has two forms: malignant and mild. The malignant form spreads among kids and is characterized by the appearance of scabs on the skin all over the body and on the mucous membranes of the respiratory and gastrointestinal tracts. The mild form, on the other hand, is characterized by a moderate rise of the body temperature and the appearance of scabs on the skin of the udder, under the tail, around the mouth and on the plantar side of the front legs.

The members of the genus *Capripoxvirus* are antigenically related and their clinical signs have many common features (Al-Bana, 1978). There are several studies using the ID test that demonstrate the immunological relationship between SPV and GPV (Bhambani and Krishna Murty, 1963; Pandey and Singh, 1972; Al-Bana, 1978; Muhamed, 1982). When antisera were absorbed with the heterologous antigens they gave only one line of precipitation with the homologous antigen (Muhamed, 1982). Kitching et al. (1985) used ^{35}S methionine to label the proteins of SPV and GPV and showed that the major structural polypeptides of these viruses co-migrate in polyacrylamide gels. By use of an AGID test with labelled antigens a major common precipitating antigen was identified.

There have been few attempts towards the production of a goatpox vaccine. Arik and Denizli (1977) produced live vaccines based on an Iranian strain and an isolate from Israel. They reported that these preparations were effective and safe. However, both strains were found to be ether resistant, in contrast to the established members of the *Capripoxvirus* genus.

Dubey and Sawhney (1978) passed the Mysore strain of GPV 25 times in goat testicular cells and used it either directly or mixed with 0.5% phenol. Both vaccines produced local reactions at the site of inoculation and gave good protection.

Suitable preparations for decontamination are formalin (1–3%), acids, caustic soda and virucidal disinfectants.

REFERENCES

Al-Bana, A.S., 1978. Purification, Physical and Chemical Analysis of Sheep Pox Virus and the Response of Sheep and Goats to Infection with Sheep and Goat Pox Viruses. Ph.D. thesis, Cornell University, Ithaca, NY.

Andrews, C.H. and Pereira, H.G., 1972. Viruses of Vertebrates. Balliere, Tindall, London, 3rd edn., p. 391.

Andrews, C.H., Pereira, H.G. and Wildy, P., 1978. Viruses of Vertebrate. Balliere, Tindall, London, 4th edn., pp. 371.

Arik, F. and Denizli, N., 1977. Effect of ether, chloroform and heat on the Iran and Israel goat pox vaccine viruses and studies on their titration, pathogenicity and antigenicity. Pendic Vet. Backt. veSer. Enst. Derg., 9: 45.

Bakos, K. and Brag, S., 1957. Studies on goat pox in Sweden. Nord. Veterinärmed., 9: 431. (Abstr. Vet. Bull., 271: 3258).

Bennett, S.C.J., Horgan, E.S. and Mansur Ali Hasseed, 1944. The pox diseases of sheep and goat. J. Comp. Pathol., 54: 131–160.

Bhambani, B.D. and Krishna Murty, D., 1963. An immunodiffusion test for laboratory diagnosis of sheep and goat pox. J. Comp. Pathol., 73: 349.

Dubey, S.C. and Sawhney, A.M., 1978. Live and inactivated tissue culture vaccines against goat pox. Indian Vet. J., 55: 925.

Hutyra, F., Marek, J. and Manninger, R., 1949. In: J.R. Greig (Editor), Special Pathology and Therapeutics of Diseases of Domestic Animals. Alexander Eger, Chicago, IL, p. 353.

Jubb, K.V.F. and Kennedy, P.C., 1970. Pathology of Domestic Animals. Academic Press, New York, 2nd edn., p. 600.

Kitching, R.P., Hammond, J.M. and Black, D.N., 1985. Studies on the major common precipitating antigen of *Capripox* virus. J. Gen. Virol., 67: 139–148.

Mohamed, K.A., Hago, B.E.D., Taylor, W.P., Nayil, A.A. and Abu-Samra, M.T., 1982. Goat pox in Sudan. Trop. Anim. Health Prod., 14: 104.

Muhamed, M.Y., 1982. Antigenic and Serologic Relationship Between Sheep and Goat Pox Viruses Isolated in Iraq. M.Sc. thesis, University of Baghdad

Pandey, R. and Singh, I.P., 1970. Cytopathogenicity and neutralization of goat pox virus in cell culture. Res. Vet. Sci., 11: 195.

Pandey, R. and Singh, I.P., 1972. Soluble antigens of sheep pox and goat pox viruses as determined by immunodiffusion in agar gel. Acta. Virol., 16: 41.

Rafyi, A. and Ramyar, H., 1959. Goat pox in Iran. Serial passage in goats and the developing egg and relationship to sheep pox. J. Comp. Path., 69: 141.

Sawhney, A.N., Singh, A.K. and Malik, B.S., 1972. Goat Pox: an anthropozoonosis. Indian. J. Med. Res., 60: 683.

Skalinskii, E.I. and Borisovich, Y.F., 1975. Structure and morphogenesis of goat pox virus. Veterinariya, Moscow, 7: 39. (Abstr. Vet. Bull., 46: 623).

Tantawi, H.H. and Falluji, M.M., 1979. Laboratory characteristics of four strains of goat pox virus. Acta. Virol., 23: 455.

Chapter 10

Lumpy Skin Disease Virus

J.A. WOODS

INTRODUCTION

Lumpy skin disease (Ngamiland disease, knopvelsiekte, maladie nodulaire cutanée) is a serious disease of cattle first seen in Northern Rhodesia (Zambia) in 1929 (Morris, 1931); since then it has spread to most parts of Africa.
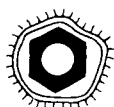
VIRUS PROPERTIES

Physical and chemical characteristics

The virus has a structure typical of other members of the Poxviridae. The surface shows a network of threads 70–90 Å wide when stained with phosphotungstic acid at pH 6.5 and a multilayered capsule at pH 8.5 (Munz and Owen, 1966). When stained with uranyl acetate the internal structure is revealed: a dumbbell-shaped core and two lateral bodies (Fig. 18). The virion contains DNA as judged by acridine orange and Feulgen staining (Weiss and Broekman, 1965) and 5-bromodeoxyuridine inhibition (Weiss, 1968).

The virus is ether and chloroform sensitive and is stable between pH 6.6 and 8.6 (Plowright and Ferris, 1959). There is no reduction in titer when it is held at 37°C for 5 days, and it remains viable at 4°C for 6 months. It has been recovered from lesions on an air-dried hide after 18 days (Alexander and Weiss, 1959).

Lumpy skin disease virus is classified as a *Capripoxvirus* along with the viruses of sheep and goat pox (Matthews, 1982). The term Neethling strain or Neethling virus has been applied to distinguish it from the herpesvirus infection causing pseudo lumpy skin disease.

Antigenic properties

Only one antigenic type of the virus exists. Strains from outbreaks in South Africa, Kenya and Nigeria have been found antigenically indistinguishable.

There is a close relationship between LSDV, SPV and GPV. Antigenic relationships and host specificity of pox viruses of sheep and goats are not exactly clear but SPV strains such as the Kedong and the Kenya SGPV O-240 strain affect both sheep and goats and also protect cattle against lumpy skin disease (Capstick, 1959; Davies and Otema, 1981).

LSDV and various strains of SPV and GPV from Africa and Europe are indistinguishable using SN and IF tests. Davies and Otema (1981) found no

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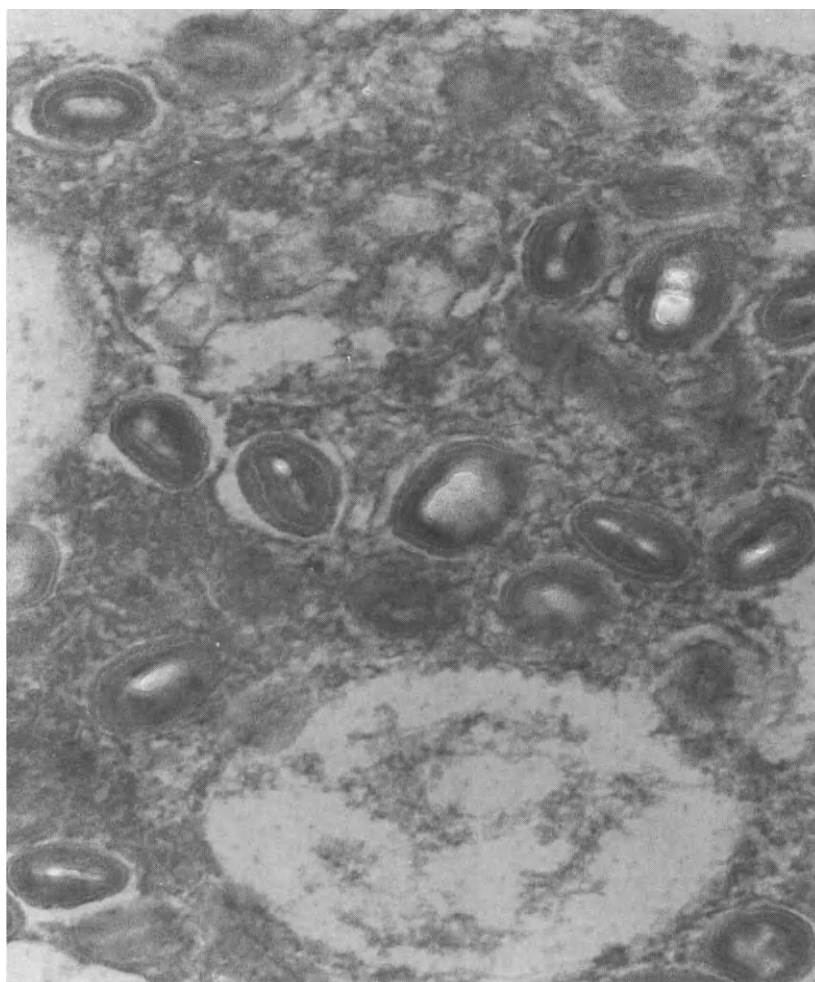
Fig. 18. Electron micrograph of LSD virus particles. Virions are still easily recognized despite widespread destruction of host cytoplasm by formalin fixation.

cross-reactivity of the capripoxvirus group to a number of orthopoxviruses, with the exception of low level reactions to cowpoxvirus using the IF test. Polyacrylamide gel electrophoresis of LSDV, SPV and GPV has revealed a common major precipitating antigen, mol. wt. 67 k, located on the outer membrane of the virus particle (Kitching et al., 1986). This antigenic conformity within the *Capripoxvirus* genus is the basis of the use of SPV as a vaccine against lumpy skin disease.

EPIZOOTIOLOGY

Distribution

The spread of the disease through Africa has been slow and tortuous. It has taken half a century to travel from central Africa southwards to the Cape, northwards to the Sahara and thence to western Africa. Its progress has been marked by a rapid spread followed by periods of up to a decade of quiescence. Most countries south of the Sahara have now experienced the infection (Fig. 19).

Morbidity varies greatly between herds and has been found in the range of 50–100% in Bechuanaland (Von Backstrom, 1945), 5–45% in South Africa (Haig, 1957), 10–50% in Madagascar (Lalanne, 1956) and 5–75% in Nigeria
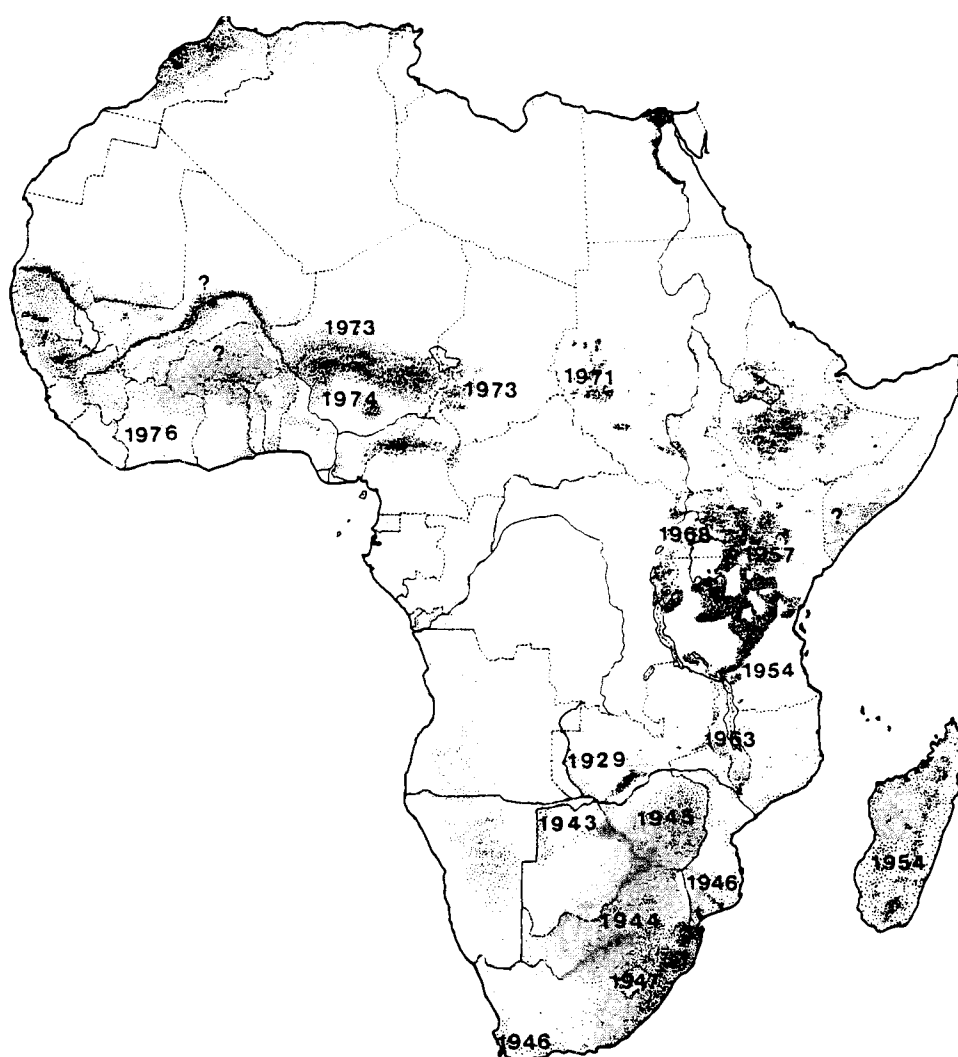
Fig. 19. Virgin outbreaks of LSD in Africa since 1929. Each dot represents 5000 cattle (based on a cattle distribution map by the Interafrican Bureau of Animal Health, Nairobi).

(Nawathe et al., 1978). The mortality rate in South African herds in the initial epizootic was around 1% (Diesel, 1949) but rising on occasions to 75%. Later outbreaks in other countries have usually shown mortality rates below 5%. *Bos indicus* and *Bos taurus* seem to be equally susceptible. There appear to be no sex, age or breed differences of significance. Of the domestic animals only cattle are susceptible, and the disease is not transmissible to man.

Nature of spread

Lumpy skin disease virus is not very contagious. Although virus is present in saliva for at least 11 days after the onset of pyrexia (Haig, 1957), experimental transmission by close contact has only been successful when cattle shared drinking bowls (Weiss, 1968).

The semen of bulls has been found to contain virus for 22 days after clinical signs have started. Virus is present in the blood for at least 4 days following the onset of pyrexia and generalization of lesions, as judged by experimental infection (Alexander and Weiss, 1959).

A number of factors are involved in the spread of infection during and

between epizootics; these include biting flies, rainfall pattern, animal movement, wild birds, game animals and susceptibility of the national herd.

Biting flies have been incriminated in most epizootics since Morris (1931) described the first outbreak in 1929. This was also noted by Haig (1957) in the case of the South African epizootic and Burdin and Prydie (1959) for the Kenyan outbreak. In Kenya, the geographic distribution of the mosquitoes *Culex mirificus* and *Aedes natronius*, present in great numbers, coincided with foci of disease. In the virgin outbreak in Chad in 1973 an exceptionally large population of biting flies of the genus *Lyperosia* was reported by Provost (1974). Virus has been isolated from *Biomyia fasciata* and *Stomoxys calcitrans* caught feeding on infected cattle (Weiss, 1963).

It has been consistently noted that disease outbreaks occur during the *rainy season* and disappear with the onset of the dry season. Heavy rains are often associated with epizootics. Disease in Kenya is not seen in the dry coastal areas but occurs in all other rainfall zones; particularly on high rainfall plateaux epizootics follow excessive rains (Davies, 1982). High savannah grasslands and lower drier guinea savannah have both been involved in Nigerian outbreaks.

Cattle movement is undoubtedly one of the most important factors in the dissemination of lumpy skin disease. Road and rail movement of cattle contributed greatly to the spread of infection in South Africa in 1946. In Kenya in 1957 spread was limited, probably due to efficient action of the veterinary services in stopping animal movement. Nomadic and trade movement of cattle in western Africa formed the basis for rapid dissemination of disease in that region. Severe droughts occurred in sub-Saharan areas in 1971 and 1972, forcing semi-nomadic cattle from Chad to migrate further south than normal. Deep in the Central African Republic they came in contact with infected cattle and brought the infection back to Chad from whence it spread to Niger and northern Nigeria. The comprehensive network of trade routes coupled with road and rail transport ensured rapid dispersal throughout Nigeria.

Naturally occurring cases of lumpy skin disease in *game animals* have not been reported to date. It can be concluded that if they do occur it is rarely. However, experimental infection of an impala (*Aepyceros melampus*) and a giraffe (*Giraffa camelopardalis*), both calves, resulted in macroscopic lesions and death (Young et al., 1970). Two adult wildebeest (*Connochaetus gnou*) and two Cape buffalo (*Syncerus caffer*) calves were refractory to infection in the same experiment. Davies (1982) found high levels of antibody to *Capripoxvirus* in Cape buffalo in Kenya, in areas where disease was endemic in cattle. He concluded that buffalo might serve as reservoir hosts during the inter-epizootic periods. A serological survey by Hedger and Hamblin (1983) covering many affected countries showed low levels of antibody in six species of game and negative titers in 38 other species, including the Cape buffalo. There was considerable uncertainty about the specificity of antibody detected. The low prevalence and generally negative results suggest that wildlife does not play a significant part in the perpetuation of the disease.

Two *birds*, the red-billed oxpecker (*Buphagus erythorhynus*) and the egret (*Bubulcus ibis*) are often seen perching on the backs of cattle throughout Africa; McOwan (1959) suggested that they might spread LSDV mechanically on their beaks.

The pattern of recurrence of epizootics (which should give some indication of *national herd susceptibility*) has varied in different parts of Africa. The disease has never caused much alarm in central Africa, and apart from the original outbreak in 1945 and resurgences in 1954 and 1976 it has made little impact in Zimbabwe, despite the presence of many exotic dairy cattle. Elsewhere the picture has been one of well-defined epizootics occurring at regular intervals. In South Africa such outbreaks occurred in 1947, 1953, 1957, 1962 and 1967, in Uganda in 1968 and 1981 and in Nigeria in 1974 and 1979. This would

suggest cycles of resistance and susceptibility of around 5 years. The extent and frequency of vaccination will of course affect the disease pattern. It is generally supposed that there are no latent virus carriers, which is supported by circumstantial evidence from South Africa (Haig, 1957).

It can be concluded that in an unvaccinated population lumpy skin disease is likely to occur in epizootics at intervals of 5 years or more, with sporadic cases in the inter-epizootic periods. Epizootics can be expected after the start of a rainy season, particularly when rains are heavy. Associated with these rains is the rapid multiplication of biting flies of which some species probably act as short-range vectors. Further extension of disease can be expected to occur following cattle movement, but animal-to-animal contact is not important in this spread. Disease prevalence will fall dramatically at the end of the wet season. There is no hard evidence for the role of any species other than cattle in the perpetuation of disease in the inter-epizootic period, but this aspect of the epidemiology is still poorly understood.

It is quite possible that the disease may appear outside Africa. Due to the nature of transmission it is unlikely that infection will be spread by meat or products. The movement of live infected animals remains the obvious risk. From time to time there is trade in live animals from eastern Africa to the Middle East. New political affiliations of some African countries raise the possibility of the disease appearing much further afield.

An assessment of the economic significance of the disease has not been made. Lumpy skin disease has always been taken more seriously in South Africa than elsewhere, though the apparent severity in that country may be a function of the concern shown by farmers with advanced husbandry systems and highly productive animals. The most important losses accrue from morbidity and not from mortality. Secondary infection causes debility and loss of weight, mastitis, a drop in milk yield and infertility. Abortions can occur in the early stages of disease but are usually not numerous. Milk yields have been seen to drop by 50% in indigenous herds in Nigeria (J.A. Woods, unpublished data, 1974); this was partly due to general malaise but also to udder lesions and purulent mastitis. Loss of quarters has been noted in Rhodesia (Anon., 1977). Damage to hides has been described (Green, 1959; De Sousa Dias and Limpo-Serra, 1956) which can be severe, as lesions affect the full thickness of the skin and a few holes can render a hide virtually useless.

PATHOGENESIS

A febrile reaction occurs about 5 days after exposure to the virus and lasts 5–12 days as judged by experimental infection. A primary nodule appears at the site of inoculation some 7 days later (Capstick, 1959; Nawathe and Asagba, 1977; Prozesky and Barnard, 1982). It would seem that virus multiplication occurs in the dermis at the sites of insect bites. Viremia follows, coinciding with the rise in temperature and lasting for about 4 days (Alexander and Weiss, 1959). Following the viremia generalized lesions occur in the skin and sometimes in the viscera. Only one "crop" of disseminated lesions is observed.

During the course of the disease, virus is present in the saliva for at least 11 days, in the semen of bulls for at least 22 days and in skin nodules for at least 33 days after generalization. Virus has also been recovered from regional lymph nodes, spleen, muscle and normal skin but not from urine or feces (Alexander and Weiss, 1959). Although saliva contains large amounts of virus, infection by direct contact and fomites is of minor importance. The period of infectivity will largely depend on the accessibility of virus to biting flies. The duration is unknown, but it can be assumed that the animal will be at its most

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infective during the short viremic period 2–3 days before and after the appearance of lesions. Since virus is present in skin nodules for 5 weeks, infected cattle are probably also a source of infection during this period.

There is no evidence for a carrier state. In South Africa, an anaplasmosis vaccine caused no ill effects despite being produced in cattle with a history of LSD (Haig, 1957).

DISEASE SIGNS

The clinical signs of lumpy skin disease have been described by Morris (1931), Von Backstrom (1945), Haig (1957), Burdin and Prydie (1959) and Provost (1974) as the condition occurred in virgin epizootics throughout Africa. Outbreaks of disease present a variety of signs which, taken together, give a quite unmistakable picture. There is a wide range of severity. Some animals show only a few skin lumps and no systemic reaction and are virtually unaffected by the disease. Others show the full spectrum of signs described. The incubation period in the field has been estimated as 2–4 weeks (Henning, 1956). A disease outbreak spread by an anaplasmosis vaccine in South Africa would indicate a period of 14 days (Diesel, 1949).

A rise in temperature of up to 41°C, a generalized lymphadenitis, anorexia and the appearance of edema in limbs, abdomen or brisket can occur in the early stages. Pyrexia can last for 2 weeks. Swelling of prescapular, precrural and popliteal lymph nodes is usually obvious. Abortion, probably due to the pyrexia, is not uncommon.

Within 48 h of the onset of pyrexia lumps appear in the skin. Most of the lumps measure 1–3 cm in diameter and extend 1–2 cm deep. They can range from a few millimetres to 10 cm or more due to nodules coalescing to form plaques. The number of lumps appearing on one animal can vary from one to many hundreds. They are hard, circular and have well demarcated edges. The full thickness of the skin and often the subcutis is involved in the lesion. Nodules protrude above the surrounding skin and in short-haired cattle are visible at a distance. The nodule can be grasped between fingers and thumb, when it will usually move easily with the skin. At this early stage the skin over and around the nodules looks quite normal and is unbroken. Some nodules remain in this stage and are slowly resorbed over a number of months. Lesions are distributed on all parts of the body from the muzzle to the tail (Figs. 20, 21).

The severity of disease generally increases with the number of skin nodules (Haig, 1957; Weiss, 1963) but severe cases can occur when lesions are restricted to the mucosae and internal organs (Provost, 1974).

Clinical examination of suspect cases, particularly of longer-haired European breeds, should be thorough. The skin should be palpated carefully, especially in the region of the perineum and posterior aspects of the thighs, as these areas are predilection sites.

Within a week of their appearance nodules begin to develop to the "sit-fast" stage. A circular line of necrosis appears around the edge of the nodule and a central cylindrical core of tissue begins to separate from the surrounding skin. The lesion now looks as though it had been hit with a circular punch. Some nodules develop no further than this and are slowly resorbed.

The core of most "sit-fast" lesions becomes necrotic in the next 5–7 weeks and sloughs, leaving a deep granulating pock involving all layers of the skin.

An affected animal in the later stages will show a mixture of unbroken skin nodules, "sit-fasts" and granulating pocks.

Sequelae to skin and lymph node lesions include sloughing of the skin, suppurative ulcers of the legs and mastitis. Sloughing of skin can occur par-
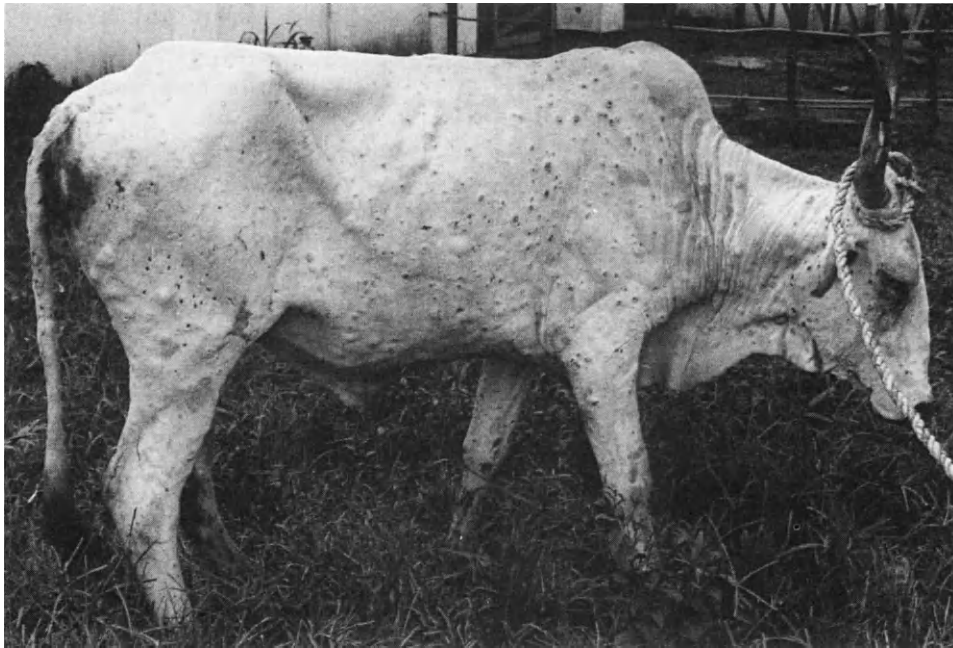
Fig. 20. A Nigerian Gudali cow with skin nodules of different sizes and stages of development.

ticularly on the lower abdomen and legs where necrosis has resulted from edema or possibly from plaques of coalesced nodules. Suppurative discharging sinuses in the lower limbs are due to abscessation of regional lymph nodes; these can persist for months. Nodules occur on the skin of the teats and udder and in the glandular substance of the udder. An accompanying purulent mastitis is common. Milk yields often fall dramatically.

Ocular and nasal discharge and occasionally drooling of saliva follow the appearance of yellowish necrotic lesions on the mucosa of the nose, mouth and

Fig. 21. Severely affected cow showing widespread distribution of nodules.

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conjunctiva. Nasal discharge may be purulent and profuse and may cake in the external nares to obstruct breathing. Necrotic foci also appear in the vagina. Lesions are common on the dental pad, at the skin–mucosal junction and at the edge of the hard palate. Most have the same "punched out" appearance of the "sit-fast" skin lesion. This is characteristic of the condition and helps to distinguish it from rinderpest and mucosal disease. The central necrotic plug of mucosa sloughs to leave a deep ulcer.

The disease affects cattle of all ages, young calves often severely. Animals in a poor nutritional state seem to be more susceptible. Chemotherapy appears to hasten recovery by countering secondary bacterial infection. Some cattle progress to a cachectic state; these animals often have lesions in the lungs and other organs not obvious from a clinical examination.

A wide range of conditions has in the past been confused with lumpy skin disease. These include Allerton virus infection (pseudo lumpy skin disease), streptothricosis, onchocercosis, globidiosis and urticarial swellings.These conditions may give rise to uncertainty when only one or two animals are affected. In practice when an outbreak occurs there should be no confusion with any other condition. Allerton virus infection is consistently mild and only the superficial skin layers are involved. The lesions have a flat surface with a slight central depression. Lumpy skin disease has on occasion mimicked rinderpest or mucosal disease. In the virgin epizootic in Chad many cattle showed mucosal lesions and few or no skin nodules (Provost, 1974). The "punched out" appearance of ulcers of the mucosa and skin-mucosal junction will help in the differentiation of these cases.

Treatment in the early stages with sulfonamides (sulfadimidine) or antibiotics (oxytetracycline) has been found to alleviate the condition by minimizing secondary bacterial infection.

PATHOLOGY

Gross changes

Skin nodules occupy the full thickness of the skin and subcutis, occasionally also the underlying muscle. The cut surface reveals a hard fibrous mass whitish in colour and centred in the dermis (Fig. 22). Early lesions show a reddish exudate in the surrounding subcutis. The yellow necrotic nodules seen in the mouth may also be present in the rumen and abomasum. Ulceration results from sloughing of the central necrotic core. Lesions can also be found through-
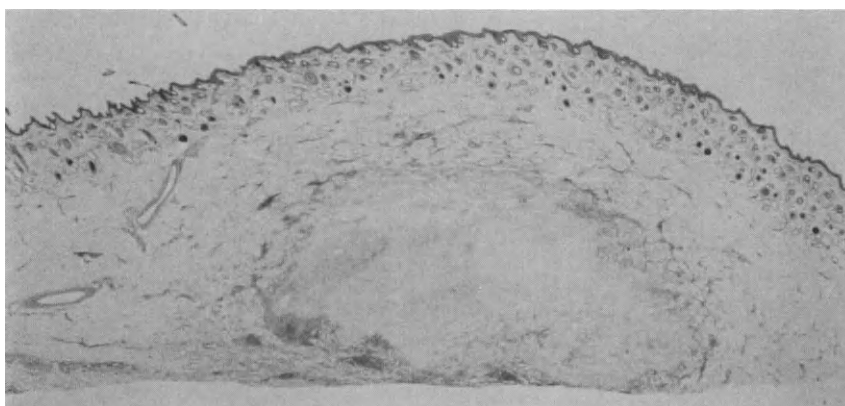
Fig. 22. A well-defined focus of necrosis centered in the dermis.

out the respiratory tract, from the nasal mucosae to the lungs. Lung lesions occur as discrete greyish-yellow spherical masses about 1 cm in diameter. They are scattered through the organ and are easily seen and palpated. They are firm in consistency and well demarcated from surrounding lung tissue.

Histopathology

The histopathology has been described in South Africa and Kenya by Thomas and Maré (1945), Burdin (1959) and Prozesky and Barnard (1982). The essential changes include a vasculitis of dermal vessels with perivascular cuffing, thrombosis and infarction leading to coagulative necrosis with cytoplasmic inclusions common in the early stages.

The basic lesion comprises a focus of necrosis in the dermis. There is an infiltration of mononuclear cells, mostly lymphocytes and macrophages with some plasma cells and fibroblasts. Neutrophils may also be present in the later stages. Cellular cuffing of arteries and veins is particularly evident. The lesion is well defined from the surrounding skin but not encapsulated, and tongues of inflammatory cells extend outwards from the lesion between the collagen bundles of the dermis. Thrombosis of blood vessels is apparent. Large intracytoplasmic inclusion bodies are present mostly in macrophages and fibroblasts and occasionally in the endothelium of blood vessels. Inclusions vary in colour from pink to purple with hematoxylin and eosin staining and are frequently of the same size as the cell nucleus or larger. A basophilic limiting membrane and internal basophilic granules can often be seen. The nucleus of the host cell usually shows well defined margination of chromatin (Fig. 23). Inclusions can sometimes be seen in an extracellular position in the necrotic debris at the edge of the lesion.

In some nodules tissue damage is restricted to the dermis and subcutis but in others changes occur concurrently in the epidermis and adnexa remote from the main focus. Epidermal cells, particularly in the basal layers, show vacuola-
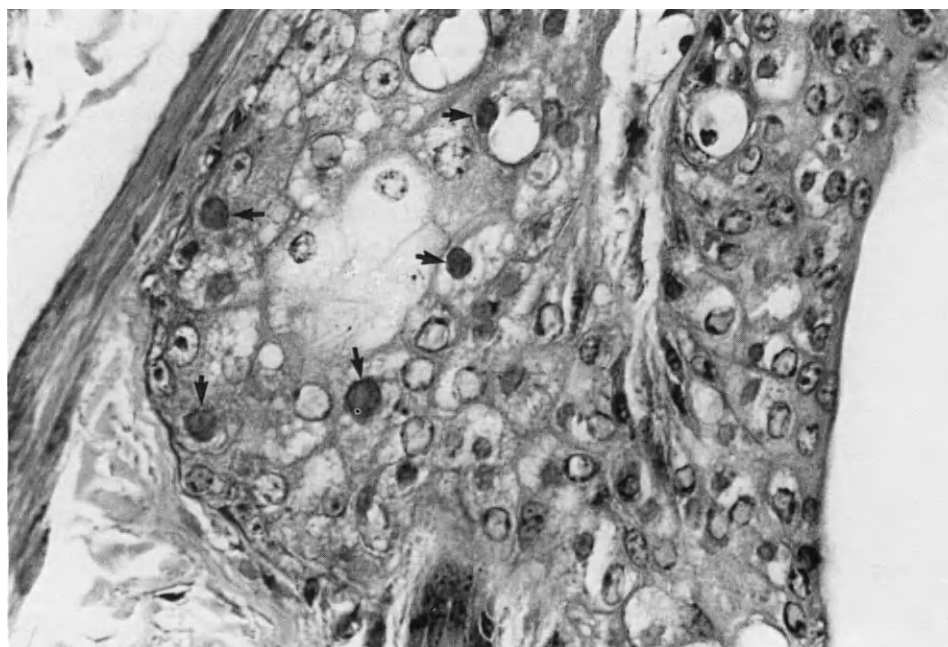
Fig. 23. Hair follicle and sebaceous gland showing vacuolation of cytoplasm, margination of nuclear chromatin and many inclusion bodies (arrows).

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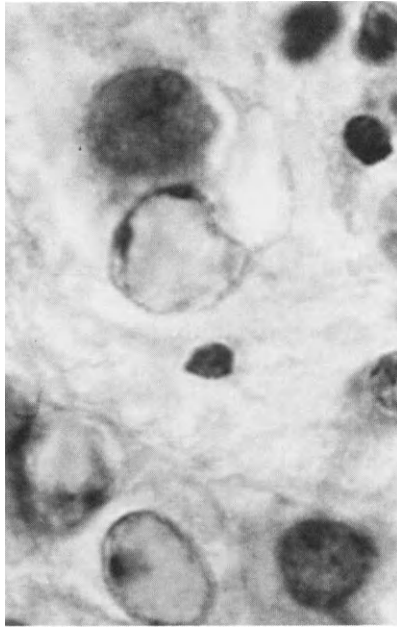
Fig. 24. Necrotic cells in the dermis, probably macrophages, showing margination of nuclear chromatin and large intracytoplasmic inclusions.

tion, margination of nuclear chromatin and many inclusion bodies. These changes are also seen in the hair follicles and sebaceous glands (Fig. 24). Foci of cellular infiltration and necrosis are often present in the dermal papillae.

The initial lesion may resolve with no serious involvement of the upper layers of the dermis and epidermis. Most lesions, however, develop to the "sit-fast" stage with an extension of necrosis to involve all layers of the skin. In these cases edema, hemorrhage and marked cellular infiltration are seen in the upper layers of the corium. The epidermis is eventually destroyed and a confluent plug of necrotic tissue then extends from the original focus in the corium to the epidermis. At this stage blood vessels in the corium are often occluded and eventually obliterated. Inclusion bodies are now less numerous. At the edges of the lesion there is proliferation of fibroblasts and formation of capillary loops as the necrotic core begins to be walled off. In most cases the layer of granulating tissue gradually extends beneath the necrotic plug, eventually expelling it and leaving a raw healing ulcer. The pathology of mucosal lesions is similar to that of the skin nodules.

Lung lesions show a central zone of coagulative necrosis in which the lobular architecture is still visible. This is surrounded by a band of mononuclear cells. An outer layer of fibrous tissue encapsulates the lesion. There is thrombosis of pulmonary blood vessels and inter-alveolar edema and hemorrhage. Intracytoplasmic inclusions are rare in chronic lesions.

Minor changes have been described in other organs but are not pathognomonic for the disease.
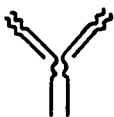
IMMUNE REACTION

It has been repeatedly observed that morbidity is variable and that innate resistance occurs in both naturally and experimentally infected animals. In South Africa about 1260 cattle were accidentally infected with LSDV through a contaminated anaplasmosis vaccine but only 493 showed clinical signs

(Diesel, 1949). A great variation also exists in the severity of clinical reactions. Capstick (1959) recorded only three cases of generalization of lesions in 56 inoculated cattle. This resistance does not seem to be associated particularly with *Bos indicus* or *Bos taurus* or with breeds within these species.

Animals which have recovered from disease develop neutralizing antibodies and are immune to reinfection. Antibody levels persist for at least 5 years (Weiss, 1963). Some cattle without skin lesions also show this response and have presumably undergone a subclinical infection. Cattle vaccinated with the attenuated Neethling strain virus mostly develop neutralizing antibody by the 10th day and a high titer by the 30th day. Titers persist for at least 3 years. About 50% of vaccinated cattle develop a reaction at the site of injection and show a serum antibody response; those without a local reaction do not develop titers but are immune to challenge (Weiss, 1968). Passive immunity (maternal antibodies) has been found to persist in calves for up to 6 months (Van der Westhuizen, 1964).

Capstick and Coakley (1962) devised an intradermal test similar to the tuberculin test. The test was used for the detection of susceptible animals for experimental use and proved accurate and specific. The nature of the histological reaction in skin lesions where cellular infiltrations are dominated by lymphocytes and macrophages would further suggest that cellular and cell mediated immunity are important. Although structural cells in the epidermis, adnexa and vascular endothelium suffer direct damage from the virus, most of the changes result from the cellular infiltration inherent in the immune reaction.

Immunity to infection is lifelong and recurrence of disease in the same animal is rare, if it occurs at all. Recurrent outbreaks in the same herd are probably due to the buildup of a susceptible population born since the previous exposure and in the absence of regular vaccination. Immunity induced by live virus vaccines (attenuated Neethling strain and the Kenya sheeppox strain) is also reckoned to be lifelong.

LABORATORY DIAGNOSIS

Electron microscopy

Examination of fresh lesion material has been applied to LSDV using the method of Parsons (1963). Samples are negatively stained with phosphotungstic acid, and the procedure takes only a few minutes. Virus particles are seen as long as inclusions are visible by light microscopy. Early lesions must therefore be selected.

EM examination 5-μm sections conventionally processed for histology from formalin fixed material has been described by Rossi et al. (1970), and the method can be applied to LSDV (J.A. Woods, unpublished data, 1980). J & E stained sections are examined under the light microscope and areas containing inclusion bodies selected for EM processing. Paraffin-embedded tissue can also be examined by removing a small portion of the block known to contain inclusion bodies and processing for EM (Lehner et al., 1966). Positive staining with uranyl acetate is used. Viroplasm zones and typical pox virions are easily and rapidly identified (see Fig. 18). The technique is very flexible. Preservation of material in 10% formalin is straightforward under field conditions. Retrospective assessment of fixed tissue or histological sections from suspect cases is possible. Transportation of H & E sections and blocks for confirmation to a laboratory in another country presents no problems. Virions remain easily recognizable for many years in paraffin-embedded tissue despite extensive damage to the cytoplasm.

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Samples taken by the field officer should include slices of skin nodule about 3 mm thick in formalin for histology and/or EM and fresh tissue on ice for EM, cryostat sectioning or virus culture depending on the facilities available. Best results for conventional EM work are obtained by preservation of tissue in 4% glutaraldehyde in sodium cacodylate buffer, pH 7.4, for 2 h followed by storage in 5% sucrose in the same buffer at 4°C.

The identity of virus grown on the CAM of embryonated eggs or in tissue culture can be confirmed by thin sectioning and positive staining and by negative staining, respectively (Munz and Owen, 1966; Van Rooyen et al., 1969).

Histology

The general histological picture is highly supportive of a diagnosis. Intracytoplasmic inclusions, however, are present only in the early stages of infection. Not all "closed" lesions are early, but unbroken nodules rather than "sit-fasts" should be sampled. Cattle known to have become recently ill and which are still pyrexic should be chosen, and biopsies should be taken from a number of animals. The complete nodule should be excised under local anesthesia, cut into slices 2–3 mm thick and preserved in 10% buffered formalin.

Immunofluorescence

The technique has been successfully used to identify virus in tissue culture. Virus antigen can be detected as early as 48 h after inoculation of the monolayer.

The indirect IF test has been used to identify antigen in cryostat sections but nonspecific fluorescence limited its usefulness when used by Davies et al. (1971).

Virus isolation

A wide variety of cell types has been used, but lamb testis and kidney and calf fetus muscle cells are particularly suitable. The CPE is characterized by rounding, shrinking and detachment of cells to give a "moth-eaten" appearance to the monolayer. Staining with H & E shows the presence of intracytoplasmic inclusions and many of the cellular changes seen in tissue sections. CPE is apparent 3 days after inoculation.

Virus can be grown by inoculating the supernatant of ground-up lesions onto the CAM of 7–9-day-old embryonated eggs incubated at 33.3°C to 35°C (Van Rooyen et al., 1969). This produces characteristic pocks on the CAM which, when sectioned, show vacuolation of ectoderm and intracytoplasmic inclusions.

Growth of the virus in tissue culture or in eggs must be regarded as supportive evidence only. Confirmation by EM, neutralization or IF tests is essential. As with histology and EM studies, all biopsy material should come from recently ill animals.

The two national laboratories holding stocks of specific antiserum and which are also most conversant with the disease are at the Department of Veterinary Services Division, Kabete, Kenya and The Veterinary Research Institute, Onderstepoort, South Africa.

PROPHYLAXIS AND CONTROL

Kenya and South Africa are alone in having shown great concern and a concerted effort to control lumpy skin disease. They have now come to terms

with the condition, accepting that it will probably continue to be endemic, and rely on a strategic vaccination policy to prevent epizootics. The disease is no longer notifiable in South Africa.

Measures that have been used with limited success include the isolation of infected herds and slaughter of clinical cases, movement control and the use of insecticides.

Quarantine measures and *movement control* did not appear to limit spread of the 1944–47 virgin epizootic in South Africa (Haig, 1957). In Kenya very stringent movement controls seemed to stop the condition becoming widespread, though it did not stop local farm-to-farm spread as would be expected with an insect-borne infection (McOwan, 1959).

Slaughter of clinical cases was discontinued in the first Kenya outbreak except for outlying foci. Since cattle are infective for some days before lesions appear this is not an efficient method of control and is no longer practiced in Africa.

Dipping and spraying of cattle with residual *insecticides* and spraying of farm buildings were practiced during the Kenya epizootic and may have assisted in limiting spread.

Vaccination is the only effective means of control. Two vaccines have been successfully used to date, a live SPV vaccine grown in tissue culture and an attenuated strain of LSDV passaged in embryonated eggs and tissue culture.

A freeze-dried live vaccine based on a local strain of SPV has been produced at the Veterinary Research Laboratory, Kabete, Kenya, and used in the field since 1959 (Capstick and Coakley, 1961; Coakley and Capstick, 1961). A dose of 2 ml is given subcutaneously in front of the shoulder. Transient local reactions and very occasional systemic reactions are seen. There is no generalization of lesions and no shedding of live virus. There is no marked thermolability but the virus is sensitive to direct sunlight, especially when reconstituted. Vaccine virus is highly virulent for sheep and goats; its use would be acceptable only in countries where sheeppox is endemic and where the sheep and goat populations show a high degree of resistance.

The Neethling strain of LSDV has been used to produce an attenuated vaccine at the Veterinary Research Institute, Onderstepoort, South Africa, since 1960. The freeze-dried vaccine is safe and effective and minor side effects include local reactions at the injection site in 50% of cattle and an occasional transient drop in milk yield in dairy cows (Weiss, 1968). The dose is 5 ml subcutaneously in calves of 6 months or older and immunity is reckoned to be life-long. Maternal antibody is considered to persist for up to 6 months in calves of immune cows.

A freeze-dried live virus vaccine derived from a virus strain isolated in Madagascar and attenuated by passage in tissue culture has been produced at the Farcha laboratory in Chad but to date has not been used in the field (Lefèvre, 1979).

The strategy used to control the disease in a country will depend on its geographical position and the risk of (re)introduction of infection.

In high-risk areas as in Africa a strategic vaccination policy is essential, particularly for valuable exotic cattle. Regular vaccination of calves at 6 months of age will ensure a resistant population. If vaccination on this scale is not possible, prompt ring vaccination around new outbreaks will help to stop spread of disease. Early and excessive rains may herald the start of an epizootic, and there may be areas of the country where past experience has shown that outbreaks are most likely to occur. In these areas clinical vigilance and limited vaccination may be adequate.

In the case of low-risk areas remote from the continent of Africa the immediate desire would be eradication. The efficiency of a policy of declared

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infected zones with movement restriction and ring vaccination might well work if carried out with thoroughness. Animals in transit through infected zones must have been vaccinated at least 2 weeks previously.

REFERENCES

Anonymous, 1977. Annual Report of the Director of Veterinary Research, Rhodesia for the year 1976–1977. Rhodesian Printing and Publishing Company, Salisbury.

Alexander, R.A. and Weiss, K.E., 1959. Unpublished data. Cited in: Weiss, K.E., 1968. Lumpy skin disease virus. Virol. Monogr., 3: 111–131.

Burdin, M.L., 1959. The use of histopathological examinations of skin materials for the diagnosis of lumpy skin disease in Kenya. Bull. Epizoot. Dis. Afr., 7: 27–36.

Burdin, M.L. and Prydie, J., 1959. Observations on the first outbreak of lumpy skin disease in Kenya. Bull. Epizoot. Dis. Afr., 7: 21–26.

Capstick, P.B., 1959. Lumpy skin disease: Experimental infection. Bull. Epizoot. Dis. Afr., 7: 51–62.

Capstick, P.B. and Coakley, W., 1961. Protection of cattle against lumpy skin disease. I. Trials with vaccine against Neethling type infection. Res. Vet. Sci., 2: 362–368.

Capstick, P.B. and Coakley, W., 1962. Lumpy skin disease: The determination of the immune state of cattle by an intradermal test. Res. Vet. Sci., 3: 287.

Coakley, W. and Capstick, P.B., 1961. Protection of cattle against lumpy skin disease. II. Factors affecting small scale production of a tissue culture propagated virus vaccine. Res. Vet. Sci. 2: 369–374.

Davies, F.G., 1982. Observations on the epidemiology of lumpy skin disease in Kenya. J. Hyg. Camb., 88: 95–102.

Davies, F.G. and Otema, C., 1981. Relationships of capripox viruses found in Kenya with two Middle Eastern strains and some orthopox viruses. Res. Vet. Sci., 31: 253–255.

Davies, F.G., Krauss, H., Lund, J. and Taylor, M., 1971. The laboratory diagnosis of lumpy skin disease. Res. Vet. Sci., 12: 123–127.

De Sousa Dias, A. and Limpo-Serra, J., 1956. La dermatose nodulaire au Mozambique. Bull. Off. Int. Epizoot., 46: 612–625.

Diesel, A.M., 1949. The epizootiology of lumpy skin disease in South Africa. Report of the 14th International Veterinary Congress, London, 1949, Vol. 2, pp. 492–500.

Green, H.F., 1959. Lumpy skin disease – its effect on hides and leather and a comparison in this respect with some other skin diseases. Bull. Epizoot. Dis. Afr., 7: 63–74.

Haig, D.A., 1957. Lumpy skin disease. Bull. Epizoot. Dis. Afr., 5: 421–430.

Hedger, R.S. and Hamblin, C., 1983. Neutralising antibodies to lumpy skin disease virus in African Wildlife. Comp. Immunol. Microbiol. Infect. Dis., 6(3): 209–213.

Henning, M.W., 1856. Animal Diseases in South Africa. Central News Agency, South Africa, Johannesburg, 3rd edn., 1239 pp.

Kitching, R.P., Hammond, J.M. and Black, D.N., 1986. Studies on the major common precipitating antigen of *Capripoxvirus*. J. Gen. Virol., 67: 139–148.

Lalanne, A., 1956. La maladie nodulaire de le peau des bovins (lumpy skin disease) a Madagascar, ses conséquences pour l'industrie des cuirs. Bull. Off. Int. Epizoot., 46: 596–611.

Lefevre, P.C., 1979. La maladie nodulaire cutanée des bovins. II. Production d'un vaccin lyophilise à virus vivant. Rev. Élev. Méd. Vét. Pays Trop., 32(3): 233–239.

Lehner, T., Nunn, R.E. and Pearse, A.G.E., 1966. Electron microscopy of paraffin embedded material in amyloidosis. J. Pathol. Bacteriol., 91: 297–300.

Matthews, R.E.F., 1982. Classification and nomenclature of viruses. Intervirology, 17: 44.

McOwan, K.D.S., 1959. Observations on the epizootiology of lumpy skin disease during the first year of its occurrence in Kenya. Bull. Epizoot. Dis. Afr., 7: 7–20.

Morris, J.P.A., 1931. Annual Report of the Department of Animal Health of the Government of Northern Rhodesia for the year 1930. Government Printer, Livingstone, pp. 20–21.

Munz, E.K. and Owen, N.C., 1966. Electron microscopic studies on lumpy skin disease virus type Neething. Onderstepoort J. Vet. Res., 33(1): 3–8.

Nawathe, D.R. and Asagba, M.O., 1977. Lumpy skin disease – experimental infection. Bull. Anim. Health Prod. Afr., 25: 313–316.

Nawathe, D.R., Gibbs, E.P.J., Asagba, M.O. and Lawman, M.J.P., 1978. Lumpy skin disease in Nigeria. Trop. Anim. Health Prod., 10: 49–54.

Parsons, D.F., 1963. Negative staining of thinly spread cells and associated virus. J. Cell Biol., 16: 620–626.

Plowright, W. and Ferris, R.D., 1959. Ether sensitivity of some mammalian pox viruses. Virology, 7: 357.

Provost, A., 1974. Rapport Annuel 1973. Laboratoire de Recherches Vétérinaires et Zootechniques de Farcha. v7 v16. Institut d'Élevage et de Médecine Vétérinaire des Pays Tropicaux, N'Djamena, Tchad.

Prozesky, L. and Barnard, B.J.H., 1982. A study of the pathology of lumpy skin disease in cattle. Onderstepoort J. Vet. Res., 49: 167–175.

Rossi, G.L., Luginbühl, H. and Probst, D., 1970. A method for ultrastructural study of lesions found in conventional histological sections. Virchows Arch. Pathol. Anat. Physiol. Klin. Med., 350: 216–224.

Thomas, A.D. and Maré, C.V.E., 1945. Knopvelsiekte. J. S. Afr. Vet. Med. Assoc., 16: 36–43.

Van der Westhuizen, B., 1964. Unpublished data. Cited in: Weiss, K.E., 1968. Lumpy skin disease virus. Virol. Monogr., 3: 111–131.

Van Rooyen, P.J., Munz, E.K. and Weiss, K.E., 1969. The optimal conditions for the multiplication of Neethling-type lumpy skin disease virus in embryonated eggs. Ondersteppoort J. Vet. Res., 36: 165–174.

Von Backstrom, U., 1945. Ngamiland cattle disease. J. S. Afr. Vet. Med. Assoc., 16: 29–35.

Weiss, K.E., 1963. Lumpy skin disease. In: Emerging Diseases of Animals. FAO Agricultural Studies, No. 61. FAO, Rome, pp. 179–201.

Weiss, K.E., 1968. Lumpy skin disease virus. Virol. Monogr., 3: 111–131.

Weiss, K.E. and Broekman, J., 1965. Unpublished data. Cited in: Weiss, K.E., 1968. Lumpy skin disease virus. Virol. Monogr., 3: 111–131.

Young, E., Basson, P.A. and Weiss, K.E., 1970. Experimental infection of game animals with lumpy skin disease virus (prototype strain Neethling). Onderstepoort J. Vet. Res., 37: 79–88.

Herpesviridae

The family has obtained its name from the multiple recurrence of fever blisters in certain persons. The term is derived from Greek herpein to creep. The family contains the subfamilies Alphaherpesvirinae (herpes simplex virus group), Betaherpesvirinae (cytomegalovirus group) and Gammaherpesvirinae (lymphoproliferative herpesvirus group). The subdivision is based mainly on biological behavior; recently, five genera have been proposed (Brown, 1986). Some 20 herpesviruses have been identified in vertebrates.

Herpesvirions are large particles, 180–200 nm in diameter when enveloped. The capsid is about 100 nm in diameter and consists of 162 capsomers, 12 pentameric and 150 hexameric. A granular zone (integument), consisting of protein, fills the space between the capsid and the envelope, which carries short projections. In the core a fibrillar protein spool is present onto which the DNA is wrapped.

The herpesvirion contains one molecule of linear double-stranded DNA with a mol. wt. between 80 and 150 $\times$ 10^6. More than 25 structural polypeptides with mol. wts. between 4 k and 200 k have been identified, some of which are glycosylated or phosphorylated. Buoyant density of the virion in CsCl is between 1.20 and 1.29 g/cm^3; sedimentation coefficients around 1800 S have been determined.

Viral DNA is transcribed in the nucleus, mRNA is translated in the cytoplasm. Viral DNA is replicated in the nucleus, too, and is spooled into preformed immature nucleocapsids. The assembled virus particles leave the nucleus by budding from the nuclear membrane which is modified by inserted viral glycoproteins and forms the envelope. Eosinophilic inclusion bodies – remnants of virus factories – are found in the nucleus; cytomegalovirus-infected cells may also contain basophilic cytoplasmic inclusion bodies.

Herpesviruses of Ruminant Cattle

A herpesvirus, mostly known as the agent of infectious bovine rhinotracheitis (IBR) or infectious pustular vulvovaginitis (IPV) is also named *bovine herpesvirus 1* (BHV-1). Another herpesvirus infecting cattle is the agent of ulcerative mammillitis, named *bovine herpesvirus 2* (BHV-2). Both BHV-1 and BHV-2 belong to Alphaherpesvirinae, BHV-2 being related to herpes simplex virus 1.

Ill-defined as agents of disease but identified as herpesviruses is a group of isolates characterized as bovine cytomegaloviruses with the Movar isolate as reference strain (Storz et al., 1983). The isolates thus belong to the Betaherpesvirinae and are named *bovine herpesvirus 3* (BHV-3).

Cattle are secondary hosts of a herpesvirus causing malignant catarrhal fever (MCF). The primary hosts are wildebeest (in Africa) and sheep (in Europe), but the European virus has not yet been isolated. In the primary hosts, the infection is inapparent, basically lymphoproliferative, the MCF virus belongs to Gammaherpesvirinae and is also named *alcelaphine herpesvirus 1* (AHV-1). This is because two additional related types have been isolated from hartebeest and topi (for references, see Rossiter, 1985).

The above naming (BHV-1, BHV-2, etc.) is presented according to the recommendations of the International Committee on Taxonomy of Viruses (ICTV).

These recommendations are, however, at variance with the opinion of some leading veterinary virologists, who proposed the name BHV-3 for the MCF virus and the name BHV-4 for the isolates of the Movar type. The recent proposal of the ICTV regarding a subdivision into genera (Brown, 1986), will probably lead to a revised naming. The last proposal by the Study Group supports the label BHV-4 for designating Movar-type BHVs (which were also named "orphan viruses" or cytomegaloviruses; Bartha et al., 1987).

Sheep and Goats

Strains of a herpesvirus, named *caprine herpesvirus 1* (CHV-1), have been isolated from cases of ovine pulmonary adenomatosis (*jaagsiekte*) which, however, appears to be caused by a retrovirus. There is a herpesvirus occurring in goats and named *caprine herpesvirus 2*. Although this virus is antigenically closely related to BHV-1, the two viruses show different restriction patterns of their genomes (Engels et al., 1983).

Pseudorabies Virus

The herpesvirus of pseudorabies (Aujeszky's disease), having the pig as the primary host, may accidentally infect cattle and sheep, which are "dead-end hosts".

REFERENCES

Bartha, A., Fadol, A.M., Liebermann, H., Ludwig, H., Mohanty, S.B., Osorio, F.A., Reed, D.E., Storz, J., Straub, O.C., Van der Maaten, M.J. and Wellemans, G., 1987. Problems concerning the taxonomy of the "Movar-type" bovine herpesviruses. Intervirology, 28: 1-7.
Brown, F., 1986. Intervirology, 25: 141–143.
Engels, M., Gelderblom, H., Darai, G. and Ludwig, H., 1983. Goat herpesviruses: Biological and physicochemical properties. J. Gen. Virol., 64: 2237–2247.
Rossiter, P.B., 1985. Immunology and immunopathology of malignant catarrhal fever. Prog. Vet. Microbiol. Immunol., 1: 121–144.
Storz, J., Ehlers, B., Todd, W.J. and Ludwig, H., 1983. Bovine cytomegaloviruses: Identification and differential properties. J. Gen. Virol., 65: 697–706.

Chapter 11

Infectious Bovine Rhinotracheitis Virus

O.C. STRAUB

INTRODUCTION

The viral agents causing infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) and infectious balanoposthitis (IBP) share a large number of properties, although they cause disease in different organ systems. In the provisional nomenclature all IBR, IPV and IBP strains have been attributed to the subfamily Alphaherpesvirinae and are designated "bovine herpesvirus 1" (BHV-1; Roizman et al., 1981). According to a more recent proposal by Ludwig (1983), these strains would form the group "bovid herpesvirus 1" (BHV-1).
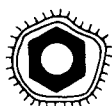
VIRUS PROPERTIES

Physical and chemical characteristics

IBR-IPV virions contain lipid in their envelope wich renders them susceptible to many disinfectants, especially solvents such as diethyl ether and chloroform. It has been demonstrated that 0.5% NaOH, 0.01% $HgCl_2$, 1% $CaOCl_2$ (chlorinated lime), 1% phenol derivatives and 1% quaternary ammonium bases inactivate BHV-1 within seconds and that 5% formalin does so within one minute (Straub, 1985). Stronger solutions were tested by Khamraev (1980), who exposed contaminated objects to an aerosol of 38% formaldehyde ($20 \, ml \, m^{-3}$) for 6 h, sodium hypochlorite solution (equivalent to 1.5% active chlorine; 200 ml per m^2 of surface) for 1 h, or 3% peracetic acid ($200 \, ml \, m^{-2}$) for 1 h. Schaller (1977), in a model experiment, sprayed daily for 4–6 weeks cattle and surroundings with a 0.5% solution of "Wofasteril" (a peracetic acid product) thus breaking the chain of infection. More recently, the effect of ozone has been studied (Anciaux et al., 1982; Bolton et al., 1982). The virus could readily be inactivated by concentrations ranging from 0.25 to $1.6 \, mg \, l^{-1}$; a temperature-sensitive (ts) strain was found to be more sensitive than a prototype IBR virus strain (LA).

BHV-1 survives in the environment for 30 days in winter, and inside buildings for 6–13 days (in winter) and 5–9 days (in spring). It will probably survive even longer under more favorable conditions, e.g. in feedstuffs. It appears unlikely, however, that virus comes into contact with feedstuffs, with the exception of grass, silage and hay. If necessary — for example for the nutrition of specific pathogen-free animals — feed can be sterilized, since the virus will be inactivated at temperatures above 63°C within seconds.

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TABLE 4

Influence of temperature on the inactivation of BHV-1 in the presence of $\leq 2\%$ serum (O.C. Straub, unpublished data)

Temperature (°C)	Time period of exposure	Initial titer ($\log_{10}TCID_{50}$/ml)	Titer reduction ($\log_{10}TCID_{50}$/ml)
56	1 hour	5.0	5.0
37	9 days	5.0	5.0
22	2 weeks	5.0	5.0
4	2 months	5.0	2.5
-30	6 months	7.0	1.0
-70	12 years	7.0	no loss

Studies on temperature sensitivity have resulted in uniform findings. Differences were only observed when field virus strains were compared with attenuated strains (Bartha et al., 1969). The virus is very stable at temperatures below $-65°C$, but less stable at $-20°C$ if storage exceeds one year (Table 4). It is slowly inactivated at $+4°C$. At $+37°C$ it survives for approximately 10 days (Hahnefeld et al., 1963; Straub, 1970). It is therefore important to note that virus will survive in semen storage containers, where it can contaminate virus-free semen when infected semen is stored in the same container (Lorrmann, 1967; Chapman et al., 1979; Guevara, 1979; Krpata, 1982).

For airborne virus survival, humidity is of special importance, but also when attenuated vaccines are used for local vaccination. From studies carried out by Elazhary and Derbyshire (1979) it can be concluded that survival is optimal at 90% RH and low temperature. With increasing temperature the required optimum humidity is lower. This may influence virus transmission under natural conditions. The virus is stable between pH 6.0 and 9.0 (Griffin et al., 1958), values that are measured in the upper respiratory (mostly below pH 7) and in the genital tract (male above pH 8, female varying; O.C. Straub, unpublished data, 1962).

Antigenic properties

A one-way serological relationship has been found between IBR-IPV virus and Aujeszky's Disease (pseudorabies) virus; it can also be demonstrated using a delayed hypersensitivity test in cattle (Aguilar-Setién et al., 1979a,b, 1980; Straub et al., 1983). This relationship is not sufficiently close to protect animals by means of heterologous vaccines. The IF test for BHV-1 antibodies in cattle that have been vaccinated with Aujeszky's disease vaccine is not affected (Wellemans et al., 1980). Bush and Pritchett (1985), who compared the genomes of BHV-1 and pseudorabies virus, concluded that the homology between these two viruses was approximately 8% as measured by liquid reassociation kinetics. Another one-way relationship exists between BHV-1 and the goat herpesvirus (BHV-6) (Engels et al., 1983).

A relationship with other bovid herpesviruses has been shown using two-dimensional immuno-electrophoresis (Levings et al., 1984). The serum neutralization test has been used in kinetic studies to demonstrate IBR-IPV virus strain differences (Potgieter and Maré, 1974).

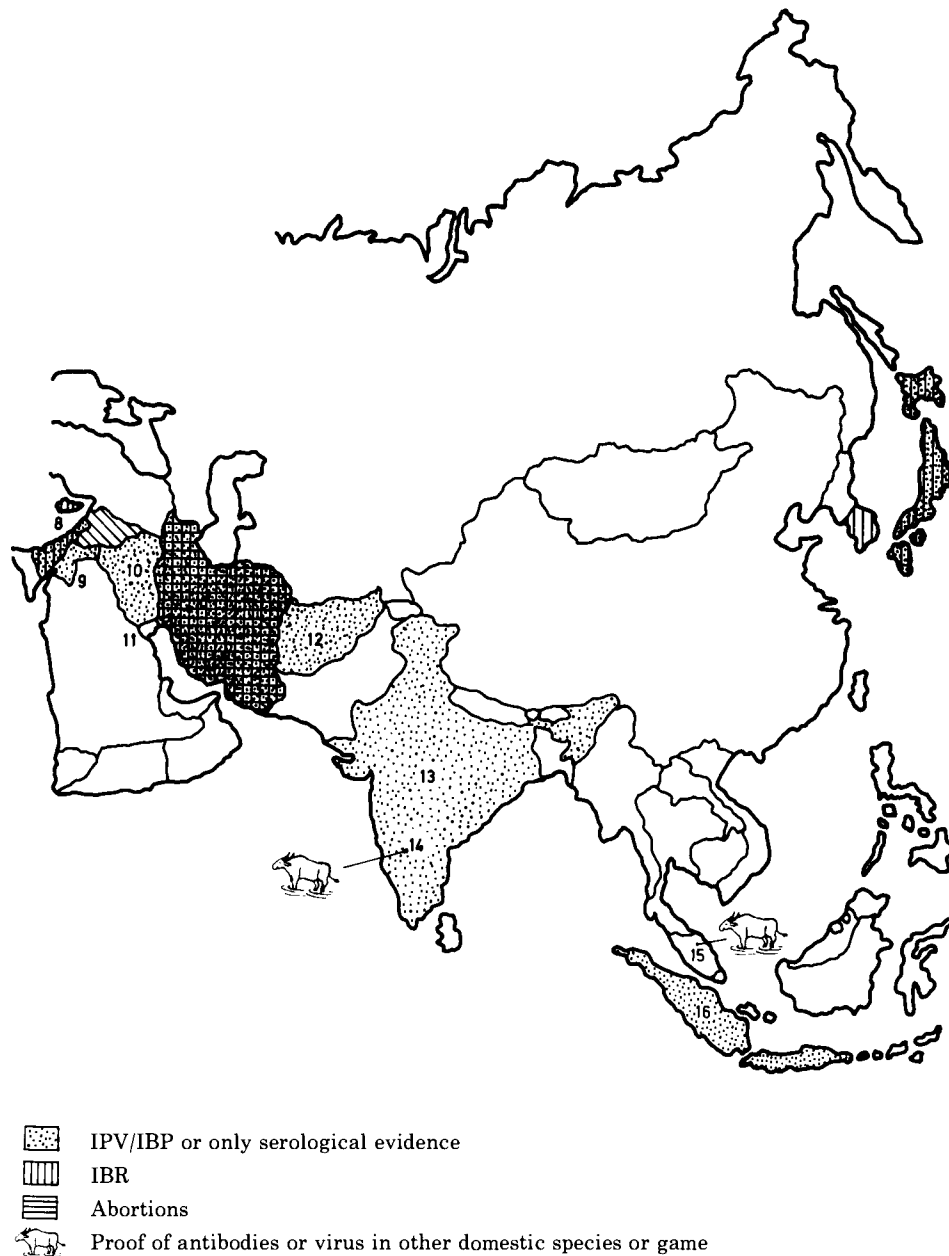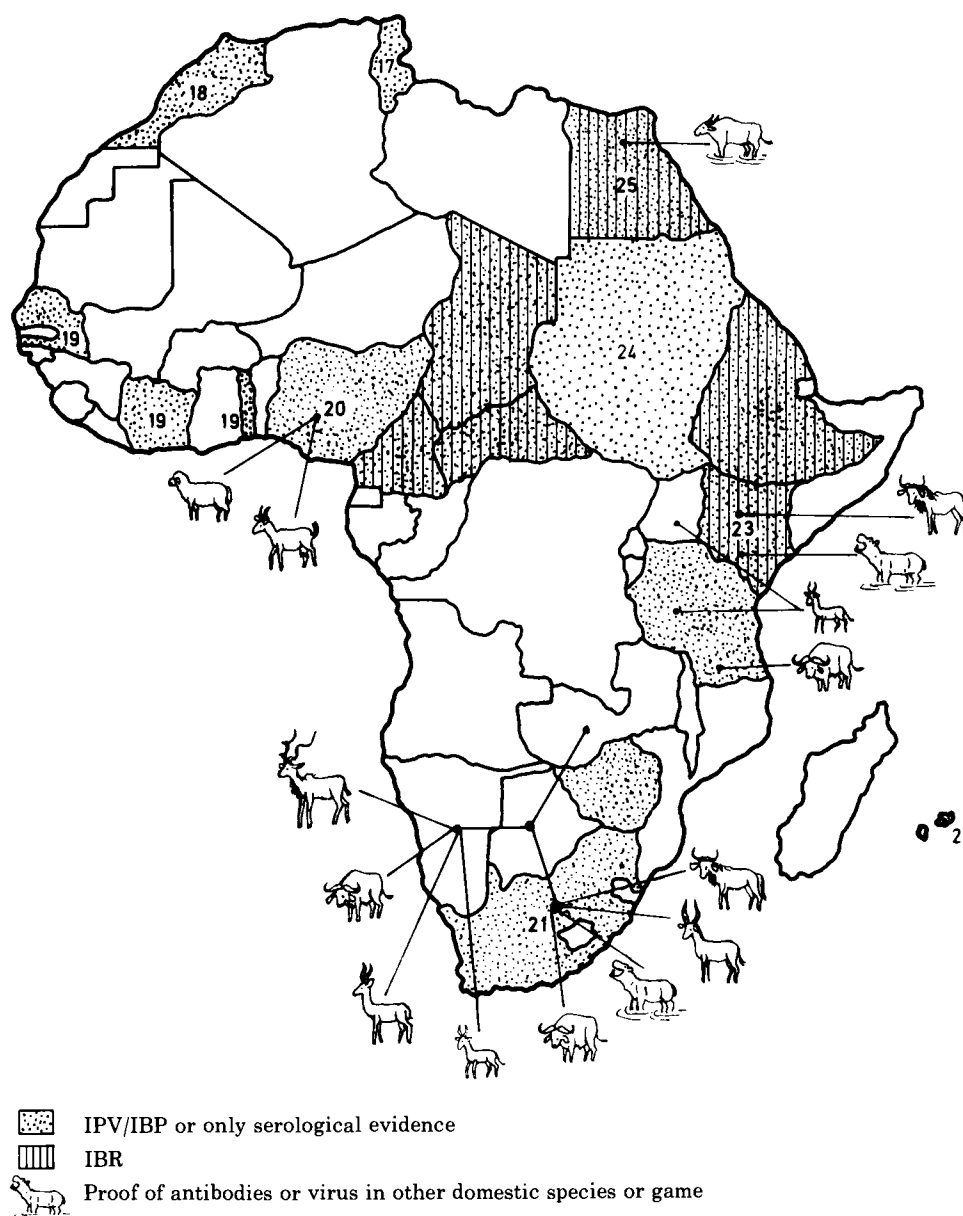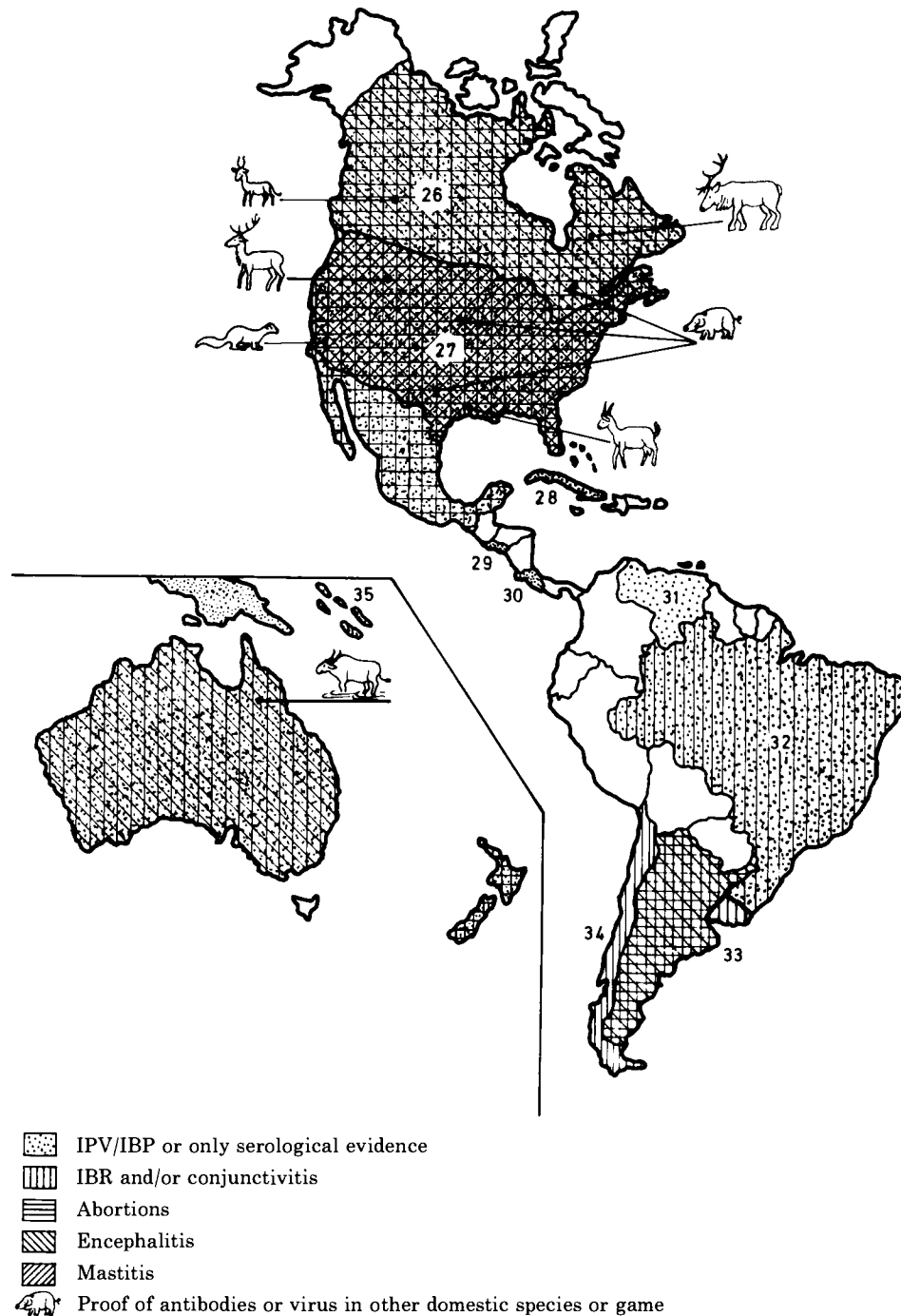|	IPV/IBP or only serological evidence
	IBR and/or conjunctivitis
	Abortions
	Encephalitis
	Mastitis
	Proof of antibodies or virus in other domestic species or game
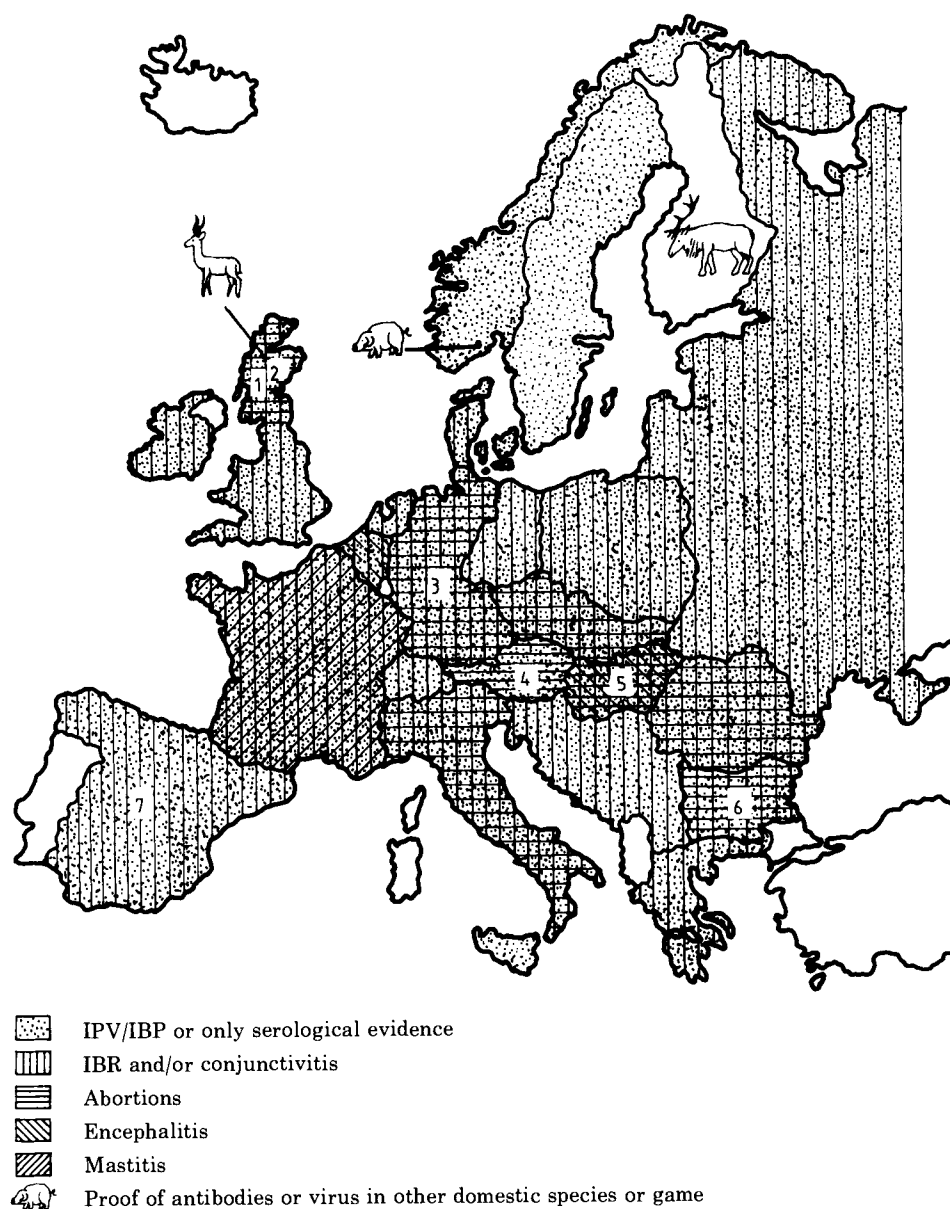
Fig. 25. Occurrence of the various diseases caused by BHV-1 in Europe. Follow-up of a 1977 review (Straub, 1978b). Numbers refer to the source of information: (1) Nettleton et al. (1981); (2) Inglis et al. (1983); (3) Straub et al. (1982b); (4) Kubin (1982); (5) Tanyi et al. (1983); (6) Karadzhov et al. (1977); (7) Rejas Garcia et al. (1979); H. Geilhausen, personal communication (1981). (Albania and Portugal have in the meantime also proven to be infected.)

EPIZOOTIOLOGY

Host range and geographic distribution

Although BHV-1 is widely distributed among cattle in all continents, its host range is limited. Many wild species have been found seropositive but distinct clinical signs have only been observed in cattle. A literature survey conducted in 1977 (Straub, 1978b) summarized the data available at that time. New data have been added to show the geographical distribution (Figs. 25–28). Differences have been found in studies with ferrets. They proved to be highly suscept-

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IPV/IBP or only serological evidence

IBR

Abortions

Proof of antibodies or virus in other domestic species or game

Fig. 26. Occurrence of the various diseases caused by BHV-1 in Asia. Follow-up of a 1977 review (Straub, 1978b). Numbers refer to the source of information: (8–12) Singh et al. (1977); (13,14) Babu et al. (1984); (15) Ibrahim et al. (1983); (16) O.C. Straub, unpublished data (1984).

ible to disease in the USA but not in Europe (Porter et al., 1975; Smith, 1978; Abraham and Straub, 1981). Lately, rabbits have sucessfully been used as experimental models (Lupton et al., 1980; Rock and Reed, 1982). Experimentally, neonatal skunks (*Mephitis mephitis*) proved also to be susceptible. This may also be true for hamsters, as judged from earlier experiments (Straub, 1978a). Experiments conducted in 1957 and confirmed in 1985 have shown that goats are not resistant to BHV-1 (McKercher et al., 1959, Wafula et al., 1985). Their clinical reaction is mild, but natural transmission also occurs (Fulton et al., 1982); in this study in Louisiana, 5 of 38 herds tested were found to contain seropositive animals (a total of 6 out of 502 animals). In Nigeria antibodies were also found in sheep, but at a lower prevalence (Taylor et al., 1977).

IPV/IBP or only serological evidence

IBR

Proof of antibodies or virus in other domestic species or game

Fig. 27. Occurrence of the various diseases caused by BHV-1 in Africa. Follow-up of a 1977 review (Straub, 1978b). Numbers refer to the source of information: (17) Cherif and M'Rabet (1984); (18) Mahin et al. (1982); (19) Espinasse et al. (1980); (20) Taylor et al. (1977); (21) Hedger and Hamblin (1978); (22) D. Sibartie, personal communication (1983); (23) Mushi and Karstad (1979); (24) Hassan and El Tom (1985); (25) Hafez (1982).

Reservoir hosts and vectors

Although IPV and IBP have been known in Europe for a century it appears that wild ruminants in Africa and in zoos (Doyle and Heuschele, 1983) are the true reservoir. Probably BHV-1 has been associated with wild ruminants in Africa for such a long time that agent and host have formed a more symbiotic association. Various virus strains can be harbored by most animals following infection. After a period of persistence they reach a state of latency from which they can emerge following natural or artificial stress.

Recently, BHV-1 was isolated from the soft-shelled tick *Ornithodorus co-riaceus*. This finding is interesting as the infected ticks had been collected from

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IPV/IBP or only serological evidence

IBR and/or conjunctivitis

Abortions

Encephalitis

Mastitis

Proof of antibodies or virus in other domestic species or game

Fig. 28. Occurrence of the various diseases caused by BHV-1 in America and Oceania. Follow-up of a 1977 review (Straub, 1978b). Numbers refer to the source of information: (26) Elazhary et al. (1981); (27) Eugster et al. (1978); (28) Centro Nacional de Sanidad Agropecuaria; (29) Rice and Jenny (1979); (30) Guevara (1979); (31) H. Geilhausen, personal communication (1984); (32) Galarza and Periolo (1983); (33) Guarino et al. (1981); Carillo et al. (1983); (34) Etchegary et al. (1977); (35) De Frederick and Reece (1980).

deer bedding areas in the Western USA on more than one occasion, in 1979 and 1981. By their restriction enzyme patterns, six out of nine strains were found to be identical with the typical IBR strain LA, the other three showing minor variations. Cattle and deer (*Odocoileus lemionus*) frequently graze together in this region, and both species proved seropositive. The report by Taylor et al.

(1982) leaves some questions unanswered because viremia, if it occurs, is very short during the overt disease; however, the tick may harbor the virus for a long period of time, or BHV-1 may even replicate in ticks. Mechanical transmission is also possible.

Zoonosis aspects

Since herpesviruses play such an important role in human disease, the risk of human infection with BHV-1 was a subject of concern. Therefore three groups of people have been examined for the presence of antibodies against BHV-1: (1) animal caretakers who have been in close contact with experimentally infected animals for many years; (2) veterinarians who have extensively used attenuated vaccines and an interferon inducer derived from an IPV strain; and (3) humans of both sexes who had suffered from a disease similar to IPV and IBP and who had contact with an artificial insemination center where an outbreak of IBP had occurred. In no instance was a positive result obtained. It may therefore be concluded that there is no risk of human infection with BHV-1.

Prevalence

Prevalence rates are usually determined only when a disease is recognized for the first time in a country or when the number of outbreaks rises suddenly. Since serological tests do not identify the disease that accompanies seroconversion the data are of limited value. Recent data are summarized in Table 5.

Economic impact

The first authors to quantify losses due to an outbreak of IBR in a herd of 156 dairy cattle were Pierson and Viar (1966). They calculated a loss of US$ 7968. A lost heat period due to IPV in the Federal Republic of Germany equals a loss of DM 150–300 depending on quality of cattle and herd management (Straub, 1978a). Barth et al., (1978) reported a non-return-rate that was 3.6–13% lower in seropositive cattle; they also reported that the interval between first insemination and conception date was longer by 3.4–5.5 days in a 1900-head cattle unit. Wiseman et al. (1979) estimated the losses caused by 15 incidents of IBR. Their total of £ 59 570 is composed of

costs due to death/culls: £ 14 750
extra feeding: 37 400
other costs: 7 420

The number of cattle at risk was 2207 and the cost per animal was £ 27.

Factors influencing spread of BHV-1

It is assumed that IPVV met favorable conditions for rapid passages in the upper respiratory tract of cattle under the feedlot conditions in the U.S.A., where IBR was first detected. It took a number of years for small herds and range cattle to become affected (McKercher and Straub, 1960). After another interval outbreaks of IBR appeared in Europe, some of which could be traced to imports from Northern America.

There is still a number of countries (Figs. 25–28) where IBR has not been diagnosed. The virus may be spread by the following types of carriers (Straub, 1978a):

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TABLE 5

Prevalences of BHV-1 seropositive cattle according to individual recent reports

Geographical area	Type of cattle	No. of herds	No. of animals	Percentage of seropositives	Author(s)
El Salvador	calves	14	393	36.6	Rice and Jenney, 1979
Argentina	cattle	4	413	48.0	Galarza and Periolo, 1983
England	dairy	83	1499	5.6	Kirby et al., 1978
	beef	37	643	6.1	Kirby et al., 1978
	AI bulls	2	177	10.2	Peters and Perry, 1983
			60	15.0	Peters and Perry, 1983
Federal Republic of Germany					
Bavaria	dairy	499	6487	9.5	Bauer et al., 1980
Hessia	dairy		1081	9.8	Frost and Wagner, 1982
Hessia	AI bulls	6	287	51.0	Gössler and Paulsen, 1975
North	AI bulls	14	334	25.4	Ziegenhagen, 1977
Northrhine	AI bulls	5	716	32.5	Zioleck, 1979
Rhineland	dairy	240	2880	4.8	Rotschuh, 1980
Wurtemberg	dairy	319	2568	34.0	Albrecht et al., 1985
France	mixed	301	2942[a]	10.8	Perrin et al., 1981
German Democratic Republic[b]	dairycalves	33	2830	0.7	Kokles, 1978
	heifers	37	5685	25.2	Kokles, 1978
	beef	21	1106	19.9	Kokles, 1978
	cows	45	6767	37.4	Kokles, 1978
	AI bulls	2	164	3.7	Kokles, 1978
	calves[c]	1	300	29.3	Barth et al., 1983
Israel	AI bulls	2	155	65.7	Abraham et al., 1982a,b
				12.2	Abraham et al., 1982a,b
Morocco	dairy	11	67[a]	60.0	Mahin et al., 1982
Netherlands	dairy	882	3130	27.9	Straver et al., 1977
	AI bulls		1330	10.8	Straver et al., 1977
	dairy	22	447[a]	76.1	Van Nieuwstadt and Verhoeff, 1983
Friesland	dairy		7229	37.2[d]	Nederlandse Gezondheidsdienst, 1979
Poland	AI bulls	2	718	46.6	Majewska et al., 1980
			275	28.0	Majewska et al., 1980
Scotland	mixed	114	1152	12.0	Msolla et al., 1981
Solomon Islands	beef	51	331	30.8	DeFrederick and Reece, 1980
Togo	mixed	5[e]	447	14.3	Espinasse et al., 1980
	mixed	1	221	79.6	Espinasse et al., 1980
Yugoslavia	dairy		2117	46	Jermolenko et al., 1978
Serbia	AI bulls	3	79	34.1	Jermolenko et al., 1978

[a]Selected.
[b]Rostock District.
[c]1-2 months old (percentage of seropositive dams 58).
[d]Regions.
[e]Based on titers of 16 and above as determined by the passive hemagglutination test.

— Animals that have become infected without clinical signs and are still shedding virus.

— Animals in which the levels of antibodies have dropped markedly. After they are reinfected, virus replicates in the mucous membranes of the upper respirat-

ory tract for a number of days without clinical signs or with signs that are attributed to transportation or some other factor.

— Animals that are latently infected; these shed virus after any stress, usually without clinical signs.

— Animals whose passive immunity has waned; virus is also readily propagated in the upper respiratory tract (up to $10^{7.5} TCID_{50}$ per ml of nasal mucus) and these calves do not exhibit any clinical signs.

Cattle of all ages are susceptible; newborns without maternal antibodies are more severely affected than older animals. In calves the infection is more systemic, involving most organ systems, including the digestive tract. This phenomenon is probably due to the fact that the abomasum of young calves resembles the stomach of nonruminants. Also, if the pH is not low enough, which might occur in older calves, then IBRV may readily pass through the stomach, whereas it is inactivated in normally functioning abomasums.

The spread of IPVV occurs by contaminated semen, natural service, the teaser bull or herdsmen (sometimes by the veterinarian, too!); occasionally, virus is transmitted on the tail from one animal to the other. Since embryo transfers are now frequently carried out, it was important to investigate the risk of this method for transmission of BHV-1. When the experiments of Singh et al. (1982) are evaluated it can be concluded that no danger exists. BHV-1 could not penetrate the zona pellucida of the 16-cell blastocyst, although it attached to this structure. Furthermore, no evidence of transmission was found when 31 eggs/embryos from seropositive donors were examined for BHV-1.

A genetic predisposition does not exist in the natural host. In the prevalence studies (Table 5) numerous breeds of cattle have been investigated. However, a low-grade immunity — passive or active — may not be detected with the routine laboratory tests but still prevents the appearance of clinical signs after infection.

It is not known why some strains of BHV-1 cause abortions and others do not. One outbreak (Straub et al., 1982b) could be traced to a newly introduced calf that passed the virus to other calves and to adult cows. Some of the young calves died after showing severe clinical signs. The adult cattle did not show any clinical signs prior to abortion; when the virus isolated from a fetus was inoculated into susceptible cattle they developed severe signs of IBR. It is also unknown why some IBRV strains pass along the nerve fibers to the central nervous system, causing encephalitis and meningitis. This form of disease occurs mainly in young animals.

Morbidity and mortality

If the morbidity rate in outbreaks of IBR is less than 100% then some animals were protected by antibodies. The same is true for IPV and IBP, provided the animals are in close contact. Frequently, cattle develop antibodies at low titers after infection with "mild" strains, most likely IPVV which replicates in the upper respiratory tract but does not cause disease. Such animals do not become sick, even if a herd is exposed to very virulent strains (Wiseman et al., 1980; Zimmermann, 1981; Straub et al., 1982a,b). Similar phenomena occur if IPVV persists in a herd in which the offspring is raised separately. When IBRV is introduced, all offspring not protected by maternal antibodies becomes severely ill, whereas calves with distinct levels of maternal antibodies do not show any signs of disease and those whose maternal antibodies have waned develop mild signs. The morbidity rates cited in the literature therefore show variations from 20 to 100% (Gibbs and Rweyemamu, 1977; Straub, 1978a; Wiseman et al., 1980; Yates, 1982).

There is general agreement that mortality rates show considerable variation

and are dependent on external factors such as crowding, food, climate and hygienic conditions. Figures available are 2–10% (Bowes et al., 1970), 7–8% (Wiseman et al., 1980), 8–12% (Andreev and Fuks, 1980) and 1–10% (Yates, 1982), which refer only to IBR. Fatalities are rare (if not absent) in cases of IPV and IBP, but death of calves suffering from encephalitis is common; the majority dies on day 5 after the first signs have been noticed (Straub, 1978a).

PATHOGENESIS

Virus entry

There is no doubt that IBRV usually enters the body via the mucous membranes of the upper respiratory tract and IPVV via the mucous membranes of the genital tract. A third possibility is the entry of IBRV by way of the conjunctival epithelium, and a fourth possibility was offered by Taylor et al. (1982) who investigated the role of the soft-shelled tick.

It is not yet established if oral infection occurs. It is known, however, that in cattle fed fluid feed the infection is most rapidly spread (Straub et al., 1965). The virus can be recovered from the unaffected buccal epithelium for at least 5 days after experimental infection (Straub and Böhm, 1964). Virus that enters the mammary gland most likely stems from a genital infection (Straub and Kielwein, 1966). Lesions in the interdigital space are certainly possible after virus entry at that site. All other manifestations of BHV-1 causing disease entities such as encephalitis and abortions are due to its spread within the animal.

Virus spread within the infected animal and organ manifestation

Two animal experiments carried out by the author were designed to detect the mode of spread after experimental infection; one involved respiratory infections of calves, the other was done with older cattle, which were inoculated by the respiratory and the genital routes (McKercher et al., 1963; Straub and Böhm, 1964). The results of both experiments are summarized in Table 6.

In the upper respiratory tract, the virus spreads along the mucous membranes, the nerve fibers and lymph vessels, thereby reaching the outer and inner ocular tissues, brain, lymph nodes and the digestive tract. The virus is inactivated in the abomasum provided the pH is normal (1.7–2.0) but may pass into the small intestine if the pH is higher. The caudal portion of the digestive tract where pH values are near 7 can possibly be infected in females from the genital tract. No virus could be detected in the genital tracts of the calves inoculated intranasally. Therefore it is not surprising that there are only few reports describing a simultaneous infection of both the genital and respiratory tracts (Collings et al., 1972; Mueller et al., 1979).

Other modes of spread must be considered. In some instances the clinical signs are limited to ocular tissues although virus must have reached the nasal cavity via the lacrimal duct; in another case virus was isolated simultaneously from clinical conjunctivitis and vaginitis (Wilke and Letz, 1970). The local tissue temperature is an important factor in the pathogenesis of BHV-2 infections (Letchworth and Carmichael, 1984); it remains to be determined whether it plays a role in BHV-1 infections, too.

Some other mode of spread must have occurred when BHV-1 is identified as the etiological agent of abortions or silent infection of the fetus (Ludwig and

TABLE 6

Results of isolation trials from various tissues after BHV-1 inoculation of 12 calves (both groups) and 11 young cattle (only older group) into the respiratory and genital tracts: samples from the genital tract of calves after the first day p.i. were all negative

Tissue	Earliest isolation (days p.i.)	Latest[a] isolation (days p.i.)	Only single isolation (days p.i.)	No isolation
Nasal mucosa	2	5		
Sinuses	2	6		
Lacrimal duct	2	6		
Olfactory bulb	2	5		
Pharyngeal lymph node	4	5		
Larynx	3	12		
Trachea	2	6		
Bronchus	3	6		
Lung	2	6		
Bronchial and medastinal lymph node			3	
Pleural fluid				x
Buccal mucosa	3	10		
Oesophagus	2	10		
Parotid gland	3	4		
Parotid lymph node			6	
Mandibular lymph node				x
Prescapular lymph node				x
Rumen				x
Abomasum				x
Caecum			4	
Mesenteric lymph node			6	
Conjunctiva	3	20		
Brain	2	5		
Inner eye	2	6		
Cerebrospinal fluid			5	
Vaginal mucosa	2	5		
Cervical mucosa	2	5		
Uterine mucosa	2	5		
Oviduct	2	5		
Ovarial tissue			2	
Peritoneal fluid			4	
Rectum	4	5[b]		
Bladder			2[b]	
Urether			2[b]	
Kidney				x
Prepuce	2	10		
Distal portion of urethra			2	
Proximal portion of urethra				x
Accessory glands				x
Epidydimis				x
Testicle				x
Blood				x
Pericardial fluid				x
Spleen			2	
Liver				x
Adrenal gland			10	
Thoracic duct				x

[a] Days p.i. of sampling were for more than one animal days 2-6, 10 and 20, for individual ones 9, 12, 24, 40, 68, 124, 204, 255, and 290.
[b] Females.

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Storz, 1973; Straub et al., 1982b). From these studies it became evident that virus may be present in organs which could only have been reached via the bloodstream. On the other hand, viremia is rarely observed. Obviously the cellular components of the blood play an important role. The distribution of the virus in the infected organism is quite different if the animal had experienced a BVDV infection (Potgieter et al. 1984), which is known to cause immunosuppression.

Nyaga and McKercher (1979) have studied the interactions of the virus with blood cells and concluded that replication occurs in monocytes; lymphocytes needed stimulation by mitogens. It must therefore be assumed that some BHV-1 strains are circulated in the animal via the bloodstream, causing disorders in the lower digestive tract in calves (Burkhardt and Paulsen, 1978; Evermann and Clemm, 1980), abortions or arthritis (Lamothe et al., 1979).

Virus has been recovered from the lung, which is not a primary site of virus replication. It reaches the bronchi and alveolar epithelium by inspiration of contaminated material from the upper respiratory tract, especially the trachea. Allan and Msolla (1980) used scanning electron microscopy to study the effect of virus replication. They found pinpoint hemorrhages on the mucosal surface on day 4 p.i. Neutrophils had migrated into the hypertrophied epithelium, which had lost its cilia, and lymphocytes had penetrated into the deeper layers. These processes continued through day 7 p.i. leaving areas covered with microvilli and the submucosal glands dilated and somewhat hypertrophied. The pathophysiologic studies by Kiorpes et al. (1978) did not fully explain the development of pneumonia. These authors considered the obstructions in the upper respiratory tract a reason for hypoventilation, increased pulmonary resistance and change in arterial carbon dioxide tension. The possible role of the alveolar macrophage was first studied by Forman and co-workers (Forman and Babiuk, 1982; Forman et al., 1982). They initially examined the susceptibility of macrophages for BHV-1 and found equal susceptibility of cells from IBR immune and nonimmune donors; in the second study they observed a rapid alteration of cell function — immune adherence, phagocytosis and complement receptor activity — following infection with IBRV. They speculated that this process favors the development of secondary lung infection. Later McGuire and Babiuk (1984) concluded that a defective neutrophil function may predispose infected animals to secondary bacterial infections, because alveolar macrophages from virus-infected animals were not able to produce neutrophil chemotactic factors.

Virus excretion and persistence

Since one replication cycle of BHV-1 requires only a few hours in the host, reisolation of the agent after experimental infection is generally possible at and after day 1 p.i. The virus is recovered from secretions for varying periods if the animals had been seronegative at the time of infection. In cases of IBR, virus excretion ceases between days 10 and 16, in IPV mostly between 8 and 14 days, and in cases of IBP in general later (14–22 days). Persistance for longer periods has been demonstrated in individual cases, especially in calves, when passively acquired antibodies are on the verge of detection (O.C. Straub, unpublished data, 1968). The virus content of secretions has been determined frequently. Maximum titers in respiratory tract infections range from $10^{8.0}$ to $10^{10.0}$ $TCID_{50}$ per g of mucus, those in the genital tract may reach 10^{11} $TCID_{50}$ per g.These are remarkable quantities considering the fact that in cattle the infectious dose is below 10^2 $TCID_{50}$ (O.C. Straub, unpublished data, 1987).

In cases of abortion the highest virus titers are present in the fetal part of the placentome, the cotyledon, where they may reach $10^{6.0}$–$10^{6.5}$ $TCID_{50}$ per g

(Kendrick and Straub, 1967; Straub et al., 1982b). If autolysis has progressed, virus cannot be recovered from any part of the fetus but isolation is still possible from the placenta.

Latency

Numerous papers have dealt with this phenomenon, which is characteristic for Herpesviridae and was also described for BHV-1: Sheffy and Davies (1972) used corticosteroids to "reactivate" the virus. Later Narita et al. (1978, 1979, 1981, 1983) demonstrated the presence of the viral genome in the ganglia and peripheral nerve fibers. They also observed degeneration, neuronophagia and trigeminal ganglionitis when they applied treatment with dexamethasone. Using hybridization in situ, Ackermann and Wyler (1984) found the genome of an IPV strain in the sacral ganglia during latency after intravaginal infection. Homan and Easterday (1982) succeeded in isolating BHV-1 from six trigeminal ganglia of slaughter cattle by 7–24-day explant cultures. The prevalence of seropositive animals in the herd was 45.4%. These authors also used neuron cultures for their in vitro studies, from which they concluded that BHV-1 did not attach to neural perikarya but did so readily to fibroblasts. The absence of receptors on neuron perikarya may be responsible for the slow spread of herpesvirus to adjacent neurons during recrudescence. Straub (1979) and Lema et al. (1981) have demonstrated that field virus genomes remained latent although animals had been repeatedly vaccinated with live and/or inactivated vaccines after the field virus infection. Although some animals show an increase in serum neutralization titers following treatment with corticosteroids, titers in other animals remain constant; clinical signs, if they occur, are insignificant (Pastoret et al., 1979; Straub, 1979; Lema et al., 1981). Recent studies have demonstrated that reactivation and shedding of BHV-1 can also be initiated by *Dictyocaulus viviparus* infection and normal parturition (Msolla et al., 1983; Thiry et al., 1985). It is interesting to note that the mean plaque size of a virus recovered after dexamethasone treatment differed from the strain used for inoculation (Pastoret et al., 1979; Curvers et al., 1985); in another publication (Pastoret et al., 1980b), the authors concluded that the virus recovered after dexamethasone treatment was identical with the one used for vaccination according to the restriction endonuclease cleavage patterns. The virus may apparently in some instances undergo changes which require further study, especially in relation to the results of restriction enzyme analysis (Thiry et al., 1984). It should be possible to determine by this method if recombinants between field and vaccine strains occur. Pastoret et al. (1980a) found cyclophosphamide not effective in stimulating the excretion of latent virus. Espinasse et al. (1983) succeeded in reactivating BHV-1 after intraruminal application of 3-methylindole. Rodriguez et al. (1984) demonstrated a different pathogenicity after inoculation of rabbits with selected isolates recovered from trigeminal ganglia from clinically healthy cattle. These isolates could also be distinguished by their plaque size — as Pastoret et al. (1979) had shown — whereas their inactivation profiles at 48°C were not different. Ts-mutants could not be detected when the isolates were grown at 41°C.

It has been demonstrated that vaccination with inactivated vaccines (locally or parenterally administered), a ts-mutant vaccine or an attenuated vaccine followed by a challenge infection with field virus leads to latency of the latter (Rossi and Kiesel, 1982; Kretzschmar et al., 1983; Lazarowicz et al., 1983; Nettleton et al., 1984; Straub, 1984a). After the administration of attenuated IPV vaccines and subsequent treatment with immunosuppressive drugs, virus recovery was reported to be negative (Kokles, 1979); Straub (unpublished data, 1982) and Nettleton et al. (1984), however, did recover BHV-1 for the vaccinees.

Miller and Van der Maaten (1984, 1985) first studied the reproductive tract lesions after intrauterine inoculation with BHV-1; they inoculated six heifers intravenously at estrus and demonstrated virus in the corpus luteum in one of them after treatment with dexamethasone.

The quantities of virus shed after reactivation do not depend on the antibody titer. Maximum titers in excretions do not reach the same levels as after the first infection, although they may be high; in our experiments, two out of eleven cattle had titers of 10^8 $TCID_{50}$ and a third one had a titer of $10^{7.5}$; some animals shed much less virus, the lowest $10^{1.5}$ $TCID_{50}$ units (O.C. Straub, unpublished results, 1984). These animals consequently are extemely dangerous for susceptible cattle, especially as they do not show distinct symptoms of disease.

No reports exist on the duration of latency. In the author's own experiments recrudescence of virus has been demonstrated 3 years following the initial infection (O.C. Straub, unpublished data, 1982). Rossi and Kiesel (1982) concluded that humoral antibodies may play a role in preventing viral shedding following the application of immunosuppressive drugs. These authors (Rossi et al., 1982) had shown that recrudescence of virus was possible only from the original site of inoculation (except following intravenous infection, where it was isolated from the respiratory and genital tract). Aguilar-Setién et al. (1979b) describe a case where an animal was found seronegative in the neutralization test but positive in the delayed hypersensitivity test. This heifer proved to be a latent carrier when she was treated with dexamethasone. We have found that application of immunosuppressive drugs interferes with the skin test (Straub, 1986b). Recently, the rabbit has been used as a model in BHV-1 latency studies (Rock, 1982; Rock and Reed, 1982).

DISEASE SIGNS

IBR

Of the clinical entities caused by BHV-1, IBR is economically the most important one and second world-wide in regard to occurrence (Figs. 25–28). The incubation period lasts from 2 to 4 days depending on the exposure. In light cattle the first sign is a pink muzzle. Nasal secretion, initially serous, is increased, food intake decreases, and as the temperature rises above 41°C the animals start strong salivation — often of a frothy appearance. The cows' milk production drops suddenly; the animals go off their feed, the nasal secretions become seromucous and then mucopurulent. At the same time the conjunctivas are reddened, ocular discharge is increased; initially also of serous consistency, it gradually becomes mucopurulent (sometimes a string of flies on the cheek is the first symptom if cattle are observed from some distance in the field). The climax is reached at days 3 and 4 after the first signs. If secondary infections are prevented by the administration of antimicrobial drugs, the temperature decreases thereafter, likewise pulse and respiration rates slow down. If bacterial infections do appear, a second rise in temperature follows or it remains constantly high. Then respiration becomes labored, hyperpnea is evident and before death occurs, open mouth breathing is common. In the recovering animals, appetite returns to normal as soon as the fever has gone. At the same time milk production starts to increase, reaching almost the same level as before if management is optimal. The weight loss caused by the lack of food intake and the energy consumption during the high fever period is gradually compensated, and a stage of restitutio ad integrum is reached by 4–5 weeks p.i.

TABLE 7

The initial clinical abnormalities recognized by farmers in 15 incidents of IBR (Wiseman et al., 1980)

Clinical signs	Number of incidents	Percentage
Reduced appetite	9	60
Serous oculonasal discharge	8	53
Coughing	7	47
Dullness	6	40
Tachypnea	5	33
Drooling saliva	4	27
Nasal discharge	3	20
Ocular discharge	2	13
Hyperpnea	2	13
Reduced milk yield	2	13
Blood on tail	1	7

Wiseman et al. (1980) painstakingly recorded the clinical abnormalities recognized by the farmers in 15 outbreaks of IBR in Scotland; a summary is presented in Table 7.

A number of reports describe unusual symptoms found in connection with outbreaks of IBR:

— In calves the disease appears to be of a more systemic type, as was first observed by Baker et al. (1960). Strikingly, the digestive tract is as much involved as the respiratory tract (Burkhardt and Paulsen, 1978; Miller et al., 1978; Ehrensperger and Pohlenz, 1979; Evermann and Clemm, 1980).

— In Australia, where 132 out of 340 animals died on one property and where BHV-1 was considered to be the etiological agent — the simultaneous infection with BVD/MD virus which had also been found could be ruled out — symptoms included ulceration of the oral cavity (tongue, dental pad, gums, buccal mucosa) and the caudal third of the esophagus, mild catarrhal enteritis and necrosis of lymph nodes draining the affected areas (Greig et al., 1981).

— In France, Dhennin et al. (1979) found, in addition to ulcerative lesions on the tongue, an ulcer in the interdigital space from which virus could be recovered.

— In the USA, BHV-1 was isolated from vesicular lesions on the udder and teats, which were up to 10 mm in diameter (Guy et al., 1984).

— In the USSR (Gunekov et al., 1982) IBR was associated with the formation of consolidated nodes in the jaw region besides the upper respiratory tract symptoms.

— Cases of abortion are often reported in connection with symptoms of IBR as e.g. by Stubbings and Cameron (1981); the typical clinical pattern of IBR abortion will be described later.

— Mixed infections worsen the clinical picture of IBR; the combination with mycoplasma leading to fatalities in 8–12% of the cases was published by Andreev and Fuks (1980) and by Rogers et al. (1980), who also isolated numerous bacteria.

— Unusually only unilateral conjunctivitis associated with pyrexia, inappetence, decreased milk production and some abortions but no clinical IBR are seen during outbreaks (Rebhun et al., 1978).

IPV

The clinical picture of this disease was, before 1958, better known as coital

exanthema (both in male and female animals). It starts 2-4 days after natural service, artificial insemination or contamination by the tail of a neighboring infected animal with an elevated temperature which usually remains undetected. Small pustules become visible in the vulvar and caudal vaginal region. They enlarge and spread over the whole epithelium in a plaque-like manner causing edema and hyperemia. The vulva is swollen; increased secretion soils the tail which is almost constantly waggling. Animals exhibit unrest and often turn their heads. At this stage the owner usually recognizes the sickness and asks for veterinary advice. Temperatures have by then reached their maximum of between 40.5 and 41.5°C, depending on the severity of secondary infections which are facilitated by the loss of epithelium. The high temperature and clinical symptoms continue for more than 8 days. Thereafter re-epithelization is rapid and a healthy stage is reached after 14 days. In general the animals do not go off their feed, and loss of milk production is less severe than in IBR unless the virus infects the mammary gland, where it may cause a catarrhal inflammation comparable to acute bacterial mastitis (Straub and Kielwein, 1966). Elevated hyperemic nodules a few millimeters in diameter are usually the last symptom after the second week p.i. These may persist for many weeks and become more distinct due to a delayed hypersensitivity type reaction when the animals are infected for a second or third time (Straub, 1970).

Reference has already been made to the involvement of reproductive organs during the infection (Table 6). The isolation of virus from ovarian tissue has lately been confirmed by Miller and Van der Maaten (1984), who also describe the histopathological changes.

IBP

The clinical symptoms are limited to the preputial, penile and sometimes the distal portion of the urethral mucosa. The progress of the disease is similar to that described for IPV, but healing of the epithelial lesions often requires more time. During the first days p.i. — usually following service of an infected female or after mechanical contamination in artificial insemination centers — the bulls serve normally, until they stop at days 3 or 4 p.i., when temperatures are very high (40.5-41.5°C) and the preputial swelling is pronounced.

The healing can be greatly disturbed if the animals are teased too early. The newly formed superficial vessels burst when erection occurs. During the peak of the inflammation process the bulls show unrest, frequently lift their hind legs and bend their heads to the side. Some animals go completely off their feed for a few days, which leads to considerable weight loss.

Four weeks p.i. small hyperemic modules are still visible on the penile mucosa; they reach pea size if secondary infections were present. A sequel may be that some bulls are reluctant to start servicing again, especially in artificial insemination centers. Secondary infections should be treated systemically rather than by local treatment, which only increases irritation.

IBR abortion

As indicated, abortions frequently occur in the course of IBR, where virus isolation from the respiratory tract is easy (see e.g. Stubbings and Cameron, 1981). This clinical entity has to be separated from those abortions where no overt IBR is noticed.

Abortions have been reported for the first time after the administration of IBR vaccine in which the virus had not been properly attenuated (Crane et al., 1964). Meanwhile virus strains are known which predominantly cause abortion during the third trimester of pregnancy. In one of the later reports (Straub et

al., 1982b) the incident is described as follows: IBRV was introduced into a dairy herd of 92 cattle by a young calf. It was transmitted to other calves, causing the death of four of them. Forty-five days passed during which six live calves were born at term from heifers and cows. Then within 56 days 12 animals (heifers and cows less than 6 years of age) aborted after 197 to 271 days of pregnancy, having shown no signs of abnormality during the preceding weeks. During the same period six animals more than 6 years of age gave birth at term to healthy calves. These animals had antibodies as a result of an outbreak of IPV several years ago. It is therefore not unexpected that both groups (aborted animals, and cows after normal parturition) were found to have antibodies when checked after the occurrence of five abortions. Virus was isolated from the ninth and tenth case with the highest titer in the cotyledon (10^6 $TCID_{50}$).

Because some time elapses between fetal death and abortion the fetuses are in general in various stages of autolysis. The general behavior of the aborting animals is hardly changed, only milk production is reduced if the abortions occurred before the normal term.

If treatment of the animals suffering from a retained placenta is carried out correctly, metritis will be avoided. Otherwise the animals may not conceive within the normal period of 60–90 days post partum. Lately Bowen et al. (1985) have shown that early embryos can be fatally infected by various strains of BHV-1.

IBR encephalitis

Encephalitis may be more frequently associated with the occurrence of IBR than is clinically recognized, as judged from the pathogenetic studies (Table 6). It must be considered as a separate clinical entity in some parts of the world such as the American continent, Australia and Hungary (Bartha et al., 1969; Baxter, 1984; Hill et al., 1984). This assumption is supported by studies of the plaque phenotype where the Australian virus strain isolated by French (1962) differed considerably from European IBR strains (Straub, 1972). This finding is supported by restriction endonuclease analysis (Wyler et al., 1989).

Laboratory data

IBR, IPV and IBP do not cause distinct changes in the cellular components of the blood, even at the time when the animals are febrile. This fact is sometimes helpful in differential diagnosis. Due to secondary infections, however, a neutrophilia can develop, as was shown by Wiseman et al. (1978); this author published clinical pathological data from two severe cases (Table 8).

Data concerning the cell content of the cerebrospinal fluid are available

TABLE 8

Hematological findings of two cases of IBR, 1 (no. 1) and 9 (no. 2) days after the detection of clinical symptoms (Wiseman et al., 1978)

Parameter	Calf no. 1	Calf no. 2
PCV (%)	28	34
Hemoglobin per 100 ml	9.1	11.0
Erythrocytes $\times$ 10^6 per μl	5.74	7.79
Leukocytes per μl	7000	10 400
Neutrophils per μl	4550	7176
Lymphocytes per μl	3224	2450
Eosinophils per μl	0	0

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TABLE 9

Cell content of cerebrospinal fluid from five cattle (Nos. 1-5) infected experimentally (Straub and Böhm, 1965)

Days p.i.	No. 1	No. 2	No. 3	No. 4	No. 5
0	0/3	0/3	0/3	0/3	0/3
2	2/3	35/3	72/3	32/3	13/3
4	10/3	712/3	1528/3	184/3	2660/3
7	20/3	X	1172/3	1136/3	†
9	1224/3	2250/3	5144/3	1216/3	
11	1920/3	X	X	328/3	
16	523/3	308/3	†	352/3	
23	423/3	90/3		12/3	
30	124/3	80/3		6/3	

X Samples were not taken because of serious condition of the animal.
† Animal died.

from experimentally induced cases of encephalitis (Table 9). Cell counts in surviving animals remain abnormal for a considerable period of time.

PATHOLOGY

IBR

During the acute stage of the infection the mucous membranes of the upper respiratory tract are swollen and congested. Pseudomembranes consisting of a mucopurulent, greenish exudate cover wide areas following secondary infection. In addition to rhinitis, laryngitis and tracheitis are usually present. The sinuses may also be affected and filled with exudate. When the pseudomembranes are removed the damage caused by the destruction of the epithelial layers is evident. Hemorrhages are also observed in the larynx and trachea. In rare cases the interlobular tissue of the lungs is edematous, but in general pneumonia does not develop.

Histologically the changes in the mucosa are characterized by a marked edema in the lamina propria and lymphocytic infiltrations; these form bandlike layers, especially along the glandular ducts, leading into the deeper layers. In the vicinity of cells destroyed by the replicating virus neutrophils are abundant. Blood vessels in affected areas are very prominent. If infected cells from the upper respiratory tract are fixed and stained during the early stages of the disease typical Cowdry A-type inclusion bodies can be observed. The latest detailed descriptions were given by Allan et al. (1980) and Narita et al. (1982). The latter author paid special attention to the changes in the neural system after experimental infection, whereas Allan et al. (1980) described the findings in field cases. Narita et al. (1982) found inflammatory changes in the peripheral and CNS, most frequently in the trigeminal ganglion and the rhombencephalon. Their histopathological findings are summarized in Table 10.

Animals in which the digestive tract is affected exhibit massive erosions and ulcerations of the buccal mucosa, lips, gums and hard palate, not unlike those occurring in cases of mucosal disease. The same type of lesions is found in the esophagus, forestomach and abomasum. The intestines show a catarrhal enteritis with involvement of the local lymph nodes.

Histologically the process starts with a ballooning degeneration of the epithelial cells leading to necrosis that is also present in the Peyer's patches. Molnár et al. (1984) found necrotic foci and intranuclear inclusions also in the

TABLE 10

Histopathological lesions in cattle after intranasal inoculation of IBRV (Narita et al., 1982)

Calf number	Age	Days after inoculation	Trigeminal ganglion	Medulla oblongata	Pons	Cerebrum	Cerebellum	Spinal cord	Other ganglia
1	1 week	8	+ +	+ +	+	−	−	−	−
2	3 weeks	8	+ +	+	−	−	−	−	−
3	5 weeks	8	+ +	+ +	−	−	−	−	−
4	1 week	14	+ +	+ + +	+ + +	+ +	−	+	−
5	3 weeks	14	+ + +	+ + +	+ +	+ +	−	+	−
6	6 years	14	+ +	+ +	−	−	−	−	−

Grade of lesion: −, no lesions; +, mild; + +, moderate; + + +, severe.

liver. The histological changes occurring in the respiratory tract after a secondary infection with *Pasteurella haemolytica* 4-5 days following BHV-1 infection are described by Jericho (1983). The bacterial colonies were seen in the turbinates, tonsils, and lung. Necrosis was present in the epithelium of nose, trachea and lung, and mucopurulent changes were particularly evident in pharyngeal tonsils and alveoli.

IPV and IBP

Typical inflammatory reactions are present in the vulval, vaginal and cervical mucosa and the preputial and penile mucosa. In some cases an endometritis occurs; in the male phimosis and paraphimosis may develop during later stages of the disease. Edema in both the male and female is a prominent feature. The changes in the epithelium have been termed "vesicles" and the disease therefore originally named coital exanthema ("Bläschenausschlag"); later pustules are prominent, which has lead to the current term IPV. In principle, this is a plaque-forming process whereby the infected cells undergo a ballooning enlargement before they die as a consequence of the virus replication. Released virus attacks neighboring cells and the process starts again. When enough cells are destroyed the lesion becomes visible, first minute, then becoming larger and confluent with other primary centers. Then the climax of the disease is reached. Bursting of capillaries in the regions of virus growth is responsible for the appearance of small focal hemorrhages. Bacterial growth occurs where the epithelium has been destroyed. If inclusion bodies are to be demonstrated, cells from the border of the plaques should be fixed. A wall of lymphocytes rapidly surrounds the foci and granulocytes phagocytize the dead epithelial cells. The fast formation of the lymphocyte barrier occurs because of the presence of large numbers of minute lymph nodes in the genital tract. When the process of virus spread is stopped by immune mechanisms, regeneration of the epithelium progresses rapidly.

IBR abortion

Characteristic changes are only recognizable in fetal organs — the uterine wall and placenta do not show any specific lesion. Even fetuses autolyzed in utero may provide evidence of the cause of death. The lesions most readily recognized are in the liver and kidneys. Macroscopically the liver appears yellowish with a rough surface, which is caused by necrotic and necrobiotic centers spread through epithelial and connective tissue. The kidneys are in most cases surrounded by a marked hemorrhagic edema. The cortex is almost completely destroyed by severe hemorrhagic processes often leaving only a thin layer attached to the capsule; the remainder including the medulla floats in a dark red fluid of necrotic debris. Histologically, focal necrosis and hemorrhages are the common finding.

IBR encephalitis

Macroscopically, hyperemia and petechiae in the brain are evident. The spinal column appears normal, only the cerebrospinal fluid is turbid due to its cell content. Histologically, most cases exhibit a perivascular edema and cuffing in the cerebrum with intranuclear inclusions in astrocytes and neurons. Degenerative processes with vacuolation around the neurons are seen in the cerebral cortex and rarefaction necrosis sometimes occurs in the superficial cortical lamina.
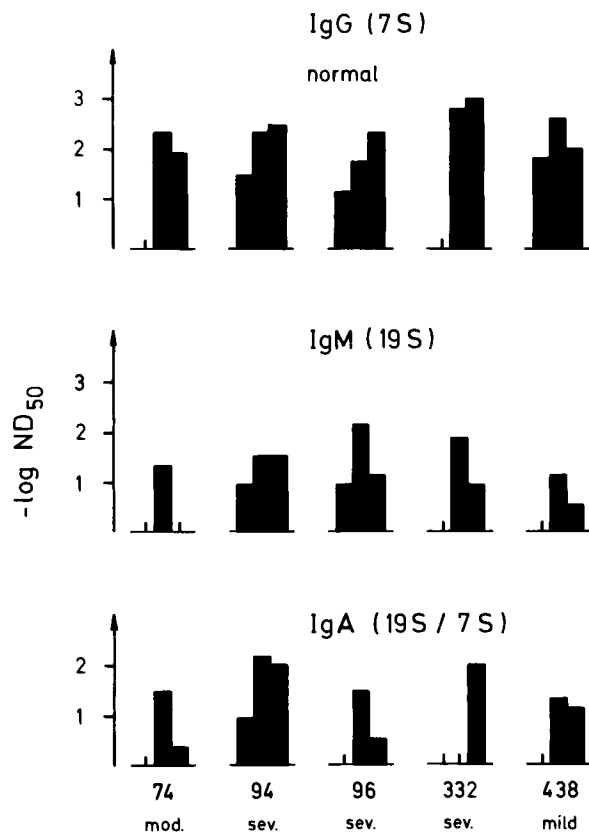
Fig. 29. Virus neutralizing titers ($-\log \mathrm{ND}_{50}$) obtained for the antibody classes IgG, IgM and IgA in gel-filtration fractions of sera from five cattle (nos. 74, 94, 96, 332 and 438) following a BHV-1 infection, and the severity of the clinical symptoms observed (mod. = moderate, sev. = severe). The sections on the base lines indicate the intervals between sampling (on days 0, 7, 14 and 28 p.i.).

IMMUNE REACTION

Immunoglobulins

The development of the different classes of immunoglobulins has been studied in detail by Matthaeus and Straub (1978). The results are depicted in Fig 29. In four of the five amimals examined maximum IgA titers were reached 2 weeks p.i. The highest IgM titers occurred after the second week p.i. IgG levels were at their maximum after the same period in two animals whereas the other three exhibited peak titers after the fourth week p.i. In pregnant animals, however, peak levels are reached earlier (Guy and Potgieter, 1985). The IgG antibody synthesis during the primary immune response is restricted to the IgG1 subclass whereas after reexposure mainly IgG2 antibodies are formed (Guy and Potgieter, 1985). No correlation exists between the levels of immunoglobulins, virus excretion and severity of clinical signs; this has been confirmed by Rüsch et al. (1981). The antibodies persist for years, but there is a difference according to the site of infection. Higher titers are usually the result of an infection of the respiratory tract, lower titers are found after genital tract infection (Straub et al., 1982a). This is of epidemiological importance, if for example a problem of infertility exists in cattle without a history of upper respiratory tract infection. The chain of genital tract infection is then maintained. As the antibody titers decrease virus latency is terminated and infectious virus is shed (Huck et al.,

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1973), which may be transmitted to neighboring cattle, e.g. via the tail (Straub and Böhm, 1963).

Another correlation exists between the level of humoral antibodies and the duration of virus excretion (Straub, 1978a) if challenge with virulent virus occurs. This correlation does not exist, however, if virus excretion is provoked by the administration of corticosteroids (Straub, 1979; Lema et al., 1981).

Immunoglobulins of all three classes are also present in the discharges of the respiratory and genital tract with higher IgG titers in the uterus during estrus than at any other time. It is interesting to note that a positive correlation exists between the serum and the follicular fluid antibody titers (Whitmore and Archbald, 1977).

Cell-mediated immunity

CMI plays a major role in the body's defense against IBR-IPV virus infections (Straub 1970, 1980). Hyperemic nodules in the genital tract of immune animals considered to be due to reinfection could be explained as evidence of CMI. Changes in the white blood counts in intravenously inoculated animals and a distinct increase in the activity of the medullary portion of the lymph nodes as early as 5 h after intranasal inoculation were described (Kendrick and Straub, 1972). Darcel and Dorward (1972) were the first to use the hypersensitivity reaction for diagnostic skin tests; later studies were conducted by Aguilar-Setién et al. (1978a,b,c) and Ivanov et al. (1982). If the tests are conducted in immunosuppressed animals the results become less pronounced (Straub, 1986b). Rouse and Babiuk (1974a,b) demonstrated that T lymphocytes play the major role. It has also been shown that besides lymphocytes the cells present in broncho-alveolar washings may be involved in this process (Bouffard et al., 1982). A series of studies was devoted to the antibody-dependent cell-mediated cytotoxicity (Rouse et al., 1976a,b; Wardley et al., 1976; Hanton and Pastoret, 1984). The mechanism is instrumental in the recovery from herpesvirus infections; the presence of polymorphonuclear leukocytes as effector cells and antibody in infected cultures markedly diminished viral expansion.

In addition to the cellular components, serotonin and dopamine play an important role in the allergic pathophysiology of the lung in ruminants (Eyre, 1978). Whether IgE antibodies are passively transferred by colostrum, leading to a sensibilization of newborn calves (Petzoldt, 1979), remains to be determined, especially in the light of a recent study conducted in rats. Jarrett and Hall (1983) discussed the possibility that the suppression of IgE antibody responsiveness by maternal IgG may represent a physiological regulatory process with a role in inhibiting allergies in the newborn.

Maternal antibodies

It has been reported that 1000 ml colostrum given within the first 12 h after birth are sufficient to attain the dam's antibody level in the newborn (Straub, 1969). In a detailed study, the absolute immunoglobulin amounts (IgG, IgM, IgA) in colostrum samples taken at different hours post partum were determined (Table 11). Peak immunoglobulin levels in the calf are reached between 6 and 9 h after parturition. If the calf does not receive sufficient colostrum its antibody levels remain below that of the dam (Fig. 30a) and production of its own antibodies starts earlier (after 6 weeks) than in calves that had received more colostrum (Fig. 30b).

Depending on the maximum levels attained, the specific antibodies against BHV-1 can be traced for up to 3–4 months, and in rare cases even longer (Straub, 1969). They can also be detected in nasal secretions, but in lower

TABLE 11

Immunoglobulin concentrations in colostrum samples taken at different hours after parturition (averages of three determinations for each time period) (Straub and Matthaeus, 1978)

Hours post partum	IgA (mg/100 ml)	IgG (mg/100 ml)	IgM (mg/100 ml)
3	333	4560	422
6	387	5250	687
9	484	5260	546
12	304	4406	425
24	288	3236	403
30	260	2530	232
36	172	1306	50

concentrations and rarely beyond the third week (Pospisil et al., 1983). Calves should therefore be vaccinated against BHV-1 infection at that age unless the history of the dam is available.

LABORATORY DIAGNOSIS

Laboratory diagnosis of BHV-1 infection is readily achieved, provided the samples are taken properly and sent under adequate conditions.

Collection of samples

For virus isolation from cases of *IBR* swabs made of gauze (not cotton) should be inserted into the nasal cavity. The gauze is preferably fixed by forceps and placed in a sterile tube after collection. The material should then be kept at approximately 4°C and transported to a diagnostic laboratory. It is advisable to send a blood sample along for all those cases where virus cannot be isolated and a later diagnosis based on serology is desirable. It is possible to take swabs from the conjunctiva also.

In cases of *IPV*, gauze swabs fixed to long forceps are placed into the vagina, left there for at least one minute and rotated during removal. Care has to be taken that the vulva is parted before insertion of the forceps, which should be covered by the gauze in order to prevent trauma.

If gauze is not available to swab the prepuce in cases of *IBP* it is possible to collect preputial washings using saline. At insemination centers the artificial vagina can be flushed after semen has been collected.

As virus in the fetus is usually inactivated at the time of abortion, a caruncle with placenta attached should be collected, cooled and dispatched to the diagnostic laboratory. In cases of mastitis, milk should be collected from individual quarters; in cases of epithelial lesions scrapings should be taken. Immediate cooling and proper shipment with an accompanying blood sample are necessary.

If there are sick animals in a herd a limited number should be sampled. Preference should be given to febrile animals showing typical clinical signs. It is advisable therefore to examine many animals of different ages before making a choice. Frequently animals are then detected where the only sign of infection is high fever. These animals shed large amounts of virus in contrast to animals with secondary infections.

Following stress, latently infected animals shed virus usually without exhibiting clinical signs.

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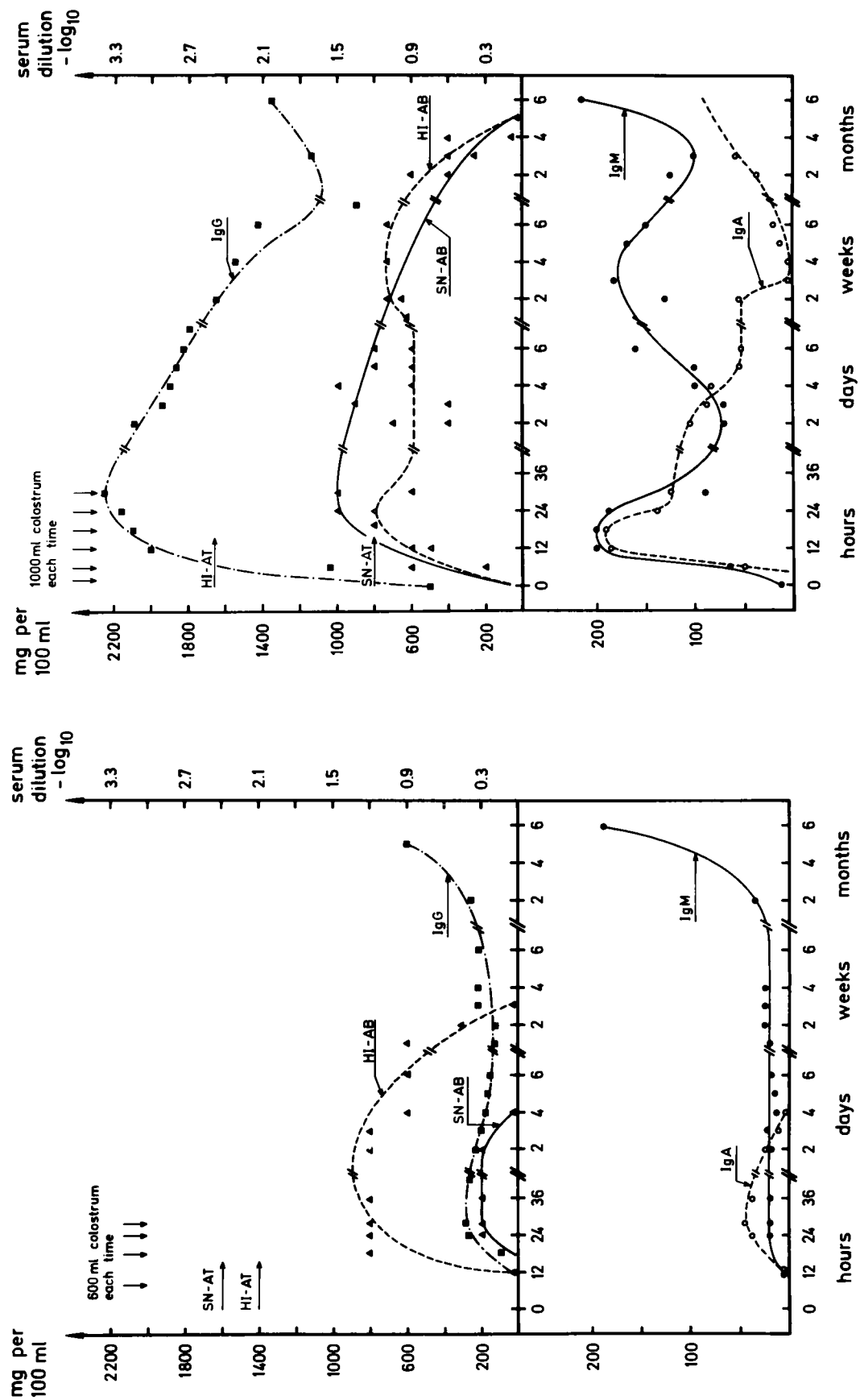
Fig. 30. Levels of immunoglobulins IgG, IgM and IgA, serum-neutralizing antibodies (SN-AB) against IBRV; hemagglutination-inhibiting antibodies (HI-AB) against parainfluenza-3 virus of calf F and the corresponding antibody titers of the dam at the time of parturition (SN-AT and HI-AT). The calf received colostrum on four occasions (a) or six occasions (b), as indicated.

Virus or antigen detection

Most commonly tissue culture is used for virus isolation and identification. Diagnostic laboratories are familiar with the procedures and are able to provide a diagnosis 24–48 h after the samples have arrived; they are immediately titrated in twofold, once in the presence of a BHV-1-negative serum and in parallel with a hyperimmune serum added. If the virus content in a sample is low it may take serial passages before it is classified as negative.

The identification of viral antigen is even more rapid. Suitable substrates are obtained by washing cells from the swabs, by scraping cells from epithelial layers (digestive tract, skin, interdigital space, fetal part of the placentome) or by using the sediment from milk after centrifugation. The method of choice is the IF technique (Wellemans and Leunen, 1974; Terpstra, 1979; Silim and Elazhary, 1983), but also the ELISA and the immunoperoxidase assay (Collins et al., 1985) have been found suitable.

When the four methods were compared, virus isolation in cell culture was found the most sensitive (Edwards et al., 1983). Special techniques are necessary if virus is to be isolated from semen samples, because undiluted semen is toxic for cell cultures (Darcel et al., 1977; Kahrs et al., 1977).

Antibody detection

The SN test is most commonly used and very reliable, but almost all other laboratory diagnostic methods have been employed. More recent reports come from Aguilar-Setién et al. (1980) concerning counterimmunoelectroosmophoresis, from Karadzhov and Khristova (1980) on the micro-CF test, and from Herring et al. (1980) on the micro-ELISA; also a passive HA test has been described (Karadzhov et al., 1979; Dannacher et al., 1979). In the author's experiments the immunodiffusion test produced acceptable results (Straub, 1986a). Usually blood serum is employed, but the tests may also be carried out with milk serum (Stuker et al., 1980; deMeuron, 1982; Perrin et al., 1984) or semen (Kharalambiev et al., 1974).

PROPHYLAXIS AND CONTROL

Numerous attenuated and inactivated BHV-1 vaccines are available all over the world. In most countries, vaccinations are carried out after outbreaks of the diseases. Before a general control or vaccination policy in a country is developed, the epidemiological pattern needs to be examined, especially with regard to the properties of the agent. If the diseases are confined to a small number of herds a strict control programme offers the best chance of eliminating the diseases. From the data collected it appears that this will be the exception, since BHV-1 carriers will remain undetected especially among wild ruminants. If a higher percentage of herds is infected or cattle are raised under feedlot conditions a prophylactic programme must be implemented. First it should be determined whether the signs of disease are to be prevented or rather spread of field virus.

Because of the potency of herpesviruses to induce cell transformation, experiments have been conducted to immunize animals with nucleic-acid-free subunit vaccines using herpes simplex type 2 virus. It proved possible to induce both humoral and CMI in mice, rabbits and monkeys. Others were less successful when they tried a subunit BHV-1 vaccine to immunize calves after they had achieved a strong response in adult cattle. Results improved when BHV-1 subunits were obtained by solubilization of infected cell cultures with Triton

X-100 and NP-40. Animals which had received antigen in Freund's incomplete adjuvant exhibited high serum titers. The current developments in genetic engineering and peptide synthesis herald a new generation of vaccines against the bovine herpesviruses.

Treatment of cattle with human leukocyte A interferon to prevent infection were unsuccessful (Roney et al., 1985). Therapy using chemical compounds may be possible in the future. Experiments using 2-deoxy-D-glucose in IBR infections in calves proved efficacious for ocular disease but did not influence respiratory tract infections (Mohanty et al., 1980).

Attenuated live vaccines

The first attenuated live vaccines were employed in the late fifties. They could be used only for intramuscular injection and some proved dangerous for pregnant cattle. Most preparations had not been properly attenuated and they proved to be virulent when tested by intratracheal inoculation. Vaccines for local application were first presented in 1970/1971 based on IPV and IBR virus strains, respectively.

Another type was the ts-vaccine, which could only be used for vaccinations of the respiratory tract; the mutant virus required lower temperatures for proper growth. This was of special advantage since field and attenuated strains could be distinguished. Nowadays restriction enzyme analysis offers this possibility.

It has not been proven that attenuated IBR or IPV vaccines will prevent the infection with field virus. In fact, it has been demonstrated that one attenuated strain and the ts-vaccine did not prevent infection. Although unlikely, recombination of an attenuated vaccine virus strain with field virus can occur. Nevertheless, the application of attenuated vaccines has proved satisfactory in most countries as a prophylactic or emergency measure. Emergency vaccinations are recommended when a herd is stricken with the disease and the diagnosis is made before all animals have contracted the field virus. Frequently vaccines against diseases caused by BHV-1 contain other components. Most common are combinations with parainfluenza-3 virus, bovine viral diarrhea/ mucosal disease virus and adenovirus. Sometimes bacteria are also included (such as *Pasteurella* or *Haemophilus*), depending on the marketing situation of the country.

Most manufacturers recommend the following vaccination scheme for attenuated products. First vaccination should be given to calves between 3 and 4 months of age, provided they had received antibody-containing colostrum. Otherwise calves may be vaccinated at any age with most vaccines. Second vaccination has to be given 4–6 weeks later. Subsequently one yearly vaccination is sufficient to maintain immunity unless severe outbreaks of disease occur in neighboring herds or if animals of unknown history are introduced. The animals should be vaccinated intranasally when infections via the respiratory tract are to be minimized. However, if all natural portals of entry of BHV-1 are to be protected it is necessary to apply vaccine virus to the mucous membranes also of the ocular and genital tissues. A single dose of vaccine is therefore not sufficient.

Intramuscular injection or only intranasal administration (it has been reported that one-nostril-application is sufficient) certainly prevents severe clinical signs but not the infection with the possibility of excretion following stress. Protection against challenge can be obtained by 48 h after injection. The local administration also leads to the production of interferon which does not occur after intramuscular administration. The best serological responses are therefore most likely achieved if no other live components are included in the

vaccine. Subsequently the IgA levels in both blood and milk are also raised. It has been shown that interferon in nasal secretions protects calves against infection with other agents. Attenuated vaccines can be given to pregnant cattle during all stages of gestation unless specified otherwise by the manufacturer. If antibodies in the colostrum are to reach high levels, dams with an unknown history should be vaccinated twice during the last 8 weeks of pregnancy, preferably 6–7 and 2–3 weeks ante partum. Under feedlot conditions it is better to vaccinate animals prior to shipment than after the occurrence of the first clinical cases of IBR. In the vaccinated animals virus clearance from the nasal mucosa is much more rapid than in nonvaccinated animals. In cases of emergency all animals in a herd without clinical signs should be vaccinated. At the second vaccination the affected animals should also be treated and preferably all mucous membranes included, because it has been demonstrated that humoral antibodies may disappear rapidly after a natural infection. It has been pointed out that a correlation exists between the number of days virus is excreted after infection with field virus and the level of humoral antibodies.

Inactivated vaccines

If the sole aim is to protect animals from clinical disease, inactivated vaccines may be administered. However, they should not be used when clinical signs are already present in a herd.

There are numerous vaccines and also vaccine combinations on the market in many countries. There is a tendency to prefer them because parenteral administration is easy. They are advantageous for administration to seronegative cattle for export to a destination with an unknown status, because no one would like to introduce even attenuated virus strains into a country considered to be free of BHV-1.

Inactivated vaccines may also be used in large-scale control programmes. Special care has to be taken in licensing. First the safety and efficacy of the inactivating compound has to be demonstrated. Also the volume to be injected and the amount of antigen per dose should be known and be above $10^{6.5}$ PFU or $TCID_{50}$ per ml prior to inactivation. Vaccines with a low antigen content are dangerous, because they introduce a low-level immunity in the recipients; these remain susceptible to field virus infections, after which they produce appreciable amounts of infectious virus in their mucous membranes without clearcut signs. They also may become latent carriers and spread the virus when introduced into herds after transport instead of being placed in quarantine for at least 3 weeks. The vaccination programme may be the same as mentioned for attenuated vaccines. It may be advantageous to repeat the vaccinations at shorter intervals (approximately every 6 months), especially when the epidemic situation shows a high prevalence in a given region.

Post vaccinal reactions

After local adminstration of *live attenuated vaccines* a slight increase in serous nasal and genital discharge may be observed, which is usually only noticed during experiments. The same is true for a minimal increase in body temperature. Other signs occur only if the vaccines are not properly attenuated or are unsuitable for local administration. The vaccine virus can be recovered from the excretions for longer periods of time (up to 16 days) after the first vaccination and for shorter periods after the second and further vaccinations. If antibody titers have reached high levels, virus can no longer be recovered. The same is true when the vaccine is injected. Attenuated live vaccines have caused abortions especially when administered during the last third of ges-

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tation. Information now provided by manufacturers indicates whether a live attenuated vaccine is suitable for parenteral injections.

After parenteral administration of *inactivated vaccines* local swelling and a slight incrase in body temperature are frequently observed. The severity depends on the adjuvant and the site of administration. The swellings are most prominent and painful after injection of the vaccine into the hind muscles or subcutaneously somewhat cranial of the prescapular lymph node. A moderate change in the white blood counts can be observed, which is correlated to the severity of the local symptoms and to the elevation of the body temperature. In some instances allergic reactions have been observed, but it is not known whether they are related to the viral or the adjuvant portion of the vaccine.

Passive immunization

Natural passive immunization of the newborn occurs via the colostrum. The antibodies (IgG_1 and IgA) are present in the colostrum at a higher concentration than in the dam's blood serum (Fig. 30). It is also possible to protect animals passively by injecting immune serum; 50 ml (or 1 ml per kg body weight) given intravenously or intratracheally are sufficient to protect a calf from disease. If the calf is older than 18 h the procedure should not be repeated because the animals are then no longer tolerant. They develop antibodies against the specific serum, and shock with immediate death may follow upon a second injection. If a second injection is indicated due to a disease such as anaplasmosis, blood from another donor should be tried in small quantities before larger amounts are injected. The passive immunity hinders the active formation of antibodies, but obviously not to the very latest stage, where the presence of minute amounts of antibodies can be proven.

Control

In infected herds all animals older than 6 months are to be tested for antibodies. It has then to be determined which aim needs to be achieved: prevention of clinical disease, elimination of field virus circulation or eradication of BHV-1.

The prevention of clinical disease can easily be achieved if the animals are vaccinated at regular intervals using attenuated, ts-, or inactivated vaccines. It has to be kept in mind that animals already infected remain latent virus carriers, because the genome of BHV-1 persists in the ganglia, as described earlier.

Elimination of field virus and prevention of clinical disease is accomplished by vaccinating all animals with attenuated live vaccines. It is important to repeat the vaccinations.

Depending on the percentage and absolute number of seroreactors in a herd an empirical programme can be applied (Table 9). Quite frequently the number of seroreactors does not change in a herd kept under conditions of low stress; transportation, introduction into new herds etc. are all dramatic events causing reactivation of BHV-1. The higher the number of seroreactors in a herd the higher is the risk that one of them might shed virus following a stress situation. On the other hand it has been shown that animals with high antibody titers shed virus less readily and in lower quantities than low-titer animals.

Therefore the scheme presented in Table 12 is recommended. Care has to be taken that replacements are only from certified BHV-1-free herds. Animals should not participate in shows and fairs. Offspring is not vaccinated and, if possible, raised separately. The vaccination has to be continued until the last seroreactor has left the premises.

TABLE 12

Scheme for the eradication of BHV-1 from infected herds[a]

Herd size	Maximal numbers of seroreactors at the various prevalence rates			
	≤ 10%	20%	40%	50%
1–10	*1*	*2*	*4*	**5**
11– 20	*2*	*4*	**5**	**10**
21– 50	*5*	**10**	**20**	**25**
51–100	**10**	**20**	**40**	**50**
> 100	**> 10**	**> 20**	**> 40**	**> 50**

[a] Italic numerals: vaccination only with inactivated vaccines; first two vaccinations at 4–6 weeks interval; subsequently one yearly vaccination.
Bold numerals: first two vaccinations at 4–6 weeks interval with attenuated live vaccines; 4 months later vaccination with inactivated vaccines; subsequently one yearly vaccination.
Stress factor increases from top to bottom.

This last section is based on the experimental work of the following authors and our own experience: Straub, 1977; Gerber et al., 1978; Mansfield et al., 1978; Straub, 1978; Latarija, 1979; Mitscherlich, 1979; Msolla et al., 1979; Schipper et al., 1979; Straub, 1979; Zuffa et al., 1979a,b; Cappel et al., 1980; Cummins and Rosenquist, 1980; Imray, 1980; Lugović and Cvetnić, 1980; Lupton and Reed, 1980; Mohanty et al., 1980; Sutton, 1980; Žuffa and Feketova, 1980; Burns and Lloyd-Evans, 1981; Darcel and Jericho, 1981; Kretzschmar, 1981; Burroughs et al., 1982; Frerichs et al., 1982; Jericho et al., 1982; Kretzschmar, 1982; Mohanty, 1982; Straub et al., 1982a,b; Vogel, 1982; Žuffa et al., 1982; Deptuła and Buczek, 1983; Jericho and Babiuk, 1983; Martin et al., 1983; Zmudziński and Baczyński, 1983; Morter et al., 1984; Nettleton et al., 1984; Straub, 1984a,b; Menanteau-Horta et al., 1985.

REFERENCES

Abraham, A. and Straub, O.C., 1981. Über die Eignung von Frettchen für die Impfstoffprüfung von IBR-IPV-Vakzinen. Berl. Münchn. Tierärztl. Wochenschr., 94: 431–432.

Abraham, A., Ayalon, N. and Marcus, S., 1982a. An outbreak of IBR/IPV infection in bulls and dairy cattle in Israel. 1. Clinical and diagnostic aspects. Refuah Vet., 39: 93–98.

Abràham, A., Ayalon, N. and Marcus, S., 1982b. An outbreak of IBR/IPV infection in bulls and dairy cattle in Israel. 2. Vaccination of bulls at artificial insemination centres. Refuah Vet., 39: 98–103.

Ackermann, M. and Wyler, R., 1984. The DNA of an IPV strain of bovid herpesvirus 1 in sacral ganglia during latency after intravaginal infection. Vet. Microbiol., 9: 53–63.

Albrecht, E., Romer, H., Reibhauer, G., Wagenseil, F. and Oesterle, P., 1985. BHV-1(IBR/IPV)-Infektion. Serologische Untersuchung über die Verbreitung im Regierungsbezirk Tübingen und den Verlauf in infizierten Beständen. Tierärztl. Umschau, 40: 652–658.

Allan, E.M. and Msolla, P.M., 1980. Scanning electron microscopy of the tracheal epithelium of calves inoculated with bovine herpesvirus. Res. Vet. Sci., 29: 325–327.

Allan, E.M., Pirie, H.M., Msolla, P.M., Selman, I.E. and Wiseman, A., 1980. The pathological features of severe cases of infectious bovine rhinotracheitis. Vet. Rec., 107: 441–445.

Anciaux, Y, Thiry, E. and Pastoret, P.-P., 1982. Effet de l'ozone sur le virus de la rhinotrachéite infectieuse bovine (Bovid Herpesvirus 1). Ann. Méd. Vét., 126: 327–333.

Andreev, E.V. and Fuks, P.P., 1980. Mixed infection with virus and mycoplasma (in calves). Vet. Bull., 51: 413 (1981).

Aguilar-Setién, A., Pastoret, P.-P., Burtonboy, G. and Schoenaers, F., 1978a. Test d'hypersensibilité retardée au virus de la rhinotrachéite infectieuse bovine (Bovid Herpesvirus 1), avec du virus purifié. Ann. Méd. Vét., 122: 193–199.

Aguilar-Setién, A., Pastoret, P.-P., Burtonboy, G., Jetteur, P. and Schoenaers, F., 1978b. Evaluation à l'aide de virus semi-purifié du test d'hypersensibilité retardée au virus de la rhinotracheite infectieuse bovine. Ann. Méd. Vét., 122: 693–698.

Aguilar-Setién, A., Correa Girón, P. and Cornejo Legorreta, G., 1978c. Correlación entre la inmunidad celulár y la serica al utilizar un antigeno de rinotraqueitis viral bovina (IBR). Tec. Pec. Mexico, 34: 80–84.

Aguilar-Setién, A., Pastoret, P.-P., Jetteur, P., Burtonboy, G. and Schoenaers, F., 1979a. Excrétion du virus de la rhinotrachéite bovine (IBR, Bovid Herpesvirus 1) après infection de dexaméthasone, chez un bovin réagissant au test d'hypersensibilité retardée, mais dépourvu d'anticorps neutralisant ce virus. Ann. Méd. Vét., 123: 93–101.

Aguilar-Setién, A., Pastoret, P.-P., Burtonboy, G., Coignoul, F., Jetteur, P., Vandeputte, J. and Schoenaers, F., 1979b. Communauté antigénique entre le virus de la rhinotrachéite infectieuse bovine (Bovid Herpesvirus 1, BHV-1) démontrée, chez le bovin, par un test d'hypersensibilité retardée. Ann. Méd. Vét. 123: 55–61.

Aguilar-Setién, A., Pastoret, P.-P. and Schwers, A., 1980. Etude chez le bovin, par neutralisation et immunoprécipitation, des réactions sérologiques croisées entre le virus de la rhinotrachéite infectieuse boving (Bovid Herpesvirus 1, BHV-1) et celui de la maladie d'Aujeszky (Sus Herpesvirus 1, SHV-1). Ann. Méd. Vét., 124: 199–209.

Babu, T.S., Mallick, B.B. and Das, S.K. 1984. Prevalence of infectious bovine rhinotracheitis virus (BHV-1) antibodies in bovines. Indian Vet. J., 61: 195–200.

Baker, J.A., McEntee, K. and Gillespie, J.H., 1960. Effects of infectious bovine rhinotracheitis-infectious pustular vulvovaginitis (IBR-IPV) virus on newborn calves. Cornell Vet., 50: 156–170.

Barth, T., Ulbrich, F., Haase, H., Streu, J., Pohle, V. and Weyhe, D., 1978. Untersuchungen über den Einfluss der IBR/IPV-Infektion auf die Reproduktion in Milchviehherden. Monatsh. Veterinärmed., 33: 538–543.

Barth, T., Ulbrich, F., Haase, G. and Fraustadt, Ch., 1983. Untersuchungsergebnisse bei Jungrindern und Färsen im Zusammenhang mit der IBR/IPV-Infektion. Monatsh. Veterinärmed., 38: 729–730.

Bartha, A., Hajdu, G., Aldasy, P. and Paczolay, G., 1969. Occurrence of encephalitis caused by infectious bovine rhinotracheitis virus in calves in Hungary. Acta Vet. Acad. Sci. Hung., 19: 145–151.

Bauer, K., Gerbermann, H., Schmittdiel, E. and Winteroll, G., 1980. Serologische Untersuchungen über den Verbreitungsgrad der IBR/IPV-Virusinfektion bei Rindern in Bayern. Tierärztl. Umschau, 35: 594–600.

Baxter, G.M., 1984. Neonatal meningoencephalitis associated with infectious bovine rhinotracheitis virus. Bovine Pract., 19: 41–44.

Bolton, D.C., Zee, Y.C. and Osebold, J.W., 1982. The biological effects of ozone on representative members of five groups of animal viruses. Environ. Res., 27: 476–484.

Bouffard, A., Derbyshire, J.B., and Wilkie, B.N., 1982. Effect of lymphocytes and broncho-alveolar washing cells on the replication of bovine herpesvirus type 1 in tracheal organ cultures. Vet. Microbiol., 7: 214–251.

Bowen, R. A., Elsden, R.P. and Seidel, G.E., 1985. Infection of early bovine embryos with bovine herpesvirus-1. Am. J. Vet. Res., 46: 1095–1097.

Bowes, N.D., Nisnovich, L. and Morgavi, M., 1970. Experimental production of bovine infectious rhinotracheitis. Gac. Vet. (Buenos Aires), 32: 183–186.

Burkhardt, E. and Paulsen, J., 1978. Nachweis von Bovinem Herpesvirus 1 (IBR-IPV) bei Rindern mit Affektionen des Verdauungstraktes. Berl. Münch. Tierärztl. Wochenschr., 91: 480–482.

Burns, P.M. and Lloyd-Evans, L.P., 1981. Effect of diluent volumes on the serological efficacy of intranasal bovine herpesvirus 1 strain RLB 106 ts in controlling infectious bovine rhinotracheitis. Vet. Rec., 109: 515.

Burroughs, A.L., Morrill, J.L., Bostwick, J.L., Ridley, R.K. and Fryer, H.C., 1982. Immune responses of dairy cattle to parainfluenza-3 virus in intranasal infectious bovine rhinotracheitis-parainfluenza-3 virus vaccines. Can. J. Comp. Med., 46: 264–266.

Bush, C.E. and Pritchett, R.F., 1985. A comparison of the genomes of bovine herpesvirus type 1 and pseudorabies virus. J. Gen. Virol., 66: 1811–1817.

Cappel, R., De Cuyper, F. and Rickaert, F., 19870. Efficacy of a nucleic acid free herpetic subunit vaccine. Arch. Virol., 65: 15–23.

Carrillo, B.J., Ambrogi, A., Schudel, A.A., Vazquez, M., Dahme, E. and Pospischil, A., 1983. Meningoencephalitis caused by IBR virus in calves in Argentina. Zentralbl. Veterinärmed. B., 30: 327–332.

Chapman, M.S., Lucas, M.H., Hebert, C.N. and Goodey, R.G., 1979. Survival of infectious bovine rhinotracheitis virus in stored bovine semen. Vet. Sci. Commun., 3: 137–139.

Cherif, A. and M'Rabet, L., 1984/1985. Infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) in Tunisia: serological survey. Maghreb Vét., 1: 19–22.

Collings, D.F., Gibbs, E.P.J. and Stafford, L.P., 1972. Concurrent respiratory and genital disease associated with infectious bovine rhinotracheitis/infectious pustular vulvoganinitis (IBR/IPV) virus in a dairy herd in the United Kingdom. Vet. Rec., 91: 214–219.

Collins, J.K., Butcher, A.C. and Riegel, C.A., 1985. Immune response to bovine herpesvirus type 1

infections: virus specific antibodies in sera from infected animals. J. Clin. Microbiol., 21: 546–552.

Crane, C.S., Lukas, G.N. and Watkins, W.W., 1964. Infectious bovine rhinotracheitis abortion in California beef cattle. J. Am. Vet. Med. Assoc., 144: 13–18.

Cummins, J.M. and Rosenquist, B.D., 1980. Protection of calves against rhinovirus infection by nasal secretion interferon induced by infectious bonine rhinotracheitis virus. Am. J. Vet. Res., 41: 161–165.

Curvers, V., Thiry, E., Leroy, P., Kinnaert, P. and Pastoret, P.-P., 1985. Effect of dexamethasone on the mean plaque size of bovine herpesvirus. 1. Ann. Rech. Vét., 16: 81–85.

Dannacher, G., Perrin, M. and Perrin, B., 1979. Utilisation d'une technique d'hémagglutination passive pour la détection des anticorps contre le virus de la rhinotrachéite bovine infectieuse. Rec. Méd. Vét., 155: 633–637.

Darcel, C. le Q. and Dorward, W.J., 1972. Skin reactivity and infectious bovine rhinotracheitis. Can. Vet. J., 13: 100–101.

Darcel, C.le Q. and Jericho, K.W.F., 1981 Failure of a subunit bovine herpesvirus 1 vaccine to protect against experimental respiratory disease in calves. Can. J. Comp. Med., 45: 87–91.

Darcel, C.le Q., Yates, W.D.G. and Mitchell, D., 1977. A technique for the isolation of bovine herpesvirus 1 from bull semen using microtiter plates. Proc. Am. Assoc. Vet. Lab. Diagn., 20: 209–214.

DeFrederick, D.F. and Reece, R.L., 1980. Diseases of cattle in the Solomon Islands. Aust. Vet. J., 56: 522–525.

De Meuron, P.-A., 1982. Recherche d'anticorps IBR/IPV dans le lait. Schweiz. Arch. Tierheilk., 124: 203–208.

Deptuła, W. and Buczek J., 1983. Efficacy of the antiviral serum PI-3 and IBR in prophylaxis of enzootic bronchopneumonia in calves kept for production. Excerpta Vet. Lublin, 22: 28–29.

Dhennin, L., Gourreau, J.M., Calvarin, R., Kaiser, C., Le Corveller, M. and Perrin G., 1979. Une nouvelle forme clinique de rhinotrachéite infectieuse bovine. Rec. Méd. Vét., 155: 851–854.

Doyle, L.G. and Heuschele, W.P., 1983. Prevalence of antibody to bovine herpesvirus 1 in wild ruminants captive in United States zoos. J. Am. Vet. Med. Assoc., 183: 1255–1256.

Edwards, S., Chasey, D. and White, H., 1983. Experimental infectious bovine rhinotracheitis: comparison of four antigen detection methods. Res. Vet. Sci., 34: 42–45.

Ehrensperger, F. and Pohlenz, J., 1979. Infektiöse Bovine Rhinotracheitis bei Kälbern. Schweiz. Arch. Tierheilk., 121: 635–642.

Elazhary, M.A.S.Y. and Derbyshire, J.B., 1979. Effect of temperature, relative humidity and medium on the aerosol stability of infectious bovine rhinotracheitis virus. Can. J. Comp. Med., 43: 158–167.

Elazhary, M.A.S.Y., Frechette, J. L., Silim, A. and Roy, R.S., 1981. Serological evidence of some bovine viruses in the caribou (*Rangifer tarandus caribou*) in Quebec. J. Wildl. Dis., 17: 609–612.

Engels M., Gelderblom, H., Darai, G. and Ludwig, H., 1983. Goat herpesviruses: biological and physical properties. J. Gen. Virol., 64: 2237–2247.

Espinasse, J., Chantal, J., Faye, P., Akakpo, H.A., Le Layec, C., L'Haridon, R. and Savey, M., 1980. Recherche par une technique d'hémagglutination passive des traces sérologiques des principaux virus respiratoires des bovins et de *Chlamydia psittaci* dans un échantillon de la population des bovidés du Togo. Rev. Elev. Méd. Vét. Pays Trop., 33: 15–20.

Espinasse, J., Viso, M., Laval, A., Le Layec, C. and Monpetit, C., 1983. Reactivation and shedding of infectious bovine rhinotracheitis virus caused by 3-methylindole. Vet. Rec., 113: 15–16.

Etchegaray, P.B., Fabia Moran, J.L. and Celedon Venegas, M.O., 1977. Caracterización del virus herpes bovino tipo 1, cepa Puento Alto Chile. Rev. Latinoam. Microbiol., 23: 181–188.

Eugster, A.K., Angulo, A.B. and Schwartz, W.L., 1978. Isolation of infectious bovine rhinotracheitis (IBR) virus from stillborn and neonatal piglets. Proc. Am. Assoc. Vet. Lab. Diagn., 21: 1–13.

Evermann, J.F. and Clemm, D.L., 1980. Re-emergence of infectious bovine rhinotracheitis virus associated with calf enteritis. Bovine Pract., 1: 45–47.

Eyre, P., 1978. Allergic pathophysiology of bovine lung. In: W.B. Martin (Editor), Respiratory Diseases in Cattle. Current Topics in Veterinary Medicine, Martinus Nijhoff, The Hague Vol. 3, pp. 129–137.

Forman, A. and Babiuk, L.A., 1982. Effect of infectious bovine rhinotracheitis virus infection on bovine alveolar macrophage function. Infect. Immunol., 35: 1041–1047.

Forman, A., Babiuk, L.A., Misra, V. and Baldwin, F., 1982. Susceptibility of bovine macrophages to infectious bovine rhinotracheitis virus infection. Infect. Immunol., 35: 1048–1057.

French, E.L., 1962. A specific virus encephalitis in calves: isolation and characterization of the causal agent. Aust. Vet. J., 38: 216–221.

Frerichs, G.N., Woods, S.B., Lucas, M.H. and Sands, J.J., 1982. Safety and efficacy of live and inactivated infectious bovine rhinotracheitis vaccines. Vet. Rec., 111: 116–122.

Frost, J.W. and Wagner, K., 1982. Zur Verbreitung der IBR-IPV-Virusinfektion bei Rindern in Hessen. Tierärztl. Umschau, 37: 176–177.

Fulton, R.W., Downing, M.M. and Hagstad, H.V., 1982. Prevalence of bovine herpesvirus-1, bovine viral diarrhea, parainfluenza-3, bovine adenoviruses-3 and -7, and goat respiratory syncytial viral antibodies in goats. Am. J. Vet. Res., 43: 1454–1457.

Galarza, J.M. and Periolo, O.H., 1983. Rinotraqueitis infecciosa bovina, prevalencia en la provincia de Formosa mediante la prueba de inmunofluorescencia indirecta. Gac. Vet., 45: 1296–1301.

Gerber, J.D., Marron, A.E. and Kucera, C.J., 1978. Local and systemic cellular and antibody immune responses of cattle to infectious bovine rhinotracheitis virus vaccines administered intranasally or intramuscularly. Am. J. Vet. Res., 39: 753–760.

Gibbs, E.P.J. and Rweyemamu, M.M., 1977. Bovine herpesviruses. Part I. Bovine herpesvirus 1. Vet. Bull., 47: 317–343.

Gössler, R. and Paulsen, J., 1975. Virologische Untersuchungen bei Besamungsbullen in Hessen. 1. Mitt.: Untersuchungen zum Vorkommen von IBR-IPV-Virusinfektionen. Berl. Münch. Tierärztl. Wochenschr., 88: 221–224.

Greig, A., Gibson, I.R., Nettleton, P.F. and Herring, J.A., 1981. Disease outbreak in calves caused by a mixed infection with infectious bovine rhinotracheitis virus and bovine virus diarrhoea virus. Vet. Rec., 108: 480.

Griffin, T.P., Howells, W.V., Crandell, R.A. and Maurer, F.D., 1958. Stability of the virus of infectious bovine rhinotracheitis. Am. J. Vet. Res., 19: 990–992.

Guarino, H., Maisonnave, J., Capano, F. and Pereira, J., 1981. Primer aislamiento e identificación del virus de la rinotraqueitis infecciosa bovina en el Uruguay. Veterinaria (Montevideo) 17: 131–134.

Guevara, M.P., 1979. Sobrevivencia del virus de la rinotraqueitis infecciosa bovina-vulvovaginitis pustular (IBR-IPV) en semen bovino congelado en forma de "pellet" y posible diseminación durante su almalcenamiento. Cienc. Vet. (Costa Rica), 1: 25–30.

Gunenkov, V.V., Sukharev, O.I. and Fomenko, N.V., 1982. New Form of clinical manifestation of infectious bovine rhinotracheitis (IBR). In: Proceedings, World Congress on Diseases of Cattle. World Association for Buiatrics, Utrecht, Vol. 1, pp. 46–48.

Guy, J.S., Potgieter, L.N.D., McCracken, M. and Martin, W., 1984. Isolation of bovine herpesvirus-1 from vesicular lesions of the bovine udder. Am. J. Vet. Res., 45: 783–785.

Guy, J.S. and Potgieter, L.N.D., 1985. Bovine herpesvirus-1 infection of cattle: Kinetics of antibody formation after intranasal exposure and abortion induced by the virus. Am. J. Vet. Res., 46: 893–898.

Hafez, M.A., 1982. IBR in Egypt. Vet. Rec., 111: 513.

Hahnefeld, E., Hantschel, H. and Hahnefeld, H., 1963. Die Stabilität des Virus des Bläschenausschlages des Rindes (Exanthema coitale vesiculosum bovis) bei verschiedenen Temperaturen und Wasserstoffionenkonzentrationen sowie bei Einwirkung organischer Lösungsmittel und Natriumdesoxycholat. Arch. Exp. Veterinärmed., 17: 433–488.

Hanton, G. and Pastoret, P.-P., 1984. La réaction de cytotoxicité à médiation cellulaire dépendante des anticorps (ADCC). Ann. Rech. Vét., 15: 443–454.

Hassan, A.K.M. and El Tom, K., 1985. Combined natural infection with infectious bovine rhinotracheitis and rinderpest viruses. Trop. Anim. Health. Prod., 17: 52.

Hedger, R.S. and Hamblin, C., 1978. Neutralizing antibodies to bovid herpes virus 1 (Infectious bovine rhinotracheitis/Infectious pustular vulvo-vaginitis) in African wildlife with special reference to the Cape Buffalo (*Syncerus caffter*). J. Comp. Path., 88: 211–218.

Herring, A.J., Nettleton, P.F. and Burrells, C., 1980. A micro-enzyme-linked immunosorbent assay for the detection of antibodies to infectious bovine rhinotracheitis virus. Vet. Rec., 107: 155–156.

Hill, B.D., Hill, M.W.M., Chung, Y.S. and Whittle, R.J., 1984. Meningoencephalitis in calves due to bovine herpesvirus type 1 infection. Aust. Vet. J., 61: 242–243.

Homan, E.J. and Easterday, B.C., 1982. Interactions of bovine herpesvirus type 1 and neurons in vitro. J. Infect. Dis., 146: 97.

Huck, R.A., Millar, P.C. and Woods, D.G., 1973. Experimental infection of maiden heifers by the vagina with infectious bovine rhinotracheitis/infectious pustular vulvo-vaginitis virus. J. Comp. Pathol. 83: 271–279.

Ibrahim, A., Saw, S.P., Fatimah, I. and Saharee, A.A., 1983. Isolation of infectious bovine rhinotracheitis virus from buffalo in Malaysia. Vet. Rec., 112: 303–304.

Inglis, D.M., Bowie, J.M., Allan, M.J. and Nettleton, P.F., 1983. Ocular disease in red deer calves associated with a herpesvirus infection. Vet. Rec., 113: 182–183.

Imray, W.S., 1980. Use of a modified live infectious bovine rhinotracheitis vaccine in the field. Vet. Rec., 107: 511–512.

Ivanov, I.E., Arsov, R. and Sizov, I., 1982. Development of the allergic response to an intradermal test for infectious bovine rhinotracheitis. Veterinarnomed. Nauki, 19: 26–31 (in Russian, with English summary).

Jarrett, E.E.E. and Hall, E., 1983. IgE suppression by maternal IgG. Immunology, 48: 49–58.

Jericho, K.W.F., 1983. Histological changes in the respiratory tract of calves exposed to aerosols of bovine herpesvirus 1 and *Pasteurella haemolytica*. J. Comp. Pathol., 93: 73–82.

Jericho, K.W.F. and Babiuk, L.A., 1983. The effect of dose. route and virulence of bovine herpesvirus 1 vaccine on experimental respiratory disease in cattle. Can. J. Comp. Med., 47: 133–139.

Jericho, K.W.F., Yates, W.D.G. and Babiuk, L.A., 1982. Bovine herpesvirus-1 vaccination against experimental bovine herpesvirus-1 and *Pasteurella haemolytica* respiratory tract infection: onset of protection. Am. J. Vet. Res., 43: 1776–1780.

Jermolenko, G., Vujović R. and Radosavljević, D., 1978. Three years of investigations into the spread of IBR/IPV infection in Serbia. Vet. Bull., 49: 25.

Kahrs, R.F., Johnson, M.E. and Bender, G.M., 1977. Studies on the detection of infectious bovine rhinotracheitis (IBR) virus in bovine semen. Proc. Am. Assoc. Vet. Lab. Diagn., 20: 187–208.

Karadjov, I., Hristov, S., Pavlov, N., Ignatov, G. and Dyakov, I., 1977. Demonstration of IBR/IPV virus in placentas and aborted fetuses from cows. Vet. Bull., 48: 579 (1978).

Karadzhov, I., and Khristova, V., 1980. Preparation of antigen from IBR/IPV virus and its use in the microcomplement fixation test for bovine infectious rhinotracheitis. Veterinarnomed. Nauki, 17: 17–22 (in Russian, with English summary).

Karadzhov, I., Ignatov, G. and Khristova, V., 1979. Serological diagnosis of IBR. Veterinarnomed. Nauki, 16: 65–71 (in Russian, with English summary).

Kendrick, J.W. and Straub, O.C., 1967. Infectious bovine rhinotracheitis–infectious pustular vulvovaginitis virus infection in pregnant cows. Am. J. Vet. Res., 28: 1269–1282.

Kendrick, J.W. and Straub, O.C., 1982. The secondary immune response of the bovine lymphnode to the infectious bovine rhinotracheitis–infectious pustular vulvovaginitis virus. Zentralbl. Bakteriol. I. Orig. A., 219: 415–419.

Khamraev, K., 1980. I. Survival of bovine infectious rhinotracheitis virus outside the host, and the development of a method of aerosol disinfection of buildings. II. Resistance. Vet. Bull., 52: 603 (1982).

Kharalambiev, Kh., Dilovski, M., Gaytandzhieva, R. and Zagorski, D., 1974. Virus-neutralizing activity of semen from bulls that have been affected with infectious rhinotracheitis-balanoposthitis. C. R. Acad. Agric. G. Dimitrov, 7: 75–77.

Kiorpes, A.L., Bisgard, G.E., Manohar, M. and Hernandez, A., 1978. Pathophysiologic studies of infectious bovine rhinotracheitis in the Holstein–Friesian calf. Am. J. Vet. Res., 39: 779–783.

Kirby, F.D., Martin, H.T. and Waring, M.J., 1978. A serological survey on the prevalence of infectious bovine rhinotracheitis in Buckinghamshire, Berkshire and Oxfordshire. Vet. Rec., 103: 134–136.

Kokles, R., 1978. Zur systematischen Überwachung landwirtschaftlicher Nutztiere auf Virusinfektionen. Tierhygiene Information, Sonderheft 10 Jahre Bezirksinstitut für Veterinärwesen, Rostock, pp. 111–120.

Kokles, R., 1979. Die Anwendung von Prednisolon zur Feststellung der Lokalisation des IBR/IPV Virus im Rinderorganismus und zur Objektivierung des Vakzinationseffektes. Monatsh. Veterinärmed., 34: 328–331.

Kretzschmar, Ch., 1981. Untersuchungen zur Tilgbarkeit der Infektion mit dem Virus der Infektiösen Bovinen Rhinotracheitis (IBR)/Infektiösen Pustulösen Vulvovaginitis (IPV). 1. Mitteilung: Über die Beeinflussung der nach natürlicher IBR/IPV-Infektion verbleibenden Viruslatenz durch anschliessende Immunisierungen mit der Riemser IBR/IPV-Lebendvakzine. Arch. Exp. Veterinärmed., 35: 535–546.

Kretzschmar, Ch., 1982. Uber die Möglichkeiten der Tilgung von Infektionskrankheiten durch Verdrängung ihrer Erreger mit Hilfe aktiver Immunisierungen. Monatsh. Veterinärmed., 37: 858–860.

Kretzschmar, C., Peters, U. and Matschei, J., 1983. Untersuchungen zur Tilgbarkeit der Infektion mit dem Virus der Infektiösen Bovinen Rhinotracheitis (IBR)/Infektiösen Pustulösen Vulvovaginitis (IPV). Untersuchungen zur Viruslatenz des Impfvirusstammes der Riemser IBR/IPV-Vakzine (Lebendvirusimpfstoff) und von Feldvirus nach Infektion immunisierter Tiere. Arch. Exp. Veterinärmed., 37: 829–835.

Krpata, V., 1982. Spread of IBR-IPV virus from contaminated to uncontaminated pellets of frozen bull semen. Vet. Bull., 53:469 (1983).

Kubin, G., 1982. Feststellung der infektiösen Rhinotracheitis (IBR) des Rindes in Österreich. Wien. Tierärztl. Monatsschr., 69: 145–148.

Lamothe, P., Larouche, Y., Marsolais, G., Montpetit, C., Breton, L., Malo R. and Saint-Pierre, H., 1979. Isolement du virus de la rhino-trachéite infectieuse bovine à partir du liquide synovial du tarse d'un taureau. Can. Vet. J., 20: 237–241.

Latarija, T.N., 1979. Cited in: Schirrmeier, A., 1982. Immunogene Eigenschaften einer Virusvakzine gegen die Infektiöse Bovine Rhinotracheitis bei Vakzination tragender Kühe. Monatsh. Veterinärmed., 37: 911.

Lazarowicz, M., Steck, F., Ackermann, M. and Kihm, U., 1983. Prüfung von zwei Impfstoffen gegen die Infektiöse Bovine Rhinotracheitis. Schweiz. Arch. Tierheilk., 125: 797–808.

Lema, B., Kokles, R. and Brüsehaber, H.-U., 1981. Untersuchungen zur Persistenz des IBR/IPV-Virus bei latent infizierten Rindern nach Immunisierung mit IBR-Lebendvirusvakzine. Monatsh. Veterinärmed., 36: 130–133.

Letchworth, G. and Carmichael, L.E., 1984. Local tissue temperature: a critical factor in the pathogenesis of bovid herpesvirus 2. Infection and Immunity, 43: 1072–1079.

Levings, R.L. Kaeberle, M.L. and Reed, D.E., 1984. Cross-reactions of bovine herpesvirus 1 antigens with those of other cattle herpesviruses. Vet. Microbiol., 9: 329–344.

Lorrmann, W., 1967. Versuche Zur Keimübertragung bie der Tiefgefrierkonservierung von Samen in Pelletform. Inaugural Dissertation, Tierärztliche Hochschule, Hannover.

Ludwig, H., 1983. Bovine herpesviruses. In: B. Roizman (Editor), The Herpesviruses. Plenum Press New York, Vol. 2, pp. 135–214.

Ludwig, H. and Storz, J., 1973. Activation of herpesvirus from normal bovine fetal spleen cells after prolonged cultivation. Med. Microbiol. Immunol., 158: 209–217.

Lugović, B. and Cvetnić, S., 1980. Serological response of calves and heifers to "Ripavak", a combined vaccine against rhinotracheitis-parainfluenza-3 viruses. Praxis Vet., 28: 9–14.

Lupton, H.W. and Reed, D.E., 1980. Evaluation of experimental subunit vaccines for infectious bovine rhinotracheitis. Am. J. Vet. Res., 41: 383–390.

Lupton, H.W., Barnes, H.J. and Reed, D.E., 1980. Evaluation of the rabbit as a laboratory model for infectious bovine rhinotracheitis virus infection. Cornell Vet., 70: 77–95.

Mahin, L., Chadli, M. and Kirmsse, P., 1982. Vorläufiger Bericht über das Vorhandensein von IBR-IPV (Bovid Herpesvirus 1) Viren bei Rindern in Marokko. Tierärztl. Umschau, 37: 264–267.

Majewska, H., Baczyński, Z. and Zmudziński, J., 1980. Investigations on infectious pustular vulvovaginitis in bulls under natural conditions. Bull. Vet. Inst. Pulawy, 24: 1–4.

Mansfield, M.E., Crandell, R.A. and Woods, G., 1978. The effect of intranasal IBR-PI₃ vaccination of feeder cattle on arrival at a feedlot compared with vaccination at the start of an outbreak of acute respiratory tract disease. Bovine Pract., 13: 83–87.

Martin, W., Willson, P., Curtis, R., Allen, B. and Acres, S., 1983. A field trial, of preshipment vaccination, with intranasal infectious bovine rhinotracheitis-parainfluenza-3 vaccines. Can. J. Comp. Med., 47: 245–249.

Matthaeus, W. and Straub, O.C., 1978. Die neutralisierenden Serumantikörper normaler und leukotischer Rinder nach der Infektion mit IBR-IPV-Virus. Zentralbl. Bakteriol. Hyg. I. Orig. A, 240: 152–164.

McGuire, R.L. and Babiuk, L.A., 1984. Evidence for defective neutrophil function in lungs of calves exposed to infectious bovine rhinotracheitis virus. Vet. Immunol. Immunopathol., 5: 259–271.

McKercher, D.G. and Straub, O.C., 1960. Isolation of the virus of infectious bovine rhinotracheitis from range cattle. J. Am. Vet. Med. Assoc., 137: 661–664.

McKercher, D.G., Saito, J.K., Wada, E.M. and Straub, O.C., 1959. Current status of the newer virus diseases of cattle. Proc. 62nd Annu. Proc. U.S. Livest. Sanit. Assoc., 1958, pp. 136–158.

McKercher, D.G., Wada, E.M. and Straub, O.C., 1963. Distribution and persistence of infectious bovine rhinotracheitis virus in experimentally infected cattle. Am. J. Vet. Res., 24: 510–514.

Menanteau-Horta, A.M., Ames, T.R., Johnson, D.W. and Meiske, J.C., 1985. Effect of maternal antibody upon vaccination with infectious bovine rhinotracheitis and bovine virus diarrhea vaccines. Can. J. Comp. Med., 49: 10–14.

Miller, J.M. and Van Der Maaten, M.J., 1984. Reproductive tract lesions in heifers after intrauterine inoculation with infectious bovine rhinotracheitis virus. Am. J. Vet. Res., 45: 790–794.

Miller, J.M. and Van Der Maaten, M.J., 1985. Effect of primary and recurrent infectious bovine rhinotracheitis virus infection on the bovine ovary. Am. J. Vet. Res., 46: 1434–1437.

Miller, R.B., Smith, M.W. and Lawson, K.F., 1978. Some lesions observed in calves born to cows exposed to the virus of infectious bovine rhinotracheitis in the last trimester of gestation. Can. J. Comp. Med., 42: 438–445.

Mitscherlich, E., 1979. Hygienische Massnahmen bei der Aufzucht eines Besamungsbullen. Tierzüchter, 31: 139–142.

Mohanty, S.B., 1982. Vaccination programs against infectious bovine rhinotracheitis, parainfluenza-3, and pasteurellae. In; Proceedings, World Congress on Diseases of Cattle. World Association for Buiatrics, Utrecht, Vol. 1., pp. 139–145.

Mohanty, S.B., Rockemann, D.D. and Tripathy, R.N., 1980. Chemotherapeutic value of 2-deoxy-D-glucose in infectious bovine rhinotracheitis viral infection in calves. Am. J. Vet. Res., 41: 1049–1051.

Molnár, T., Glávits, R. and Pálfi, V., 1984. An unusual form of IBR in a young calf. Magy. Allatorv. Lapja, 39: 661–665 (in Hungarian).

Morter, R.L., Amstutz, H.E. and Crandell, R.A., 1984. Experimental exposure of feedlot cattle to infectious bovine rhinotracheitis virus. Bovine Pract., 19: 38–40.

Msolla, P.M., Wiseman, A., Selman, I.E., Pirie, H.M. and Allan, E.M., 1979. Vaccination against infectious bovine rhinotracheitis (correspondence). Vet. Rec., 104: 423–536.

Msolla, P.M., Wiseman, A. and Selman, I.E., 1981. The prevalence of serum neutralizing antibodies to infectious bovine rhinotracheitis virus in Scotland. J. Hyg., 86: 209–215.

Msolla, P.M., Allan, E.M., Selman, I.E. and Wiseman, A., 1983. Reactivation and shedding of bovine herpesvirus 1 following *Dictyocaulus viviparus* infection. J. Comp. Pathol., 93: 271–274.

Mueller, S.B.K., Ikuno, A.A., Campos, M.T.G.R., Ribeiro, L.O.C., Barbosa, H.S.Q. and Oliveira, B.O.A., 1979. Ocorrencia simultanea de alterações respiratorias e genitais asociadas a rinotraqueite infecciosa dos bovinos/vulvovaginite pustular infecciosa (IBR/IPV) em um rebanho no Estado de Sao Paula. Biologico, 45: 55–60.

Mushi, E.Z. and Karstad, L., 1979. Experimental infection of wildebeest with the herpesvirus of

infectious bovine rhinotracheitis/infectious pustular vulvovaginitis. J. Wildl. Dis., 15: 579–583.

Narita M., Inui, S., Namba, K. and Shimuzu, Y., 1978. Neural changes in recurrent infection of infectious bovine rhinotracheitis virus in calves treated with dexamethasone. Am. J. Vet. Res., 39: 1399–1403.

Narita, M., Inui, S., Yabuki, Y., Namba, K. and Shimizu, Y., 1979. Neural changes in recurrent genital infection of infectious bovine rhinotracheitis virus in calves treated with dexamethasone. Br. J. Exp. Pathol., 60: 111–119.

Narita, M., Inui, S., Namba, K. and Shimizu, Y., 1981. Recrudescence of infectious bovine rhinotracheitis virus and associated neural changes in calves treated with dexamethasone. Am. J. Vet. Res., 42: 1192–1197.

Narita, M., Inui, S., Murakami, Y., Namba, K. and Shimizu, Y., 1982. Pathological changes in young and adult cattle after intranasal inoculation with infectious bovine rhinotracheitis virus. J. Comp. Pathol., 92: 41–49.

Narita, M., Inui, S., Namba, K. and Shimizu, Y., 1983. Detection of virus particles in trigeminal ganglion cells in a calf recurrently infected with infectious bovine rhinotracheitis virus. Jap. J. Vet. Sci., 45: 691–693.

Nederlandse Gezondheidsdienst voor Dieren in Friesland, 1979. I.B.R./I.P.V. in dairy farms. In: 60e Jaarverslag. NGDF, Leeuwarden, pp. 44–46 (in Dutch).

Nettleton, P.F., Herring, J.A., Hogg, R.A. and Nicolson, T.B., 1981. Laboratory confirmation of IBR virus induced abortion. Vet. Rec., 108: 243.

Nettleton, P.F., Sharp, J.M., Herring, A.J. and Herring, J.A., 1984. Infectious bovine rhinotracheitis virus excretion after vaccination challenge and immunosuppression. In: G. Wittmann, R.M. Gaskell and H.-J. Rziha (Editors), Latent Herpesvirus Infections in Veterinary Medicine (Current Topics in Veterinary Medicine and Animal Science, Vol. 27). Martinus Nijhoff, Dordrecht, pp. 191–209.

Nyaga, P. and McKercher, D.G., 1979. Pathogenesis of bovine herpesvirus-1 (BHV-1) infection: interactions of the virus with peripheral bovine blood cellular components. Comp. Immunol. Microbiol. Infect. Dis., 2: 587–602.

Pastoret, P.-P., Jetteur, P., Aguilar-Setién, A., Leroy, P. Schwers, A., Godart, M. and Schoenaers, F., 1979. Effet de la dexaméthasone sur le titre et la taille des plages du virus de la rhinotrachéite infectieuse bovine (Bovid Herpesvirus 1, IBR). Ann. Méd. Vét., 123: 397–402.

Pastoret, P.-P., Aguilar-Setién, A., Burtonboy, G. and Schwers, A., 1980a. Effet de la cyclophosphamide sur la latence du virus de la rhinotrachéite infectieuse bovine (Bovid Herpesvirus 1). Ann. Méd. Vét., 124: 55–67.

Pastoret, P.-P., Burtonboy, G., Aguilar-Setién, A., Godart, M., Lamy, M.E. and Schoenaers, F., 1980b. Comparison between strains of infectious bovine rhinotracheitis virus (Bovid Herpesvirus 1), from respiratory and genital origins, using polyacrylamide gel electrophoresis of structural proteins. Vet. Microbiol., 5: 187–194.

Perrin, B., Tixier, G., Dannacher, G., Soula, A., Moussa, A., Goyon, M., Legardinier, J.-C., Millet A. and Protin, P., 1984. Rhinotrachéite bovine infectieuse: utilisation d'un "kit ELISA" pour la détection des anticorps dans les sérums et dans les laits. Rec. Méd. Vét., 160: 755–761.

Perrin, M., Dannacher, G., Coudert, M. and Fedida, M., 1981. La rhinotrachéite bovine infectieuse: résultats de l'enquête nationale 1978. Rec. Méd. Vét., 157: 485–490.

Peters, A.R. and Perry, C.T., 1983. Serological survey on the prevalence of bovine herpesvirus 1 antibodies in pedigree beef bulls. Vet. Rec., 113: 237–238.

Petzoldt, K., 1979. Anaphylaktische Reaktivität bei neugeborenen Kälbern und Lämmern durch passiv erworbene Antikörper. XXI. Welt-Tierärztekongress Moskau, Thesen, Vol. 3, p. 42.

Pierson, R.E. and Viar, C.A., 1966. The economic loss associated with infectious bovine rhinotracheitis in a dairy herd. J. Am. Vet. Med. Assoc., 147: 350–352.

Porter, D.D., Larsen, A.E. and Cox, N.A., 1975. Isolation of infectious bovine rhinotracheitis virus from Mustelidae. J. Clin. Microbiol., 1: 112–113.

Pospisil, Z., Krejci, J. and Rodák, L., 1983. Demonstration of colostral antibodies in the nasal secretions of calves and their protective effect against infection. Acta Vet. Brno, 52: 59–65.

Potgieter, L.N.D. and Maré, C.J., 1974. Differentiation of strains of infectious bovine rhinotracheitis virus by neutralization kinetics with late 19S rabbit antibodies. Infect. Immun., 10: 520–527.

Potgieter, L.N.D., McCracken, M.D., Hopkins, F.M. and Walker, R.D., 1984. Effect of bovine viral diarrhea virus infection on the distribution of infectious bovine rhinotracheitis virus in calves. Am. J. Vet. Res., 45: 687–690.

Rebhun, W.C., Smith J.S., Post J.E. and Holden, H.R., 1978. An outbreak of the conjunctival form of infectious bovine rhinotracheitis. Cornell Vet., 68: 297–307.

Rejas Garcia, F., Garrido Pertierra, A., Pérez Huertes, A. and Argüello Villares, J.L., 1979. Immunización contra la rinotraqueitis infecciosa bovina con vacunas atenuadas. An. Fac. Vet. Leon, 25: 63–68.

Rice, D.A. and Jenny, E.W., 1979. Serological evidence of infectious bovine rhinotracheitis, bovine virus diarrhoea and parainfluenza-3 viruses in calves in El Salvador. Trop. Anim. Health Prod., 11: 123–124.

Rock, D.L., 1982. Persistent infection with bovine herpesvirus-1: a rabbit model. Diss. Abstr. Int., B, 42: 4321.

Rock, D.L. and Reed, D.E., 1982. Persistent infection with bovine herpesvirus type 1: Rabbit model. Infect. Immun., 35: 371–373.

Rodriguez, L.L., Homan, E.J. and Easterday, B.C., 1984. Characterization of bovine herpesvirus-1 isolated from trigeminal ganglia of clinically healthy cattle. Am. J. Vet. Res., 45: 1069–1072.

Rogers, R.J., McCubbin, I.K., McKenzie, R.A., Chung, Y.S. and Rosenfeld, L.E., 1980. Severe rhinotracheitis and pneumonia of feedlot cattle due to bovine herpesvirus type 1 infection. Aust. Vet. J., 56: 147–148.

Roizman, B., Carmichael, L.E., Deinhardt, F., De-The, G., Nahmias, A.J., Plowright, W., Rapp, F., Sheldrick, P., Takahashi, M. and Wolf, K., 1981. Herpesviridae. Definition, provisional nomenclature, and taxonomy. Intervirology, 16: 201–217.

Roney, C.S., Rossi, C.R., Smith, P.C., Lauerman, L.C., Spano, J.S., Hanrahan, L.A. and William, J.C., 1985. Effect of human leukocyte A interferon on prevention of infectious bovine rhinotracheitis virus infection of cattle. Am. J. Vet. Res., 46: 1251–1255.

Rossi, C.R. and Kiesel, G.K., 1982. Effect of infectious bovine rhinotracheitis virus immunization on viral shedding in challenge-exposed calves treated with dexamethasone. Am. J. Vet. Res., 43: 1576–1579.

Rossi, C.R. Kiesel, G.K. and Rumph, P.F., 1982. Association between route of inoculation with infectious bovine rhinotracheitis virus and site of recrudescence after dexamethasone treatment. Am. J. Vet. Res., 43: 1440–1442.

Rothschuh, L., 1980. Serologische Untersuchungen über den Verbreitungsgrad der IBR-IPV-Virusinfektion bei Rindern im Rheinland. Tierärztl. Umschau, 35: 289–292.

Rouse, B.T. and Babiuk, L.A., 1974a. Host defense mechanisms against infectious bovine rhinotracheitis virus: in vitro stimulation of sensitized lymphocytes by virus antigen. Infect. Immun., 10: 681–687.

Rouse, B.T. and Babiuk, L.A., 1974b. Host responses to infectious bovine rhinotracheitis virus. III. Isolation and immunologic activities of bovine T lymphocytes. J. Immunol., 113: 1391–1398.

Rouse, B.T., Wardley, R.C. and Babiuk L.A., 1976a. The role of antibody-dependent cytotoxicity in recovery from herpesvirus infections. Cell. Immunol., 22: 182–186.

Rouse, B.T., Wardley, R.C. and Babiuk, L.A., 1976b. Antibody-dependent cell-mediated cytotoxicity in cows: comparison of effector cell activity against heterologous erythrocyte and herpesvirus-infected bovine target cells. Infect. Immun., 13: 1433–1441.

Rüsch, P., Engels, M., Berchtold, M. and Wyler, R., 1981. Untersuchungen über den Titerverlauf virusneutralisierender Antikörper nach akuter IBR. Schweiz. Arch. Tierheilk., 123: 419–427.

Schaller, I., 1977. Labordiagnostische Untersuchungen im Rahmen eines IBR/IPV-Sanierungsversuches in einem Milchviehstall. Monatsh. Veterinärmed., 32: 731–733.

Schipper, I.A., Kelling, C.L., Mayer, J. and Pfeiffer, N.E., 1979. Effects of passive immunity on immune response in calves following vaccination for IBR. N. Dakota Farm Res., 36: 20–21.

Sheffy, B.E. and Davies, D.H., 1972. Reactivation of a bovine herpesvirus after corticosteroid treatment. Proc. Soc. Exp. Biol., 140: 974–976.

Silim, A. and Elazhary, M.A.S.Y., 1983. Detection of infectious bovine rhinotracheitis and bovine viral diarrhea viruses in the nasal epithelial cells by the direct immunofluorescence technique. Can. J. Comp. Med., 47: 18–22.

Singh, E.L., Thomas, F.C., Papp-Vid, G., Eaglesome, M.D. and Hare, W.C.D., 1982. Embryo transfer as a means of controlling the transmission of viral infections. II. The in vitro exposure of preimplantation bovine embryos to infectious bovine rhinotracheitis virus. Theriogenology, 18: 133–140.

Singh, K.V., Hajj, A. and Barghout, R., 1977. A survey of neutralizing antibodies to infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea mucosal disease (BVD-MD) and parainfluenza type 3 (PI$_3$) viruses in cattle in Lebanon and some other countries of the Middle East. Bull. Anim. Health. Prod. Afr. 25: 85–89.

Smith, P.C., 1978. Experimental infectious bovine rhinotracheitis virus infections of English ferrets (*Mustela putorius furo* L.). Am. J. Vet. Res., 39: 1369–1372.

Straub, O.C., 1965. In vitro-Untersuchungen über die Wirkung einiger Desinfektionsmittel auf ein zur Herpesgruppe gehörendes Virus der IBR-IPV-Virusgruppe (Rhinotracheitis- und Bläschenausschlagviren der Rinder). Tierärztl. Umschau, 20: 568–573.

Straub, O.C., 1969. Die Übertragung viraler Antikörper durch das Kolostrum. Tierärztl. Umschau, 24: 571–573.

Straub, O.C., 1970. Allergische Sofort- und Spätreaktionen bei IBR-IPV-immunen Rindern. Zentralbl. Bakteriol. I. Orig., 214: 483–494.

Straub, O.C., 1972. Die durch IBR-IPV-Viren hervorgerufenen Krankheiten beim Rind und ihre Bekämpfung mit einem neuen Lebendimpfstoff. Bundesforschungsanstalt für Viruskrankheiten der Tiere, Tubingen, 161 pp.

Straub, O.C., 1977. Erfahrungen bei der Bekämpfung der Infektiösen bovinen Rhinotracheitis (IBR) und der Infektiösen Pustulösen Vulvovaginitis (IPV). Tierärztl. Umschau, 32: 107–111.

Straub, O.C. 1978a. Bovine Herpesvirusinfektionen. VEB Gustav Fischer Verlag, Jena, 211 pp.

Straub, O.C., 1978b. Vorkommen der durch IBR-IPV-Viren hervorgerufenen Krankheiten und mögliche differentialdiagnostische Probleme in den verschiedenen Kontinenten und deren Länder. Dtsch. Tierärztl. Wochenschr., 85: 84–90.

Straub, O.C., 1979. Persistence of infectious bovine rhinotracheitis–infectious pustular vulvovaginitis virus in the respiratory and genital tract of cattle. Comp. Immunol. Microbiol. Infect. Dis., 2: 285–294.

Straub, O.C., 1980. Der Bläschenausschlag und die Rhinotracheitis. Tierärztl. Umschau, 35: 746–748.

Straub, O.C., 1984a. The establishment of IBR-IPV vaccine. In: G. Wittmann, R.M. Gaskell and H.-J. Rziha (Editors), Current Topics in Veterinary Medicine and Animal Science. Martinus Nijhoff Publishers, The Hague pp. 241–249.

Straub, O.C., 1984b. Lokale oder parenterale Anwendung von viralen Impfstoffen beim Rind. In: Collegium Veterinarium XIV Prakt. Tierarzt, Sondernummer April 1984: 98–100.

Straub, O.C., 1986a. Über die Eignung des Immundiffusionstests zur Bestimmung humoraler Antikörper gegen bovines Herpesvirus (BHV1). Berl. Münch. Tierärztl. Wochenschr. 99: 424–427.

Straub, O.C., 1986b. Über die Eignung eines Intrakutantests als diagnostisches Hilfsmittel bei der Bekämpfung der durch BHV1 hervorgerufenen Krankheiten. Prakt. Tierarzt 67: 1043–1048.

Straub, O.C., 1987. Untersuchungen zur Bestimmung der Rinder-infektiösen Dosis bei einem virulenten und einem avirulenten BHV1-Stamm sowie zur Ermittlung der Viruslatenz. Tierärztl. Umschau, 42: 231–234.

Straub, O.C. and Böhm, H.O., 1963. Zur Epidemiologie der infektiösen Rhinotracheitis (IBR) und des Bläschenausschlags (IPV) beim Rind. In: Kongressberichte XVII, Welttierärztekongress, Hannover, Vol. 1, pp. 455–458.

Straub, O.C. and Böhm, H.O., 1964. Untersuchungen über die Lokalisation und Persistenz des Virus der infektiösen Rhinotracheitis und des Bläschenausschlages in experimentell infizierten Rindern. Berl. Münchn. Tierärztl. Wochenschr., 77: 458–462.

Straub, O.C. and Böhm, H.O., 1965. Experimentelle Infektion des Zentralnervensystems durch das Virus der Bovinen Rhinotracheitis. Dtsch. Tierärztl. Wochenschr., 72: 124–128.

Straub, O.C. and Kielwein, G., 1966. Experimentelle Mastitiden durch das Bläschenausschlagvirus des Rindes. Berl. Münchn. Tierärztl. Wochenschr., 79: 310–312.

Straub, O.C. and Matthaeus, W., 1978. The immunoglobulin composition of colostrum and the persistence of acquired immunoglobulins and specific antibodies in the calf. Ann. Rech. Vét., 9: 269–275.

Straub, O.C., Plab, M.del C. and Kleine, B., 1965. Infektiöse Rhinotracheitis in einem Milchviehbestand. Tierärztl. Umschau, 20: 23–25.

Straub, O.C., Schmidt, B. and Liebke, H., 1982a. Ein Beispiel zur Sanierung IBR-IPV-Virusverseuchter Bestände. Tierärztl. Umschau, 37: 319–324.

Straub, O.C., Wettke, K. and Weiland, F., 1982b. Seuchenhaftes Auftreten von IBR-IPV-Virus-Aborten. Tierärztl. Umschau, 37: 613–617.

Straub, O.C., Ohlinger, V. and Wittmann, G., 1983. Die Impfung von Rindern mit einer inaktivierten Aujeszkyvirusvakzine. I. Homologe und heterologe (IBR-IPV) Antikörperbildung. Tierärztl. Umschau, 38: 528–534.

Straver, P.J., Terpstra, C. and Van Nieuwstadt, A., 1977. IBR. In: Jaarverslag 1977. Stichting voor Diergeneeskundig Onderzoek, Lelystad, Netherlands, 147 pp.

Stubbings, D.P. and Cameron, I.R.D., 1981. Bovine abortion associated with infectious bovine rhinotracheitis virus infection. Vet. Rec., 108: 101–102.

Stuker, G., Haab, P. and Giger, T., 1980. Nachweis von IBR/IPV-Antikörpern aus der Milch. Schweiz. Arch. Tierheilk., 122: 707–710.

Sutton, M.L., 1980. Rapid onset of immunity in cattle after intramuscular injection of a modified-live-virus IBR vaccine. Vet. Med. Small Anim. Clin., 75: 1447–1452.

Tanyi, J., Bajmócy, E., Fazekas, B. and Kaszanyitzky, E.J., 1983. Mass abortion caused by infectious rhinotracheitis (IBR/IPV) virus in a beef cattle herd. Acta. Vet. Hung., 31: 135–143.

Taylor, W.P., Okeke, A.N.C. and Shidali, N.N., 1977. Prevalence of bovine virus diarrhoea and infectious bovine rhinotracheitis antibodies in Nigerian sheep and goats. Trop. Anim. Health Prod., 9: 171–175.

Taylor, R.E.L., Seal, B.S. and St. Jeor, S., 1982. Isolation of infectious bovine rhinotracheitis virus from the soft-shelled tick, *Ornithodoros coriaceus*. Science, 216: 300–301.

Terpstra, C., 1979. Diagnosis of infectious bovine rhinotracheitis by direct immunofluorescence. Vet. Q., 1: 138–144.

Thiry, E. and Pastoret, P.-P., 1984. Le virus de la rhinotrachéite infectieuse bovine (Bovine Herpesvirus 1): aspects biochimiques. Ann. Rech. Vét., 15: 455–465.

Thiry, E., Pastoret, P.-P., Kettmann, R. and Burny, A., 1984. Use of restriction analysis for the study of bovid herpesvirus 1 latency. In: G. Wittmann, R.M. Gaskell and H.-J. Rziha (Editors), Latent Herpes Virus Infections in Veterinary Medicine. Martinus Nijhoff, The Hague, pp. 251–256.

Thiry, E., Saliki, J., Schwers, A. and Pastoret, P.-P., 1985. Parturition as a stimulus of IBR virus reactivation (correspondence). Vet. Rec., 166: 599–600.

Thumann, D., 1980. Klinische, bakteriologische, virologische und serologische Untersuchungen an mittels Paramunitätsinducers (HP-Präparat) prophylaktisch bzw. therapeutisch behandelten Mastbullen- und Aufzuchtkälbern. Ein Beitrag zur Vorbeuge und Behandlung der Enzootischen Bronchopneumonie des Rindes. Inaugural Dissertation, Tierärztliche Hochschule, Hannover.

Van Nieuwstadt, A.P.K.M.I. and Verhoeff, J., 1983. Aspects of the epizootiology of infections with Infectious Bovine Rhinotracheitis virus on dairy farms. Tijdschr. Diergeneeskd., 108: 383–391 (in Dutch).

Vogel, H., 1982. Untersuchung über den Einfluss der Grundimmunisierung mit Riemser IBR-IPV-Vakzine in einem Milchviehbestand auf den Antikörperverlauf bei Muttertieren und Kälbern sowie ausgewählte zuchthygienische Parameter. Monatsh. Veterinärmed., 37: 355.

Wafula, J.S., Mushi, E.Z. and Wamwayi, H., 1985. Reaction of goats to infection with infectious bovine rhinotracheitis virus. Res. Vet. Sci., 39: 84–86.

Wardly, R.C., Rouse, B.T. and Babiuk, L.A., 1976. Antibody dependent cytotoxicity mediated by neutrophils: a possible mechanism of antiviral defense. J. Reticuloendothelial Soc. 19: 323–332.

Weinmaster, G.A., Misra, V., McGuire, R., Babiuk, L.A. and DeClercq, E., 1982. Bovid herpesvirus type-1 (infectious bovine rhinotracheitis virus)-induced thymidine kinase. Virology, 118: 191–201.

Wellemans, G. and Leunen, J., 1974. Le tropisme digestif du virus IBR. II. Examens virologiques. Ann. Méd. Vét., 118: 243–251.

Wellemans, G., Biront, P. and Van Opdenbosch, E., 1980. Spécificité du test d'immunofluorescence indirecte (I.F.I.) dans la recherche des anticorps IBR chez des bovins vaccinés avec un vaccin Aujeszky. Rec. Méd. Vét., 156: 911–914.

Whitmore, H.L. and Archbald, L.F., 1977. Demonstration and quantitation of immunoglobulins in bovine serum, follicular fluid, and uterine and vaginal secretions with reference to bovine viral diarrhea and infectious bovine rhinotracheitis. Am. J. Vet. Res., 38: 455–457.

Wilke, I. and Letz, W., 1970. Gleichzeitiges Auftreten von Konjunktivitis und Vulvovaginitis, verusacht durch IBR/IPB-Virus, in einer Färsenherde. Fortpfl. Haustiere, 6: 256–263.

Wiseman, A., Msolla, P.M., Selman, I.E., Allan, E.M., Cornwell, H.J.C., Pirie, H.M. and Imray, W.S., 1978. An acute severe outbreak of infectious bovine rhinotracheitis: Clinical, epidemiological, microbiological and pathological aspects. Vet. Rec., 103: 391–397.

Wiseman, A., Selman, I.E., Msolla, P.M., Pirie, H.M. and Allan, E., 1979. The financial burden of infectious bovine rhinotracheitis. Vet. Rec., 105: 469.

Wiseman, A., Msolla, P.M., Selman, I.E., Allan, E.M. and Pirie, H.M., 1980. Clinical and epidemiological features of 15 incidents of severe infectious bovine rhinotracheitis. Vet. Rec., 107: 436–441.

Wyler, R., Engels, M. and Schwyzer, M., 1989. Herpesvirus Diseases of Cattle, Horses and Pigs. Kluwer Academic Publishers, Boston, 345 pp.

Yates, W.D.C., 1982. A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral–bacterial synergism in respiratory disease of cattle. Can. J. Comp. Med. 46: 225–263.

Ziegenhagen, T., 1977. Erhebungen über Massnahmen zur Bekämpfung der IPV-Virusinfektion in norddeutschen Besamungsstationen. Inaugural Dissertation, Tierärztliche Hochschule, Hannover.

Zimmermann, F., 1981. Bericht über einen IBR-Ausbruch im Neckar–Odenwald-Kreis. Tierärztl. Umschau, 36:126–130.

Zioleck, M., 1979. Untersuchungen über das Vorkommen neutralisierender Antikörper (nAK) gegen IBR/IPV-Virus bei Besamungsbullen mit besonderer Berücksichtigung des Fruchtbarkeitsgeschehens. Tierärztl. Umschau, 34: 160–170.

Zmudziński, J. and Baczyński, Z., 1983. Immunogenic properties of the Riemser IBR/IPV vaccine. Bull. Vet. Inst. Pulawy, 26: 22–26.

Žuffa, A. and Feketová, N., 1980. Protection of cattle vaccinated with inactivated oil-adjuvant IBR-vaccine against experimental infection. Zentralbl. Veterinärmed. B, 27: 725–733.

Žuffa, A., Brányik, A. and Salaj, J., 1979a. Resistance of the mucous membranes of the upper respiratory tract of cattle to infection with IBR virus in relation to the content of humoral and secretory antibodies and the local production of interferon. Vet. Med. (Praha), 24: 193–207 (in Czech).

Žuffa, A., Brányik, A., Salaj, J. and Mrácek, K., 1979b. Immunogenicity of an inactivated vaccine with an oil adjuvant against infectious bovine rhinotracheitis (IBR). Vet. Med. (Praha), 24: 257–268 (in Czech).

Žuffa, A., Brányik, A., Cernik, K. and Salaj, H., 1982. Protection against experimental infection of calves vaccinated intranasally by three attenuated strains of IBR virus. Zentralbl. Veterinärmed. B, 29: 413–425.

Chapter 12

Bovine Mammillitis Virus

W.B. MARTIN

INTRODUCTION

Several viruses are now recognized as causing specific infections of the teats of cows. One of them, cowpox, is now rarely seen, and with the cessation of vaccination infection with vaccinia virus should cease. Probably the most severe virus infection of cows' teats is caused by a herpesvirus, bovine mammillitis virus. Infection of the teat and udder with this virus has only been recognized within the past 25 years.

The first isolation was made in 1957 from cattle in South Africa, where it was called the "Allerton" strain after the farm of origin. The virus was cultured from an excised skin nodule associated with a generalized skin infection known now as pseudo lumpy skin disease. A further isolation of this herpesvirus from skin lesions on cattle was made in West Africa a few years later. In 1964 the virus was recovered in Great Britain from the teats of cattle with severe skin ulcers. Additional isolations have been made subsequently in several European countries, America and Australia.

Depending on the country of origin the isolates have been given different names: Allerton virus, bovine ulcerative mammillitis virus, bovine herpes mammillitis virus, bovine dermopathic herpesvirus.

Useful reviews of this herpesvirus and the associated disease syndromes have been given by Cilli and Castrucci (1976) and Gibbs and Rweyemamu (1977).
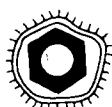
VIRUS PROPERTIES

The virus of herpes mammillitis has been classified as a member of the family Herpesviridae, subfamily Alphaherpesvirinae, genus *Simplexvirus*, with the proposed name of bovine herpesvirus 2 (BHV-2). It is ether- and chloroform-labile and is readily inactivated by UV light, acidic conditions (pH3) and by heat ($\geqslant 4$ log reduction in 60 min at 50°C). Halogen disinfectants will destroy BHV-2 rapidly; thus an iodophore disinfectant diluted according to the manufacturer's instructions completely inactivated the virus in 20 s.

EPIZOOTIOLOGY

BHV-2 can affect cattle of all ages and has been recovered from African buffalo (*Syncerus caffer*) in which it produces disease. Serological evidence of natural infection has been obtained from other free-living large mammals in Africa.

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Experimentally, infection with BHV-2 has been produced in sheep, goats, pigs and laboratory animals, e.g. rabbits, guinea-pigs and other rodents. Subcutaneous or intraperitoneal inoculation of mice 1–5 days old will cause severe or fatal disease. Domestic poultry appear to be refractory. There is no confirmed evidence that man can be infected by BHV-2.

Both ticks (*Rhipicephalus appendiculatus*) and biting flies (*Stomoxys calcitrans* and *Biomyia fasciata*) can carry the virus, although transmission of infection by these vectors has not been shown.

BHV-2 infection in cattle has been reported from South, West and East Africa, the USA, several European countries (e.g. Britain, Italy, France, Yugoslavia and the Netherlands) and from Australia. One survey of 400 randomly selected sera from cattle in West England showed that 19.5% had antibodies to BHV-2. In Italy, where 1079 sera from cows in five dairy herds in different provinces were examined, 0–41.6% were positive, whereas in the FRG a survey of 1000 cattle sera did not detect a single one with antibody. BHV-2 infection is prevalent in Africa; 95% of 317 cattle sera in Somalia were shown to have antibodies, over 85% of cattle sera in East Africa and from 65–93% in Zambia, depending on the area. Also a serological survey of game animals in East Africa has shown a high prevalence of infection in buffalo, and positive sera have been obtained from giraffe, hippopotamus and several species of gazelle. In contrast, antibodies were not found in 190 sera from five species of deer in Britain in one survey.

The infrequency with which outbreaks of disease due to BHV-2 have been reported would suggest that it is not a common or economically important problem in most countries. Individual cattle herds may, however, experience serious occurrence of the disease which can greatly reduce the milk output for several weeks. In one series of outbreaks in a small area of western Scotland, 24 dairy herds were affected in 3 months. An analysis of a number of these herds, and others affected the following year, showed that about 50% (range 18–90%) of the milking cows had lesions, of which 22% developed mastitis. The course of the clinical disease in individual animals varied from 2 to 10 weeks and in the herds from 5 to 15 (average 9) weeks. No breed incidence is recognized.

As will be described later, two main syndromes (pseudo lumpy skin disease and mammillitis) are caused by this virus. The disease as seen in temperate regions is a mammillitis which has an interesting epizootiological pattern not yet fully understood.

Mammillitis outbreaks in Europe are sharply confined to the autumn and early winter seasons (between September and November). It is of interest to note that outbreaks described in Australia also occurred between October and mid-December, which are the spring months in that country (Turner et al., 1976).

Irrespective of the seasonal incidence the pattern of farms affected is random. Thus the cattle on several farms within an area of several square kilometers may be affected about the same time, though the disease may not occur on contiguous farms but has a sporadic distribution. The disease generally does not recur in the same herd, at least for some years. Indeed there is considerable variation in the prevalence within areas from year to year.

Most outbreaks have been reported in dairy herds and many start with one or a few cows, usually primiparous heifers, developing lesions within a few days of calving. Other cows may develop lesions on calving and lateral spread through the herd can result, so that a variable number of cows, some of which will be in mid-lactation, develops lesions. Sometimes cases are confined to a few newly calved heifers, whereas in other herds most of the cows can become affected, presumably due to spread of infection during milking. Serous fluid

exuding from lesions contains high concentrations of BHV-2 ($\geqslant 10^6$ TCID$_{50}$/ml) and virus has been recovered from milking machine cups for several days. Although BHV-2 does not appear to penetrate intact skin, minor abrasions, which are common on teats, will allow entry of the virus. In some herds lesions of pseudo cowpox caused by *Parapoxvirus* occur about the same time and may well be a factor in allowing BHV-2 to gain access to the deeper epithelial cells.

Other features of the epizootiology of BHV-2 infection are less well understood. No explanation is available for the sudden appearance of disease in many herds, but occasionally outbreaks coincide with the introduction of a newly purchased cow. Generally the source of infection in the first affected cows cannot be explained, as no evidence of infection will have been obtained in that or previous years.

One fact which is now accepted and which may be important in understanding the epidemiology of this disease is that BHV-2 can become latent in cattle, and remain so for at least 18 months. Following the administration of corticosteroid, recrudescence of disease can occur (Martin and Scott, 1970). Spread of infection has also been shown to occur among bulls housed together and to others in a separate shed. Although the bulls were being inspected daily, no clinical disease was noted and it was concluded that spontaneous reactivation of latent virus with subsequent transmission had occurred (Letchworth, 1980).

This information, combined with the knowledge that biting flies can carry BHV-2, had led some workers to consider that infection in dry cows occurs during the summer months, possibly spread by flies from cows with minor lesions. The virus is then retained as a latent infection until permitted to replicate and to produce lesions. The reduction in CMI that occurs in cows at parturition may permit the replication of the herpesvirus, and this has been suggested as the reason why lesions appear shortly after calving. No site for the latency of the virus is known; during acute infections, virus has been recovered from the central nervous system and the skin, among other tissues.

Another school of thought holds that disease occurs in the first cows by spread from clinically unrecognized cases. However, this hypothesis is unlikely to explain outbreaks for such reasons as the rapidity of onset after calving, the severity of lesions in the early cases, the subsequent spread through the herd, the lack of evidence of infection in previous years and the seasonal prevalence.

BHV-2 virus appears to replicate most readily in skin with somewhat lower than normal temperature, as can occur post-calving in the edematous area around the udder.

Calves receiving milk from infected cows may become infected. The part played by such calves in the epidemiology of the disease is uncertain, but presumably they may carry the virus as a latent infection for long periods.

Although herpes mammillitis virus can produce severe mammary lesions it will not cause systemic illness or death. A variable percentage of an affected dairy herd may have to be disposed of because of mastitis which can develop due to the teat damage and the consequent difficulty in milking adequately.

PATHOGENESIS

During the acute phase of disease the fluid which exudes from skin lesions has a high virus content, e.g. $\geqslant 10^{6.0}$ TCID$_{50}$/ml. Virus may also be present in lesions and fluid during reactivation. Lateral spread may therefore occur.

Experimental inoculations have shown three important facts. First, lesions occur at the site of intradermal or subcutaneous inoculation, but dissemination of lesions to other parts of the skin does not occur. Second, generalized infection with the development of widespread skin nodules (pseudo lumpy skin

disease), follows the intravenous inoculation of BHV-2, whether the virus is of European or African origin. Third, the virus replicates to high titer and produces severe lesions in the cooler areas of the skin (Letchworth and Carmichael, 1984), which may explain the lesions on the thin skin of the udder and teats during cold weather, particularly when subcutaneous edema is present.

Following local or systemic inoculation of virus, lesions usually occur within 3–8 days and reach maximum size 3–4 days after they first appear. Virus can be recovered from circulating leukocytes for a few days after intravenous inoculation and from skin lesions for about 10 days after the reaction becomes visible. Titers of virus in lesions, and in the serous fluid from them, are between 10^6 and $10^7 TCID_{50}/ml$ initially but reduce as the reaction regresses and antibodies develop.

During the early phase of experimental intravenous infection, virus has been recovered from the skin, superficial and deep lymph nodes, cutaneous nerves, spinal ganglia and even the central nervous system. Virus has also been isolated from nasal fluid and the milk from teats with lesions. In experiments involving reactivation, BHV-2 has also been recovered from some of these sites. The sites of virus latency are believed to be the skin and possibly nervous tissue. Only in generalized infections with the Allerton strain of BHV-2 has virus been recovered from semen, urine and feces.

Horizontal spread of infection may occur through viral contamination of wounds or abrasions, but seroconversion has been recorded even in the absence of clinical disease.

DISEASE SIGNS

A mild febrile reaction of short duration has been noted in cattle following intravenous inoculation of BHV-2 and occasionally in heifers during natural infection, but other systemic illness does not occur. Cattle continue to eat and do not appear dull.

In classical herpes mammillitis, lesions appear on the skin of the teats and udder. Sometimes the lesions extend up the skin covering the posterior part of the udder to the perineum and exceptionally may involve the vulva.

Lesions sometimes appear quickly. Though the mature lesion is an ulcer, a variety of earlier lesions can occur. Probably the most common is a local, thickened plaque which involves the wall of the teat. Such plaques are usually oval or circular and about 0.5–2.0 cm in diameter so that the contour of the teat is distorted. The overlying skin either becomes dark purple or black and necrotic or moist and ulcerated.

Ulceration may be shallow but in the latter case the ulcer is deep with an irregular edge that is sharply demarcated from the surrounding skin (Fig. 31). Such lesions are generally painful and sensitive to manipulation, so that milking becomes difficult.

Infection causes the entire teat to become grossly swollen and inflexible. The skin becomes light yellow or blue and hard before breaking to leave most of the teat ulcerated. Vesiculation is rare but a single large vesicle may occasionally be seen.

Lesions on the skin of the udder are present only in a minority of cases. In the most severely affected udders, widespread necrosis of the more superficial layers of the skin accompanied by exudation occur over all quarters; such cases have been termed "gangrene of the udder". In milder cases the affected area is smaller (Fig. 32). The necrotic skin gradually separates in dry sheets 1–2 mm thick and of varying size, beneath which there may be moist, serous or purulent foci. Ulceration of the vulvo–vaginal mucosa has been recorded.

Fig. 31. Deep ulceration of a teat caused by BHV-2.

Calves sucking milk from infected cows can become infected. The buccal mucosa may show a general erythema which may last for several days, and circular ulcers can appear on the lips, nostrils or muzzle, from which virus can be recovered. On the muzzle thick crusts form which on removal reveal a red, granulating ulcer.
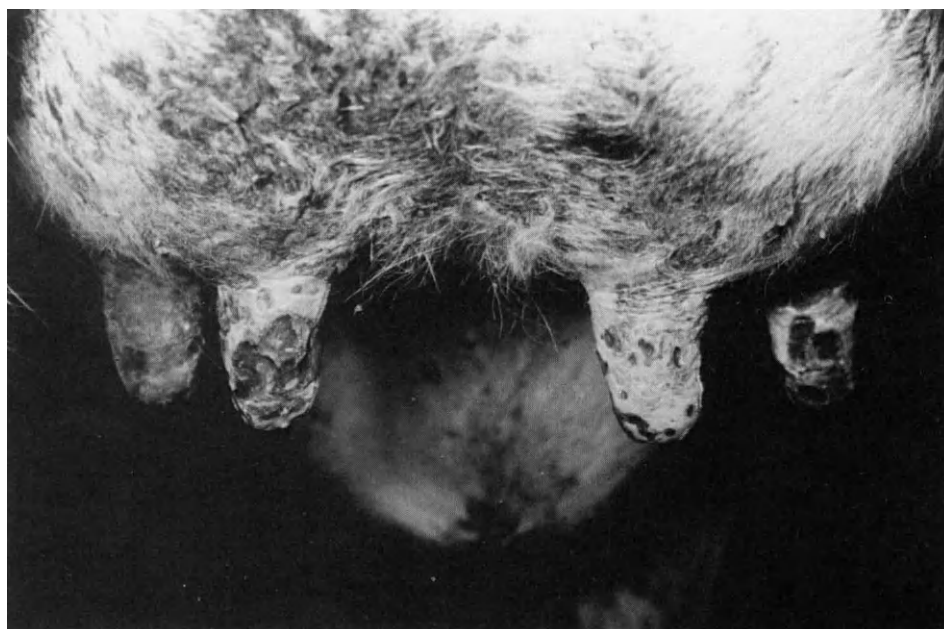
Fig. 32. Extensive BHV-2 lesions affecting skin of udder and teats.

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Nodules resembling those of lumpy skin disease have very occasionally been recorded on areas of the body during outbreaks of mammillitis. However, the pseudo lumpy skin disease syndrome has generally been reported from tropical or subtropical countries (Weiss, 1963). After a slight febrile reaction, skin nodules develop which may be numerous and generalized or few and localized. Sites most frequently affected are the skin over the neck, shoulders, back and perineum. Nodules are hard, circular or oval raised areas of thickened skin 2-3 cm in diameter with a depressed centre. Palpation is resented. Initially the hair over the nodules appears raised, fixed by the necrosis of the more superficial layers of the epidermis and the dried exudate. This crust gradually separates from the underlying tissue and drops off within about 2 weeks, leaving a healed, thickened, pigmented, oval or circular area devoid of hair for several months. Some enlargement of the local lymph nodes may be observed.

A leukopenia has been described in cattle experimentally infected with BHV-2, but even if confirmed in the field this alteration would be of little diagnostic value.

PATHOLOGY

The histopathological changes present in the early lesions are those of a severe inflammatory reaction in the epidermis. Affected areas show hydropic degeneration, necrosis and intercellular edema resulting in the formation of microvesicles. Cells in the deeper layers of the epidermis contain single acidophilic or lightly basophilic inclusion bodies in their nuclei, separated from the nuclear membrane by a clear halo. The formation of syncytia containing intranuclear inclusion bodies is a characteristic feature of the BHV-2 lesion.

The necrotic epidermis is infiltrated with masses of leukocytes. Hair follicles and sebaceous glands are involved in the necrotizing process. The dermis is infiltrated with polymorphonuclear leukocytes and mononuclear cells are present especially around blood vessels.

The focal areas of necrosis enlarge to involve a wider area of the epidermis and ulceration results. Hemorrhage and exudate cover the dermis at the base of the ulcer and form a coagulated scab. By the tenth day of infection surviving epithelial cells begin to show increased mitotic activity and regeneration takes place over the granulating tissue beneath the scab.

Inclusion bodies are no longer evident after about the fifth day of the reaction.

Using EM the inclusion bodies are seen to be composed of *paracrystalline* aggregations of virions in the nuclei. Occasional enveloped virus particles are present in the cytoplasm of cells and in the intercellular stroma.
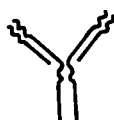
IMMUNE REACTION

Neutralizing antibodies first appear in the serum of experimentally infected cows about 5-7 days after the development of lesions. Titers reach a maximum about 3-4 weeks later and are rarely greater than about 100; they decrease to low levels after several weeks. In herds, neutralizing antibody levels have been claimed to persist for at least 2 years; inapparent infections or reactivation of virus may explain the disparity between the field and experimental observations.

Immunity does not correlate with the presence of neutralizing antibodies in serum, and recrudescence of disease or reinfection with the production of lesions can occur even in the presence of circulating antibody. Further, calves with no demonstrable antibodies can be refractory to experimental infection.

Antisera to BHV-2 will significantly neutralize herpes simplex virus types 1 and 2 (HSV-1, 2). Immunodiffusion tests also show a common line of precipitation between BHV-2 and HSV-1, 2. Mice immunized with BHV-2 will resist an otherwise fatal infection with HSV-1, 2. BHV-2 is thus clearly related antigenically to HSV-1, 2.

Peripheral blood leucocytes, probably monocytes, from both immune and susceptible cattle are reported as suppressing the growth of BHV-2 nonspecifically in in vitro culture systems. Cultures of bovine thyroid cells or monocytes have been shown to produce interferon following infection with BHV-2 (Letchworth, 1980).

LABORATORY DIAGNOSIS

Irrefutable diagnosis of BHV-2 infection is achieved by the isolation of virus, which is most readily recovered during the acute phase of disease. Fluid from the somewhat rare teat vesicles is a particularly rich source of virus. Alternatively, small drops of fluid may be expressed from a lesion and drawn up into a syringe or adsorbed onto a swab. Small fragments of tissue from the edge of active ulcerated lesions are also sources of virus. Where ulcers are present in the mouth, nostril etc. swabs can be taken.

Dried crust or scab material, although most readily obtained, is a poor source of virus. Biopsy samples of lesions, from any skin site, are valuable specimens for virus isolation, and, if fresh, may be used to prepare explant cultures.

Swabs, fluid or pieces of tissue should be placed in virus transport medium and kept at about + 4°C in ice (vacuum flask) or in a refrigerator. Ideally, transportation to a laboratory should be made at this temperature, but virus – if present in high concentration – can still be recovered following about 24 h of postal transport, provided this takes place in a suitable medium and at moderate temperatures.

BHV-2 will replicate in a variety of cells, but for primary isolation cells of bovine origin, e.g. calf testes or kidney, are preferred. Reducing the temperature of incubation to 32°C appears to aid replication of virus isolates.

Fluid, suspensions of tissue and sections can be examined by EM for herpesvirus particles which may provide rapid confirmation where the lesions are characteristic.

Histological examination of tissues from lesions may be useful if characteristic lesions with intranuclear inclusions can be seen.

SN, CF, AGID and IF tests can be used to confirm the presence of antibody to BHV-2 or BHV antigen. Paired sera are obviously essential to establish that a current infection is caused by BHV-2.

PROPHYLAXIS AND CONTROL

No commercial vaccine is available for the control of disease caused by BHV-2. Live, unattenuated virus has been given by intramuscular injection to prevent serious disease, but such methods are best confined to infected herds because of the likely spread of infection. Inactivated vaccines have so far not been effective.

As the mode of transmission between herds is unknown, so are the measures to control the infection. When disease occurs in a herd some control of spread may be attempted. Where possible, infected cows should be milked last. As virus is readily inactivated by halogen disinfectants these should be used to

disinfect milking machine teat clusters between cows. Udder cloths should be discarded, boiled or thoroughly disinfected and the use of iodophore teat dips is recommended.

Treatment is usually based on the use of topical emollients and antibiotics to reduce trauma, infection and mastitis. To avoid transmission to calves, fresh milk from infected dams should not be fed, nor should calves be allowed to suck cows with teat lesions. None of the newer antiviral preparations has been found to be effective in treating BHV lesions.

REFERENCES

Cilli, V. and Castrucci, G., 1976. Infection of cattle with bovid herpesvirus 2. Folia. Vet. Lat., 6: 1–44.

Gibbs, E.P.J. and Rweyemamu, M.M., 1977. Bovine herpesviruses. Part II. Bovine herpesvirus 2 & 3. Vet. Bull., 47(6): 411–425.

Letchworth, G.J., 1980. Bovid Herpesvirus 2. Epizootiology, Persistence and Effects of Temperature on Pathogenesis. PhD Thesis. Cornell University, Ithaca, NY.

Letchworth, G.J. and Carmichael, L.E., 1984. Local tissue temperature: a critical factor in the pathogenesis of bovid herpesvirus 2. Infect. Immunol., 43: 1072–1079.

Martin, W.B. and Scott, F.M.M., 1979. Latent infection of cattle with bovid herpesvirus 2. Arch. Virol., 6: 51–58.

Turner, A.J., Morgan, I.R., Sykes, W.E. and Nicholls, W.A., 1976. Bovine herpes mammillitis of dairy cattle. Aust. Vet. J., 52: 170–173.

Weiss, K.E., 1963. Lumpy Skin Disease. In: Emerging Diseases of Animals. FAO Agricultural Studies No. 61. FAO, Rome, pp. 179–201.

Chapter 13

Pseudorabies Virus

SASHI B. MOHANTY

INTRODUCTION

Pseudorabies (Aujeszky's disease, mad itch, infectious bulbar paralysis) is primarily a viral infection of swine but occurs naturally in a wide range of animals (Mohanty and Dutta, 1981). In all animals but adult swine the disease is fatal. It was first described by Aujeszky (1902) in Hungary; he demonstrated the etiology and originally isolated the virus from a naturally infected ox, a dog, and a cat.

VIRUS PROPERTIES

Pseudorabies virus (PRV, porcine herpesvirus 1), a member of the subfamily Alphaherpesvirinae in the virus family Herpesviridae, is more thermostable and resistant to changes in pH than other herpesviruses (Mohanty and Dutta, 1981). The survival of the virus is influenced by temperature, moisture, and pH (Davis and Beran, 1981). It may survive for 2–7 weeks in infected premises, and for up to 5 weeks in meat. In clean damp bedding, the virus may remain infectious for 140 days at 4°C, and for 24 h at 37.5°C. It survives only a few hours on material contaminated with feces and urine and is rapidly inactivated by sunlight, heat, and dry conditions. The virus survives less than 48 h in pit and lagoon water, and is rapidly destroyed on clean concrete and well-cured hay (Thawley et al., 1982). It is sensitive to ether and chloroform, and may be destroyed by ultraviolet and gamma irradiations. Recommended disinfectants include 5% phenol, sodium and calcium hypochlorites, 2% formalin, 2% sodium hydroxide, trisodium phosphate, and quarternary ammonia. For the disinfectants to be most effective, it is important that surfaces be free of organic matter.

PRV grows and produces a rapid CPE in cell cultures derived from swine, cattle, rabbits, dogs, monkeys, and chicken embryos. Cowdry type A intranuclear inclusions and polykaryocytes are seen in infected cells and pocks are produced on the CAM of embryonating chicken eggs (Mohanty and Dutta, 1981). Only one antigenic type with no subtypes has been recognized.

EPIZOOTIOLOGY

Naturally occurring fatal non-suppurative encephalomyelitis is produced in cattle, sheep, goats, dogs, cats, deer, foxes, coyotes, opposums, rabbits, rats and mice (Gustafson, 1980). The morbidity rate is very high in swine. Pigs in the

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first few weeks of life usually succumb to pseudorabies, but mortality is neglig-
ible in older pigs. Many mammals and birds have been experimentally infected.
Domestic swine are the principal reservoirs (Gustafson, 1980), and almost all
cases of pseudorabies in cattle are due to cohabitation with swine. Latent
infection occurs in swine and inapparently infected pigs are responsible for
most infections in domestic ruminants.

The disease has a wide geographical distribution, including New Zealand,
but it has not been reported in Australia and Japan. It causes serious economic
losses in swine and is of growing importance in swine, cattle, and sheep in the
USA and in many European countries. In cattle, the disease is of economic
significance because of the occurrence of clustered fatal cases.

The nasal discharge of infected pigs is probably the source of infectious
virus. Cattle of all ages, sexes, and breed are susceptible. Mechanical, accident-
al transmission by man may occur when veterinarians and herdsmen working
with infected swine handle cattle immediately afterwards (Bitsch, 1975). Cattle
are considered dead-end hosts of PRV and there is no clear evidence of inter-
bovine transmission. However, a transmission of this nature in crowded quar-
ters with limited ventilation is not impossible (Gustafson, 1980). Infected
domestic ruminants rarely transmit the disease to other animals unless their
tissues are ingested by susceptible carnivors. The virus causes a short viremia
in the early stages of the disease, but no vectors are involved in its trans-
mission.

Although rodents have been incriminated in the transmission of PRV, it
appears that rats and mice are quite resistant to infection and are poor shed-
ders of the virus. Thus, they are considered poor reservoirs and do not play a
role in the spread of the virus (McFerran and Dow, 1970). The importance of
wild animals in pseudorabies is not clear. However, carnivores can serve as a
source of infection of other species after they have become infected by ingest-
ing infected carcasses (Kirkpatrick et al., 1980). Reports of human infection are
sketchy and of questionable validity (Trainer, 1981). Although the threshold of
infection for humans is very high, one should have respect for PRV, and
infected animals and their tissues should be handled with caution.

PATHOGENESIS

PRV can naturally enter into domestic ruminants by several routes, most
commonly via a break in the skin caused by bite or other wounds. Occasional
aerosol infection may occur. Experimentally, cattle can be infected by sub-
cutaneous, intramuscular, intravenous, oral, and intranasal routes. In the
prodromal phase, infected swine may shed the virus in the saliva and transmit
it to cattle while wandering among them, nuzzling or biting the flank, vulva,
and anal region (Gustafson, 1980). Pruritus usually occurs at the site of viral
inoculation. Peroral infection in cattle, sheep, and goats may occur via conta-
minated feed and water. Respiratory transmission of PRV from swine to cattle
may result especially in poorly ventilated, crowded areas. Pruritus may not be
seen in these cases.

PRV enters the central nervous system via peripheral nerves, moving along
them centripetally toward the brain, causing neuronal damage, and resulting
in local pruritus and encephalomyelitis. The virus has been isolated from the
nasopharynx, lungs, and vagina of affected cattle. It appears that it replicates
not only in nerve endings or terminal fibers, but also in epithelial cells to a
limited degree. In domestic ruminants, pseudorabies appears to be strictly
neurotropic and spread of the virus in cattle along peripheral afferent and
efferent nerves has been suggested (Ohshima et al., 1976). Persistent latent

infections capable of reactivation are common in adult swine but have not been reported in cattle.

DISEASE SIGNS

In cattle, the disease is referred to as "mad itch" and affected animals develop a fatal encephalomyelitis characterized by intense pruritus of some portion of the skin, leading to self-mutilation. The onset of clinical signs varies somewhat according to the route of infection. The incubation period in naturally infected animals is usually between 4 and 7 days. The signs are often short, lasting from 10 to 24 h, and some animals may die without any sign of illness. The syndromes in experimentally infected animals range from 8 to 72 h. Uneasiness, evidenced by wandering about the pen followed in some instances by ataxia, are the first signs of the disease. The body temperature may range from 39.5 to 41°C and the animals may continue to eat despite having a fever and pruritus at the site of viral entry (Gustafson, 1980). The signs vary widely among individual animals as the disease progresses. Itching may be localized to any part of the body, but most commonly affected areas are the head, shoulder, flank, hind quarters, and anal region, the most likely sites of viral inoculation. At this stage, there is intense excitement and convulsion. Grinding of teeth and constant bellowing may occur. Affected cattle rub the itching area against firm objects to the point of causing bruises and lacerations. If the part is accessible, they lick and bite it incessantly until it becomes reddened and abraded. The itching is so intense that the animals become frenzied (Mohanty and Dutta, 1981). Occasionally, cattle may die without having pruritus (Blood et al., 1979).

Cutaneous tremor often occurs in the early phase of the disease, followed by neurologic signs due to the involvement of the medulla. Excess salivation and frothing are usually seen at this point. This may be followed by spasms of muscles in the neck and thorax that give rise to hiccoughs. The animals breathe rapidly, their eyes have a staring or wild appearance, and trembling, opisthotonos, and ataxia are evident. Later, convulsions are more frequent and pronounced, accompanied by paralysis, sweating, and atony of the rumen with bloating and straining. The animals usually have a fever, often as high as 41–43°C, and shallow, rapid respiration occurs, immediately followed by death in 6–48 h after the first appearance of illness. Cattle do not exhibit aggressive behavior toward other animals or people (Gustafson, 1980). Young calves have encephalitis, erosion of the oral cavity and esophagus, no pruritus, but a high case fatality rate (Blood et al., 1979).

The clinical signs in sheep are similar to those observed in cattle.

In goats, the central nervous system is largely involved and the signs are restlessness, incessant screaming, profuse sweating, and, in the terminal stages, spasms and paralysis (Blood et al., 1979). Death usually occurs in 24–48 h. Pruritus may be absent.

PATHOLOGY

There is a general lack of typical and constant gross lesions (Mohanty and Dutta, 1981). The site of pruritus is often lacerated, contains exudate, and has subcutaneous edema. There are accumulations of cerebrospinal fluid and meningeal congestion, but lesions in the viscera are insignificant. The lungs may show some congestion, edema, and hemorrhages. Microscopically, significant lesions in the central nervous system consist of nonsuppurative ence-

phalomyelitis, meningitis, and neuronal damage. Perivascular cuffing, focal necrosis, and type A intranuclear inclusions may also be present (Mohanty and Dutta, 1981).
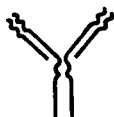
IMMUNE REACTIONS

Since infected domestic ruminants succumb rapidly, immunity of these animals to PRV and serologic procedures have not been described.

LABORATORY DIAGNOSIS

Pseudorabies is usually fatal to domestic ruminants within 3 days after the onset of signs, and serologic tests are not employed for its diagnosis. The disease is clinically characteristic and presumptive diagnosis can be made by the history, syndromes (particularly severe pruritus), and its short course. Pruritus, however, may not be seen in a few cattle, sheep, and goats. His-topathologic changes may aid in the diagnosis. The nervous form of the disease must be differentiated from listeriosis, rabies, acetonemia, lead poisoning, and the rare encephalitic form of IBR.

A definitive diagnosis can be made by virus isolation and identification. The virus is readily isolated in a variety of cell cultures. The brain is the specimen of choice, otherwise samples from the site of pruritus may be used for virus isolation.

Animal inoculation can be used when laboratory facilities are not available; rabbits are most sensitive to PRV. Subcutaneous injection of homogenate in the flank (1 ml of 1:10 dilution of the tissue suspension) results in signs within 48 h after inoculation. The rabbits become agitated, bite at the site of inocula-tion, breathe rapidly, and die of respiratory failure within 3–5 days (Gustafson, 1980). The virus can be isolated from the cord and spinal ganglia of rabbits, but not from the brain. Guinea pigs may also be used; the signs are similar to those in rabbits. Mice are less sensitive.

The IF test on cryostat section of fresh tissues is extremely rapid and accurate.

PROPHYLAXIS AND CONTROL

Vaccines for use in cattle are not available in the USA. Attenuated and inactivated vaccines made for swine have been used in cattle in Europe. In a limited study, cattle vaccinated with two doses of a killed pseudorabies swine vaccine developed a marginal serologic response but were resistant to subse-quent challenge (Muhn and Beard, 1977).

Once clinical signs appear, there is no effective treatment. Homologous hyperimmune serum and concentrated gamma globulin have been successfully used to treat calves in Europe. Antisera made in horses or pigs produce anaphy-lactic reactions in cattle.

Pseudorabies in cattle and other domestic ruminants is prevented by keep-ing pigs from contact with these animals. Animals showing clinical signs should be separated from those that are normal. Carcasses of affected animals should be burned or buried. Where the disease is enzootic, veterinarians and other animal handlers should be aware of their potential role in transmitting the disease from swine to cattle (Gustafson, 1980).

Eradication by slaughter and quarantine of infected pigs may be considered after the incidence of pseudorabies in swine is reduced to a manageable level.

REFERENCES

Aujeszky, A., 1902. Ueber eine neue Infektionskrankheit bei Haustieren. Zentralbl. Bakteriol. I. Abt. Orig. A, 32: 353–373.

Bitsch, V., 1975. A study of outbreaks of Aujeszky's disease in cattle. Acta Vet. Scand., 16: 402–455.

Blood, D.C., Henderson, J.A. and Radostits, O.M., 1979. Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, and Horses. Lea and Febiger, Philadelphia, PA, 5th edn., pp. 686–689.

Davis, E.B. and Beran, G.W., 1981. Influence of environmental factors upon the survival of Aujeszky's disease virus. Res. Vet. Sci., 31: 32–36.

Gustafson, D.P., 1980. Pseudorabies. In: H.E. Amstutz (Editor), Bovine Medicine and Surgery, 2nd edn., Vol. 1. American Veterinary Publications, Santa Barbara, CA, pp. 190–194.

Kirkpatrick, C.M., Kanitz, C.L. and McCroklin, S.M., 1980. Possible role of wild mammals in transmission of pseudorabies in swine. J. Wildl. Dis., 164: 601–604.

McFerran, J.B. and Dow, C., 1970. Experimental Aujeszky's disease (pseudorabies) in rats. Br. Vet. J., 126: 173–178.

Mohanty, S.B. and Dutta, S.K., 1981. Veterinary Virology. Lea and Febiger, Philadelphia, PA, pp. 187–189.

Muhn, R.L. and Beard, P.D., 1977. Lesions of pseudorabies in several species of animals. Proc. Am. Assoc. Vet. Lab. Diag., 20: 1–10.

Ohshima, K., Gorham, J.R. and Henson, J.B., 1976. Pathologic changes in ferrets exposed to pseudorabies virus. Am. J. Vet. Res., 37: 591–596.

Thawley, D.G., Gustafson, D.P. and Beran, G.W., 1982. Procedures for elimination of pseudorabies virus from herds of swine. Am. J. Vet. Res., 181: 1513–1518.

Trainer, D.P., 1981. Pseudorabies. In: J.W. Davis, L.H. Karstad and D.O. Trainer (Editors), Infectious Diseases of Wild Mammals. Iowa State University Press, Ames, IA, 2nd edn., pp. 102–107.

Chapter 14

Malignant Catarrhal Fever Virus

WALTER PLOWRIGHT

INTRODUCTION

Malignant catarrhal fever (MCF; malignant catarrh; bösartiges Katarrhal-fieber; coryza gangréneux) is a sporadic disease of domestic cattle and buf-faloes, as well as of many other wild Bovidae. It has a characteristic, severe, clinical course, leading almost invariably to death. The distribution of MCF is virtually worldwide and the clinico–pathological features observed do not differ significantly from one continent to another.

Cases of MCF outside Africa can usually be associated with close contacts between presumed "carrier" sheep and the susceptible species; occasionally this is also true in Africa. This form of the disease has long been known to be transmissible in series, albeit often with difficulty (Götze and Liess, 1929) but the causal agent has not yet been identified.

In Africa, and occasionally elsewhere in zoological gardens, the source of infection is known to be one of two species of wildebeest (*Connochaetes tauri-nus* and *C. gnu*) and a lymphoproliferative herpesvirus is the established cause (Plowright et al., 1960). The wildebeest-derived virus and the disease it causes forms a basis for this description, but the "sheep-associated" condition is probably caused by another related herpesvirus for which permissive cell cultures are not yet available (Reid and Buxton, 1985; Plowright, 1986).

VIRUS PROPERTIES

Physical and chemical characteristics

As a typical herpesvirus, cell-free MCFV is inactivated by lipid solvents and detergents; it is relatively stable in culture media containing serum. Thus, high-passage levels of the WC-11 strain in culture medium with 5% serum had a half-life of 11–14 days at 4°C and 9.3 h at 37°C. At 56°C all infectivity was lost in 9–15 min. Stocks of virus at −70°C or lower retained their original titer for many months (Plowright, 1981). The Ololossos strain used by Harkness and Jessett (1981) had a half-life of 33 h at 37°C; their medium contained 2% serum only. Mushi et al. (1980) found that 90% of cell-free infectious virus survived in the laboratory for at least 30 days at 22°C, so long as the relative humidity was high (100% RH); they suggested that these findings would be reflected in stability in the natural environment. The virus was stable for 30 min at 37°C in phosphate buffers at pH 6.0–8.0; stability was increased by higher concentra-tions (150 mM vs. 100 mM) of sodium ions, whilst potassium or magnesium ions were also protective at 45°C (Schloer and Breese, 1982).

Antigenic properties

Wildebeest isolates of MCFV appear to be indistinguishable antigenically. Thus in indirect IF or cross-neutralization tests there were no appreciable differences between the WC-11 (high-passage attenuated) and a virulent (C500) isolate (Rossiter et al., 1977). Two of three isolates from hartebeest (see below), were antigenically reactive with wildebeest virus at a low level only, but the other (K30) was judged to be identical to the WC-11 strain in neutralization tests using experimental antisera (Reid and Rowe, 1973). Later, higher neutralizing titers were recorded in hartebeest sera against homologous (K30) virus than against the WC-11 (wildebeest) isolate. All the antelopes (hartebeest, topi, oryx) which possess neutralizing antibody to wildebeest (MCFV show low titers, which possibly reflect minor antigenic differences in the viruses which induce them (Reid et al., 1975). Virus isolates from topi also cross-react in IF tests with antisera to wildebeest virus (Mushi et al., 1981).

It is of interest that the majority (> 95%) of sheep from many countries possess antibodies which react in indirect IF tests with antigens of wildebeest strains of MCFV. These antibodies can be absorbed by infected cells and blocked by rabbit antiserum to MCFV, not by normal rabbit serum (Rossiter, 1981b); they are not induced by the ovine herpesvirus associated with jaagsiekte (Martin et al., 1979).

The very few cattle which recover from infection with African MCFV are all immune for at least 8 years to repeated parenteral challenge with homologous or heterologous isolates and possess low but persistent levels of neutralizing antibody (Plowright, 1963, 1964, 1968). There is, therefore, no evidence for significant immunological variation.

A summary of the more recent evidence of the relationship of the wildebeest-derived (WD) and sheep-associated (SA) viruses is given by Plowright (1986).

Cultivation

In both wildebeest and indicator hosts, such as cattle and buffaloes, the infectivity in tissues is strictly cell-associated. Disruption or death of cells brought about, for example, by ultrasonic treatment or by freezing and thawing in the absence of cryoprotectants, leads to complete loss of infectivity (Plowright, 1963). The only exception to this generalization is that cell-free infectious virus is found in the ocular and nasal secretions of wildebeest calves. The richest sources of virus are the lymphoreticular tissues, such as lymph nodes, thymus, spleen, bone marrow and the buffy coat separated from peripheral blood; the highest titers are about $10^5-10^6\,TCID_{50}$/g in bovine lymph node and thymus, somewhat lower ($10^2-10^4\,TCID_{50}$/g) in spleen and about $10^2-10^3\,TCID_{50}$/ ml in the blood of reacting cattle. It is now established that the cells which carry infectivity are medium-sized lymphocytes.

In SA-MCF infectivity is associated with cultivable cells, designated large granular lymphocytes of T-cell lineage and with natural killer-cell properties (Reid and Buxton, 1985).

The most sensitive cell cultures for virus isolation from animals were, for many years, considered to be primary or secondary monolayers prepared from calf thyroid (Plowright et al., 1960), but more recently it has been found that some strains of serially cultivated calf testis cells are more satisfactory. These cultures have the additional advantages that a CPE is more easily detected in them and accompanied, even at low passage levels, by high yields of cell-free infectivity — $10^5-10^6 TCID_{50}$/ml (R.G. Watt and W. Plowright, unpublished results, 1977). The thyroid cell culture systems have about the same sensitivity as cattle inoculation for detection of cattle tissue virus (Plowright, 1963, 1964);

strains of bovine testis cells are approximately 100 times more sensitive than rabbits for demonstration of cultured virus.

Cytopathic effects can be detected in unstained cultures by light microscopy; the most characteristic change is the development after 3 days to 3 weeks of syncytia (multinucleate cells) with a variable degree of vacuolation (Fig. 33). Rounded, refractile or stellate cells may also be seen. There is little tendency for the foci to extend or disseminate in bovine thyroid monolayers at 37°C, presumably because very little cell-free virus is produced at low passage levels; on the contrary, syncytia may detach and the CPE is then difficult to recognize. At 32°C, however, CPE appears more slowly but spreads more extensively and cell-free infectivity is released into the medium in greater quantities at low passage levels; yields of $10^{4.5}$–$10^{5.9}$/ml in comparison with trace quantities at 37°C, have been recorded, and maximum titers are attained at 8–10 days (Harkness and Jessett, 1981).

In fixed and stained cultures affected cells show "beading" of the nuclear membrane and large intranuclear inclusions which are increasingly DNA-positive as they mature (Fig. 34; in some cells DNA-positive, small, granular intracytoplasmic inclusions may also occur, especially in the juxtanuclear region (Figs. 35, 36). Morphologically, released virus has a typical herpesvirus structure, with a capsid about 100 nm in diameter, a nucleoid about 40 nm across and hollow capsomers (Fig. 37). Some particles have an outer envelope and a diameter of 140–220 nm with an inner nucleocapsid of 85–100 nm (Fig. 38) (Plowright et al., 1965; Castro and Daley, 1982; Schloer and Breese, 1982).

EPIZOOTIOLOGY

Host range

The disease, MCF, is primarily one of domesticated cattle (*Bos taurus* and *B. indicus*) and buffaloes (*Bubalus bubalis*), but it is now being increasingly encountered in farmed deer, for which it sometimes represents a major disease hazard (see Reid et al., 1979, Reid and Buxton, 1984, and Fletcher, 1982, for red deer, *Cervus elaphus*; Westbury and Denholm, 1982, for Rusa deer, *C. timorensis*). Multiple cases of MCF have also been reported in free-living, indigenous North American cervids, such as white-tailed and mule deer (*Odocoileus virginianus* and *O. hemionus*, Clark et al., 1970; Pierson et al., 1974). Finally, there is a wide range of other species, belonging to the subfamilies Bovinae, Cervinae and Odocoileinae, in which cases of the disease have been reliably reported in zoological collections; tabulated lists were given by Plowright (1981), and further information was summarized by Plowright (1986). On the other hand, it was found that the African buffalo (*Syncerus caffer*) and the camel were not susceptible to experimental infection with wildebeest strains (Plowright, 1981). The evidence for susceptibility of sheep to parenteral inoculation with the latter is conflicting; attempts to infect East African sheep failed (Plowright, 1964), but Kalunda et al. (1981b) obtained some evidence for infrequent establishment of "Africa" virus in North American sheep, and Straver and Van Bekkum (1979) recovered a probable wildebeest isolate from Dutch sheep which had been inoculated 20–23 days previously.

The most important experimental host for MCFV is the domestic rabbit (*Oryctolagus cuniculus*), which is susceptible to parenteral inoculation by a number of routes, with material from naturally infected cattle, deer or with culture-propagated agents; it invariably succumbs to the disease if infected (for WD-MCFV see Daubney and Hudson, 1936; Piercy, 1955; Plowright et al., 1960; Plowright, 1964; Rossiter et al., 1977; Edington et al., 1979; Straver and Van

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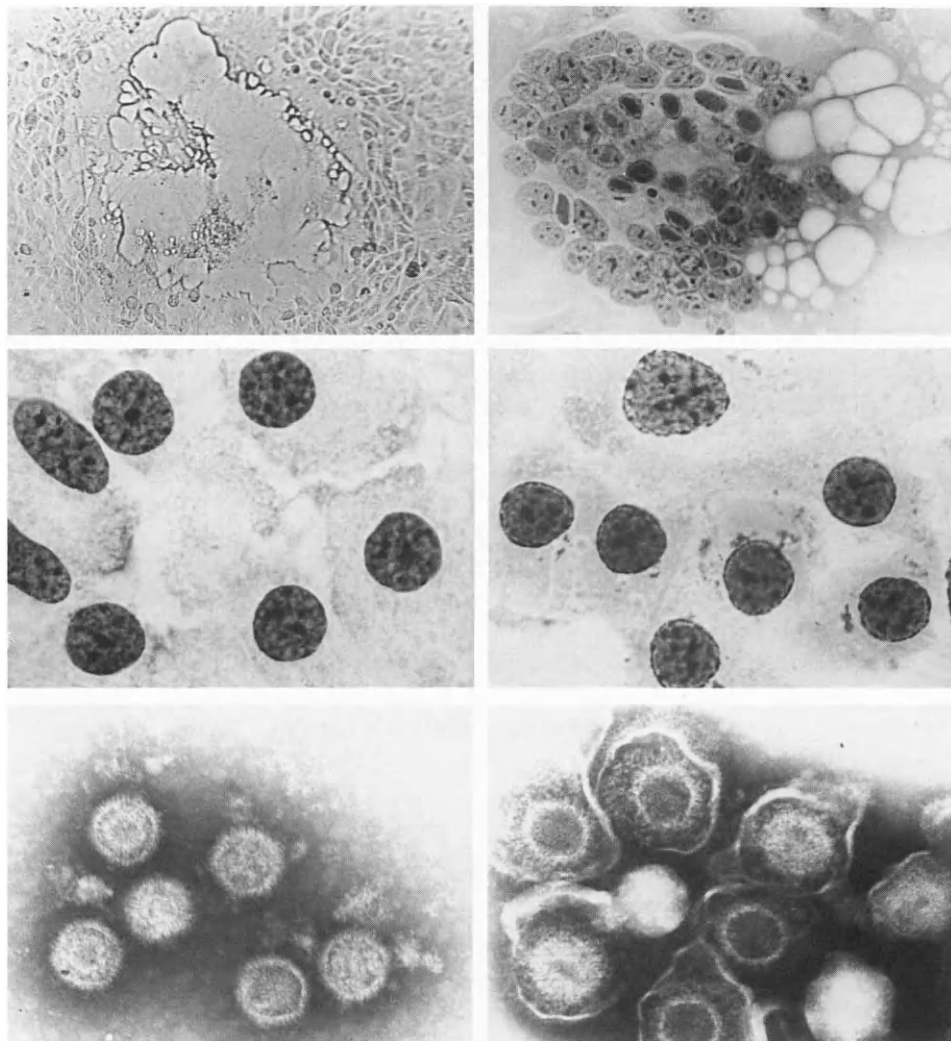
Fig. 33 (top, left). Unstained monolayers of calf thyroid cells. Large syncytium with peripheral vacuolation, 3 days after inoculation of MCFV-infected bovine leukocytes (× 150).

Fig. 34 (top, right). Calf kidney cells with typical vacuolated syncytium and numerous intranuclear inclusion bodies, 7 days p.i.; Bouin: May–Grunwald–Giemsa (× 500).

Fig. 35 (middle, left). Uninfected bovine thyroid monolayer; Carnoy: May–Grunwald–Giemsa (× 1000).

Fig. 36 (middle, right). Bovine thyroid monolayer, 4 days after infection with high passage MCFV (strain WC-11); all cells show beading of nuclear membranes, obscured chromatin pattern and numerous DNA-positive cytoplasmic granules, mainly paranuclear; Carnoy: May–Grunwald–Giemsa (× 1000).

Fig. 37 (bottom, left). Non enveloped virions of the WC-11 strain of MCFV; hollow capsomeres clearly visible on the surface and in profile at the periphery; potassium phosphototunstate pH 6.8 (× 150 000) (with thanks to Drs. R.F. Macadam and J.F. Armstrong).

Fig. 38 (bottom, right). Enveloped virions of WC-11 strain of MCFV, with two nonenveloped capsids not penetrated by stain; potassium phosphototunstate pH 6.8 (× 150 000) (with thanks to Drs. R.F. Macadam and J.F. Armstrong).

Bekkum, 1979; for SA-MCFV see De Kock and Neitz, 1950; Daubney, 1959; for deer-pathogenic virus see Huck et al., 1961; Buxton and Reid, 1980; Westbury and Denholm. 1982). The rabbit provides a model system in which to study the disease. The infection is not contagious in this species and suggestions that rabbits may act as a natural source of infection for cattle (Kalunda et al., 1981b) are unreasoned.

Recently, it has been reported that both newborn guinea pigs and hamsters (55% and 100% respectively) succumb to inoculation of cultured WD-MCFV (Kalunda et al., 1981b). More recently hamsters and rats have been successfully used to passage the agent from continuous lines of SA-MCF-infected lymphoblasts (Reid and Buxton, 1985; Reid et al., 1986). Embryonated eggs are not susceptible to inoculation according to most observers, but Danskin (1955) claimed to have propagated virus in the yolk-sac of 8-day embryonated eggs.

Reservoir hosts and transmission

Sheep-associated cases

The majority of cases of MCF, particularly when multiple incidents occur in any species, can be associated with close contact between sheep and the "indicator" hosts. The disease has been reported most frequently when cattle are housed with sheep over long periods, and the classical experiments of Götze and Liess (1930) and Jesse (1930) showed that three carrier sheep transmitted MCF to 18 out of 50 cattle which were placed in continuous close contact over a period of 10 months. In 17 of these cases the maximum incubation period, estimated from the time of introduction of the sheep, must have been 96–185 days. Cases of MCF continued to appear up to 3 or 4 months after removal of sheep from effective contact with cattle; exposure needed to be very close, preferably in a stable with common food and water troughs (Götze, 1932). Similar experiments, occasionally successful (e.g. Magnusson, 1939; De Kock and Neitz, 1950; Piercy, 1954; Snowdon, 1972; Daniels et al., 1988), sometimes failures (e.g. Wyssmann, 1933, 1934; Piercy, 1954; Plowright, 1964; Pierson et al., 1974), have been reported. In no case, however, has it been possible to induce typical MCF in cattle by the inoculation of tissues from naturally infected sheep.

Whilst recent reports of multiple cases of MCF have supported the thesis that sheep are probably reservoir hosts of the virus (e.g. Pierson et al., 1973, in the USA; James et al., 1975, in New Zealand; Selman et al., 1974, and Reid et al., 1979, in Great Britain), there are also experienced investigators who have failed to incriminate sheep. The possibility remains that other species act as inapparent reservoirs, but these will not be easily identified until the agent(s) of MCF outside Africa has (have) been cultivated and serological tests for it (them) have been developed. Some investigators assert that SA-MCF is more common during the lambing season (Buxton and Reid, 1980).

Whilst many observers agree that indirect contagion is an unlikely method of transmission of SA-MCF to cattle, Reid et al. (1979) reported that intermediate contagion was likely in one outbreak involving red deer. There is some evidence that infection may, unusually, spread amongst highly susceptible deer by direct contact (McCallum et al., 1982; Reid et al., 1986), but evidence for this in cattle is either lacking or unconvincing (Maré, 1977).

Wildebeest-derived MCF

Although two species of wildebeest are mentioned in the introduction, only one, the blue or white-bearded variety (*Connochaetes taurinus*), has been investigated adequately and this only in East Africa; the pioneer work of Mettam (1923) was, however, carried out with virus derived from the black wildebeest

(*C. gnu*) in South Africa, and virus derived from this species has been isolated in an Oklahoma zoo (Castro et al., 1982, 1984). There is no epidemiological evidence that serologically related viruses in hartebeest (Reid and Rowe, 1973), topi (*Damaliscus korrigum*) (Mushi et al., 1981) and probably oryx (Reid et al., 1975; Mushi and Karstad, 1981) are naturally transmissible to cattle. The hartebeest viruses are, however, pathogenic for them under experimental conditions.

Many significant features of virus maintenance and transmission to cattle are now known, and this work has been reviewed recently by Mushi and Rurangirwa (1981) and Plowright (1984, 1986). All the geographically distinct populations of East African wildebeest are infected with MCFV and serologically negative animals are very rare. The virus crosses the placenta in some cows (Plowright et al., 1960) and the calf is born infected; Mushi and Rurangirwa (1981) recorded nasal excretion at the age of 4 days, and Plowright (1965a, b) postulated that congenitally infected calves may disseminate virus among other members of the annual calf crop, which is normally born over a period of about 2 months. Approximately 30% of calves in Tanganyika were viremic in cell culture tests when sampled at ages up to 3 months, but the rate of virus recovery then fell to 7% and 2% in the second and third trimesters, respectively; by 18 months virus was not demonstrable by the same technique in 20-ml volumes of blood. Inoculation of larger quantities into cattle showed that virus was present in blood or lymphoid tissues in animals of all ages, including — and perhaps particularly — in pregnant adults; it probably persists for life in many of them. The duration of viremia in calves which were sampled continuously by cell culture techniques varied from 3 to 36 weeks and the infectivity was associated entirely with circulating leukocytes. Passively acquired neutralizing antibody is present in the majority of calves but does not prevent infection, which leads to increased titers up to about 18 months, followed by a slight decline. Neither free-living nor captive wildebeest infected with MCFV show clinical or pathological signs attributable to the virus.

Natural transmission of MCFV from wildebeest to cattle takes place almost entirely during the first 3–4 months of life of the annual calf crop, i.e. during the period of first viremia. Transfer occurs on common grazing lands and sometimes in zoological gardens. Experimentally, the incubation period for "contact" infections was 30–81 days (Plowright, 1965b). Very occasionally transmission also takes place from adult wildebeest, at least under laboratory conditions of housing or paddocking (Mushi and Rurangirwa, 1981; Plowright, 1981).

Virus excretion by wildebeest is through nasal and ocular secretions, apparently not through urine or saliva. In calves up to 3 months old, particularly those in the 6–8 weeks age group, virus titers in excretions may be $\geqslant 10^{3.2} \mathrm{TCID}_{50}/\mathrm{ml}$ and part of the infectivity is cell-free virus; it is probably derived from the turbinate mucosa and cornea, which appear to represent predilection sites for superficial virus replication. Explant cultures of cornea tissue frequently yield virus up to and including 4 months of age. After 3 months the secretions contain neutralizing antibody, predominantly IgA, which accounts for the rapid decline in virus excretion and loss of ability to transmit infection (Mushi and Rurangirwa, 1981).

The infectivity found in the nasal secretions of adult wildebeest after stress (Rweyemamu et al., 1974) and virus in the nasal and oral secretions of reacting cattle is probably associated with living infected cells and therefore unlikely to infect susceptible species (Kalunda et al., 1981a). There are very few records indeed of contact infection amongst cattle, although De Kock and Neitz (1950) suspected it had occurred; the disease is generally regarded as incapable of spreading among them, even after very close and prolonged exposure (Plow-

right, 1968, 1981). For this reason and the observed seasonal incidence, the inability to spread at a distance and the generally very low prevalence, vectors such as flies, ticks or lice are unlikely to play a role in the transmission of MCF.

There is no evidence that MCFV is infectious for man.

Incidence

Neither the widely distributed SA nor the restricted, WD forms of the disease are commonly encountered, and it is seldom that they become economically important. SA-MCF can, however, be serious for individual properties. Roderick (1958) described "problem" farms in North Dakota where owners had been forced out of business by losses of up to 50% of the herd, and Pierson et al. (1973) reported the death of 87 out of 231 cattle (37%) within 68 days in a herd in Colorado. Clark et al. (1970) recorded the loss of 26 out of 40 axis deer (*Axis axis*) in the course of a year on one property.

Both SA-MCF and WD-MCF are of some importance in Africa. Piercy (1954) and De Kock and Neitz (1950) described serious outbreaks of the former and the latter has produced annual losses in Kenya Masailand which may reach 7% (Plowright et al., 1975); one outbreak in 1960 resulted in the death of about 100 of a herd of 500 cattle (Plowright, 1964). A similar loss has been reported from Indonesia (Daniels et al., 1988).

In spite of these incidents, the majority of cases of MCF are sporadic in occurrence; the SA cases have a tendency to recur at intervals over many years, but only one or two animals are affected at a time. Some authors report a higher incidence of SA-MCF in the spring and early summer. The WD form, as encountered in East Africa, is seen almost exclusively in the months January–July, from the time wildebeest calves are born up to 2–3 months thereafter.

The majority of cases of MCF occur in young adults, but animals of all ages are susceptible under experimental conditions. All breeds of cattle (*Bos indicus*) and water buffaloes are apparently susceptible; the indigenous cattle of Bali, an Indonesian island, are extremely susceptible (Daniels et al., 1988).

Susceptibility

The low incidence of MCF could suggest that subclinical immunizing infections of cattle occur on exposure to reservoir or even indicator hosts. This hypothesis is not supported by the extremely high morbidity and case mortality rates which are observed following experimental infection with African MCF (Plowright, 1968). Cattle which have been held for many weeks in contact with clinical cases are still susceptible to challenge, and in two areas of Kenya where the WD form of the disease is commonly encountered, six different serological tests failed to produce evidence of subclinical infection in "normal" cattle (Rossiter et al., 1980).

The case mortality rate in cattle infected experimentally with WD-MCF is extremely high. The mean for 9 isolates in 311 cattle in Kenya was 94%, survival being favoured by concurrent anaplasmosis (Plowright, 1968). The mortality rate recorded for a single isolate in the USA was comparable; thus only 3 of 50 cattle (6%) developed mild disease; 28 died and the rest were killed when sick (Kalunda et al., 1981a). The lethal effects of "non-African" MCF are probably equally great, but it is difficult to collect comparable figures due to difficulties in transmission and a tendency to kill some animals for collection of materials, especially in an experimental series. Liggitt et al. (1978) reported that "once clinical signs became obvious death or a moribund state occurred within 5 days".

PATHOGENESIS

Route of infection

The presumed natural portal of entry in WD-MCF is the upper respiratory tract: the precise site is unknown but it is probably the nasal mucosa and/or tonsil; instillation of infectious tissue suspensions or low-passage culture virus into the nares sometimes results in transmission to cattle (Plowright, 1963, 1964, 1968). Aerosols or the intratracheal route have been used with consistent success in cattle (Kalunda et al., 1981a) and in rabbits (Mushi and Rurangirwa, 1981). The nasal and salivary secretions of reacting cattle do contain virus infectivity (Kalunda et al., 1981a) but contact transmission is virtually unknown in this species, presumably because there is no stable, cell-free virus in excretions but only living, infected lymphocytes (see below).

Congenital transmission of WD-MCFV takes place frequently in the reservoir host but has been demonstrated only once in cattle; the animal concerned was a cow that had a subclinical infection following experimental inoculation and produced, over a period of 80 months, six calves, of which four were infected *in utero* (Plowright et al., 1972). Evidence suggestive of congenital transmission has been seen in a fetus in one outbreak in New Zealand (James et al., 1975).

Incubation period and death time

A long incubation period is characteristic for both forms of MCF. Thus, in East African cattle infected with about 10^3 ID_{50} of WD virus, it was 19.5 ± 3.7 days (11-34 days; Plowright, 1968). Previously published figures from Africa were similar and, more recently, those obtained elsewhere have been comparable (Table 13); the shorter periods observed by Straver and Van Bekkum (1979) may have been due to a very large dose of blood. In practice it is essential to wait at least 2 months before concluding that an animal has not been infected by a parenteral inoculation, since a few previously unexposed animals do not show recognizable reactions until 9-10 weeks have passed.

In the experimental SA form transmission has almost invariably been by intravenous or subcutaneous inoculation of large volumes of blood, and the incubation period has ranged from 11 to 63 days (Plowright, 1968). In a more recent passage series, figures of 30.2 ± 13.6 days (range 19-73) were recorded in Colorado, but the amount of infectivity was clearly marginal, since only 19 out of 28 (68%) of animals inoculated developed the disease during twelve passages (Liggitt et al., 1978). Other figures include means of 27 days (range 15-49) (Pierson et al., 1974) and 20-36 days (Selman et al., 1978).

The death time in MCF, i.e. the time from the beginning of pyrexia to death,

TABLE 13

The incubation period and death time in WD-MCF of cattle

Infective materials	Incubation period		Death time		Mortality rate	Reference
	Range	Mean ± S.D.	Range	Mean ± S.D.		
Blood, lymph node	11-34	19.5 ± 3.7	3-22	8.2 ± 2.9	291/311 (94%)	Plowright, 1968
Blood, culture virus	14-46	22.3 ± 6.6 ($n = 47$)	4-52	12.9 ± 10.3 ($n = 28$)	ND	Kalunda et al., 1981a
Blood	7-16	10.6	ND	ND	ND	Straver and van Bekkum, 1979

ND = not determined.

is somewhat variable, as is evident from Table 13. Pierson et al. (1979) remarked that the incubation period in "American" MCF was longer than that in the "African" form and that the course of the disease was shorter; their data revealed death times of 4 ± 6 days (range 1–9) and 12 ± 4 days (range 6–23), respectively, for the two forms. In deer shorter death times have been reported (1–4 days; Reid et al., 1979).

Rabbits are important experimental hosts; Table 14 gives some data for the incubation periods and death time in this species. Continuous passage of WD-MCFV up to 75 times in rabbits, using either spleen or mesenteric lymph node inoculated intraperitoneally, did not materially change the incubation period or course of the disease. The mortality rate in infected rabbits is unfortunately 100%.

The primary site of MCFV replication

It is not known whether the primary site of MCFV replication is at a mucosal surface and includes epithelial cells. The difficulty, even with WD strains, lies in the fact that histological, EM and IF studies failed, until recently, to reveal convincing evidence of infection by a herpesvirus at any stage of the disease, in either rabbits or cattle (see Plowright, 1984). However, by subinoculation of tissues of rabbits it was shown that WD-MCFV infectivity was present in pooled lymphoid tissues of rabbits at 2 and 4 days after intraperitoneal infection and in the spleen at 4 days after intravenous inoculation. From 8 days to death, spleen and lymph nodes were continuously infective (Edington et al., 1979; Edington and Patel, 1981).

Furthermore, some "mononuclear" cells developed specific MCFV fluorescence in the spleen, lymph nodes and thymus of rabbits at 4–6 days. Sites particularly involved were the splenic red pulp, paracortical areas of lymph nodes and cortex and medulla of the thymus. All these locations suggest an involvement of T lymphocytes, and the first two of these sites are also areas where lymphoblastic infiltration and focal lymphocytic necrosis occur early. Hence it appears that, in the rabbit, there is rapid establishment of infection in the spleen and lymph nodes, but no evidence of epithelial involvement at the site(s) of mucosal penetration.

The "mononuclear" cells which express viral antigens, are medium-sized lymphocytes, 11 ± 0.6 μm in diameter and with little cytoplasm. They contrast with the infiltrating lymphoblasts, for long regarded as typical of the lesions, which are somewhat larger (10–15 μm), have more cytoplasm, a large vesicular nucleus and often a reticulated nucleolus. It is not yet clear whether the cells which contain viral antigen are of B or T lineage.

When lymphopoietic tissues from reacting rabbits were dispersed and the cell suspensions used to prepare smears for IF staining, it was found that only 1–4 in 10^6 expressed viral antigen(s). However, after 48–72 h in culture in RPMI 1640 medium (with 20% fetal calf serum) the number of antigen-positive cells increased 50–1000-fold, as did the number of cells which registered as infectious centers in culture assays. Small numbers of herpes-like virions appeared simultaneously in both nucleus and cytoplasm of 0.1–0.3% of cultured cells (Patel and Edington, 1980). Productively infected cells also developed membrane antigens demonstrable by IF of living cells (Patel and Edington, 1982a).

Similar methods were applied to infected bovine lymphopoietic tissues and, whilst differentiated medium-size lymphocytes (11–13 μm diameter) were again virus "carriers", important differences were observed; the number of infectious centers was initially much higher ($\bar{x}$ = $1000/10^6$) and increased little on culture, whilst cells expressing antigens in IF tests rose from ≤ 2 to $300–3000/10^6$ (Patel and Edington, 1981). The apparent anomaly may be explained by the

TABLE 14

The incubation period and death time in rabbits infected with MCFV

Type of agent	Infective materials	Incubation period[a]		Death time		No. of passages	Reference
		Range	Mean	Range	Mean		
WD	Spleen (i.p.)	9-24	13.25 (*n* = 159)	1-7	3.59 (*n* = 77)	75	Plowright, 1964
WD	Spleen (i.p.)	NR	15.8 (± 3.7)	NR	3.0 (± 1.2)	NR	Wilks and Rossiter, 1978
WD	Spleen (i.p.)	5-32	14.1	1-3	NR	≥ 8	Straver and Van Bekkum, 1979
WD	Spleen (i.p.)	NR	15.6	NR	2.7	NR	Rossiter et al., 1977
SA	Mixed tissues	2-33	(3)11[b]	1-3	NR	125	Buxton and Reid, 1980 Reid et al., 1986
SA	Mixed tissues	8-12	12 (*n* = 16)	NR	NR	11	Westbury and Denholm, 1982

NR = Not recorded.
[a] Calculated from pyrexia (≥ 40°C).
[b] Two modal periods, not means.

ability of the bovine complement/antibody system to destroy cells expressing viral antigens on their plasma membranes, whereas the rabbit complement/ antibody system lacks this ability (Patel and Edington, 1982a). The inference was that the proportion of cells incorporating viral genomes at the time of explantation was similar in both species and probably did not increase in culture. There is little evidence for the infection by WD-MCFV of other cell types, except perhaps macrophages (see Plowright, 1984).

Viremia in MCF

In cattle WD-MCFV produces a viremia detectable from 9 to 17 days after inoculation of virulent blood, i.e. on average 7 days (range 3–15) before the onset of pyrexia (Plowright, 1964, 1968). Similar delays between the onset of viremia and clinical signs have been reported by Rweyemamu et al. (1976) and Kalunda et al. (1981a). Once viremia is detected it persists throughout the course of the disease; the titer increases during the late incubation period to reach levels of $\geqslant 10^{2.0}$ TCID$_{50}$/ml at the onset of fever or even 3–4 days earlier. Peak mean viremia titers ($10^{2.6}$ TCID$_{50}$/ml) are recorded at about day 3–7 of pyrexia, and high levels are usually maintained to death (Fig. 39). In the few animals which recover, blood titers decline during convalescence but virus is usually detectable for up to 50 days by isolation in cell culture; by inoculating 5 ml of blood into cattle persistence of viremia for 3–6 months was demonstrated (Plowright, 1964).

The viremia in cattle and wildebeest is associated entirely with cellular fractions of the blood and not the plasma, erythrocytes or platelets. "Mononuclear" cells, devoid of neutrophils and eosinophils, have the highest infectivity, and hematological monitoring reveals in some animals that increasing viremia is associated with a rise in the numbers of medium-sized and large lymphocytes and with a terminal decline in small lymphocytes (Plowright, 1953b, 1964; Fig. 40). It can be assumed that the infectious elements in the blood are derived from lymphopoietic tissues such as lymph nodes and thymus, which have titers of $\geqslant 10^5$ TCID$_{50}$/g, or from spleen and bone-marrow, which also often contain more infectivity (per gram) than blood.

No quantitative data on the viremia in SA-MCF are available, but the
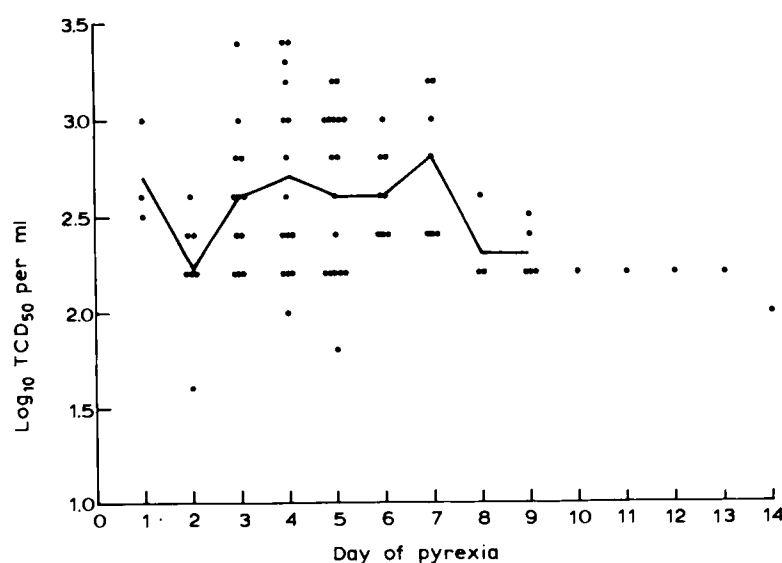
Fig. 39. The titer of WD-MCFV in the blood of reacting cattle; all titrations were performed in calf thyroid monolayers (from Plowright, 1964); line indicates mean titer.
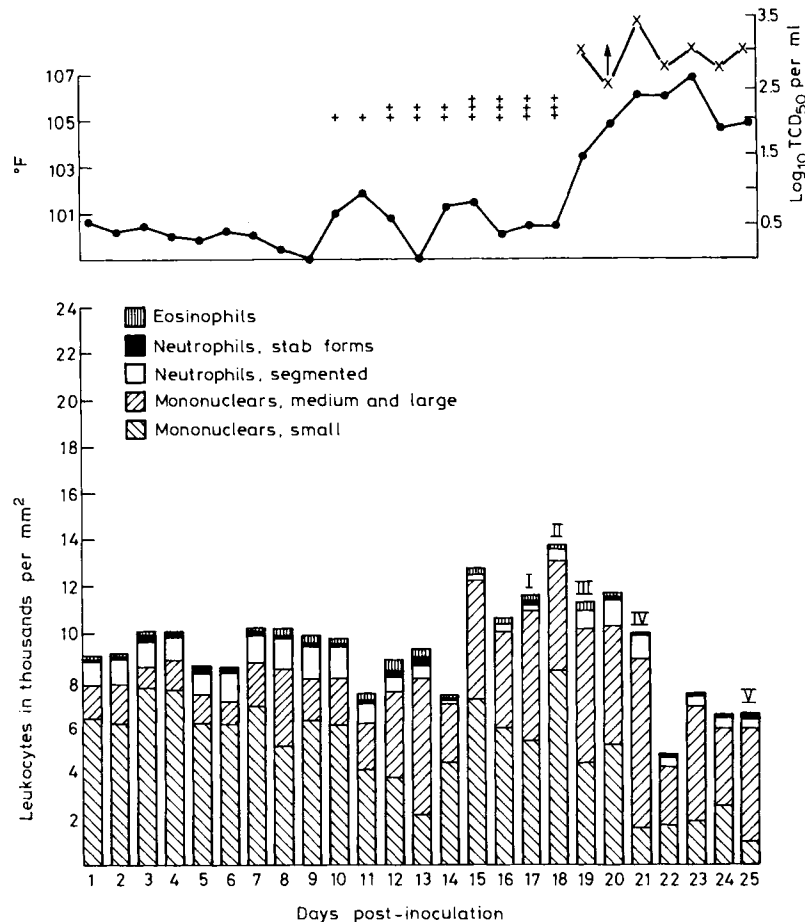
Fig. 40. Correlation of viremia, clinical signs and hematological changes in an ox infected with WD-MCFV by intravenous inoculation of virulent blood (from Plowright, 1964); (+) viremia, not titrated; (×—×) viremia; (●—●) rectal temperature (in °F); I, lymph node enlargement; II, oral congestion and necrosis; III, nasal discharge; IV, peripheral corneal opacity; V, complete corneal opacity.

majority of experimental transmission series in cattle have relied on subinoculation of large volumes (usually 0.5-1) of freshly collected blood or, in one case, of separated "mononuclear" cells (Liggitt et al., 1978). In rabbits mixed tissues, including blood, have usually been employed for transmission (Buxton and Reid, 1980; Westbury and Denholm, 1982; Reid et al., 1986).

Immune pathogenesis

There is increasing evidence that the disease MCF is an immunopathological condition. The main arguments for this are:

— The lesions are not attributable directly (in WD-MCF) to the causal herpesvirus, as shown by the virtual absence of viral antigens and virions.

— The rather prolonged incubation period and prepatent viremia may suggest a requirement for sensitization to viral or virus-associated antigens.

— The lesions show some similarity to those of graft-versus-host disease and other possibly virus-induced autoimmune and lymphoproliferative conditions such as Behcet's syndrome (Liggitt et al., 1978; Liggitt and DeMartini, 1980a, b).

— The invariable temporal and spatial association of lymphocytic infiltration with the vascular and epithelial lesions (Liggitt and DeMartini, 1980a, b) fits in well with the demonstration by Reid et al. (1983) that SA-MCF infectivity is

present in lines of cultured rabbit T lymphocytes, which also have natural killer cell activity and large dense cytoplasmic granules (Reid et al., 1983, 1986; Reid and Buxton, 1985). According to these workers the essential defect in MCF pathogenesis appears to be immune deregulation attributable to virus infection causing dysfunction of natural killer cells and uncontrolled proliferation of lymphoblastoid elements in many tissues.

Others have suggested that the lesions of MCF may be due to a type III, or Arthus-like, hypersensitivity reaction with deposition of antigen–antibody complexes, e.g. in vessel walls (Rweyemamu et al., 1976) or to a combination of this with a type IV, delayed T-cell mediated response. With respect to the former suggestion, the minor participation of polymorphs, the absence of a hypergammaglobulinemia and failure to demonstrate IgG or complement in the walls of blood vessels and glomeruli do not lend support (see Plowright, 1984). However, Patel and Edington (1982b) found immune complexes in both sites, containing IgG, C3 and conglutinin, as also a depletion of circulating levels of the two latter. These authors suggested that extensive binding of conglutinin masked the complement which had been sought by others; their observations and conclusions have yet to be confirmed.

DISEASE SIGNS

Götze (1930) described four clinical forms of SA-MCF in Europe, termed peracute, "head-and-eye", intestinal and mild; whereas all of these undoubtedly occur, the majority of naturally occurring cases probably belongs to the "head-and-eye" category. The disease produced by WD-MCFV is overwhelmingly of this type, but "mild" cases have been reported by several workers and some of the diagnoses are supported by virological data (e.g. Plowright et al., 1972; Kalunda et al., 1981a). In addition there is a category of "delayed" disease which is encountered in experimental animals rendered partially resistant by previous exposure to African MCFV; it is characterized not only by incubation periods of 2 months and more but particularly by an ulcerative enteritis with perforating peritonitis (W. Plowright, unpublished data, 1966–1970; Rweyemamu et al., 1976). There is no doubt that the proportion of peracute and enteric cases is higher in cattle infected naturally or experimentally with SA strains (James et al., 1975; Liggitt et al., 1978; Pierson et al., 1979) and in several species of deer (Huck et al., 1961; Reid et al., 1979). Bali cattle (*Bos javanicus*) are especially prone to develop very rapid disease with poorly developed clinical signs at death (Daniels et al., 1988).

In the most easily recognized "head-and-eye" form, the disease is usually sudden in onset and manifested by serous or sero–mucoid ocular and nasal discharges, severe congestion of the scleral vessels and congestion of the conjunctival, nasal and oral mucosae. The rectal temperature is raised and palpation often reveals a firm, slightly painful enlargement of the superficial lymph nodes; in cattle, chains of hemolymph nodes may also be detected. Anorexia is present but usually not complete until late in the clinical course. In hyperacute cases, particularly in deer, there may be sudden death, without premonitory signs, but severe depression with diarrhea or dysentery of sudden onset, accompanied by rapid loss of weight is more common. In these cases fever, lymph node enlargement and mucous discharges are less marked, but there is sometimes frequent, painful micturition. In mild cases there is moderate pyrexia, accompanied by discharges and congestion of the visible mucosae, sometimes also enlargement of lymph nodes; all these signs gradually regress during the convalescent period.

The time of onset of the lymph node enlargement is a matter of controversy

and of importance in relation to pathogenesis but of limited value in diagnosing the natural disease. In experimental infection by WD-MCFV, Daubney and Hudson (1936) and Plowright (1964) noted that enlargement occasionally became palpable 1–4 days before the onset of pyrexia; Pierson et al. (1979) stated that this event occurred regularly 5–10 days before pyrexia of ≤ 39.4°C. In the experimental SA form, Pierson et al. (1974) often noted a delay of 2–3 days after onset of fever before superficial lymphodenopathy was noted, but for Selman et al. (1978) it was the first detectable clinical abnormality, occurring in 6 out of 10 calves at 7 days after inoculation, although the "incubation period" was at least 20 days. The disparity between these results could be due to the subjective nature of assessments of the size of lymph nodes.

When inocula are injected subcutaneously, the homolateral node frequently shows distinct enlargement several days before the onset of fever (Piercy, 1952a, b; Plowright, 1964). Whatever the time of its appearance, lymph node enlargement tends to increase progressively during the clinical course of the disease. Even in the WD form it is not invariable, only 82% of 233 animals having shown this sign, 57% to a marked extent (Plowright, 1964); it is similarly variable in the SA form.

The ocular and nasal discharges which appear early in the disease become more profuse and mucopurulent as the lesions develop; they leave trails of matted hair on the cheeks or hang in viscid strings, sometimes blood-streaked, from the nostrils (Fig. 41). Examination of the muzzle often reveals congestion at an early stage, followed by exudation of serum, which coagulates and forms tightly adherent scabs, to which food and bedding may stick. Similar congestion, together with necrosis, erosion and fibrino–necrotic deposits, are seen in and around the nares. Again, these deposits are removed with difficulty and may gradually block the openings, leading to noisy dyspnea, extended head and even mouth-breathing (Fig. 42). In advanced cases the discharges and expirations are foul-smelling, containing necrotic mucosal fragments. A slight, harsh

Fig. 41. Head of an experimental case of WD-MCFV on day 11 of the disease; note ocular discharge, slight diffuse corneal opacity, mucopurulent nasal discharge and dry adherent scabs on the muzzle (ox 8522).
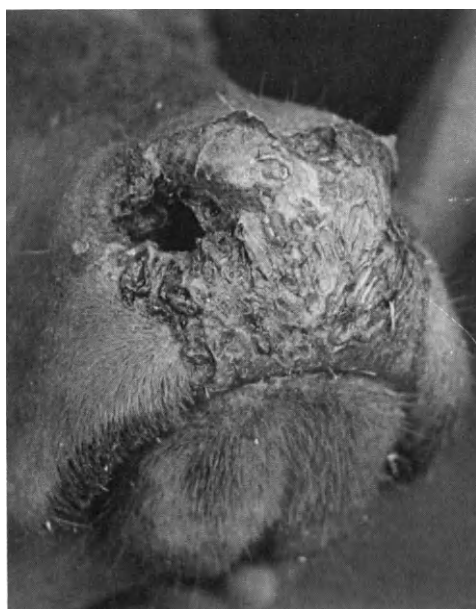
Fig. 42. Muzzle of an experimental case of WD-MCFV on day 8 of the disease; there are extensive scabs on the muzzle and around the nares, the latter partially occluded (ox 8871).

cough may be heard, presumably due to the necrotic pharyngitis, laryngitis and tracheitis which are commonly present.

The ocular discharges draw attention to the eyes where an opthalmia usually develops which is characteristic of the disease; it affected 97% of 234 WD cases (Plowright, 1964) but may be much less frequently recorded in SA outbreaks. A corneal opacity begins near the limbus as a thin grey line, usually on day 2–5 of the fever (Table 15). It spreads centripetally (Fig. 43) and may eventually involve the whole of the cornea, although this is by no means constant; an iridocyclitis with exudate in the anterior chamber is frequently seen and sometimes anterior or posterior synechiae occur. In a few cases staphyloma, glaucoma or corneal vascularization develop late in the disease. The eye lesions are accompanied from the beginning by intense photophobia and pain on handling the head.

The early congestion of the visible oral mucosae is accompanied or followed by foci of superficial necrosis and erosion (see Table 15), especially seen on the gums and buccal papillae. The latter are often intensely congested or hemorrhagic with necrosis of the tips, followed by erosion and blunting. No animal with the fatal WD form failed to develop this form of stomatitis (Fig. 44). Epithelial necrosis and erosions can also be found on the undersurface of the free part of the tongue, the hard palate and dental pad. The oral lesions lead to excessive, often frothy, salivation, less profuse and "ropy" than that in

TABLE 15

The frequency and time of appearance of clinical signs of WD-MCF in experimental cattle (from Plowright, 1964)

Clinical sign	No. of animals	Cumulative % with lesion on given day of disease						
		1	2	3	4	5	6	⩾7
Corneal opacity	234	4	25	55	80	90	94	97
Oral necrosis and erosion	116	58	76	91	97	99	100	-

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Fig. 43. Eye lesions in ox 8651, on day 5 of WD-MCFV reaction; ocular discharges adhere to the eyelids and stream down the face; corneal opacity and hypopyon are extensive.
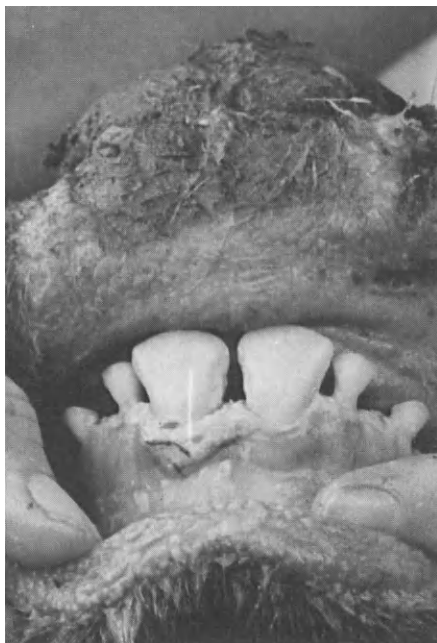
Fig. 44. Necrotic deposits on epithelia around incisors and on lower lip and gum; dried deposits on muzzle and erosions on dental pad; day 8 of disease (ox 9158).

foot-and-mouth disease; there is also pain when the mouth is examined and some difficulty with eating, but complete anorexia is not usually recorded until near death.

Diarrhea or dysentery are very infrequent in WD-MCF but may be signs in peracute SA-MCF, especially in deer (James et al., 1975; Pierson et al., 1979; Reid et al., 1979). Constipation is a common feature of WD-MCF, persisting throughout the course of the disease.

Skin lesions were frequently recorded in SA-MCF in Europe but were not observed by the majority of those working with the WD form; only two cases of extensive dermatitis in calves (0.25% of all) were seen by Plowright (1964) in many hundreds of experimental cattle. An exudative dermatitis affecting the skin between the accessory digits and around the bulbs of the heels was reported by Selman et al. (1978) before the onset of pyrexia in experimental calves. In naturally occurring SA cases the dermatitis affected the skin of the vulva, scrotum, body, teats and lower parts of the limbs (Selman et al., 1974); these authors, incidentally, published excellent color photographs of many clinical signs. Laminitis or separation of the hooves and loss of the horns have been observed in a few animals.

Nervous signs are not infrequent in both WD- and SA-MCF. They usually take the form of extensive muscular tremors, readily seen in muscles behind the shoulder and in front of the femur. Incoordination, head pressing, nystagmus, twitching of the ears and aggressive behaviour have been reported and torticollis is occasionally seen. These signs probably occur more frequently in natural than in experimental cases.

The hematological changes in MCF of cattle include a leukopenia, with total counts beginning to fall at about the time of or just before the onset of pyrexia and sometimes sinking within 4–5 days to 2000–3000 cells/mm^3. There is a relative lymphocytosis in the majority of animals, which may attain 95–97%, accompanied by a decrease in the number of small lymphocytes and a marked increase in "immature" lymphoblastoid cells. The small lymphocytes which persist are often shrunken and pyknotic with very little cytoplasm; the large forms resemble those which occur in enormous numbers in many tissues, especially the lymph nodes. Rabbits sometimes exhibit massive terminal leukocytosis, with flooding of the circulation by lymphoblastoid cells and up to 5% of macrophages, some laden with debris. There are no important changes in erythrocyte count, unless severe diarrhea or dysentery intervene (Plowright, 1953b).

The urine frequently contains protein and blood as a result of the mucosal petechiae and erosions present in the bladder and the extensive tubular and glomerular changes which often develop in the kidneys.

In the cerebrospinal fluid there is a marked pleocytosis due to enlarged mononuclear cells, and occasionally some neutrophils; there is also an increased total protein content (Liggitt et al., 1978).

PATHOLOGY

Gross changes

In addition to the clinically detectable lesions of MCF, autopsy reveals the following:

Alimentary tract. Epithelial necrosis and erosions, sometimes followed by ulceration, occur in the pharynx, on the soft palate, esophagus, on the folds of the reticulum and the pillars of the rumen. The palatal tonsil is usually enlarged and the crypts filled with mucopus. In the abomasum, congestion of the fundic folds and hemorrhagic erosions of the pyloric region are common. The intestines, particularly the cecum and upper colon, exhibit congestion and mucosal petechiae, which turn black along the crests of folds. In the intestinal form the contents are blackened or contain fresh blood and an excess of mucus. Congestion of the omentum and a slight peritoneal exudate are frequently

encountered. The liver usually shows a fine regular and greyish mottling or small (1–2 mm) rounded foci attributable to cellular infiltration.

Respiratory tract. Congestion, with necrosis, diptheritic membranes, erosion and hemorrhagic areas are present on the turbinates and septum in the great majority of cases of the head-and-eye form. Pseudomembranes also form on the vocal cords of about 80–90% of such cases. A similar change with hemorrhages is not infrequent in the trachea. Congestion and edema of the lungs and a patchy bronchopneumonia, with slight pleural exudate, are present in about 40% of animals which die.

Urogenital tract. In natural cases especially, the kidneys are swollen with irregular cortical mottling and wedge-shaped infarct-like areas of dense, cellular infiltration or necrosis. The mucosa of the urinary bladder is usually congested with areas of hemorrhage and erosion. A vaginitis with congestion, necrosis and erosion of the mucosa and slight mucopurulent exudate is also seen.

Lymphopoietic system. About 80% of all cases probably show generalized lymph node enlargement, which is particularly marked in the head and neck region and in visceral nodes such as the bronchials or renals. The glands are firm, fleshy, sometimes surrounded by a gelatinous edema; in cattle they usually show little congestion or hemorrhage, except sometimes in the medulla and in the nodes of the pharyngeal region. On incision, an excess of lymph escapes, sometimes forming a soft fibrinous clot. The spleen is slightly to moderately enlarged, the Malpighian corpuscles frequently prominent and up to 2–3 mm in diameter; the same applies to hemolymph nodes.

Miscellaneous. The adrenal glands often show a greyish infiltration, with hemorrhage, in the capsular and cortical areas. Occasionally the organ is visibly enlarged and friable. An extensive synovitis has also been reported in some cases (Liggitt et al., 1980).

Histopathology

There are three essential components in the genesis of the very widespread and severe lesions which characterize MCF of whatever origin in any indicator host (Plowright, 1953a). These are:

(i) a destruction of smaller lymphocytes, especially in the lymphopoietic tissues;

(ii) a proliferation and infiltration in many organs of larger lymphoblastoid cells, primarily in perivascular locations; and

(iii) an angiitis, affecting all components of the walls of arteries and veins. There is no histological or cytological evidence that any of these processes is due directly to a herpesvirus in either form of the disease.

Lymphocyte destruction is particularly marked in germinal follicles of the cortex of lymph and hemolymph nodes (Fig. 45). It is manifested particularly by karyorrhexis and by macrophages ingesting cell debris; in some cases large areas of the outer cortex show a diffuse necrosis. The destruction in these areas contrasts with a dense, diffuse proliferation of lymphocytic and lymphoblastoid cells in the interfollicular and paracortical areas (Fig. 46). In the medulla of nodes the cell cords are thickened and the sinuses engorged with increased numbers of enlarged reticulum and lymphoid elements as well as numerous macrophages.

The angiitis is segmental and irregular in distribution, most readily seen in
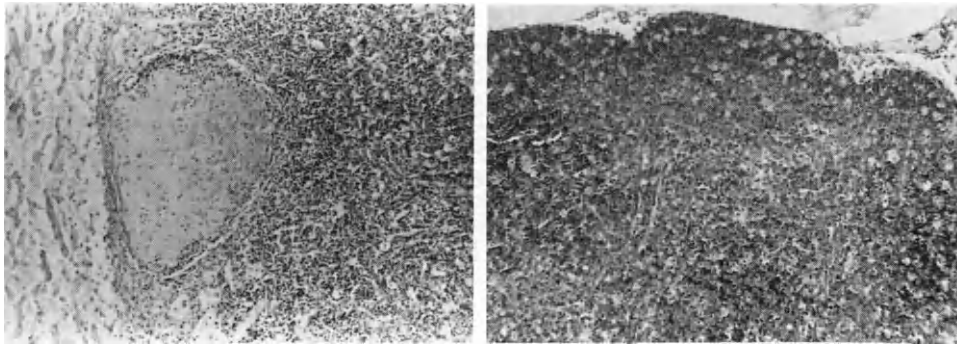
Fig. 45 (left). Diffuse necrosis of a lymphoid follicle in a bovine hemolymph node (ox 7129); H & E (× 120).

Fig. 46 (right). Depletion of small lymphocytes in subcortical areas with numerous macrophages appearing as partially cleared holes; dense lymphoblastoid infiltration centrally (rabbit 91); H & E (× 120).

medium-sized arteries (Figs. 47, 48) invariably accompanied by perivascular and intramural infiltration of mononuclear cells — lymphocytes, lymphoblasts and macrophages — but with few polymorphs. There is frequently a striking degeneration, often fibrinoid in character, of infiltrated connective tissue and smooth muscle elements of vessel walls (Fig. 49). The endothelial cells are enlarged and/or hyperplastic, forced up into irregular projections by an underlying infiltration of lymphoid elements or cells orientated radially into the lumen; occasionally lymphoid cells accumulate in the lumen (Fig. 50). The increased cellularity of the intimal layer is, according to Denholm and Westbury (1979) and Liggitt and DeMartini (1980a), due to lymphocytes rather than endothelial elements, a statement confirmed through EM by the latter authors. Previous reports emphasizing endothelial hyperplasia (e.g. Selman et al., 1974, Plowright, 1981) are probably misleading. In spite of these changes in the intima, thrombosis is a relatively rare event, though it does occur and may lead to infarction.

Changes similar to those seen in the walls of blood vessels also occur in connective tissues of the capsule and trabeculae of lymph nodes and spleen; they may even extend into the perinodal connective and adipose tissues. In hollow viscera, such as the intestines, similar perivascular infiltration and degeneration occur in smooth muscle layers.

Vascular lesions appear to have predilection sites, namely the kidney (the
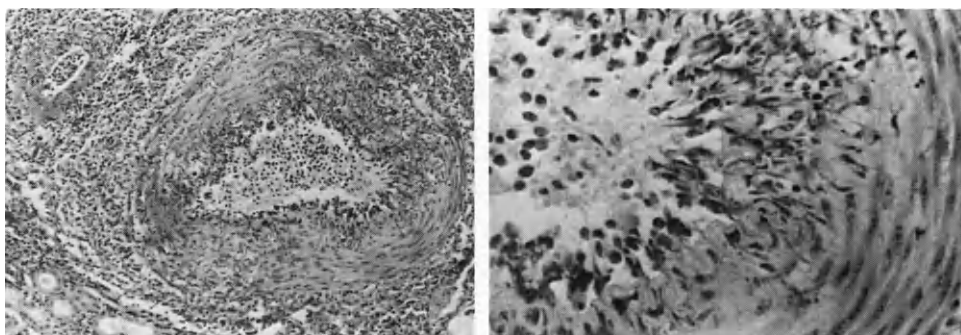
Fig. 47 (left). An arcuate artery in the kidney of an ox (7129) with severe changes in all layers of the wall; H & E (× 120).

Fig. 48 (right). An arcuate artery in the kidney of an ox (7129); intimal lesions, including radial orientation of infiltrating cells and disruption of endothelium; H & E (× 500).
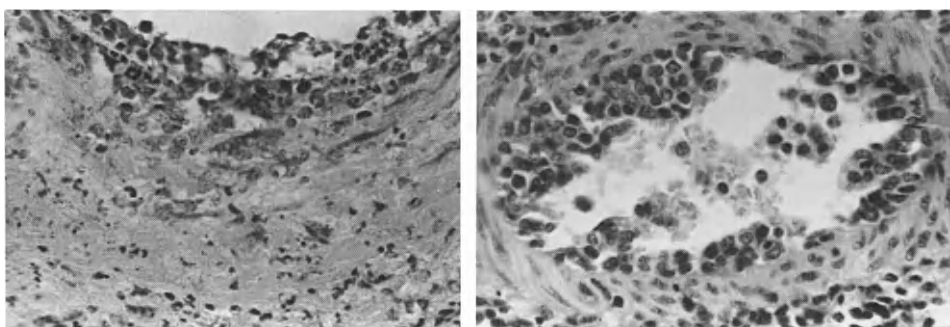
Fig. 49 (left). Diffuse fibrinoid change in the tunica media of a medium-sized artery in the adrenal capsule (ox N41, SA-MCFV); infiltration and irregular thickening of intima; H & E (× 500).

Fig. 50 (right). A small artery in the submucosa of the cecum of a calf (7006); irregular protrusions into lumen formed by lymphoid and endothelial cells; H & E (× 500).

arcuate vessels particularly), brain and meninges, the portal triads, lung, capsule of lymph nodes and adrenal gland and the carotid rete (Liggitt et al., 1978; Liggitt and DeMartini, 1980a).

The processes (ii) and (iii) occur in the lamina propria and submucosa of many body surfaces, where they are associated with the degeneration and necrosis of surface epithelia (Figs. 51, 52) and subjacent glands. In parenchyma-
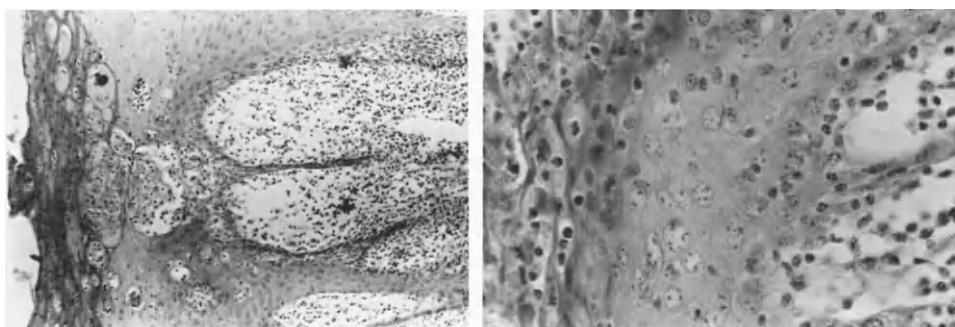
Fig. 51 (left). Tip of the tongue of a calf (7136) with subepithelial edema and cellular infiltration; acantholytic foci with degenerating and lymphoid cells in the epithelium; H & E (× 125).

Fig. 52 (right). Enlargement of Fig. 51, showing mononuclear cells invading the lamina propria, basement membrane and epithelium; a few polymorphs are present towards the surface.
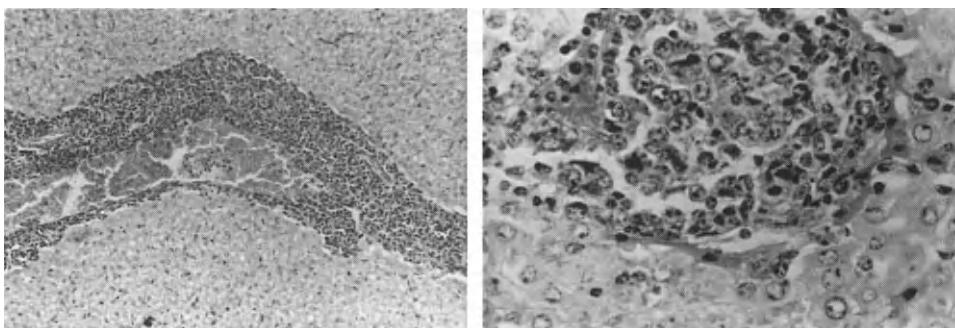
Fig. 53 (left). Liver of a calf (7006) with heavy infiltration by lymphoid cells, virtually virtually obliterating the walls of a verin; H & E (× 100).

Fig. 54 (right). Liver of an ox (7204) with lymphoblastoid infiltration of the portal tract accompanied by compression and degeneration of adjacent liver parenchymal cells (× 500).

tous organs, such as the liver and kidneys, they are accompanied by similar changes in adjacent specialized structures, e.g. kidney tubules and, to a lesser extent, hepatic cell cords (Figs. 53, 54).
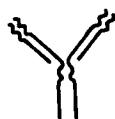
IMMUNE REACTION

The few cattle that survive an infection with virulent WD-MCFV are resistant to parenteral challenge for several years, if not for life; there are apparently no immunological differences between isolates. In a few cases, animals apparently protected by attenuated live virus (Reid and Rowe, 1973) succumbed to a modified form of the disease after a prolonged interval (Rweyemamu et al., 1976). There is only one record of susceptible rabbits having survived an infection with cell-associated MCFV.

Studies of neutralizing antibodies were first made possible by the development of cell-free strain of WD-MCFV, propagated in calf thyroid monolayers (Plowright et al., 1965; Plowright, 1967); the technique was subsequently adapted to microtiter trays and embryonic calf kidney cells (Mushi and Plowright, 1979). Cattle which recover from infection develop neutralizing antibodies in their serum, the titer of which is usually low ($\leqslant 20$) unless the animals are challenged parenterally, in which case there is frequently a considerable boost (Reid and Rowe, 1973). Neutralizing antibody appears towards the end of the incubation period in the majority of rabbits infected intraperitoneally with virulent spleen suspensions (Rossiter et al., 1977). Experimental cattle usually do not develop this antibody before convalescence from the experimental disease (Plowright, 1968; Rossiter et al., 1977). Nevertheless, it was detected in some reacting American cattle (Kalunda et al., 1981a) and in 6 of 13 naturally occurring fatal cases (Rossiter et al., 1980). It is evident that the development of VN antibodies does not avert the fatal outcome of the disease.

Tests for neutralizing antibody have been employed to study the host range of WD-MCFV and related viruses in wild and captive ungulates, including particularly members of the subfamilies Alcelaphinae, Hippotraginae and Caprinae (Ramsey et al., 1982; Hamblin and Hedger, 1984; Heuschele et al., 1984; Plowright, 1984, 1986). Passively acquired (colostral) antibody does not protect the young against natural infection.

Indirect IF tests are more sensitive than VN for detecting antibodies to WD-MCFV. Using productively infected calf kidney or thyroid cells as antigen, antibodies are demonstrable by IF in cattle or rabbits 5–7 days before the onset of pyrexia and increase continuously until death. However, there are low-level reactions between antisera to the bovine viruses IBRV (BHV-1) and BHV-3 (Movar type) and MCFV antigens, but the titers are 16–32 times lower than against the homologous antigens. Serological relatedness of BHV-3 and WD-MCFV has been confirmed by Rossiter et al. (1988), who also demonstrated an immunological cross-reaction between these viruses (see also Rossiter, 1985).

If cultured cells are treated with cytosine arabinoside (Ara-C) no infectious virus is produced but two "early" antigens develop, one of which is diffuse and found throughout the cell (DEA), the other being particulate and intranuclear (PEA). Antibody to DEA is found only in hyperimmunized animals, whereas activity against PEA appears later and to lower titer than that to "late" antigens in cattle, whilst in wildebeest it was transitory in calves (Rossiter et al., 1978, 1983).

By indirect IF test, Rossiter et al. (1980) found higher levels of antibodies in the sera of natural bovine cases in Kenya than in any other group of normal cattle and suggested that this test could have diagnostic value. It was later reported that the IIP technique, with infected calf kidney cells as antigen

substrate, detected antibody in all of 23 naturally infected or experimental bovine cases, titers being 8-fold higher than the corresponding indirect IF figures (Rossiter, 1981a).

Sheep sera from Australia, Great Britain, Austria and Kenya possessed indirect IF (late antigen) activity in 162/167 cases. It was present even in some gnotobiotic and colostrum-deprived lambs, which suggests widespread perinatal or in utero infection; antibody to the PEA component was also demonstrated. Blocking and adsorption experiments, as well as the similarity in distribution of the antibody in sheep and wildebeest sera, suggested that it was induced by a virus related to that of WD-MCFV (Rossiter, 1981b).

The class-specific antibody responses to infection in rabbits were investigated by Rossiter (1982a) using IIP tests; both IgG and IgM appeared 2–6 days before pyrexia, the former increasing at least four times more quickly as the disease progressed. Complement-fixing antibodies developed in the late incubation period both in cattle and rabbits; their demonstration may require a normal calf serum (Clq) supplement (Hamdy et al., 1980; Rossiter and Jessett, 1980).

Little is known about CMI in MCF, although the failure of neutralizing antibodies to protect suggests that it is the most important part of the immune response. The response of whole-blood leukocytes of reacting rabbits to nonspecific B- or T-cell mitogens was depressed but washed lymphocytes reacted as expected, also to viral antigens (Wilks and Rossiter, 1978).

Delayed hypersensitivity to MCFV antigens injected intradermally has been reported and also lymphocyte-mediated cytolysis of MCFV-infected cells (see Rossiter, 1985).

LABORATORY DIAGNOSIS

The initial diagnosis of MCF rests on clinicopathological data which have been described; the histopathology is fortunately unique and pathognomonic (see, for example, Jubb and Kennedy, 1976), as virological confirmation is still impossible for the SA form.

Virological diagnosis of WD-MCF is now well-established and based on the recovery of virus from sick or moribund, killed animals, and on the demonstration of specific antibodies in the serum — especially if collected late in the disease course or during convalescence in the very few survivors.

In live animals blood taken at any time during the clinical course of the disease should contain virus; the titer is usually in excess of $10^{2.0}$ TCID$_{50}$/ml at collection. When animals are killed, or dead for periods not exceeding 1–2 h, the solid tissues of choice for virus isolation are lymph nodes (such as prescapular, which may have titers $\geq$ 10^5 TCID$_{50}$/g) thymus or spleen. For blood, an anticoagulant devoid of preservatives should be employed; a solution of EDTA gives better preservation than heparin or sodium citrate. One-third of the final volume of 1.5% (w/v) EDTA in 0.7% NaCl, with antibiotics, should be used and the mixture placed immediately on ice (4°C). The buffy coat should be separated by centrifugation as soon as possible and resuspended in the maintenance medium used for roller tube cultures of bovine thyroid or testis cells.

Solid lymphoid tissues should be collected aseptically, finely chopped with scalpels, and reduced to a well-dispersed suspension in culture maintenance medium, using a glass tissue grinder. Samples kept for more than 3 days at 4°C are unlikely to yield virus. The thyroid cells should be dense primary or secondary monolayers; testis cells should be of a strain which had been passaged approximately 5–20 times in vitro.

In the USA a strain of fetal kidney cells from a Barbary sheep (aoudad) was

reported to be sensitive for virus isolation (Heuschele and Fletcher, 1984).

Whether buffy-coat cells or solid tissues are used for virus isolation, it is advisable to use a series of dilutions of the material, as heavy concentrations may be inhibitory to virus replication (Plowright, 1986). Some workers have advocated the fusion by polyethylene glycol of suspect cell suspensions and permissive cell cultures as a means of facilitating virus isolation (Castro et al., 1984).

A CPE, consisting of syncytial (multinucleate cell) foci, is detectable microscopically after as little as 3 days at 37°C with large inocula, but may be delayed for up to 3 weeks when infectivity is minimal. In thyroid cultures the syncytia detach, leaving a narrow border, and the number of foci does not increase on prolonged incubation. In testis cell monolayers the retracting syncytia have long, refractile processes and secondary foci appear; detection of virus is, therefore, facilitated. The identity of any viral agent isolated is confirmed by specific IF tests using acute-phase, immune or "hyperimmune" sera prepared in rabbits or cattle. Neutralization is not possible until cell-free virus is available; this is the case earlier when calf testis monolayers are used for propagation, or a lower temperature of inoculation, 32°C (see p. 126).

Where cell cultures are not available, rabbit inoculation can be employed, the intraperitoneal and intracerebral routes being most frequently successful. Large volumes can be injected into the abdominal cavity, e.g. 2–5 ml of a 10% w/v suspension of lymph node. A record of rectal temperature should be maintained until the animal dies or for up to 2 months in any case. The clinical signs in rabbits, which should be examined daily, include ocular and nasal discharges, enlargement of superficial lymph nodes and death within 3–5 days. Calf inoculation is occasionally carried out using blood intravenously or lymphoid tissue suspensions subcutaneously. Intraglandular inoculations, as sometimes practiced, are unnecessary.

When rabbits or cattle show typical reactions blood can be taken for virus isolation in cell culture, or an animal can be killed to harvest tissues for passage or storage with cryoprotectants at −70°C or below. After fixation, it is necessary to confirm the presence of a characteristic histopathology.

Serum obtained during the acute, usually fatal, course of the clinical disease contains specific antibodies in a proportion of cases, IIP tests being the most sensitive (Rossiter, 1981a). The earliest to appear is antibody reacting in IIP or IF tests, but care must be taken to exclude low dilution reactions with other herpesviruses. Virus-neutralizing antibody is present in a proportion of cattle which die of the disease; similarly, complement-fixing antibodies have been demonstrated in many reacting cattle (Rossiter and Jessett, 1980).

There are no diagnostic tests at present which depend on the demonstration by serological means of viral antigens in infected tissues. It is possible that DNA probes will soon become available for the detection of viral genomes in tissues infected by either WD- or SA-MCFV (Bridgen et al., 1986). It is now also possible to isolate lines of large granular lymphocytes with NK (natural killer cell) activity from tissues of cattle and deer with SA-MCF, especially lymphoid tissues, cornea and cerebrospinal fluid. Unfortunately no antigens of MCFV were detectable in them and they were irregularly infectious for deer and rabbits only (Reid et al., 1989).

PROPHYLAXIS AND CONTROL

Nearly all attempts to immunize cattle against WD-MCF, using virus which may have been attenuated, have essentially failed. A claim by Hamdy et al. (1978) to have isolated a virus from a fatal case of SA-MCF in Minnesota and

to have immunized cattle with it after a few culture passages awaits confirma-
tion. After serial passaging for up to 75 times in rabbits, or in bovine thyroid
and kidney-cell cultures for up to 59 times, the virus either failed to infect and
immunize or, occasionally, caused only delayed but still fatal reactions (Plow-
right, 1968). Reid and Rowe (1973) investigated a hartebeest isolate which after
30 monolayer passages did not cause reactions in six inoculated cattle; two out
of three of them were later immune to homologous virulent virus, but the other
three were susceptible to a wildebeest isolate. One of the two survivors, how-
ever, when challenged with wildebeest virus, appeared at first to be resistant
but later succumbed to delayed disease (Rweyemamu et al., 1976).

A number of attempts have been made to protect cattle and rabbits against
challenge with virulent WD-MCFV, using inactivated virus preparations (see
Plowright, 1968). The antigens employed in cattle were culture-propagated
"attenuated" or virulent virus, formalin-inactivated and combined with an
oil/water adjuvant; although very high levels of neutralizing antibody de-
veloped, there was no resistance to parenteral challenge by cell-free or cell-
associated virus (Plowright et al., 1975). In rabbits, similar inactivated anti-
gens have provided protection against cell-free virus given intravenously, but
not against virulent spleen-cell suspensions (Edington and Plowright, 1980;
Russell, 1980). Rossiter (1982b) found that several inoculations of fixed or living
cultured cells (RK13) expressing viral antigens on their membranes and com-
bined with "reversed" Freund's adjuvant (double emulsion), immunized rabbits
against challenge at 47 weeks with virulent cell suspensions. At 90 weeks
protection was no longer demonstrable. Attempts to protect cattle and rabbits
against parenteral challenge using cell-associated virus by vaccination with
fixed virulent tissue suspensions, or even living "attenuated" cells, have con-
sistently failed, in spite of the development of high-titer antibodies and delayed
cutaneous hypersensitivity (Plowright, 1968; Rossiter, 1982b, 1985).

To conclude, inactivated or live viral preparations with suitable adjuvants
do induce antibodies to MCFV, including those neutralizing the virus. How-
ever, whilst protection against parenteral challenge with cell-free virus is
sometimes demonstrable in rabbits, there is little evidence for protection
against cell suspensions infected with virulent virus. Claims to have isolated
viruses safely attenuated for cattle, which confer a regular immunity to nat-
ural or experimental challenge, have yet to be substantiated. Similarly the
resistance of cattle (2/3) given multiple inoculations of BHV-3 (bovine
cytomegalovirus) needs to be confirmed (Rossiter et al., 1988).

In the absence of vaccines, the use of which could seldom be justified by the
losses incurred, the prevention of MCF rests simply and reliably on the preven-
tion of close contacts between susceptible and "reservoir" hosts. In the major-
ity of cases outside Africa, this means the avoidance of housing or herding
together of sheep and cattle or deer; in a small minority there may be a
possibility of indirect contagion over short distances (Reid et al., 1979) or of
reservoir hosts other than sheep.

In Africa, the seasonal problem of contact on extensive grazings — between
migratory wildebeest herds and the cattle of semi-nomadic pastoralists or
ranchers — is much more difficult to combat. The costs of effective fencing to
separate the species are very high, and objections to the solution of eliminating
the wildebeest are increasingly voiced by conservationists.

Less frequently, severe and important losses due to MCF occur in zoological
collections, where again the only practical solution is to separate the reservoir
host, usually wildebeest, from the many susceptible species (Heuschele, 1982;
Heuschele et al., 1984).

REFERENCES

Bridgen, A., Herring, A.J. and Reid, H.W., 1986. The molecular biology of alcelaphine herpesvirus 1 (AHV-1). 11th Int. Herpesvirus Workshop, Cambridge, Abstr. 345.

Buxton, D. and Reid, H.W., 1980. Transmission of malignant catarrhal fever to rabbits. Vet. Rec., 106: 243–245.

Castro, A.E. and Daley, G.G., 1982. Electron microscopic study of the African strain of malignant catarrhal fever virus in bovine cell cultures. Am. J. Vet. Res., 43: 576–584.

Castro, A.E., Daley, G.G., Zimmer, M.A., Whitenack, D.L. and Jensen, J., 1982. Malignant catarrhal fever in Indian gaur and greater kudu. Experimental transmission, isolation and identification of a herpesvirus. Am. J. Vet. Res., 43: 5–11.

Castro, A.E., Ramsey, E.C., Dotson, J.F., Schramke, M.L., Kocan, A.A. and Whitenack, D.L., 1984, Characteristics of the herpesvirus of malignant cararrhal fever isolated from captive wildebeest calves. Am. J. Vet. Res., 45: 409–415.

Clark, K.A., Robinson, R.M., Marburger, R.G., Jones, L.P. and Orchard, J.H., 1970. Malignant catarrhal fever in Texas cervids. J. Wildl. Dis., 6: 373–383.

Daniels, P.W., Sudurisman, Wiyono A. and Ronohardjo, P., 1988. Epidemiological aspects of malignant catarrhal fever in Indonesia. In: Malignant Catarrhal Fever in Asian Livestock. Australian Centre for International Agricultural Research, Canberra, pp. 20–31.

Danskin, D., 1955. Elementary bodies in bovine malignant catarrh. Nature, 176: 518.

Daubney, R., 1959. Suspected bovine malignant catarrh. Vet. Rec., 71: 493.

Daubney, R. and Hudson, J.R., 1936. Transmission experiments with bovine malignant catarrh. J. Comp. Pathol., 49: 63–89.

De Kock, G. and Neitz, W.O., 1950. Sheep as a reservoir host of snotsiekte (or bovine malignant catarrh of cattle) in South Africa. S. Afr. J. Sci., 46: 176–180.

Denholm, L.J. and Westbury, H.A., 1982. Malignant catarrhal fever in farmed rusa deer (*Cervus timorensis*). Clinico-pathological observations. Aust. Vet. J., 58: 81–87.

Edington, N. and Patel, J., 1981. The location of primary replication of the virus of bovine malignant catarrhal fever in rabbits. Vet. Microbiol., 6: 107–112.

Edington, N. and Plowright, W., 1980. The protection of rabbits against the herpesvirus of malignant catarrhal fever by inactivated vaccines. Res. Vet. Sci., 28: 384–388.

Edington, N., Patel, J., Russell, P.H. and Plowright, W., 1979. The nature of the acute lymphoid proliferation in rabbits infected with the herpesvirus of bovine malignant catarrhal fever. Eur. J. Cancer, 15: 1515–1522.

Fletcher, T.J., 1982. Management problems and disease in farmed deer. Vet. Rec., 111: 219–223.

Götze, R. and Liess, J., 1929. Erfolgreiche Übertragungsversuche des bösartigen Katarrhalfiebers von Rind zu Rind. Indentität mit der Südafrikanischen Snotsiekte, Dtsch. Tierärztl. Wochenschr., 37: 433–437.

Götze, R., 1930. Untersuchungen über das bösartige Katarrhalfieber des Rindes III. Dtsch. Tierärztl. Wochenschr., 38: 487–491.

Götze, R., 1932. Bösartiges Katarrhalfieber IV. Mitteilung. Berl. Tierärztl. Wochenschr., 53: 848–855.

Götze, R. and Liess, J., 1930. Untersuchungen über das bösartige Katarrhalfieber des Rindes II. Schafe als Überträger. Dtsch. Tierärztl. Wochenschr., 37: 433–437.

Hamblin, C. and Hedger, R.S., 1984. Neutralizing antibodies to wildebeest-derived malignant catarrhal fever virus in African wildlife. Comp. Immunol. Microbiol. Inf. Dis., 7: 195–199.

Hamdy, F.M., Dardiri, A.H., Mebus, C., Pierson, R.E. and Johnson, D., 1978. Aetiology of malignant catarrhal fever outbreak in Minnesota. Proc. 82nd Annu. Meet. U.S. Anim. Health Assoc., pp. 248–267.

Hamdy, F.M., Dardiri, A.H. and Ferris, D.H., 1980. Complement fixation test for diagnosis of malignant catarrhal fever. Proc. 87th Annu. Meet. U.S. Anim. Health Assoc., pp. 329–338.

Harkness, J.W. and Jessett, D.M., 1981. Influence of temperature on the growth in cell culture of malignant catarrhal fever virus. Res. Vet. Sci., 31: 164–168.

Heuschele, W.P., 1982. Malignant catarrhal fever in wild ruminants — a review. Proc. 86th Annu. Meet. U.S. Anim. Health Assoc., Nashville, TN, pp. 552–550.

Heuschele, W.P. and Fletcher, H.R., 1984. Improved methods for the diagnosis of malignant catarrhal fever. 27th Annu. Proc. Am. Assoc. Vet. Lab. Diagnosticians, pp. 137–150.

Heuschele, W.P., Fletcher, H.R., Oosterhuis, J., Janssen, D. and Robinson, P.T., 1984. Epidemiological aspects of malignant catarrhal fever in the USA. Proc. 88th Annu. Meet. U.S. Anim. Health Assoc., pp. 640–651.

Huck, R.A., Shand, A., Allsop, P.J. and Paterson, A.B., 1961. Malignant catarrh of deer. Vet. Rec., 73: 457–465.

James, M.P., Neilson, F.J.A. and Stewart, W.J., 1975. An epizootic of malignant catarrhal fever. I. Clinical and pathological observations. N.Z. Vet. J., 23: 7–12.

Jesse, F., 1930. Experimentelle Übertragung des bösartigen Katarrhalfiebers durch gesunde Schafe auf Rinder. Versuch auf dem Lehrgut Adendorf. Inaug. Diss., Univ. Hannover, 67 pp.

Jubb, K.V.F. and Kennedy, P.C., 1976. Pathology of Domestic Animals. Vol. 2. Academic Press, New York, London, 2nd edn., pp. 27–34.

Kalunda, M., Dardiri, A.H. and Lee, K.M., 1981a. Malignant catarrhal fever. 1. Response of American cattle to malignant catarrhal fever virus isolated in Kenya. Can. J. Comp. Med., 45: 70–76.

Kalunda, M., Ferris, D.H., Dardiri, A.H. and Lee, K.M., 1981b. Malignant catarrhal fever. III. Experimental infection of sheep, domestic rabbits and laboratory animals with malignant catarrhal fever virus. Can. J. Comp. Med., 45: 310–314.

Liggitt, H.D. and DeMartini, J.C., 1980a. The pathomorphology of malignant catarrhal fever. I. Generalized lymphoid vasculitis. Vet. Pathol., 17: 59–73.

Liggitt, H.D. and DeMartini, J.C., 1980b. The pathomorphology of malignant catarrhal fever. II. Multisystemic epithelial lesions. Vet. Pathol., 17: 74–84.

Liggitt, H.D., DeMartini, J.C., McChesney, A.E., Pierson, R.E. and Storz, J., 1978. Experimental transmission of malignant catarrhal fever in cattle: Gross and histopathological changes. Am. J. Vet. Res., 39: 1249–1257.

Liggitt, H.D., DeMartini, J.C., Storz, J. and Coulter, G.R., 1980. Synovitis and bovine syncytial virus isolation in experimentally induced malignant catarrhal fever. J. Comp. Pathol., 90: 519–533.

McCallum, H.J.F., Mavor, N.M. and Hemmingsen, P., 1982. A malignant catarrhal fever-like disease in red deer (*Cervus elaphus*) in New Zealand. N.Z. Vet. J., 39: 99–101.

Magnusson, H., 1939. Experimental transmission of bovine malignant catarrh. Abstract in Vet. Bull. (1942), 12: 213.

Maré, C.J., 1977. Malignant catarrhal fever. An emerging disease of cattle in the U.S.A. Proc. 81st Annu. Meet. U.S. Anim. Health Assoc., pp. 151–157.

Martin, W.B., Angus, K.W., Robinson, G.W. and Scott, F.M., 1979. The herpesvirus of sheep pulmonary adenomatosis. Comp. Immunol. Microbiol. Inf. Dis., 2: 313–325.

Mettam, R.W.M., 1923. Snotsiekte in cattle. Dir. Vet. Educ. Res. Union S. Africa, Report Nos. 9 and 10, pp. 395–432.

Mushi, E.Z. and Karstad, L., 1981. Prevalence of virus neutralising antibodies to malignant catarrhal fever virus in oryx (*Oryx beisa callotis*). J. Wildl. Dis., 17: 467–470.

Mushi, E.Z. and Plowright, W., 1979. A microtitre technique for the assay of malignant catarrhal fever virus and neutralising antibody. Res. Vet. Sci., 27: 230–232.

Mushi, E.Z. and Rurangirwa, F.R., 1981. Epidemiology of malignant catarrhal fever, a review. Vet. Res. Commun., 5: 127–142.

Mushi, E.Z., Karstad, L. and Jessett, D.M., 1980. Isolation of bovine malignant catarrhal fever virus from ocular and nasal secretions of wildebeest calves. Res. Vet. Sci., 29: 168–171.

Mushi, E.Z., Rossiter, P.B. and Jessett, D.M., 1981. Isolation and characterisation of a herpesvirus from the topi (*Damaliscus korrigum* Ogilby). J. Comp. Pathol., 91: 63–68.

Patel, J.R. and Edington, N., 1980. The detection of the herpesvirus of bovine malignant catarrhal fever in rabbit lymphocytes in vivo and in vitro. J. Gen. Virol., 48: 437–444.

Patel, J.R. and Edington, N., 1981. The detection and behaviour of the herpesvirus of malignant catarrhal fever in bovine lymphocytes. Arch. Virol., 68: 321–326.

Patel, J.R. and Edington, N., 1982a. The effect of antibody and complement on the expression of herpesvirus of bovine malignant catarrhal fever in cultured rabbit lymphocytes. Vet. Microbiol., 7: 325–333.

Patel, J.R. and Edington, N., 1982b. Immune complexes associated with infection of cattle by the herpesvirus of malignant catarrhal fever. Vet. Microbiol., 7: 335–341.

Piercy, S.E., 1952a. Studies on bovine malignant catarrh. I. Experimental infection in cattle. Br. Vet. J., 108: 35–47.

Piercy, S.E., 1952b. Studies on bovine malignant catarrh. II. Experimental infection in cattle. Br. Vet. J., 108: 214–220.

Piercy, S.E., 1954. Studies on bovine malignant catarrh. V. The role of sheep in the transmission of the disease. Br. Vet. J., 110: 508–516.

Piercy, S.E., 1955. Studies on bovine malignant catarrh. VI. Adaptation to rabbits. Br. Vet. J., 111: 484–491.

Pierson, R.E., Thake, D., McChesney, A.E. and Storz, J., 1973. An epizootic of malignant catarrhal fever in feed-lot cattle. J. Am. Vet. Med. Assoc., 163: 349–350.

Pierson, R.E., Storz, J., McChesney, A.E. and Thake, D., 1974. Experimental transmission of malignant catarrhal fever. Am. J. Vet. Res., 35: 523–525.

Pierson, R.M., Hamdy, R.M., Dardiri, A.H., Ferris, D.H. and Schloer, G.M., 1979. Comparison of American and African forms of malignant catarrhal fever: Transmission and clinical signs. Am. J. Vet. Res., 40: 1091–1095.

Plowright, W., 1953a. The pathology of infectious bovine malignant catarrh in cattle and rabbits. Proc. XVth Int. Vet. Congr., Stockholm, 1: 323–328.

Plowright, W., 1953b. The blood leucocytes in infectious bovine malignant catarrh of the ox and rabbit. J. Comp. Pathol., 63: 318–334.

Plowright, W., 1963. Studies on the virus of malignant catarrhal fever in Africa. Proc. XVIIth World Vet. Congr., Hannover, 1: 519–523.

Plowright, W., 1964. Studies on bovine malignant catarrhal fever of cattle. DVSc. Thesis, University of Pretoria, 336 pp.

Plowright, W., 1965a. Malignant catarrhal fever in East Africa. I. Behaviour of the virus in freeliving populations of blue wildebeest (*Gorgon taurinus taurinus*, Burchell). Res. Vet. Sci., 6: 56–68.

Plowright, W., 1965b. Malignant catarrhal fever in East Africa. II. Observations on Wildebeest calves at the laboratory and contact transmission of the infection to cattle. Res. Vet. Sci., 6: 69–83.

Plowright, W., 1967. Malignant catarrhal fever in East Africa. III. Neutralising antibody in freeliving wildebeest. Res. Vet. Sci., 8: 129–136.

Plowright, W., 1968. Malignant catarrhal fever. J. Am. Vet. Med. Assoc., 152: 795–804.

Plowright, W., 1981. Herpesviruses of wild ungulates including malignant catarrhal fever virus. In: J.W. Davis, L.H. Karstad and D.O. Trainer (Editors), Infectious Diseases of Wild Mammals. Iowa State University Press, Ames, IA, 2nd edn., pp. 126–146.

Plowright, W., 1984. Malignant catarrhal fever virus: a lymphotropic herpesvirus of ruminants. In: G. Wittmann, Rosalind M. Gaskell and H.J. Rziha (Editors), Latent Herpesviruses in Veterinary Medicine. Martinus Nijhoff, The Hague, pp. 279 305.

Plowright, W., 1986. Malignant catarrhal fever. Rev. Sci. Tech. Off. Int. Epiz., 5: 897–918.

Plowright, W., Ferris, R.D. and Scott, G.R., 1960. Blue wildebeest and the aetiological agent of bovine malignant catarrhal fever. Nature, Lond., 188: 1167–1169.

Plowright, W., Macadam, R.F. and Armstrong, J.A., 1965. Growth and characterisation of the virus of bovine malignant catarrhal fever in East Africa. J. Gen. Microbiol., 39: 253–266.

Plowright, W., Kalunda, M., Jessett, D.M. and Herniman, K.A.J., 1972. Congenital infection of cattle with the herpesvirus causing malignant catarrhal fever. Res. Vet. Sci., 13: 37–45.

Plowright, W., Herniman, K.A.J., Jessett, D.M., Kalunda, M. and Rampton, C.S., 1975. Immunisation of cattle against the herpesvirus of malignant catarrhal fever: failure of inactivated culture vaccines with adjuvants. Res. Vet. Sci., 19: 159–166.

Ramsey, E.R., Castro, A.E. and Banmeister, B.M., 1982. Investigations of malignant catarrhal fever in ruminants at the Oklahoma City Zoo. Proc. 86th Annu. Meet. U.S. Anim. Health Assoc., Nashville, TN, pp. 571–582.

Reid, H.W. and Buxton, D., 1984. Malignant catarrhal fever of deer. Proc. Roy. Soc., Edinburgh, 82B: 261–293.

Reid, H.W. and Buxton, D., 1985. Immunity and pathogenesis of malignant catarrhal fever. In: P.-P. Pastoret, E. Thiry and J. Saliki (Editors), Immunity to Herpesvirus Infections of Domestic Animals. Rep. EUR 9737 EN, CEC, Brussels, pp. 117–130.

Reid, H.W. and Rowe, L., 1973. The attenuation of a herpesvirus (malignant catarrhal fever virus) isolated from hartebeest (*Alcelaphus buselaphusz cokei*, Gunther). Res. Vet. Sci., 15: 144–146.

Reid, H.W., Plowright, W. and Rowe, L., 1975. Neutralising antibody to herpesviruses derived from wildebeest and hartebeest in wild animals in East Africa. Res. Vet. Sci., 18: 269–273.

Reid, H.W., Buxton, D., Corrigal, W., Hunter, A.R., McMartin, D.A. and Rushton, R., 1979. An outbreak of malignant catarrhal fever in red deer (*Cervus elaphus*). Vet. Rec., 104: 120–123.

Reid, H.W., Buxton, D., Pow, I., Finlayson, J. and Berrie, E.L., 1983. A cytotoxic T-lymphocyte line propagated from a rabbit infected with sheep-associated malignant catarrhal fever. Res. Vet. Sci., 34: 109–113.

Reid, H. W., Buxton, D., Pow, I., Brodie, T.A., Holmes, P.D. and Urquhart, G.M., 1986. Malignant catarrhal fever: experimental transmission of the "sheep-associated" form of the disease from cattle and deer to cattle, deer, rabbits and hamsters. Res. Vet. Sci., 41: 76–81.

Reid, H.W., Buxton, D., Pow, I. and Finlayson, J., 1989. Isolation and characterisation of lymphoblastoid cells from cattle and deer affected with sheep-associated malignant catarrhal fever. Res. Vet. Sci., 47: 90–96.

Roderick, L.M., 1958. Malignant Catarrhal Fever. Technical Bulletin No. 97, Agricultural Experimental Station, Kansas State College of Agriculture and Applied Science, Manhattan, 16 pp.

Rossiter, P.B., 1981a. Immunofluorescence and immunoperoxidase techniques for detecting antibodies to malignant catarrhal fever in infected cattle. Trop., Anim. Health Prod., 13: 189–192.

Rossiter, P.B., 1981b. Antibodies to malignant catarrhal fever virus in sheep sera. J. Comp. Pathol., 91: 303–311.

Rossiter, P.B., 1982a. Immunoglobulin response of rabbits infected with malignant catarrhal fever virus. Res. Vet. Sci., 33: 120–122.

Rossiter, P.B., 1982b. Attempts to protect rabbits against challenge with virulent cell-associated malignant catarrhal fever virus. Vet. Microbiol., 7: 419–425.

Rossiter, P.B., 1985. Immunology and immunopathology of malignant catarrhal fever. Progr. Vet. Microbiol. Immunol., 1: 121–144.

Rossiter, P.B. and Jessett, D.M., 1980. A complement fixation test for antigens of and antibodies to malignant catarrhal fever virus. Res. Vet. Sci., 28: 228–233.

Rossiter, P., Mushi, E.Z. and Plowright, W., 1977. The development of antibodies in rabbits and cattle infected experimentally with an African strain of malignant catarrhal fever virus. Vet. Microbiol., 2: 57–66.

Rossiter, P.B., Mushi, E.Z. and Plowright, W., 1978. Antibody response in cattle and rabbits to early antigens of malignant catarrhal fever in cultured cells. Res. Vet. Sci., 25: 207–210.

Rossiter, P.B., Jessett, D.M. and Mushi, E.Z., 1980. Antibodies to malignant catarrhal fever virus antigens in the sera of normal and naturally infected cattle in Kenya. Res. Vet. Sci., 29: 235–239.

Rossiter, P.B., Jessett, D.M., Mushi, E.Z. and Karstad, L., 1983. Antibodies in carrier wildebeest to the lymphoproliferative herpesvirus of malignant catarrhal fever. Comp. Immunol. Microbiol. Inf. Dis., 6: 39–43.

Rossiter, P.B., Gaman, I.D. and Mirangi, P.K., 1988. Immunological relationships between malignant catarrhal fever virus alcelaphine herpesvirus 17 and bovine cytomegalovirus (bovine herpesvirus 3). Vet. Microbiol., 16: 211–218.

Russell, P.H., 1980. Malignant catarrhal fever in rabbits — reproduction of clinical disease by cell-free virus and partial protection against such disease by vaccination with inactivated virus. Vet. Microbiol., 5: 161–163.

Rweyemamu, M.M., Karstad, L., Mushi, E.Z., Otema, J., Jessett, D.M., Rowe, L., Drevemo, S. and Grootenhuis, J.G., 1974. Malignant catarrhal fever virus in nasal secretions of wildebeest: A probable mechanism for virus transmission. J. Wildl. Dis., 10: 478–487.

Rweyemamu, M.M., Mushi, E.Z., Rowe, L. and Karstad, L., 1976. Persistent infection of cattle with the herpesvirus of malignant catarrhal fever and observations on the pathogenesis of the disease. Br. Vet. J., 132: 393–400.

Schloer, G.M. and Breese, S.S., 1982. Purification of African malignant catarrhal fever virus using a two-phase aqueous polymer system. J. Gen. Virol., 59: 101–110.

Selman, I.E., Wiseman, A., Murray, M. and Wright, N.G., 1974. A clinicopathological study of bovine malignant catarrhal fever in Great Britain. Vet. Rec., 94: 483–490.

Selman, I.E., Wiseman, A., Wright, N.,G. and Murray, M., 1978. Transmission studies with bovine malignant catarrhal fever. Vet. Rec., 102: 252–257.

Snowdon, W.A., 1972. Bovine malignant catarrh. Annu. Rep. Anim. Health, CSIRO, Australia, p. 16.

Straver, P.J. and Van Bekkum, J.G., 1979. Isolation of malignant catarrhal fever virus from a European bison (*Bison bonasus*) in a zoological garden. Res. Vet. Sci., 26: 165–171.

Westbury, H.A. and Denholm, L.J., 1982. Malignant catarrhal fever in farmed Rusa deer (*Cervus timorensis*). Aust. Vet. J., 58: 88–92.

Wilks, C.R. and Rossiter, P.B., 1978. An immunosuppressive factor in serum of rabbits lethally infected with the herpesvirus of bovine malignant catarrhal fever. J. Inf. Dis., 137: 403–409.

Wyssmann, E., 1933. Zur Frage der Übertragung des bösartigen Katarrhalfiebers des Rindes durch Schafe. Schweiz. Arch. Tierheilk., 75: 577–591.

Wyssmann, E., 1934. Weitere Mitteilungen zur Katarrhalfieberfrage. Schweiz. Arch. Tierheilk., 76: 509–523.

Chapter 15

Other Herpesviruses

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INTRODUCTION

Of the other herpesviruses isolated from domesticated ruminants, the Movar type of isolates (BHV-3) has been associated with a variety of disease syndromes in cattle. A herpesvirus of sheep, caprine herpesvirus 1 (CHV-1), has been associated with ovine pulmonary adenomatosis, but its role in this or any other disease is unclear. A herpesvirus of goats, caprine herpesvirus 2 (CHV-2), is known to cause either a generalized and enteric infection in kids or a genital infection in adult goats.

THE MOVAR TYPE OF BOVINE HERPESVIRUSES (BHV-3)

Bartha et al. (1967) isolated a new serotype of bovine herpesvirus (strain Movar 33/63) that was nonpathogenic to calves. Subsequently, agents similar to this virus were isolated in the USA, Africa and the FRG (Mohanty, 1978). Although their pathogenic role is either minor or insignificant, they are often isolated from a variety of clinical samples. Strain DN-599 was isolated from cattle with respiratory infection (Mohanty et al., 1971), as were strains FTC-1 and FTC-2 (Smith et al., 1972). Other strains include isolates from lymphosarcomatous tissues, bovine metritis (strain DDV-71, Parks and Kendrick, 1973), lactating dairy cows with mammary pustular dermatitis (strains 3374, 4112, and 7952, Reed et al., 1979), and bovine abortion (strain 9583, Reed et al., 1979). It now appears that these strains, along with virus isolates from Africa and the FRG, have characteristics similar to those of strain Movar 33/63; all are antigenically related, all have an identical restriction pattern, and should be classified as BHV-4 (Gibbs and Rweyemamu, 1977; Mohanty, 1978; Ludwig, 1983). The ICTV proposed the name BHV-3 (see Roizman, 1982).

Virus properties
Assigned originally to the subfamily Alphaherpesvirinae, it now appears that this virus may have characteristics of cytomegaloviruses and therefore be a member of the subfamily Betaherpesvirinae (Ludwig, 1983). The virus is heat and acid labile, sensitive to lipid solvents, and is destroyed by detergents and common disinfectants. It replicates with CPE in cell cultures of bovine, ovine, and caprine origin and in rabbit kidney cells. Numerous type A intranuclear inclusions are produced, but syncytia are rarely seen (Mohanty, 1978).

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Epizootiology

Cattle of all ages, sexes, and breeds are susceptible. It is probably worldwide in distribution and cattle are the principal reservoir. The morbidity rate may approach 20–30%, but mortality is usually very low. In a serologic survey of Oklahoma cattle for respiratory infection, BHV-3 was involved in 2% of the population (Potgieter and Aldridge, 1977). Its economic importance is unknown. Transmission is usually by direct contact. The virus has been associated with the bovine respiratory disease (BRD) complex; aerosol infection can occur, and in crowded conditions with limited ventilation it could spread rapidly. Although it has been isolated from enzootics of mammary pustular dermatitis in lactating cows, it is not known if transmission occurs during milking via contaminated hands or milking machines. The virus is non-pathogenic to man.

Pathogenesis

The principal route of entry of the virus is the respiratory tract which is mainly involved in the disease process, and the nasal discharge of infected cattle is the usual source. BHV 3 has been associated with bovine abortion and has been isolated from bovine metritis. Thus, a genital tract infection and vertical transmission are possible. The virus may be shed for up to 17 days in nasal secretions (Mohanty, 1978), and it causes a latent infection as demonstrated by its isolation from trigeminal ganglia of abattior cattle (Homan and Easterday, 1981). It can also establish a persistent infection in lymphoid tissues, and during a prolonged viremia it is associated with the mononuclear cell fraction (Osorio and Reed, 1983).

Disease signs

The incubation period in experimental infections (with strain DN-599) is 5–7 days (Mohanty, 1978). The disease may be inapparent, mild or acute. Although BHV-3 induced mild clinical signs of tracheitis in young calves (Smith et al., 1972), the respiratory illness caused by the virus in association with *Pasteurella multocida* may be quite severe (Mohanty, 1978). The acute disease is characterized by fever (39.5–41.5°C), dyspnea, cough, hyperpnea, and a copious nasal discharge. Some animals may develop conjunctivitis. The hemogram is essentially unaltered.

The role of BHV-3 in bovine mammary pustular dermatitis, abortion, and metritis is not clear. Experimental inoculation of bovine fetuses in the third month of gestation had no adverse effect (Kendrick et al., 1976). In experimental respiratory infection, the lesions were confined to the respiratory tract (Mohanty et al., 1971; Mohanty, 1978). There are areas of consolidation, atelectasis, and emphysema in the lungs. Histologically, proliferative bronchiolitis and in most severe cases, peribronchial aggregates of lymphocytes are seen. The causal role of BHV-3 in the BRD complex has not been fully elucidated.

Immune reaction

BHV-3 is a poor immunogen and it has been almost impossible to prepare VN antibodies against it (Mohanty et al., 1971). Although little or no serum antibodies are detected in naturally or experimentally infected cattle, the animals remain solidly immune to challenge. Cell-mediated immunity probably plays an important role in this infection. Neutralization of the virus with homologous antiserum is extremely slow, and incubation of the serum–virus mixture for 12–24 h at 4°C is recommended for conducting VN tests (Mohanty, 1978).

Laboratory diagnosis

The diagnosis of BHV-3 infection is difficult because the virus induces a poor serologic response, if any, and respiratory syndromes induced by most respiratory viruses along with the gross and histopathologic lesions are similar. The VN test generally cannot be used for determining seroconversion. However, indirect IF (Sass et al., 1974), immunoferritin, and immune EM techniques are quite sensitive for detecting viral antibodies (Mohanty, 1978). Sera that are negative by the VN test are found positive in these tests. The indirect IF test can be routinely used for detection of antibodies and identification of the virus. Isolation of virus from clinical cases, along with seroconversion using the indirect IF test are probably the only means of diagnosing the disease in the laboratory. On cryostat sections of infected tissues the direct IF test may be helpful.

Prophylaxis and control

There is no vaccine available.

HERPESVIRUS OF SHEEP (CHV-1)

A herpes-like virus has been associated with pulmonary adenomatosis (chronic progressive pneumonia, jaagsiekte) of sheep in Europe, South Africa, Iceland, Israel, and the USA. The disease has a long incubation period of 6–9 months, and the infection is characterized by a chronic progressive pneumonia with the development of adenomatous ingrowths of the alveolar walls (Buxton and Fraser, 1977). EM studies revealed a herpes-like virus in macrophages cultured from lungs of infected sheep (Mackay, 1969a, b). The role of this agent in pulmonary adenomatosis was not clear and the jaagsiekte agent has now indeed been found to be a retrovirus. [Martin et al. (1976) proposed that in pulmonary adenomatosis the oncogenic transformation might result from a synergetic effect of the herpesvirus (which alone is not pathogenetic) with a retrovirus (see also chapter 14 on jaagsiekte)]. There is no vaccine, and eradication by slaughter of sheep with pulmonary adenomatosis is recommended.

HERPESVIRUS OF GOATS (CHV-2)

Herpesviruses have been isolated from kids with severe generalized infections (Saito et al., 1974; Mettler et al., 1979), from genital lesions in goats (Horner et al., 1982; Rosadio et al., 1984; Hyllseth et al., 1985; Grewal and Wells, 1986), as well as from clinical conditions such as acute and chronic pneumonia, wart-like lesions of the eyelid and proliferative lesions around mouth and hard palate (Rosadio et al., 1984) and dermal lesions (Waldvogel et al., 1981). Experimental infections have caused abortions (Saito et al., 1974; Berrios et al., 1975b; Waldvogel et al., 1981), but there is little evidence in the literature that natural infections cause abortion. Serologic evidence indicates that herpesvirus infections occur worldwide (Taylor et al., 1977; Fulton et al., 1982; Plebani et al., 1983; Elazhary et al., 1984; Horner and Tisdall, 1985; Hyllseth et al., 1985; Kao et al., 1985).

Restriction enzyme mapping studies support the previous findings that cleavage patterns of goat herpesvirus DNA do not correspond to those of any other herpesvirus. Whetstone and Evermann (1988) examined virus isolates from sheep and goats and found differences both between and within species. Engels et al. (1987) showed that although CHV-2 had a restriction profile different from BHV-1, their DNAs shared a high degree of base sequence homology. Brake and

Studdert (1985) found that isolates from goat, buffalo, bovine encephalitis and BHV-1 had distinct DNA profiles. E. Rimstad and B. Hyllseth (unpublished data, 1989) compared isolates from goat, reindeer and cattle and found that their DNA profiles were different.

Studies carried out on antigenic relationships between herpesviruses from goat and other animals support and enlarge previous findings. Nixon et al. (1988) found that both goat and red deer viruses appeared to be more closely related to BHV-1 than they were to each other. Whetstone and Evermann (1988) showed that goat and sheep viruses were antigenically related; both were related to BHV-1. Sanvittore (1986) showed that there is a distinct antigenic relationship between CHV-2 and BHV-1, and Rimstad and Hyllseth found that BHV-1, goat and reindeer viruses were all antigenically related (unpublished data, 1989). Using monoclonal antibodies, Friedly and Metzler (1986) established that BHV-1 and CHV-2 both share and have unique antigenic determinants.

An extensive survey in Greece showed that more than 50% of examined goats had antibodies (Koptopoulos et al., 1988). The bucks were found to have a higher rate of infections than the female goats. The kids possessing maternal antibodies became seronegative at the age of about 4 months and "new" antibodies appeared at the age of about 7–8 months. Observations in two closed goat herds showed that the virus does not spread during the mating and lactation periods. Infections in seronegative animals occurred after the summer when breeding took place.

A small number of British goat sera (40) were all negative in ELISA (Nixon et al., 1988).

In Australia, genital tracts of 76 mature feral bucks were examined at an abattoir (Tarigan et al., 1987). Two bucks had hyperemia of the penis and acute ulcerative lesions on the prepuce. The latter consisted of numerous small (1–5 mm) discrete punctate areas of epithelial desquamation on the preputial mucosa together with associated petechial and ecchymotic hemorrhage; caprine herpesvirus was isolated from the lesions.

In New Zealand it is reported that the virus may cause sporadic abortion (Horner, 1987). A serosurvey in 1985 showed that 6.3% of tested goats and 27% of the tested flocks were positive. As the genital caprine herpesvirus lesions heal quickly and the virus does not cause infertility, it could be argued that the disease is not important. However, occurrence of the disease can delay mating and lengthen the reproductive cycle. In addition, if more virulent strains emerge, abortion outbreaks and kid deaths could cause substantial financial losses for individual farmers.

In Djibouti, Africa, a serosurvey of several virus infections showed i.a. that 34% of 439 examined goats had antibodies to BHV-1 (Bohrman et al., 1988).

In Switzerland the question whether goats could be a source of herpesvirus infection in cattle was examined experimentally using both animal species and their respective viruses CHV-2 and BHV-1 (Ackermann et al., 1986). Although goats were susceptible to BHV-1, this species is of no importance in the transmission of BHV-1 infection to cattle in Switzerland. Antibodies to BHV-1 were detected in 13% of 1700 goat sera; it was shown by homologous and heterologous serologic tests that goats had been infected with the antigenically related CHV-2 (Hasler and Engels, 1986). There was thus no evidence for a BHV-1 infection in goats.

The *generalized and enteric form* of the infection affects 1–2-week-old kids and is characterized by fever, inappetance, abdominal pain, nasal and ocular discharges, diarrhea and depression. Gross lesions consist of necrosis and ulceration of mucous membranes in the digestive tract, particularly in the cecum and the ascending colon, and sometimes in the bladder and in the skin. Virus has been isolated from bone marrow, spleen, lung and liver organs; in

experimental infections virus has been isolated from nasal, oral, conjunctival, rectal and vaginal samples as well as from skin lesions (Saito et al., 1974; Berrios et al., 1975b; Mettler et al., 1979, Waldvogel et al., 1981; Plebani et al., 1983). Experimentally infected goats remained normal in appearance and behavior, but some of them had fever and three does aborted 20–46 days p.i. Experimental infection of kids was quite severe as described above (Berrios et al., 1975b). Histological examination of experimentally infected animals showed necrosis, tissue infiltrations with macrophages, monocytes, neutrophils and lymphocytes. Intranuclear inclusions could be seen in cells lining areas of necrosis (Waldvogel et al., 1981). The principal route of infection is probably via respiratory tract. The "enteric" goat herpesvirus was not pathogenic for lambs or calves (Berrios et al., 1975b).

The *genital form* of the infection is characterized by fever, varying degrees of vulval edema and erythema with shallow erosions in the vulval and posterior vaginal mucosa. Yellowish necrotic material attached to lesions and clear yellowish vaginal discharges have been described (Horner and Tisdall, 1985). Balanoposthitis with lesions similar to those found in vulvovaginitis are found in the buck. Virus has been isolated both from genitalia and from leukocytes. In experimental topical infection of genitalia of goats, lesions were observed from day 4 p.i. and lasted for 1–2 weeks; mild fevers were recorded on days 4–6 p.i.. Neutralizing antibodies were first detected on days 5–7 p.i. and highest titers (128–512) were recorded 2–3 weeks p.i. Virus was reisolated during the first $1\frac{1}{2}$ weeks. Histological examination of preputial mucosa 5 weeks p.i. showed marked infiltrations with mononuclear cells, including plasma cells, but no inclusions. Lambs that were experimentally infected showed milder clinical signs without fever. Antibody responses appeared at the same time as in goats, but the titers were 2–4-fold lower. Inoculation of calves gave no clinical signs and no immune response (B. Hyllseth et al., unpublished data, 1985). The principal mode of transmission appears to be venereal (Horner et al., 1982, Hyllseth et al., 1985).

Virus replicates with a CPE in cells of bovine, caprine, ovine, equine, canine and feline origin (Saito et al., 1974; Berrios and McKercher, 1975). For virus isolation bovine embryonic lung cells have been used by several workers (Mettler et al., 1979; Horner et al., 1982); bovine turbinate cells (Rosadio et al., 1984) and caprine chorioid plexus cells are also suitable. In the neutralization test several of the above mentioned cells have been used. The monkey kidney cell line MA104 is less permissive than some other cells tested (Engels et al., 1983), but this line is useful for the neutralization test (B. Hyllseth et al., unpublished data, 1985).

A diagnosis can be made by virus isolation and serological identification, seroconversion, and by demonstration of herpesvirus/viral antigen in infected tissue or cell cultures immunohistochemically and by EM (Waldvogel et al., 1981; Ludwig, 1983, 1984; Plebani et al., 1983).

There is a very close antigenic relationship between enteric and genital goat herpesviruses and they have a "one-way" serologic relationship with BHV-1, i.e they are neutralized by antisera to BHV-1 (Engels et al., 1983). The goat strains are, however, serologically unrelated to human, canine, feline and other bovine herpesviruses as well as to pseudorabies virus and herpes B-virus (Saito et al., 1974; Berrios et al., 1975; Horner et al., 1982; Engels et al., 1983; Gregersen et al., 1983; Ludwig, 1983; Plebani et al., 1983; Rosadio et al., 1984; B. Hyllseth et al., unpublished data, 1985). The restriction enzyme cleavage pattern of goat herpesvirus DNA does not correspond to those of any other herpesvirus, including BHV-1 (Ludwig, 1983). Small differences in patterns have been shown for enteric strains (Engels et al., 1983) and for genital strains (Tisdall et al., 1984).

The common herpesvirus feature of latency and reactivation (Ludwig, 1983, 1984) has been shown using corticosteroid treatment in goats (Plebani et al., 1983; Hyllseth et al., 1985).

There are no recommendations given for prophylaxis and control.

REFERENCES

Ackermann, M., Metzler, A.E., McDonagh, H., Bruckner, L., Müller, H.K. and Kihm, U., 1986. Stellen nichtbovine Paarhufer ein IBR-Virus-Reservoir dar? I. BHV-1-und CapHV-1-Infektions- und Reaktivierungsversuche an Ziegen, Virustyp-Spezifität der humoralen Antikörper und Charakterisierung der viralen Antigene. Schweiz. Arch. Tierheilk., 128: 557–573.

Bartha, A., Juhasz, M., Liebermann, H., Hantschel, H. and Schulze, P., 1967. Isolierung und Eigenschaften eines bovinen Herpesvirus vom Kalb mit repsiratorischer Krankheit und Keratokonjunktivitis. Arch. Exp. Vet. Med., 21: 615–623.

Berrios, P.E. and McKercher, D.G., 1975. Characterization of a caprine herpesvirus. Am. J. Vet. Res., 36: 1755–1762.

Berrios, P.E., McKercher, D.G. and Knight, H.D., 1975. Pathogenicity of a caprine herpesvirus. Am. J. Vet. Res., 36: 1763–1769.

Bohrman, R., Frey, H.R. and Liess, B., 1988. Survey of the prevalence of neutralizing antibodies to bovine viral diarrhea (BVD) virus, bovine herpes virus type 1 (BHV-1) and parainfluenza virus type 3 (PI-3) in ruminants in the Djibouti Republic. Dtsch Tierärztl. Wochenschr., 95: 99–102.

Brake, F. and Studdert, M.J., 1985. Molecular epidemiology and pathogenesis of ruminant herpes-viruses including bovine, buffalo and caprine herpesviruses 1 and bovine encephalitis herpes-virus. Aust. Vet. J., 62: 331–334.

Buxton, A. and Fraser, G., 1977. Animal Microbiology. Vol. 2, Rickettsias and Viruses. Blackwell Scientific Publications, Oxford, pp. 765–767.

Elazhary, M.A.S.Y., Silim, A. and Dea, S., 1984. Prevalence of antibodies to bovine respiratory syncytial virus, bovine viral diarrhea virus, bovine herpesvirus-1, and bovine parainfluenza-3 virus in sheep and goats in Quebec. Am. J. Vet. Res., 45: 1660–1662.

Engels, M., Gelderblom, H., Daraj, G. and Ludwig, H., 1983. Goat herpesviruses: Biological and physicochemical properties. J. Gen. Virol., 64: 2237–2247.

Engels, M., Loepfe, E., Wild, P., Schraner, E. and Wyler, R., 1987. The genome of caprine herpes-virus 1: Genome structure and relatedness to bovine herpesvirus 1. J. Gen. Virol., 68: 2019–2023.

Friedly, K. and Metzler, A.E., 1986. Bovine herpesvirus 1 and caprine herpesvirus 1: viral polypep-tides exhibiting shared and unique antigenic determinants. Experientia, 42: 93.

Fulton, R.W., Downing, M.M. and Hagstad, H.V., 1982. Prevalence of bovine herpesvirus-1, bovine viral diarrhea, parainfluenza-3, bovine adenoviruses-3 and -7, and goat respiratory syncytial viral antibodies in goats. Am. J. Vet. Res., 43: 1454–1457.

Gibbs, E.P. and Rweyemamu, M.M., 1977. Bovine herpesviruses. II. Bovine herpesviruses 2 and 3. Vet. Bull., 47: 411–425.

Gregerson, J.-P., Pauli, G. and Ludwig, H., 1983. Major immunogenic components of the bovid herpesviruses 1 and 6 and their cross-reaction. Abstract. Zentralbl. Bakteriol., Mikrobiol. Hyg., I. Abt., Orig. A, 255: 171.

Grewal, A.S. and Wells, R., 1986. Vulvovaginitis of goats due to a herpesvirus. Aust. Vet. J., 63: 79–82.

Hasler, J. and Engels, M., 1986. Stellen nichtbovine Paarhufer ein IBR-Virus-Reservoir dar? II. Seroepidemiologische Untersuchungen an Ziegen, Schafen, Schweinen und Wildpaarhufern in der Schweiz. Schweiz. Arch. Tierheilk., 128: 575–585.

Homan, E.J. and Easterday, B.C., 1981. Further studies of naturally occurring latent bovine herpesvirus infection. Am. J. Vet. Res., 42: 1811–1813.

Horner, G.W., 1987. Herpesvirus vulvovaginitis in goats. Proceedings from goat seminar. Veterin-ary Continuing Education, Massey University, Palmerston North, New Zealand, Publication 115, pp. 73–77.

Horner, G.W., Hunter, R. and Day, A.M., 1982. An outbreak of vulvovaginitis in goats caused by a caprine herpesvirus. N.Z. Vet. J., 30: 150–152.

Horner, G.W. and Tisdall, D., 1985. Genital disease in goats caused by caprine herpesvirus. In: A.J. Della-Porta (Editor), Veterinary Viral Diseases, Their Significance in South-East Asia and the Western Pacific. Academic Press, pp. 459–462.

Hyllseth, B., Andresen, Ø. and Larsen, H.J., 1985. Genital goat herpesvirus infection in Norway. The 1985 Symposium, European Society against Virus Diseases, Oslo, Norway, June 17–19, Abstract 44.

Kao, M., Leiskau, T., Koptopoulos, G., Papadopoulos, O., Horner, G.W., Hyllseth, B., Fadel, M., Gedi, A.H., Straub, O.C. and Ludwig, H., 1985. Goat herpesvirus infections: A survey on specific

antibodies in different countries. Commission of the European Communities. Agriculture: Immunity to herpesvirus infections of domestic animals. Report EUR 9737 EN, Luxembourg, pp. 93–97.

Kendrick, J.W., Osburn, B.I. and Kronlund, N., 1976. Pathogenicity study on a bovine herpesvirus. Theriogenol., 6: 447–462.

Koptopoulos, G., Papanastasopoulou, M., Papadopoulos, O. and Ludwig, H., 1988. The epizootiology of caprine herpesvirus (BHV-6) infections in goat populations in Greece. Comp. Immunol. Microbiol. Infect. Dis., 11: 199–205.

Ludwig, H., 1983. Bovine herpesviruses. In: B. Roizman (Editor), The Herpesviruses. Vol. 2. Plenum Press, New York, London, pp. 135–214.

Ludwig, H., 1984. Herpesvirus of bovidae: The characterization, grouping and role of different types, including latent viruses. In: G. Wittmann, R.M. Gaskell and H.J. Rziha (Editors), Latent Herpesvirus Infections in Veterinary Medicine. Martinus Nijhoff Publishers, The Hague, pp. 171–189.

Mackay, J.M.K., 1969a. Tissue culture studies of sheep pulmonary adenomatosis (Jaagsiekte). I. Direct culture of affected lungs. J. Comp. Pathol. Therap., 79: 141–146.

Mackay, J.M.K., 1969b. Tissue culture studies of sheep pulmonary adenomatosis (Jaagsiekte). II. Transmission of cytopathic effects to normal cultures. J. Comp. Pathol. Therap. 79: 147–154.

Martin, W.B., Scott, F.M.M., Sharp, J.P., Angus, K.W. and Norwal, M., 1976. Experimental production of sheep pulmonary adenomatosis (jaagsiekte). Nature, 264: 183–185.

Mettler, F., Engels, M., Wild, P. and Bivetti, A., 1979. Herpes-virus-Infektionen bei Zicklein in der Schweiz. Arch. Tierheilkd., 121: 655–662.

Mohanty, S.B., 1978. Bovine respiratory viruses. Adv. Vet. Sci. Comp. Med., 22: 83–109.

Mohanty, S.B., Lillie, M.G. and Hammond, R.C., 1971. A new bovine herpesvirus and its effect on experimentally infected calves. Arch. Ges. Virusforsch., 34: 394–395.

Nixon, P., Edwards, S. and White, H., 1988. Serological comparisons of antigenically related herpesviruses in cattle, red deer and goats. Vet. Res. Commun., 12: 355–362.

Osorio, F.A. and Reed, D.E., 1983. Experimental inoculation of cattle with bovine herpesvirus-4: Evidence for a lymphoid-associated persistent infection. Am. J. Vet. Res., 44: 975–980.

Parks, J.B. and Kendrick, J.W., 1973. The isolation and partial characterization of a herpesvirus from a case of bovine metritis. Arch. Ges. Virusforsch., 41: 211–215.

Plebani, G.F., Engels, M., Metzler, A.E. und Wyler, R., 1983. Caprines Herpesvirus in der Schweiz: Verbreitung, Häufigkeit und Latenz der Infektion. Schweiz. Arch. Tierheilkd., 125: 395–411.

Potgieter, L.N.D. and Aldridge, P.L., 1977. Frequency of occurrence of viruses associated with respiratory tract disease of cattle in Oklahoma: Serologic survey for bovine herpesvirus DN 599. Am. J. Vet. Res., 38: 1243–1245.

Reed, D.E., Langpap, T.J. and Bergeland, M.E., 1979. Bovine abortion associated with Movar 33/63 type herpesvirus and bovine viral diarrhea virus infection. Cornell Vet., 69: 54–66.

Roizman, B., 1982. The family Herpesviridae: General description, taxonomy and classification. In: B. Roizman (Editor), The Herpesviruses. Vol. 1. Plenum Press, New York and London, pp. 1–23.

Rosadio, R.H., Evermann, J.F. and Muller, G.M., 1984. Spectrum of naturally occurring disease associated with herpesvirus infections of goats and sheep. Agri-Practice, 5: 20–27.

Saito, K., Gribble, D.H., Berrios, P.E., Knight, D. and McKercher, G., 1974. A new herpesvirus isolate from goats; Preliminary report. Am. J. Vet. Res., 35: 847–848.

Sanvittore, E., 1986. Serologische Untersuchungen zur antigenen Beziehung zwischen den Bovinen Herpesviren 1 und 4 sowie dem Caprinen Herpesvirus 1. Schweiz. Arch. Tierheilk., 128: 375.

Sass, B., Mohanty, S.B. and Hetrick, F.M., 1974. Fluorescent antibody study of a new bovine herpesvirus (strain DN-599). Am. J. Vet. Res., 35: 1343–1346.

Smith, P.C., Cutlip, R.C., Ritchie, A.E. and Young, J.K., 1972. A bovine herpesvirus associated with a disease of the upper respiratory tract of feedlot cattle. J. Am. Vet. Med. Assoc., 161: 1134–1141.

Tarigan, S., Webb, R.F. and Kirkland, D., 1987. Caprine herpesvirus from balanoposthitis. Aust. Vet. J., 64: 321.

Taylor, W.P., Okeke, A.N. and Shidali, N.N., 1977. Prevalence of bovine virus diarrhea and infectious bovine rhinotracheitis antibodies in Nigerian sheep and goats. Trop. Anim. Health Prod., 9: 171–175.

Tisdall, D.J., Bentley, C.B., Collins, D.M. and Horner, G.W., 1984. New Zealand caprine herpesvirus: Comparison with an Australian isolate and with bovine herpesvirus type 1 by restriction endonuclease analysis. N.Z. Vet. J., 32: 99–100.

Waldvogel, A., Engels, M., Wild, P., Stünzi, H. and Wyler, R., 1981. Caprine herpesvirus infection in Switzerland: Some aspects of its pathogenicity. Zentralbl. Veterinärmed. B, 28: 612–623.

Whetstone, C.A. and Evermann, J.F., 1988. Characterization of bovine herpesviruses isolated from six sheep and four goats by restriction endonuclease analysis and radioimmunoprecipitation. Am. J. Vet. Res., 49: 781–789.

Adenoviridae

The first adenovirus was discovered in uninoculated cultures of human adenoid tissue (Greek "aden" means gland).

Adenovirions are naked icosahedral particles 60–90 nm in diameter. Their capsid is composed of well-discernable protein subunits (capsomeres) surrounding a core with DNA and internal, arginine-rich proteins. There are in total 252 capsomeres termed hexons (each surrounded by six neighbours) and pentons (surrounded by five hexons, at the twelve verticles of the icosahedron). To each penton base capsomer an antenna-like fiber is attached, which may carry a hemagglutinating activity.

The adenoviral genome is a single linear molecule of double-stranded DNA of a mol.wt. between 20 and 25×10^6. The terminal nucleotide sequences of each strand are inverted repetitions — if the DNA is denatured both strands form single stranded circles. A small protein of about 55 kd is covalently linked to the 5' end of each strand. There are at least ten polypeptides in the virion with mol.wts. ranging from 5 k to 120 k. Buoyant density of the mammalian viruses in CsCl is 1.33–1.34 g/cm^3.

After uncoating the viral DNA is transcribed into early nonvirion polypeptides (before initiation of DNA synthesis) and "late" proteins (when viral DNA and structural polypeptides are synthesized). DNA replication occurs by strand displacement; transcription in the nucleus is followed by splicing into mRNAs (the splicing mechanism has been discovered in adenovirus-infected cells). The mRNAs migrate into the cytoplasm, where the structural polypeptides are synthesized. Assembly of the virus particles again takes place in the nucleus, and release takes place by disintegration of the damaged cells. The virus infection efficiently shuts off host cell DNA, RNA and protein synthesis. Intranuclear inclusions may contain nucleic acid, viral protein and virions in paracrystalline arrangement.

As regards ruminants, there are bovine, ovine, and caprine serotypes. The nine bovine serotypes are divided into two subgroups mainly on the basis of replication in either calf kidney and testicle cells (types 1–3 and 9) or only in calf testicle cells (types 4–8). The two subgroups are antigenically distinct (see below). Serological evidence suggests a high incidence of infection with certain strains which might be replaced by other serotypes from time to time. The six ovine serotypes replicate in ovine kidney cells and seem to belong to a single subgroup. The two known caprine serotypes differ from both the bovine and ovine strains.

The viral proteins bear determinants which are family-, subgroup- or type-reactive and are detectable by appropriate serological techniques. Mammalian adenoviruses form the genus *Mastadenovirus* ("mastos" means breast). They share common determinants which differ from those of the genus *Aviadenovirus*. However, "certain bovine adenovirus species contain little or none of the genus-specific antigen from hexons of other mammalian adenoviruses" (Wigand et al., 1982); this refers to the types 4–8, replicating preferably in calf testicle cell culture. Adenoviruses with a lower percentage

of G + C content are tumorigenic for newborn hamsters but could not be associated with naturally occurring tumors.

In ruminants there is no virus of any serotype known to be the agent of a well-defined disease. Instead, adenoviruses are pathogens either solitary or as members of a miscellaneous microbial "flora" in acute respiratory/enteric disease.

REFERENCE

Wigand, R., Bartha, A., Dreizin, R.S., Esche, H., Ginsberg, H.S., Green, M., Hierholzer, J.C., Kalter, S.S., McFerran, J.B., Pettersson, U., Russell, W.C. and Wadell, G., 1982. Adenoviridae: Second Report. Intervirology, 18: 169–176.

Chapter 16

Bovine Adenoviruses

F. BÜRKI

INTRODUCTION

Bovine adenoviruses (BAV) are classified into nine serotypes (species) so far (Wigand et al., 1982). These nine serotypes are divided into two subgroups (Bartha, 1969) mainly on the basis of replication in either calf kidney or testicle cells (subgroup I, serotypes 1–3 and 9) or only in testicle cells (subgroup II, serotypes 4–8; see Table 16). The two subgroups also differ in their antigenic properties.
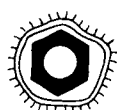
VIRUS PROPERTIES

Physical and chemical properties

Bovine adenoviruses show a remarkable stability outside their hosts. Rondhuis (1970) tested representative strains of several BAV types and found them resistant to pH 2, pH 11, 0.25% trypsin, heating for 30 min at 50°C, partially even for 7 days at 41°C.

Inactivation took place at pH 12 (therefore, NaOH at 0.5–2.0% concentrations acts as a fast disinfectant), as well as by 0.5% chloramine within 30 min. The Adenovirus Study Group (Wigand et al., 1982) confirms that chlorine preparations are effective disinfectants at moderate concentrations.

TABLE 16

Prototype strains of bovine adenoviruses

BAV type	Prototype strain[a]	Reference[b]	Subgroup
1	B-10	Klein et al., 1959	I
2	B-19	Klein et al., 1960	I
3	WBR-1	Darbyshire et al., 1965	I
4	THT/62	Bartha and Aldásy, 1966	II
5	B 4/65	Bartha and Aldásy, 1966	II
6	671130	Rondhuis, 1968	II
7	Fukuroi	Matumoto et al., 1970	II
8	Misk/67	Bartha et al., 1970	II
9	Sofia-4/67	Guenov et al., 1970	I

[b] Available from: American Type Culture Collection, Virus Repository, 12301 Parklawn Drive, Rockville, MD 20852, USA; and WHO Collaborating Centre for Collection and Evaluation of Data on Comparative Virology, Institute for Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, Ludwig Maximilians University, Königinstr, 49, 8000 Munich 22, FRG.
[b] The complete references are given in Benkö et al. (1988).

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Antigenic properties

Typing of bovine adenoviruses is based first of all on cross-neutralization tests. The Adenovirus Study Group requests that "a species (type) has either no cross-reaction with other adenoviruses or shows a homologous titer ratio of > 16 in both directions". Low-grade cross-SN which does not disturb classification has been reported between BAV types 1 and 2, and between types 4, 5 and 8 (Bartha, 1969; Rondhuis, 1970; Adair and McFerran, 1976; Bürki et al., 1978).

Only some serotypes show hemagglutinating activity. Erythrocytes from rats were agglutinated by types 1, 2, 4 and 7 (Messner, 1978). Bovine erythrocytes were agglutinated at low titer by types 1, 2 and 4 (Messner et al., 1978; Belák et al., 1983). Hemagglutination titers are generally so low that HI is not used for typing.

The two subgroups are differentiated by CF or ID tests. Subgroup I viruses share antigens in common with all mammalian adenoviruses except for those of subgroup II.

Taxonomically unresolved appears the position of bovine subgroup II, for which subgroup-reactive soluble antigens were found by Bürki et al. (1979), who also proposed the name of a third genus — *Paramastadenovirus* — for this subgroup. The "recall phenomenon" (Pálfi and Belák, 1979) supports the concept of a common protective subgroup antigen. However, in subgroup II there are probably family-reactive determinants detectable by other methods than those listed above. Adair et al. (1983) reported that in cross-IF tests between subgroup II viruses and conventional adenoviruses (e.g. viruses of subgroup I, several ovine, porcine and human serotypes) a sharing of antigens was demonstrated. However, the low-level cross-reactions were predominantly one-way. Thus, it is not easy to determine the final taxonomical position of bovine subgroup II viruses. Even the use of monoclonal antibodies does not furnish exclusively type-specific and unequivocal results for classification of bovine types (Rusvai et al., 1988). Furthermore, monoclonals produced against bovine types partly cross-reacted with human types (Adám et al., 1988). Recently, Benkö et al. (1988) showed that bovine adenoviruses of subgroup I have larger genomes and fewer Eco RI recognition sites than those belonging to subgroup II.

Another point of importance is that at least one of the bovine serotypes crosses the host species barrier. Belák et al. (1977) have shown that certain of their ovine isolates cross-neutralized at high levels with BAV type 2 and infected both sheep and cattle under experimental conditions. Upon closer examination, specifically of the different HA and DNA restriction enzyme patterns, Belák et al. (1983) proposed to subdivide BAV-2 into two subtypes. Subtype B is present both in cattle and sheep, whereas subtype A strains were isolated only from cattle so far. Another example is a New Zealand ovine isolate being antigenically related to BAV-7 (Adair et al., 1982).

Cultivation

In suitable cell cultures all laboratory-adapted BAV serotypes produce a CPE (Fig. 55). Subgroup I viruses replicate in a wide range of cultured mammalian cell types, whereas subgroup II viruses grow only in calf testicle cells (Bartha, 1969). In stained tissue cultures characteristic nuclear inclusion bodies are seen, single ones for subgroup I types (Fig. 55e), multiple inclusions for subgroup II types (Fig. 55f, g). Plaques with diameters of 0.5–4 mm can be seen within 7–12 days (Rondhuis, 1970). It is important to use only cultures which are free from latent adenovirus infection. This can be achieved by preparing subcultures, in which the CPE may appear. Fetal organs can be transplacentally infected (Bartha and Máté, 1983). The use of conventional calf sera in the
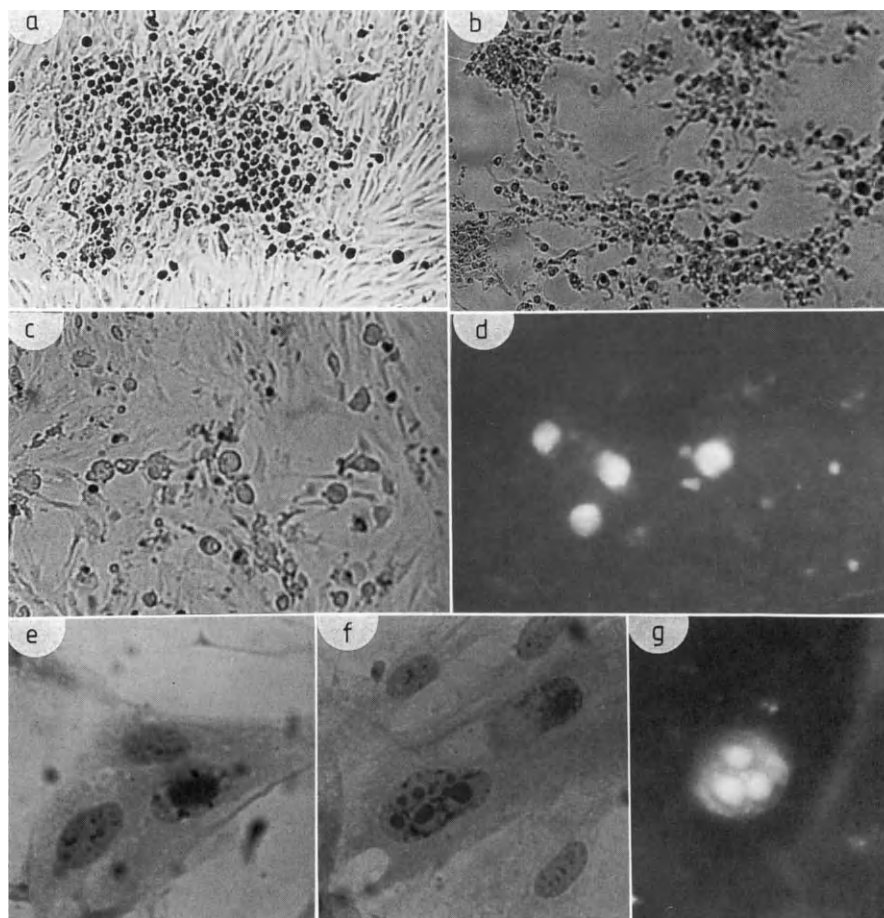
Fig. 55. (a) CPE of BAV-4 in cultured testicle cells. Only epitheloid cells become affected, go through a very short phase of swelling, later of shrinking (this phase shown), and finally detach from culture vessel; fibroblastic cells remain unaltered; living cells, × 250. (b) CPE of BAV-1 in cultured calf testicle cells; epitheloid as well as fibroblastic cells become affected, very briefly show swelling, followed by lysis (this phase shown); living cells, × 250. (c) CPE of BAV-3 in calf testicle cells; affected cells show pronounced rounding and swelling (this phase shown), before lysis; grape-like clusters become less apparent on calf testicle cells than on calf kidney cells; living cells, × 300. (d) Cryostat section of tonsils of a calf experimentally infected with BAV-4; aceton fixed, stained by direct IF, × 700. (e) A single nuclear inclusion body formed by BAV-1 in cultured calf kidney cells; fixed by Carnoy, stained H & E, × 1000. (f) Multiple nuclear inclusion bodies formed by BAV-4 in cultured calf testicle cells; fixed by Carnoy, stained H & E × 1200 (g) Multiple nuclear inclusion bodies formed by BAV-4 in cultured calf testicle cells; aceton-fixed, stained by IF, × 1400.

culture medium should be avoided, because they may contain adenovirus or antibodies to it. Fetal calf sera should be preferred and tested for virus and antibody.

EPIZOOTIOLOGY

The distribution of BAV in cattle populations is worldwide. Serological evidence suggests a high incidence of infection, with certain serotypes being replaced by others from time to time. In a given geographical region a serotype may disappear and a newly introduced serotype may suddenly dominate, e.g. BAV-7 replaced BAV-6 in a certain region of Austria (Table 17). In Bulgaria, BAV-1 was the dominating type; in Great Britain it was BAV-3; in Hungary BAV-4, to be replaced later by BAV-5 and 8; in the USA BAV-3 and 5 (for references, see Bürki et al., 1980).

Virus can be excreted during disease or latency of infection. Acutely infected animals may shed the virus in nasal and conjunctival secretions, feces and

TABLE 17

Distribution of adenovirus serotypes in Austria and the Netherlands

	Percentage of SN-positive sera with BAV types							
	1	2	3	4	5	6	7	8
Austria								
1976[a]	68	68	48	83	–	23	–	48
1978[b]	41	67	–	–	–	–	81	–
The Netherlands								
1970[c]	47	84	74	38	–	43	–	–

[a]Böckmann and Bürki (1976).
[b]Messner (1978).
[c]Rondhuis (1970).
– not determined.

urine (Aldásy et al., 1965; Rondhuis, 1970; Bürki et al., 1980). Excretion in the urine may follow the infection of kidneys for 10 weeks or longer, in spite of clinical recovery and high titers of circulating antibodies (Aldásy et al., 1965).

As outlined in the section on pathogenesis, maternal antibodies provide protection against the homologous BAV type (Bürki et al., 1980). In Central Europe, however, where calves originating from different producers are re-grouped at 2–3 weeks (Baumgartner et al., 1979), passive immunity is non-functional against heterologous BAV types that are introduced into groups by latently infected animals. This became most obvious in Hungary, where up to 4000 calves from different producers are accumulated on state farms. Under such conditions, pneumoenteritis, the most common clinical form of BAV infection, used to start with a few isolated cases, to sweep thereafter through infected premises within 1–2 weeks (Aldásy et al., 1965).

In Austria, private farmers usually purchase groups of 30–50 calves and stable three different age groups under one roof. They never practice the all-in-all-out system and neglect disinfection. This allows BAV and other copathogens to stock up in the older groups, with the effect that the newly purchased group usually falls ill 1–2 weeks after having been added (Baumgartner et al., 1979). In a study conducted over several months on fattening calves originating from the same producers, Böckmann and Bürki (1976) showed that over the winter months the reactor rate among groups sold for slaughter rose from 58% to 90%. This illustrates the stocking-up effect of such husbandry conditions.

Pneumoenteritis has often a multiple etiology. It is in such cases difficult to decide which agent is the primary cause of disease and which is involved in complications. Results of serological screening, in particular on paired sera, can provide information about the spread of a given agent in a certain cattle population. This means that the laboratory can diagnose the infection, but not the disease. It is therefore difficult to relate morbidity and mortality rates to a certain agent. There are exceptions, however, when the agent is solitary, or when losses can be estimated indirectly, by means of the efficacy of vaccination.

PATHOGENESIS

If a calf has no adenovirus antibodies it may be infected by various inoculation routes; the route profoundly influences the incubation period. Respiratory, enteral and parenteral infection all lead to viremia and systemic infection (Aldásy et al., 1965; Tanaka et al., 1968; Rondhuis, 1970; McClurkin and Coria, 1975; Bürki et al., 1980). In the calves infected by the Viennese group of workers (Bürki et al., 1980), fever developed 2–3 days after intravenous infection, 7–8 days after nasal or tracheal infection with a fluid inoculum and only after 2

weeks when nasal infection was practiced by aerosol. In newborn, colostrum-free calves various BAV serotypes often induced severe disease (Bürki et al., 1980). Usually, the symptoms seen in experimentally infected calves are less pronounced than those in natural cases (Tanaka et al., 1968; Bürki et al., 1980). Irrespective of its age, a susceptible calf develops viremia, accompanied by moderately high fever that lasts for 2–6 days. Thereafter, the virus may be demonstrated by isolation or, more easily, by IF test in any organ or tissue examined, particularly in respiratory mucosae, fecal samples, lung, liver, kidney, tonsils and testicles (Aldásy et al., 1965; Bürki et al., 1980). Wilcox (1970) transmitted several BAV isolates from Australian outbreaks of enzootic keratoconjunctivitis but could reproduce this syndrome in only 10% of his experimental animals. McClurkin and Coria (1975) were unable to reproduce experimentally the poly-arthritic "weak calf syndrome".

DISEASE SIGNS

Adenoviruses are generally present in large herds. Viruses are excreted by different forms of shedding and by coughing. Accordingly, peroral as well as respiratory infections ensue. Infection occurs most frequently when calves are 3 weeks to 4 months old. Under natural conditions, the incubation period varies from 7 to 10 days (Aldásy et al., 1965). In diseased calves respiratory and enteric symptoms, fever and anorexia can be observed. The disease usually starts with respiratory symptoms. Serous excretions from the nose and conjunctivae are accompanied by coughing. Enteric infection becomes manifest by salivation and thin, greyish–yellow feces. There may be fever of 39.5–40.5°C and anorexia. The disease can be aggravated by secondary bacterial infections.

Outbreaks of adenoviral pneumoenteritis may cause severe losses among calves, especially in large herds (Aldásy et al., 1965), but even in small farms losses can be high; a farmer in Austria lost 20 animals in a group of 30 calves. Infection is followed by reduced weight gain due to malabsorption (Bürki et al., 1980).

PATHOLOGY

There are neither gross nor histopathological changes pathognomonic for disease syndromes caused by BAV. It remained undetermined whether the interstitial nephritis found in Hungarian calves was etiologically connected with a long-term urinary shedding of adenoviruses, as observed by Aldásy et al. (1965).

Intranuclear inclusion bodies are often present in the organs a few days after infection, but they are essentially absent later on (Rondhuis, 1970; Bürki et al., 1980). In Australia, however, during outbreaks of enzootic bovine keratoconjunctivitis, nuclear inclusion bodies could be frequently demonstrated in cells from conjunctival scrapings.
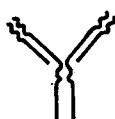
IMMUNE REACTION

Adenoviruses are potent antigens. At least 14 polypeptides are associated with well-defined virion structures (Norrby, 1969; Wigand et al., 1982). After natural infection VN titers of > 1000 were noticed. Maternal antibodies mediate passive protection against the homologous virus type. The protection by heterologous antibodies is unknown.

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LABORATORY DIAGNOSIS

Virus isolation must always be attempted, preferably from nasal and rectal swabs. Urine can also be tested. In this way the serotype can be determined and additional ones may be discovered. Post mortem various organs (e.g. mucous membranes of the head, the bronchi, small intestine, tonsils, lungs, liver, kidneys and testicles) can be homogenized and used for virus isolation. The chance of virus isolation decreases when the antibodies appear; therefore it is preferable to collect samples during the first week of the disease. For virus isolation calf testicle or a recently described permanent calf thyroid cell line (Benkö et al., 1989) should be used. In this way the subgroup I as well as II viruses can be demonstrated. Isolation and typing sometimes takes weeks, especially when blind passages have to be made.

Demonstration of viral antigens can be performed by CIEP of fecal samples. Direct IF tests on cryostat sections (Fig. 55d) will uncover the presence of antigen in cells. This will speed up the virological diagnosis of the fastidious subgroup II types. A potent antiserum to type 4 will reveal the presence of all bovine adenovirus types (Weber, 1976). On the other hand, the ELISA using polyclonal or monoclonal antibodies on swab material proved unsatisfactory (Anderson et al., 1983). In the case of an acute disease, paired serum samples should be taken from calves. The acute-phase sera are collected from at least 4–6 recently diseased animals. The convalescent sera are collected from the same calves 2–3 weeks later. If no acute disease is observed but infection of the herd is suspected, a serological survey can be performed. In this case single serum samples are collected from adult animals representing 5–10% of the herd.

Adenoviruses contain type-specific as well as group-specific antigens, and induce the corresponding categories of antibodies following infection. Preferably, serodiagnosis should start using CF (Messner et al., 1979), ID (Messner et al., 1983) or group-reactive ELISA (Anderson et al., 1983; Rossmanith and Horvath, 1988). These methods are *subgroup-specific* if antigen representing each of the two subgroups is used. The results will show to which subgroup the virus belongs. Thereafter, the *serotype* can be determined using virus types of the given subgroup. Serotyping is performed using VN or type-reactive ELISA. In the case of a hemagglutinating virus the HI test can also be applied (Rosen, 1960). The use of subgroup-reactive tests makes the diagnosis fast and economical. These tests demonstrate whether there has been an adenovirus infection or not. However, if vaccination is considered, serotyping must be performed.

Sequential serotesting of experimentally infected calves has shown that SN is positive first, followed by CF and ID, in this order (Bürki et al., 1980). HI has not been systematically investigated but probably turns positive in parallel to SN. It may be predicted that ELISA, owing to its high sensitivity, will parallel SN in becoming positive. WIth homologous antigen, SN, HI and ELISA titers of BAV-infected calves reach levels of 500 to several thousands. Group-reactive CF titers frequently reach up to 128, rarely higher; ID is a qualitative test.

PROPHYLAXIS AND CONTROL

Immunoprophylaxis against BAV is needed especially under the husbandry conditions practiced in large parts of Europe. Calves are often weaned at the tender age of around 8 days and sold to fattening lots when 2–5 weeks old. There they are exposed to one, frequently to several BAV types carried in latent form by contact calves. Transportation, change of feed, crowding and such are additional stressors. As a consequence, respiratory and/or enteric

outbreaks commonly start within 1–2 weeks after new calf groups are introduced for fattening (Baumgartner et al., 1979). Immunoprophylaxis should therefore be functional within 1–2 weeks after arrival on fattening lots. Ideally, cows in their home herds should be immunized while pregnant, using a multicomponent vaccine that is composed of the geographically prevailing BAV types (plus additional viral agents affecting young calves). For economical reasons this is not practiced in the field as long as pregnancy in cattle is not synchronized, because it would require too frequent visits by a veterinarian. Therefore, efforts were made to quickly immunoprotect very young calves traded at auctions or upon arrival on fattening lots. Haralambiev et al. (1972) and Baumgartner et al. (1979) tried the intratracheal application of hyperimmune sera. The method was not satisfactory under field conditions.

Several producers have marketed vaccines, notably all in Europe. In the USA calves are 5 months old when alotted to fattening farms. At this age calves are already less susceptible to the disease. They have lost maternal antibodies but might have developed postinfectional immunity to types prevailing on their home premises. At present no adenovirus vaccine is licensed in the USA (Gillespie and Timoney, 1981).

Calves are passively protected by colostral antibodies. This is one of the reasons why the disease generally does not appear on small home premises. Vaccination of pregnant cows is recommended on large farms, when protection of very young calves is necessary. Hyperimmune sera are predominantly monotypic and too large quantities would be needed. This procedure would be costly and the protection limited both in range and duration.

In Hungary, a bivalent adenovirus vaccine has been developed, containing representatives of both subgroups. Five commercial vaccines containing BAV components have appeared on the Western European market. Invariably viruses were used in inactivated form, β-propiolactone or formalin serving as inactivants. Aluminum hydroxide, either alone or in combination with saponin was added as an adjuvant. All vaccines marketed in Western Europe were of the multifactorial type, five containing BAV-3 as the only component of the adenovirus spectrum, one containing BAV-1, 3 and 5. Individual doses measure 2–5 ml, depending on the total number of viruses incorporated. All vaccines have been specified for subcutaneous use. No local or systemic side effects have been reported from any vaccine used. Two of the products licensed in Western Europe have been withdrawn from the market by their respective firms because of poor performance.

At present, all vaccine producers are aware that at least two, better four, doses are needed to confer immunity. Basically, two different approaches have been recommended: combined pregnant cow/calf vaccination, or calf vaccination only. According to the first scheme, cows are vaccinated twice during the last trimester of pregnancy; their calves are vaccinated at the age of 6–8 weeks. Vaccination is repeated when the calves are 10–12 weeks old. Ideal as this quadruple vaccination might be, its high cost impedes its use. Vaccination of only the calves is actually practiced in Western Europe with two different approaches and highly variable success. Some producers recommend that the first dose be given immediately after calves arrive on fattening lots, i.e. when 8–10 (!) or 14–30 days old — according to local trading habits — followed 6 weeks later by a booster dose. Others specifically reject vaccination during the weeks of adaption and recommend a first dose at 6 weeks and the booster at 10–12 weeks.

Baumgartner et al. (1979) made experiments with a combined immunoprophylaxis. On auction day the calves were injected intratracheally with 10 ml of hyperimmune serum plus 5 ml of a tailored inactivated and adjuvanted vaccine given intramuscularly. Preliminary tests had disclosed

that such application did not weaken the immune response to the inactivated vaccine injected intramuscularly on the same day. Nevertheless, calves were merely partially protected from disease. More impressive than numerical reduction in disease incidence (2.5-fold) was that bronchopneumonia was cut 6-fold and veterinary treatment 8-fold.

Neither researchers nor vaccine producers have ever tested whether a BAV type incorporated into a vaccine exerted cross-protection against heterologous BAV types. As long as this information is lacking, market vaccines should be so composed as to contain the BAV types which actually prevail in the cattle population, in which they are to be used.

BAV-3 is incorporated into most vaccines, frequently as the only BAV representative. However, nobody has actually proven that this type causes severe enzootics in the field nor studied its pathogenicity in calves of the susceptible age. Furthermore, type 3 antigenically occupies an isolated position, as described above; it is therefore certainly not the most plausible BAV type for active vaccination in the field. A more careful selection of virus types incorporated into adenovirus vaccines is recommended to improve the result of vaccination.

Statistics show that one out of ten calves born in Austria is lost at a young age. The losses are even higher in other countries where the majority of young calves is collected on large farms. This means that special attention should be paid to the proper and fast diagnosis of calf diseases. In addition, the factors that contribute to the spread of viruses (e.g. crowding, mixing of animals) have to be controlled. Attention must also be paid to hygienic conditions (e.g. temperature, ventilation). However, on large industrialized farms vaccination is unavoidable. The best results are obtained when the vaccines are tuned to the infections present on a given farm. For large companies it would be desirable to produce such "farm-specific" vaccines. Since vaccines containing adenovirus components have been used with good result in the sheep industry, there is a chance to decrease economical losses in a similar way among young calves.

REFERENCES

Adair, B.M. and McFerran, J.B., 1976. Comparative serological studies with mammalian adenoviruses. Arch. Virol., 51: 319–325.

Adair, B.M., McFerran, J.B. and McKillop, E.R., 1982. A sixth species of ovine adenovirus isolated from lambs in New Zealand. Arch. Virol., 74: 269–275.

Adair, B.M., McKillop, E.R., McFerran, J.B. and Todd, D., 1983. Studies on the antigenic relationship between bovine subgroup 2 and conventional mammalian adenoviruses using immunofluorescence. Vet. Microbiol., 8: 121–128.

Adám, E., Rusvai, M., Lengyel, A., Belák, S. and Nasz, I., 1988. Antigenic relationship between human and animal adenovirus hexons determined by means of monoclonal antibodies directed against bovine adenovirus type 2 hexon. Arch. Virol., 100: 9–15.

Aldásy, P., Csontos, L. and Bartha, A., 1965. Pneumo-enteritis in calves caused by adenoviruses. Acta Vet. Acad. Sci. Hung., 15: 167–175.

Anderson, L.J., Godfrey, E., McIntosh, K. and Hierholzer, J.C., 1983. Comparison of a monoclonal antibody with a polyclonal serum in an enzyme-linked immunosorbent assay for detecting adenovirus. J. Clin. Microbiol., 18: 463–468.

Bartha, A., 1969. Proposal for subgrouping of bovine adenoviruses. Acta Vet. Acad. Sci. Hung., 19: 319–321.

Bartha A. and Máté, S., 1983. Transplacental transmission of bovine adenoviruses. Comp. Immunol. Microbiol. Infect. Dis., 6: 189–192.

Baumgartner, W., Hinaidy, B. and Bürki, F., 1979. Feldversuche zur Prophylaxe virusbedingter Krankheiten des Respirationstraktes bei Mastkälbern. Wien. Tierärztl. Monatschr., 66: 291–297.

Belák, S., Pálfi, V., Szekeres, T. and Tury, E., 1977. Experimental infection of calves with an

adenovirus isolated from sheep and related to bovine adenovirus type 2. Clinical and Virological Studies. Zentralbl. Veterinärmed. B., 24: 542–547.

Belák, S., Berencsi, G., Rusvay, M., Lukács, K. and Nász, I., 1983. DNA structure, and hemaglutination properties of bovine adenovirus type 2 strains which bypass species specificity. Arch. Virol., 77: 181–194.

Benkö, M., Bartha, A. and Wadell, G., 1988. DNA restriction enzyme analysis of bovine adenoviruses. Intervirology, 29: 346–350.

Benkö, M., Bartha, A., Möstl, K. and Bürki, F., 1989. A heteroploid permanent cell line originating from embryonic calf thyroid supporting the replication of all known bovine adenovirus serotypes. Vet. Microbiol., 19: 317–324.

Böckmann, J. and Bürki, F., 1976. Serologische Bestimmungen über die Häufigkeit respiratorisch-pathogener Virusarten unter österreichischen Schlachtkälbern. Wien. Tierärztl. Monatschr., 63: 117–120.

Bürki, F., Wege, H., Reich-Rohrwig, Chr. and Hinaidy, B., 1978. Bovine adenoviruses. I. Characterization and serological classification as types 4 of two isolates from latently infected calf testicles. Zentralbl. Veterinärmed. B, 25: 555–565.

Bürki, F., Hinaidy, B. and Böckmann, J., 1979. Bovine adenoviruses. III. Subclassification into two subgroups by means of immunodiffusiontest. Microbiologica, 2: 65–74.

Bürki, F., Schlerka, G., Burtscher, H. and Hinaidy, B., 1980. Infektionsversuche an Mastkälbern mit dem bovinen Adenovirus Typ 4. Dtsch. Tierärztl. Wochenschr., 87: 80–88.

Haralambiev, H., Draganov, M. and Zwetkov, P., 1972. Hyperimmunserum against myxovirus parainfluenza-3 and bovine adenovirus in prophylaxis of respiratory diseases in calves. Arch. Exp. Vet. Med., 26: 273–276.

Gillespie, J.H. and Timoney, J.F., 1981. In: Hagan and Bruner's Infectious Diseases of Domestic Animals. Cornell University Press, Ithaca, NY, 7th edn., pp. 515–517.

McClurkin, A.W. and Coria, M.F., 1975. Infectivity of bovine adenovirus type 5 recovered from a polyarthritic calf with weak calf syndrome. J. Am. Vet. Med. Assoc., 167: 139–141.

Messner, A., 1978. Empfindlichkeit und diagnostiche Aussage verschiedener serologischer Testverfahren für bovine Adenovirusinfektionen. Vet. Thesis, Veterinary University, Vienna, 77 pp.

Messner, A., Hinaidy, B. and Bürki, F., 1979. Bovine adenoviruses. IV. Two mixed antigens for routine serodiagnosis by complement fixation reaction. Comp. Immunol. Microbiol. Infect. Dis., 1: 351–359.

Messner, A., Hinaidy, B. and Bürki, F., 1983. Bovine adenoviruses. V. Diagnostic efficiency of dual-antigen immuno-diffusion test using two different group-reactive antigens in parallel. Zentralbl. Veterinärmed. B, 30: 762–774.

Norrby, E., 1969. The structural and functional diversity of adenovirus capsid components. J. Gen. Virol., 5: 221–236.

Pálfi, V. and Belák, S., 1979. Recall phenomenon induced with ovine adenoviruses. Arch. Virol., 61: 169–173.

Rondhuis, P.R., 1970. Bovine Adenoviruses. A Comparative, Serological and Experimental study. Centraal Diergeneeskundig Instituut, afdeling Rotterdam. Drukkerij-Uitgeverij G. van Dijk, Breukelen, 83 pp.

Rosen, L., 1960. A hemagglutination-inhibition technique for typing adenoviruses. Am. J. Hyg., 71: 120–128.

Rossmanith, W. and Horvath, E., 1988. Bovine adenoviruses VI. An enzyme-linked immunosorbent assay for detection of antibodies to bovine adenovirus types belonging to subgroups I and II. Microbiologica, 11: 387–394.

Rusvai, M., Belák, S., Stolz, B., Adám, E. and Nász, I., 1988. Antigenic structure of mammalian adenoviruses studied by monoclonal antibodies against bovine adenovirus type 2. Acta Microbiol. Hung., 35: 417–422.

Tanaka, Y., Inaba, Y., Ito, Y., Omori, T. and Matumoto, M., 1968. Bovine adenovirus. I. Recovery of a serotype, Nagano, from Japanese cattle. Jap. J. Microbiol., 12: 77–95.

Weber, P., 1976. Einsatz der Immunofluoreszenz bei virologischen Forschungs- und Diagnostikfragen. Vet. Thesis, Veterinary University, Vienna, 66 pp.

Wigand, R., Bartha, A., Dreizin, R.S., Esche, H., Ginsberg, H.S., Green, M., Hierholzer, J.C., Kalter, S.S., McFerran, J.B., Pettersson, U., Russell, W.C. and Wadell, G., 1982. Adenoviridae: Second Report. Intervirology, 18: 169–176.

Wilcox, G.E., 1970. The aetiology of infectious bovine kerato-conjunctivitis in Queensland. 2. Adenovirus. Aust. Vet. J., 46: 415–420.

Chapter 17

Ovine Adenoviruses

S. BELÁK

INTRODUCTION

Sheep adenoviruses have been classified into six serotypes so far (Adair et al., 1982; Wigand et al., 1982). McFerran et al. (1969, 1971) isolated ovine adenovirus (OAV) strains in Northern Ireland identified as serotypes 1–3 (OAV-1–3) from apparently healthy lambs, from lambs with enteritis and from animals with enteric and respiratory symptoms. Serotype 4 (OAV-4) was first isolated by Sharp et al. (1974) in Scotland from 4–10-week-old lambs recovered from respiratory disease. Bauer et al. (1975) first isolated OAV-5 in Turkey from the feces of a clinically healthy animal. OAV-6 was isolated from a lamb in New Zealand (Davies and Humphreys, 1977b; Thurley et al., 1977) and characterized by Adair et al. (1982).

In Hungary it has been proven that sheep may be naturally infected not only with OAV but also with BAV-2 (Belák and Pálfi, 1974a; Belák et al., 1983).

The serotypes and reference strains of sheep adenoviruses are shown in Table 18.

VIRUS PROPERTIES

Physical and chemical characteristics

All ovine strains examined resisted 30 min exposure to 56°C, although with a notable loss of titer. Bivalent cations significantly decreased heat resistance. All strains were resistant to pH 3.0, chloroform and sodium deoxycholate treatment (McFerran et al., 1971; Bauer et al., 1975; Belák, 1978).

Sodium hypochlorite and formalin produced complete inactivation of OAV-1, Iosan, an iodophore, exerted much weaker effect (Fig. 56; Belák, 1980).

TABLE 18

Serotypes and reference strains of adenoviruses isolated from sheep

Serotype	Reference strain	Original references
OAV-1	S1	McFerran et al., 1969
OAV-2	PX 515	McFerran et al., 1969
OAV-3	PX 611	McFerran et al., 1969
OAV-4	7769	Sharp et al., 1974
OAV-5	SAV	Bauer et al., 1975
OAV-6	WV 419	Davies and Humphreys, 1977b
BAV-2B	Het/3	Belák and Pálfi, 1974a
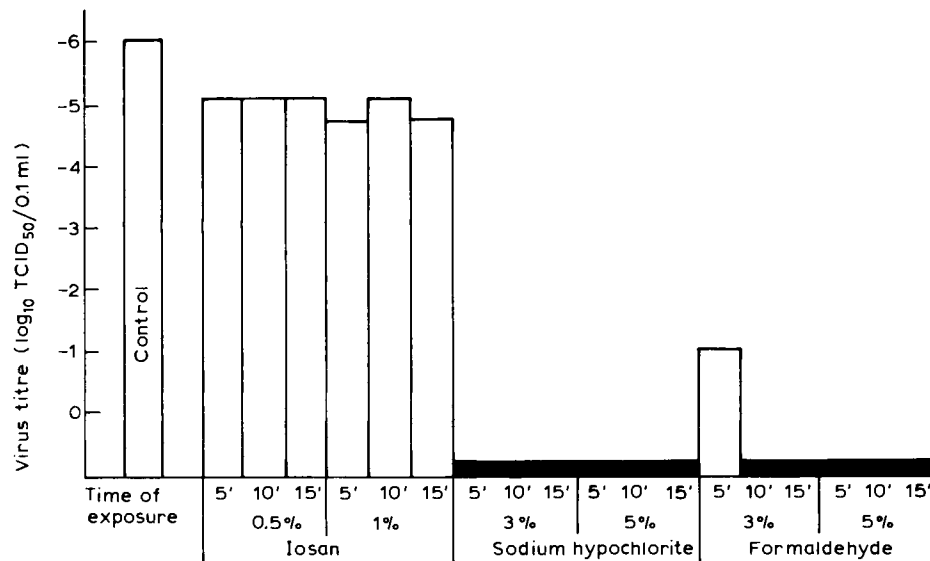
Fig. 56. Effect of different disinfectants on the infectivity of ovine adenovirus.

Antigenic properties

McFerran (1981) concluded that OAV-1 agglutinates some batches of rat cells. Strain WV757/75 isolated in New Zealand (typing still not completed; Adair et al., 1982) agglutinates chicken erythrocytes (Davies and Humphreys, 1977b). Belák (1980) described that rat erythrocytes are agglutinated by OAV-1–OAV-5 and also by bovine and ovine strains of BAV-2B.

With AGID, CF and IF tests all viruses isolated from sheep share a soluble group specific antigen with human adenoviruses, but not to the same extent (McFerran et al., 1971; Belák, 1980; McFerran, 1981).

The six serotypes and BAV-2B strains of sheep could be clearly distinguished in cross-neutralization tests (Adair and McFerran, 1976; Belák, 1980; Adair et al., 1982); HI tests confirmed the results of the neutralization tests (Belák, 1980), which revealed cross-reactions of a low degree between OAV-2 and OAV-3 and viruses of the bovine subgroup I (Adair and McFerran, 1976). Some cross-reaction with caprine adenoviruses was also observed (Gibbs et al., 1977). Strains of BAV-2B isolated from sheep showed a complete two-way reaction with the reference strain of BAV-2 allowing designation as the same serotype (Belák, 1978). Strain WV757/75 isolated in New Zealand cross-reacted with BAV-7 to an extent that there was insufficient difference to describe it as a separate serotype (Adair et al., 1982).

Cultivation

Ovine adenoviruses readily replicate in various ovine and other mammalian cell cultures, such as lamb kidney, testis, thyroid and lung cells (McFerran et al., 1971; Sharp et al., 1974; Dubey et al., 1986), sheep chorioid plexus cells (S. Belák and B. Klingeborn, unpublished data, 1983), in calf kidney and testis cells, pig kidney cells, and at varying rates in the MDBK cell line (Belák, 1980). The only exception is the collection of New Zealand isolates, including OAV-6, which preferably multiply in lamb testis cell cultures (Davies and Humphreys, 1977b; Adair et al., 1982).

CPE appears generally 14–24 h p.i. (Belák et al., 1978; Adair et al., 1979). Increased refractility and rounding of cells are the earliest changes noted in

monolayer cultures. Comparison of replication cycles in stationary and rotated cultures revealed considerable inter- and intratypic differences. Intra- and extracellular virus titers generally culminate around 40–58 h after inoculation (Belák et al., 1978; Belák, 1980).

The earliest inclusions in the nucleus are observed 14 h p.i. Enlargement of the nucleus is accompanied with deformation and the appearance of pleiomorphic inclusion bodies (Adair et al., 1979). Nearly all cells are affected 52 to 72 h p.i. (Belák et al., 1978). Using EM, Müller (1974) observed virus particles in the nucleus in the 36th hour of infection. Other investigators showed the presence of newly formed virions as soon as 10 h (Belák, 1980) and 24 h (Adair et al., 1979) after infection.

Viral antigen in the nucleus was demonstrated by IF test 8–14 h p.i. (Fig. 57; Belák et al., 1978; Adair et al., 1979).

EPIZOOTIOLOGY

As adenoviruses in general, ovine strains seem to be largely confined to their host species. However, fatal outbreaks of pneumoenteritis caused by BAV-2 were also repeatedly observed among lambs (Belák and Pálfi, 1974a,b). The BAV-2 viruses are similarly pathogenetic for lambs and calves.

The presence of adenoviruses or adenoviral antibodies in sheep populations has been proven in England (Darbyshire and Pereira, 1964), Northern Ireland (McFerran et al., 1969, 1971), Hungary (Belák and Pálfi, 1974a), Scotland (Sharp et al., 1974), Turkey (Bauer et al., 1975), New Zealand (Davies and Humphreys, 1977b), France (Russo et al., 1978), Australia (Peet et al., 1983), the USA (Lemkuhl and Cutlip, 1984a, b), Nigeria (Obi and Taylor, 1984), Sweden (Belák et al., 1985), India (Dubey and Sharma, 1985) and Bulgaria (Sizov et al., 1988). One of the New Zealand isolates was found serologically related to
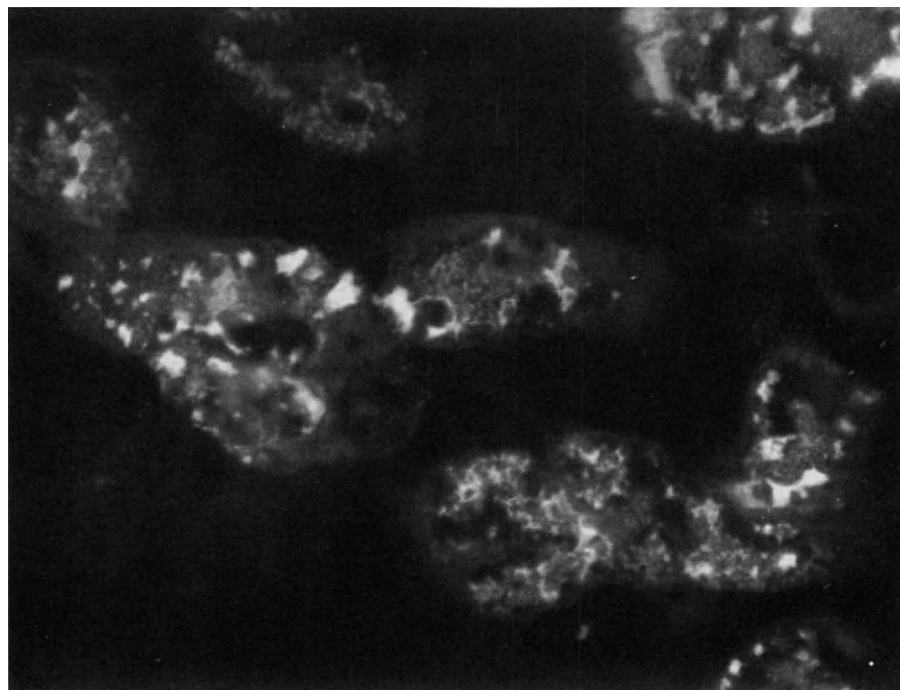
Fig. 57. Intranuclear fluorescence in lamb kidney cells 14 h after infection with a sheep isolate of BAV-2B (× 800).

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BAV-7. An American isolate was identified as OAV-5, the other as OAV-6, although the serological cross-reaction was largely one-sided (Adair et al., 1985). The Swedish and Indian isolates proved to be OAV-1 (Belák et al., 1985; Dubey and Sharma, 1985, 1986).

To learn about the geographic distribution within a country a serological survey was done in Hungary. Of 50 large farms, 43 were found to be affected generally by more than one serotype (Pálfi and Belák, 1978). Seroepidemiological surveys in Northern Ireland, the USA and India showed similar high prevalence of adenovirus antibodies in sheep populations (Adair et al., 1984; Dubey et al., 1985; Lemkuhl et al., 1985). Antibodies against BAV subgroup II antigen were also found in sheep (Bélak, 1980; Adair et al., 1984). This shows that the bovine is not the only animal species which is infected with subgroup II adenoviruses, which differ from all other mammalian adenoviruses in antigen composition. Experiments in cattle show that subgroup II adenoviruses may have great economical importance, but their unique cell spectrum (preferential growth in testicle but not in kidney cells) makes detection difficult.

Adenoviral pneumoenteritis may cause great losses both in suckling and fattening lambs (Bélak and Pálfi, 1974b). This affects mainly large, industrialized farms, where many lambs of different immunological background are collected. Economical losses are caused not only by death and emergency slaughter, but also by increased food consumption and retarded growth rate of lambs. Preventive vaccination showed that rentability of fattening may be substantially lowered even by clinically inapparent infections.

Virus is shed mainly with the nasal discharge, feces and urine. Direct contact between animals is the most important factor in the spread of infection. Fattening lambs in units with continuous replacement are most likely to develop overt disease. This is the reason why the most serious cases of pneumoenteritis have been observed in fattening plants where several thousand lambs are continuously housed. Under such conditions the passage of the virus is uninterrupted, resulting in a permanent chain of infection with adenoviruses of enhanced virulence (Belák et al., 1976). Endemics with clinical symptoms generally prevail in a large farm for 1–2 months, depending upon its size and the degree of close contact between lambs. The recovered animals may shed the virus permanently, passing the infection to newly arrived lambs.

Crowding, insufficient ventilation, heat stress, etc. may aggravate the outcome of the disease. Inadequate feeding of ewes may contribute to the severity of the disease in suckling lambs. The effect of these factors was directly proven in immunization experiments (Pálfi et al., 1980a).

The disease is frequently accompanied by infection with other viruses and bacteria. Simultaneous infection with reovirus and/or parainfluenza-3 virus is common. Bacterial complications are generally caused by *Pasteurella haemolytica* or corynebacteria (Bélak, 1980; Leamaster et al., 1987). Infection with *Mycoplasma ovipneumoniae* is also common (Stipkovits et al., 1975).

As mentioned above, inapparent infections occur very often. However, in flocks where the infection results in clinical disease, morbidity may reach 100%. The disease occurs in 2–12-week-old animals and may cause losses as high as 30–40% in suckling lambs and 10–15% in fattening lambs (Belák et al., 1976). Approximately 10% of the losses occur in the acute phase of the disease and about 90% in the second phase, when bacterial infections complicate the process (Belák et al., 1976).

PATHOGENESIS

The disease may be reproduced by intranasal and intratracheal inoculation of the virus into lambs. The virus replicates in the respiratory and intestinal

tract; from about 4 days p.i. and onward pathological changes may appear in other organs, indicating that viremia has developed. Lesions were found in the nasal mucous membranes, lungs, intestines, lymph nodes, spleen, kidneys and liver (Belák et al., 1975, 1980; Tury et al., 1975; Palya et al., 1977; Pálfi et al., 1982). Sharp et al. (1976) demonstrated virus in various organs, but characteristic pathological changes were not observed. Rushton and Sharp (1977) later continued the studies with OAV-4 and reported pulmonary and hepatic lesions. Experimental infections with New Zealand isolates revealed virus replication in the respiratory tract and appearance of the virus in the kidneys (Davies and Humphreys, 1977a; Davies et al., 1982). Australian investigators observed adenovirus inclusions and virus particles in areas of hepatic lesions (Peet et al., 1983). Experimental infection with the OAV-6 and OAV-5 USA isolates resulted in lesions in the respiratory tract, most severe in the terminal airways (Cutlip and Lemkuhl, 1983, 1986; Lemkuhl and Cutlip, 1986). Indian investigators produced experimental pneumoenteritis in seronegative young lambs by inoculating a local isolate of OAV-1 (Dubey et al., 1987).

The discrepancies found by various groups may be due to differences in virulence of the viruses studied. Hungarian authors described, for example, that two strains of OAV-5, one of them isolated from the nasal discharge of an acutely diseased lamb, the other from the feces of an apparently healthy animal, differed in their invasiveness, although both belong to the same serotype (Belák et al., 1980).

The virus is shed with the nasal discharge and feces generally from day 2–3 p.i. onward (Belák et al., 1975, 1980; Davies et al., 1982; Pálfi et al., 1982; Lemkuhl and Cutlip, 1984a). Davies et al., (1982) reported peak virus titers 4–6 days p.i., which then decreased rapidly until only traces were present in the nasal secretions on day 8. Other authors were able to reisolate the virus 3–14 days p.i. from feces, 3–11 days p.i. from nasal discharge and 3–9 days p.i. from the urine of experimentally infected lambs (Pálfi et al., 1982).

Field investigations proved that the virus can be readily isolated from naturally infected lambs in the acute phase of the disease (Belák et al., 1976). Later, lambs may become carriers, and virus can be isolated only occasionally from animals with antibodies; in these cases virus may be shed intermittently with the feces (Pálfi and Belák, 1978). Carrier sheep were also reported by Sharp et al. (1976), who were able to reisolate the virus from the feces of an animal 80 days p.i. Pálfi et al. (1982) studied chronic infection and found that histopathological lesions in the respiratory and renal epithelium persisted throughout the observation period of 75 days. In this experiment acute lesions reappeared after cortisone treatment; however, no virus excretion could be demonstrated in the chronic period of the infection.

OAV-1 strains were isolated from the kidneys of sheep fetuses, and VN antibodies were detected in the fetal blood samples (Belák and Rusvai, 1986). These data indicate the possibility of a transplacental infection.

DISEASE SIGNS

In the field, adenoviral disease may start with diarrhea after an incubation period of 7–8 days; respiratory symptoms follow 2–3 days later. Sneezing, seromucous nasal discharge, conjunctivitis and lachrymation are common. Breathing may be superficial, accelerated and forced. Diarrhea usually stops after one week. Respiratory symptoms persist, turning chronic in most cases. The chronic form is characterized by seropurulent nasal discharge, respiratory disorders and coughing. Temperature may rise to 41–42°C. Chronic respiratory symptoms may prevail for months. Spontaneous, intermittent diarrhea may
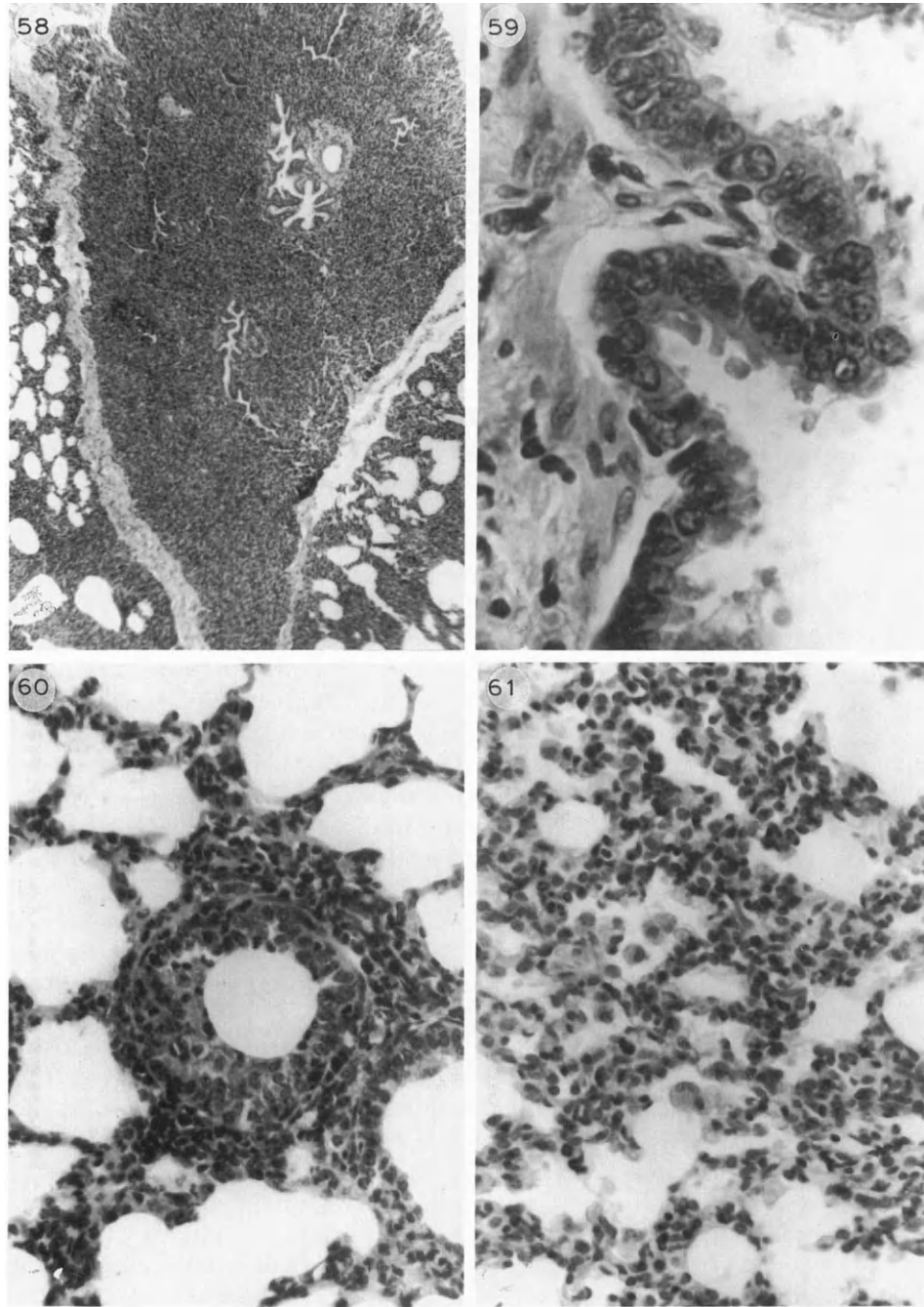
Fig. 58. Atelectasis in a pulmonary lobule of a lamb infected with BAV-2B; H & E, × 40 (courtesy of Dr. E. Tury. Department of Anatomy, University of Veterinary Sciences, Budapest).

Fig. 59. Swollen and proliferating epithelial cells in a bronchiolus of a lamb infected with BAV-2B; H & E, × 690 (courtesy of Dr. E. Tury).

Fig. 60. Epithelium cell proliferation in a bronchiolus of a lamb infected with BAV-2B; H & E, × 275 (courtesy of Dr. E. Tury).

Fig. 61. Intralobular interstitial pneumonia in a lamb infected with OAV-5; in the alveoli a large number of detached, degenerated alveolar epithelial cells are present; H & E, × 400 (courtesy of Dr. F. Vetési, Department of Pathology, University of Veterinary Sciences, Budapest).

occur. Sometimes the disease occurs only in the respiratory form, without intestinal disorders.

During the acute phase of pneumoenteritis the lambs loose weight. A retarded growth rate as a consequence of anorexia is common in both phases of the disease (Belák et al., 1976). The same symptoms could be reproduced by experimental infection (Belák et al., 1975; Tury et al., 1975; Palya et al., 1977). In an experiment by Pálfi et al. (1982) early signs of respiratory tract involvement (rales) and swelling of retropharyngeal and submandibular lymph nodes were seen. In several lambs percussion revealed blunt areas in the retrocubital zone and crepitation could be heard. Moderate leukocytosis developed on days 10–13 p.i. The differential leukocyte count showed a significant increase in neutrophils and a decrease of lymphocytes. Kidney involvement resulted in the appearance of protein, blood and pus in the urine. The urine sediment contained renal epithelial cells, erythrocytes, leukocytes and various cylinders.

Experimental infection with the New Zealand isolates produced mild respiratory illness (Davies et al., 1981, 1982). Lambs experimentally infected with a USA virus strain developed a mild febrile response and serous nasal discharge; they were inactive and their respiratory rates and efforts were increased (Lemkuhl and Cutlip, 1984a).

PATHOLOGY

Gross changes

In cases of pure viral infection, lesions of focal interstitial pneumonia are common, mainly in the apical, cardiac and accessory lobes. Bacterial complications are indicated by lesions of croupous pneumonia. In some cases chronic catarrhal bronchopneumonia can be seen. Lesions in the tracheal mucous membrane are connected with bacterial invasion. The surface of the mucous membrane appears swollen and reddish, covered by catarrhal exudate. The nasal mucous membrane is swollen and reddish already at the very beginning of the disease and is covered by seromucous, later by mucopurulent exudate. The mucous membrane of the small intestines is generally congested and covered by viscous exudate. The lymphoid patches are swollen and may contain pinpoint hemorrhages. The retropharyngeal, peribronchiolar and especially the mesenteric lymph nodes are markedly swollen. In the cortex of the kidneys sometimes greyish-white foci are seen.

Histopathology

Lesions in the lungs can be detected as early as 4 days p.i. The endothelial cells of the capillaries are swollen and marked histiocytic proliferation and infiltration of the alveolar septa by lymphocytes and neutrophilic granulocytes can be seen. Degeneration and desquamation of the alveolar epithelial cells can be demonstrated (Figs. 58–61). Peribronchial lymphoid follicles generally show hyperplasia. In some cases proliferation of the lymphoid cells results in peribronchial cuffing. In complicated cases croupous pneumonia and chronic catarrhal pneumonia may characterize the histopathological lesions.

In the submucosa of the trachea of animals succumbed in the advanced stage of the disease hyperemia and edema can be present. In the epithelial and propria layers massive infiltration with neutrophils can be observed. In the lamina propria and submucosa of the nasal mucous membrane edema and hyperemia are common. In some foci epithelial degeneration and necrosis are accompanied by infiltration of neutrophilic granulocytes. In addition, par-
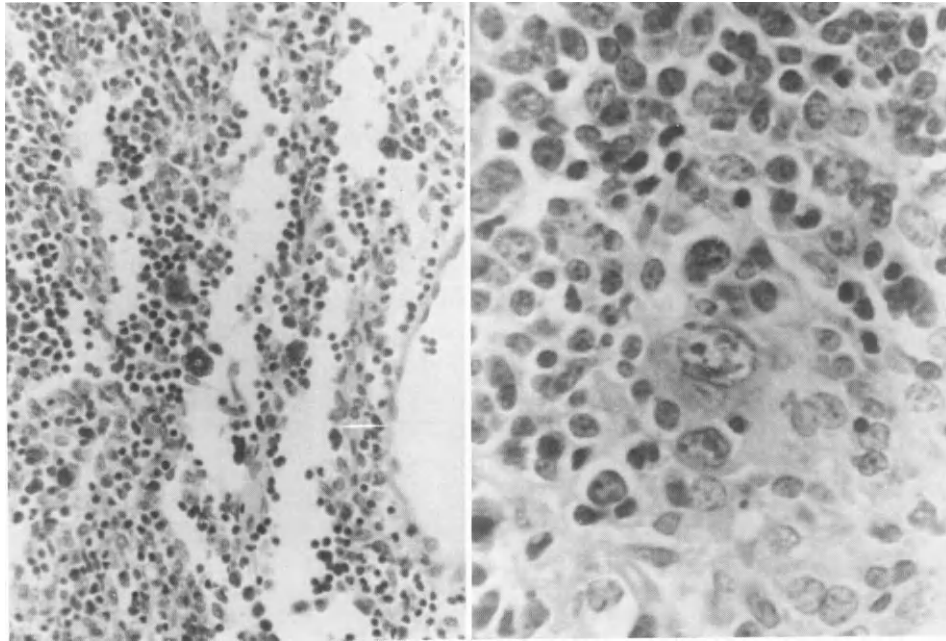
Fig. 62 (left). Peribronchial lymph node of a lamb infected with OAV-5; so-called smudge cells and polynuclear giant cells; H & E, × 400 (courtesy of Dr. F. Vetési).

Fig. 63 (right). Mononuclear giant cell in the peribronchial lymph node of a lamb infected with OAV-5; inclusion bodies in the nuclei of proliferating reticular cells; H & E, × 900 (courtesy of Dr. F. Vetési).

ticularly around the blood vessels of the propria, infiltration with histiocytes and lymphoid cells can be observed. Hyperplasia of the lymphoid follicles can also be present.

Similar changes are seen in the retropharyngeal, peribronchial and mesenteric lymph nodes; their structure is changed due to proliferation of follicles and thickening of medullary cords. Sinuses are dilated and filled with proliferating endothelial and reticular cells, lymphocytes, plasma cells and neutrophilic granulocytes (Figs. 62, 63).

In the small intestines infiltration of the mucous membrane with eosinophilic and neutrophilic granulocytes and to a lesser degree with lymphocytes and plasma cells can be observed. Hyperemia of the lymphoid patches is also seen.

In the kidneys the tubular cells, especially those in the proximal convoluted tubules, show degeneration. Some epithelial cells are enlarged, their cytoplasm is foamy. Signs of edema can be found by EM in the tubular epithelial cells (Fig. 64). Pyknosis in the nuclei of tubular epithelial cells can be seen, mitochondria can be disarranged and accumulated around the nucleus. By 13–15 days p.i. destruction or, in other areas, proliferation of the tubular epithelial cells can be seen as well as interstitial infiltration of lymphocytes and histiocytes around the glomeruli and tubules (Fig. 65).

Activation of the reticuloendothelial system is general in the liver; hepatitis can also be found. Hyperplasia of the reticular cells of the spleen may occur. Degeneration and neutrophil granulocytic infiltration can be seen in all three layers of the conjunctiva.

In cases uncomplicated by bacterial infection, especially when lambs are emergency slaughtered, basophilic nuclear inclusions can be seen in the bronchiolar epithelial cells, alveolar septal cells, reticulum cells of lymph nodes and
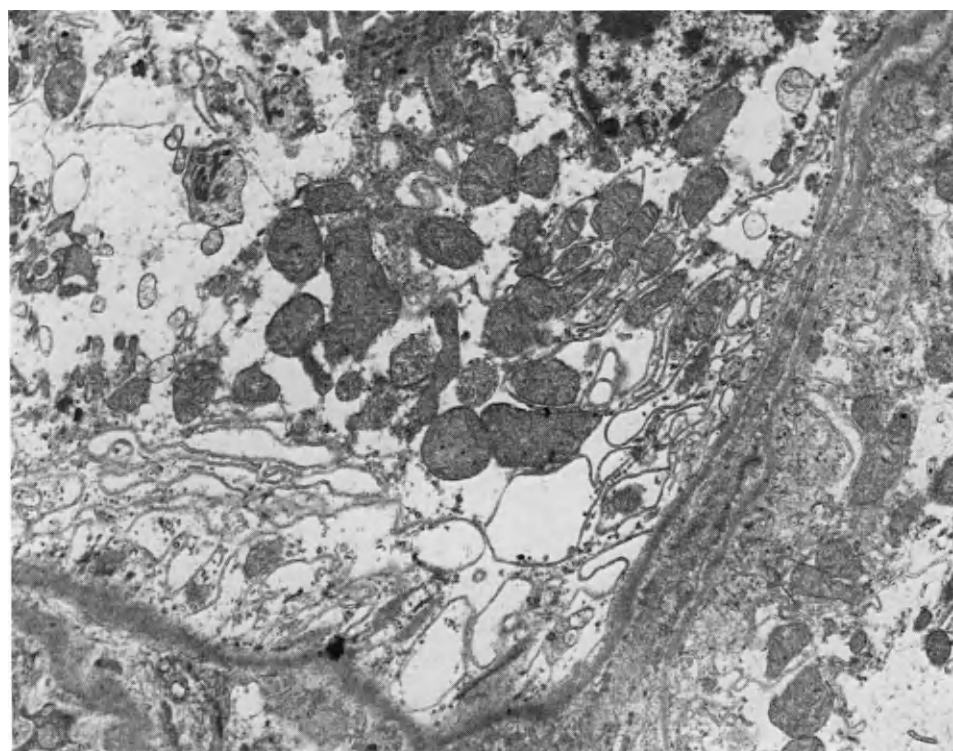
Fig. 64. Lesion in the kidney of a lamb infected with OAV-5; degeneration of epithelial cells of the proximal tubules; EM, × 2000 (courtesy of Dr. F. Vetési).

epithelial cells of the nasal mucosa (Fig. 66). Inclusion bodies generally disappear by day 10 p.i.

The lesions listed above were reported after infection with OAV-1 and OAV-5, and BAV-2B (Belák et al., 1975, 1980; Palya et al., 1977; Pálfi et al., 1982). Pulmonay and hepatic lesions were found in specific pathogen free (SPF) lambs
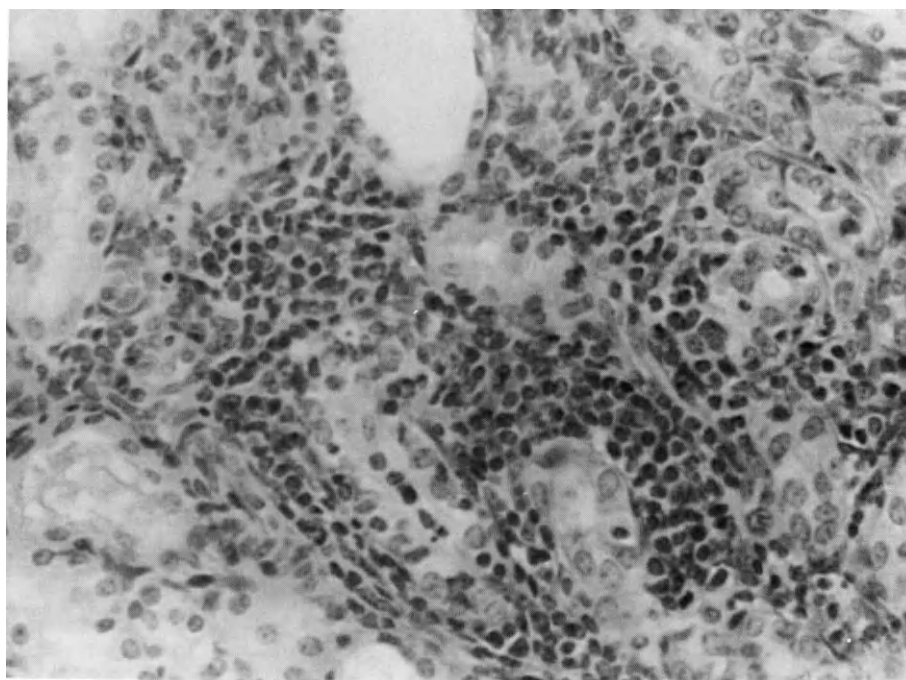
Fig. 65. Chronic nephritis in a lamb infected with OAV-1; intralobular and periglomerular cell infiltration in the renal cortex; H & E, × 160 (courtesy of Dr. F. Vetési).

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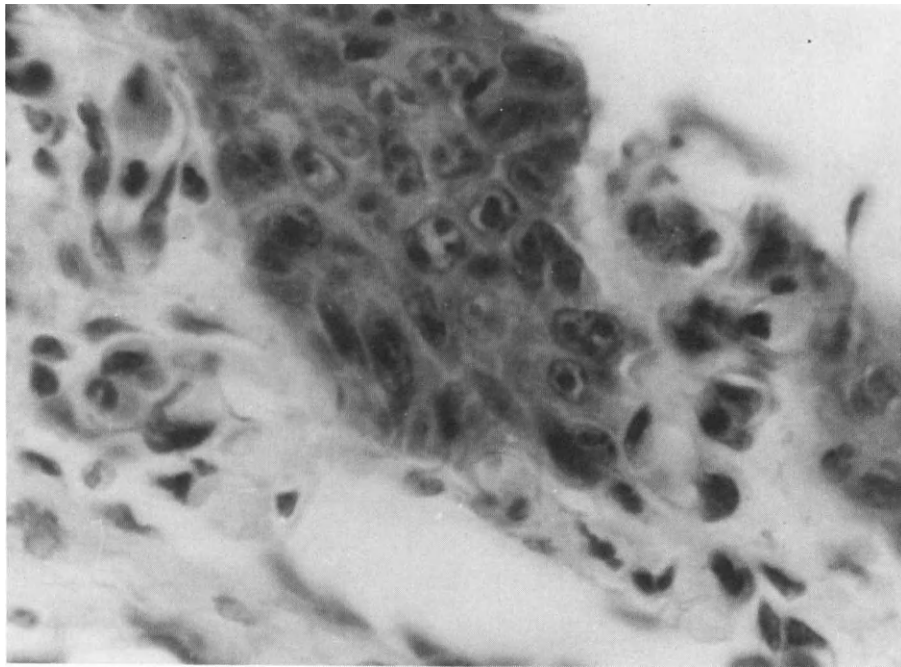
Fig. 66. Fully developed intranuclear inclusions in nasal mucous epithelial cells; BAV-2B infection; H & E × 690 (courtesy of Dr. E. Tury).

infected with OAV-4 (Rushton and Sharp, 1977). Pulmonary lesions were also described by New Zealand, American and Indian investigators (Davies and Humphreys, 1977a; Davies et al., 1981, 1982; Cutlip and Lemkuhl, 1983, 1986; Dubey et al., 1987). It worth noting that BAV-2B isolated from lamb caused similar disease and lesions in both lambs and calves (Figs. 67, 68; Belák et al., 1975, 1977; Tury et al., 1975, 1978).
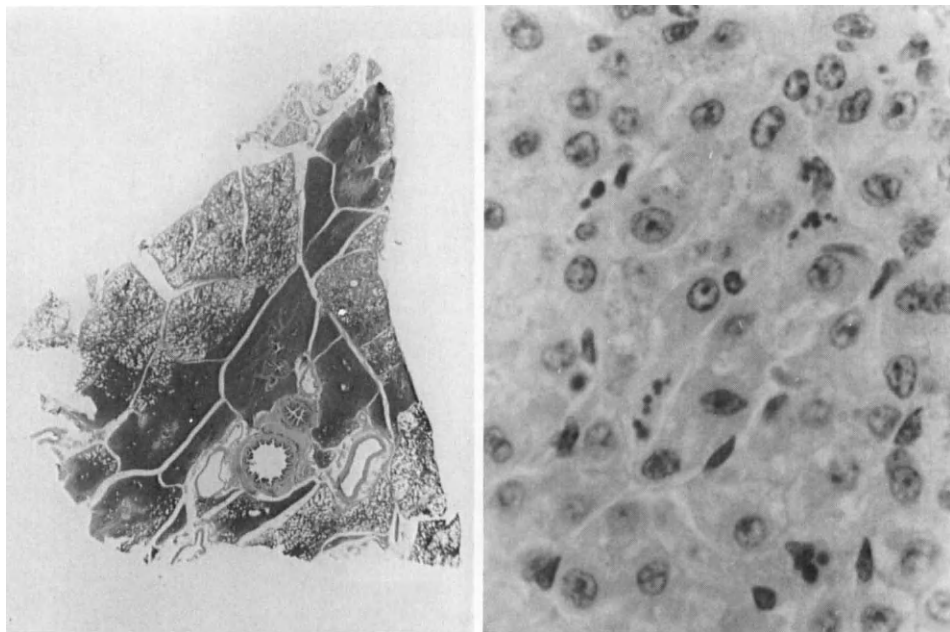
Fig. 67 (left). Collapsed lobules in the lungs of a calf infected with BAV-2B isolated from a lamb; H & E (courtesy of Dr. E. Tury).

Fig. 68 (right). Karyopyknosis and karyorrhexis in the Kupffer cells of a calf infected with BAV-2B isolated from lamb; H & E, × 690 (courtesy of Dr. E. Tury).
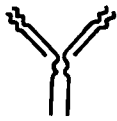
IMMUNE REACTION

When antibody-free lambs are infected, neutralizing antibodies appear 5–7 days p.i., reaching maximal titers of 32–256 by days 16–21 (Belák et al., 1975; Davies et al., 1982; Lemkuhl and Cutlip, 1984a). Subsequently the antibody titer gradually decreases (Pálfi et al., 1982).

Sharp et al. (1976) studied the development of precipitating antibodies. On day 20 p.i. these were not demonstrable; they appeared around day 31 and were still present 90 days p.i. Increase in antibody titers was observed following the antigenic stimulus with a different serotype, indicating that virus belonging to one serotype is able to boost the antibodies against another serotype (Pálfi and Belák, 1979).

The adenovirus-induced immune response has also been studied in 70–80-day-old ovine fetuses. They were experimentally infected, removed from the uterus 19 days p.i. and examined for pathological changes and immune reactions. Fetuses (and their noninfected twins) had neutralizing serum antibodies against the inoculated virus in a titer range of 32–128. The antibodies were identified as belonging to the subclasses IgG_1 and IgG_2 (Tuboly and Belák, 1980). Tuboly (1987) reported that adenovirus-induced immune-complexes might be important in the pathogenesis of fetopathies.

LABORATORY DIAGNOSIS

Differentiation of adenoviral pneumoenteritis from other viral diseases of very similar symptoms (like reovirus or parainfluenza-3 virus infection) has to be based on laboratory examination.

Nasal secretion, feces and urine are used for virus isolation. Samples must be collected early in the acute period of the disease, before virus shedding significantly decreases (around 10 days p.i.). Within this period organ homogenates (lung, kidney, liver, intestine, lymph nodes) may also serve as sources for virus isolation. After appearance of antibodies the change of virus isolation decreases. However, protracted shedding of virus via the feces may occur.

Lamb kidney, testicle and thyroid cells are equally suitable for virus isolation. After one or two passages a CPE usually appears. Viral antigen can be detected in the organs of acutely diseased lambs and in the inoculated tissue cultures by direct IF test, making a rapid diagnosis possible. EM may reveal adenovirions in the nuclei of infected cells. The viral DNA can be rapidly detected in nasal cells by DNA-hybridization, using cloned viral DNA fragments or synthetic oligonucleotide molecules as probes (Belák et al., in preparation).

Serologic screening should be done on about 5–10% of the flock. It is advisable to check the sera first by gel precipitation, IF test or ELISA; those reacting may be assayed in the neutralization test against different serotypes of ovine and also bovine adenoviruses.

When studying acute disease, paired sera are of great value to demonstrate the rise of neutralizing and HI antibodies to the given serotype.

PROPHYLAXIS AND CONTROL

Since predisposing conditions play an important role in the manifestation of adenoviral pneumoenteritis of lambs, elimination of these factors is of great importance. Adequate feeding and management conditions for the preparation

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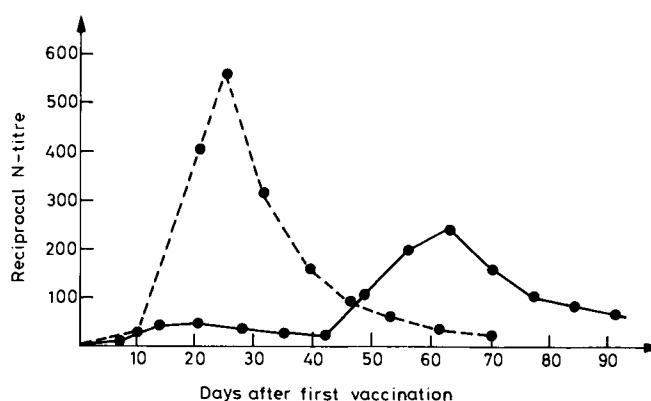
Fig. 69. Comparison of the serological response after revaccination on day 10 (---) or day 42 (—); geometric mean titers of sera from ten sheep.

of ewes for lambing must be assured. Crowding of fattening lambs and mixing of large populations must be prevented.

In farms where thousands of lambs are collected it is difficult to prevent adenovirus infection. Detailed studies have shown that the infection may manifest itself as a pronounced respiratory and/or enteric disease, but in the majority of the cases it occurs in a clinically inapparent form. Since both forms cause important economic losses, it is necessary to prevent infection.

In Hungary an inactivated, bivalent sheep adenovirus vaccine (OVIVAC) is commercially available which has been tested in many large farms. After administration, neutralizing antibody titers of 128–512 appear in the sera of lambs. Experimental infections showed that viremia was prevented, whereas the nonvaccinated controls developed signs of disease and shed virus. Neither clinical disease nor pathological lesions developed in the vaccinated lambs (Pálfi and Belák, 1978/79; 1979). Vaccination resulted in increased homologous (type-specific) and heterotypic antibody levels in the sera of pregnant ewes, with passive immunity conferred to their lambs. Challenge of suckling lambs with homologous and heterologous virus showed significant resistance to development of lesions by homologous virus (present in the vaccine); partial resistance was observed against heterologous virus (Pálfi et al., 1980b).

Factors affecting antibody production after vaccination were studied. Antibodies produced by a single vaccination of seronegative lambs were detectable only for a short time at a sufficient level to prevent virus shedding and development of clinical symptoms. This indicated the need for two vaccinations. The time of the second vaccination significantly influences its efficacy. The level and persistence of antibodies produced by the booster dose given 10 or 42 days
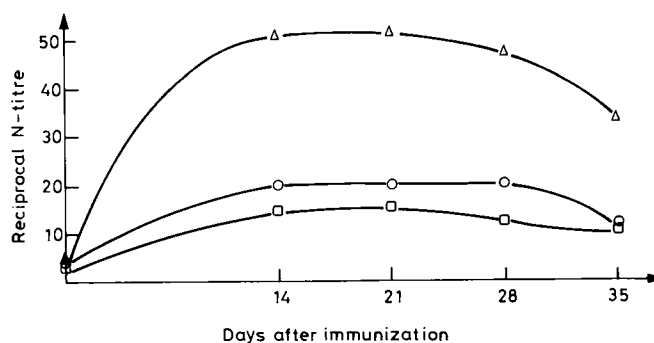
Fig. 70. The influence of high and low temperature stress on the immunological response of lambs; (—o—) 5–15°C, varying; (—▲—) 15°C, constant; (—□—) from 15 to 30°C, varying; geometric mean titers of sera from eight animals.

following the first dose significantly exceeded those when the booster dose was given at a 21-day interval. Revaccination while antibody production was increasing gave the highest titers; revaccination done in the phase of decreasing antibody production gave lower titers but longer persistence (Fig. 69; Pálfi et al., 1980a).

Cold and heat stress also affect antibody production after vaccination. Antibody titers were higher in vaccinated lambs kept at a favourable ambient temperature than in those kept at varying low and high temperatures (Fig. 70; Pálfi et al., 1980a).

In farms where regular immunization had been introduced, losses due to pneumoenteritis were markedly reduced. An economic effect of the vaccine was also noted on farms where no clinical disease but only seropositivity had indicated adenovirus infection. On these farms food consumption was reduced by 10–15% in the vaccinated fattening lambs. Also, daily weight gain was higher and fattening completed faster (Szilágyi et al., 1981). It is interesting to note that the occurrence of urolithiasis was also reduced in vaccinated fattening lambs. Considering the kidney lesions induced by adenovirus infection, a preventive effect of vaccination cannot be excluded, but further studies are needed to answer this question.

Pregnant ewes are vaccinated twice with a 6-week interval between the injections. The second vaccination should be given no later than 3 weeks before lambing. In this way suckling lambs are passively protected. Lambs are actively immunized around 5 weeks of age and given a booster vaccination 10 days later (the dosage of the OVIVAC vaccine is 2 ml subcutaneously, both for ewes and lambs). Immunization of lambs provides lifelong immunity. Since the vaccine causes a good booster effect, a single revaccination of the ewes during each subsequent pregnancy is sufficient to guarantee high colostral antibody levels.

REFERENCES

Adair, B.M. and McFerran, J.B., 1976. Comparative serological studies with mammalian adenoviruses. Arch. Virol., 51: 319–325.

Adair, B.M., Curran, W.L. and McFerran, J.B., 1979. Light and electron microscope studies of the growth of ovine adenoviruses in primary lamb kidney cell cultures. Vet. Microbiol., 4: 293–303.

Adair, B.M., McFerran, J.B and McKillop, E.R., 1982. A sixth species of ovine adenovirus isolated from lambs in New Zealand. Arch. Virol., 74: 269–275.

Adair, B.M., McFerran, J.B., McKillop, E.R. and McCullough, S.J., 1984. Survey for antibodies to respiratory viruses in two groups of sheep in Northern Ireland. Vet. Rec., 115: 403–406.

Adair, B.M., McKillop, E.R. and McFerran, J.B., 1985. Serologic classification of two ovine adenovirus isolates from the central United States. Am. J. Vet. Res., 46: 945–946.

Bauer, K., Müller, H. and Gürtürk, S., 1975. Isolierung eines Virus von Schafen und seine Einordnung als neuer Serotyp oviner Adenoviren. Zentralbl. Veterinärmed. B, 22: 556–665.

Belák, S., 1978. Characteristics of Ovine Adenoviruses. Thesis, Univ. Budapest, 155 pp. (in Hungarian).

Belák, S., 1980. Properties of ovine adenoviruses. Acta Vet. Acad. Sci. Hung., 28: 47–55.

Belák, S. and Pálfi, V., 1974a. An adenovirus isolated from sheep and its relationship to type 2 bovine adenovirus. Arch. Ges. Virusforsch., 46: 366–369.

Belák, S. and Pálfi, V., 1974b. Pneumoenteritis of lambs caused by adenovirus. Acta Vet. Acad. Sci. Hung., 24: 327–328.

Belák, S. and Rusvai, M., 1986. In utero adenoviral infection in sheep. Vet. Microbiol., 12: 87–91.

Belák, S., Pálfi, V. and Tury, E., 1975. Experimental infection of lambs with an adenovirus isolated from sheep and related to bovine adenovirus type 2. Virological studies. Acta Vet. Acad. Sci. Hung., 25: 91–95.

Belák, S., Pálfi, V. and Palya, V., 1976. Adenovirus infection in lambs. I. Epizootiology of the disease. Zentralbl. Veterinärmed. B, 23: 320–330.

Bélak, S., Pálfi, V., Szekeres, T. and Tury, E., 1977. Experimental infection of calves with an adenovirus isolated from sheep and related to bovine adenovirus type 2. I. Clinical and virological studies. Zentralbl. Veterinärmed. B, 24: 542–547.

Belák, S., Pálfi, V. and Tuboly, S., 1978. Replication of ovine adenoviruses. Acta Microbiol. Acad. Sci. Hung., 25: 253–264.

Belák, S., Vetési, F., Pálfi, V. and Papp, L., 1980. Isolation of a pathogenic strain of ovine adenovirus type 5 and a comparison of its pathogenicity with that of another strain of the same serotype. J. Comp. Pathol., 90: 169–176.

Belák, S., Berencsi, Gy., Rusvai, M., Lukács, K. and Nász, I., 1983. DNA structure, and hemagglutination properties of bovine adenovirus type 2 strains which bypass species specificity. Arch. Virol., 77: 181–194.

Belák, S., Thunegard, E. and Rusvai, M., 1985. Occurrence of adenoviral pneumoenteritis among sheep kept under extensive conditions in Sweden. Nordisk Veterinärmed., 37: 249–250.

Cutlip, R.C. and Lemkuhl, H.D., 1983. Experimental infection of lambs with ovine adenovirus isolate RTS-151: Lesions. Am. J. Vet. Res., 44: 2395–2402.

Cutlip, R.C. and Lemkuhl, H.D., 1986. Pulmonary lesions in lambs infected with ovine adenovirus 5 strains RTS-42. Vet. Pathol., 23: 589–593.

Darbyshire, J.H. and Pereira, H.G., 1964. An adenovirus precipitating antibody present in some sera of different animal species and its association with bovine respiratory disease. Nature, 201: 895–897.

Davies, D.H. and Humphreys, S., 1977a. Experimental infection of lambs with an adenovirus of ovine origin. Vet. Microbiol., 2: 67–72.

Davies, D.H. and Humphreys, S., 1977b. Characterization of two strains of adenovirus isolated from New Zealand sheep. Vet. Microbiol., 2: 97–107.

Davies, D.H., Dungworth, D.L. and Mariassy, A.T., 1981. Experimental adenovirus infection of lambs. Vet. Microbiol., 6: 113–128.

Davies, D.H., Herceg, M. and Thurley, D.C., 1982. Experimental infection of lambs with an adenovirus followed by *Pasteurella haemolytica*. Vet. Microbiol., 7: 369–381.

Dubey, S.C. and Sharma, S.N., 1985. Ovine adenovirus (OAV) pneumoenteritis in lambs in India. Ind. J. Anim. Sci., 55: 878–879.

Dubey, S.C. and Sharma, S.N., 1986. Isolation and characterization of ovine adenovirus (OAV) type 1 from lambs. Ind. J. Anim. Sci., 56: 301–305.

Dubey, S.C., Shrivastava, C.P., Lonkar, P.S. and Maru, A., 1985. Prevalence of oviadenovirus antibodies in sheep and goats of semiarid Rajasthan. Ind. J. Comp. Microbiol., Immunol. Infect. Dis., 6: 167–169.

Dubey, S.C., Kumar, N. and Sharma, S.N., 1986. Cytopathic effect of ovine adenovirus type 1 in the primary cell-cultures of ovine and caprine origin. Ind. J. Anim. Sci., 56: 385–387.

Dubey, S.C., Kumar, N. and Sharma, S.N., 1987. Experimental pneumoenteritis in lambs with local isolate of ovine adenovirus type 1. Ind. J. Anim. Sci., 57: 787–792.

Gibbs, E.P.J., Taylor, W.P. and Lawman, M.J.P., 1977. The isolation of adenoviruses from goats affected with peste des petits ruminants in Nigeria. Res. Vet. Sci., 23: 331–335.

Leamaster, B.R., Evermann, J.F. and Lemkuhl, H.D., 1987. Identification of ovine adenovirus types five and six in an epizootic of respiratory tract disease in recently weaned lambs. J. Am. Vet. Med. Assoc., 190: 1545–1547.

Lemkuhl, H.D. and Cutlip, R.C., 1984a. Experimental infection of lambs with ovine adenovirus isolate RTS-151: Clinical, microbiological, and serological responses. Am. J. Vet. Res., 45: 260–262.

Lemkuhl, H.D. and Cutlip, R.C., 1984b. Characterization of two serotypes of adenovirus isolated from sheep in the central United States. Am. J. Vet. Res., 45: 562–566.

Lemkuhl, H.D. and Cutlip, R.C., 1986. Inoculation of lambs with ovine adenovirus 5 (Mastadenovirus ovi 5) strains RTS-42. Am. J. Vet. Res., 47: 724–726.

Lemkuhl, H.D., Cutlip, R.C., Bolin, S.R. and Brogden, K.A., 1985. Seroepidemiological survey to selected viruses in the respiratory tract of lambs. Am. J. Vet. Res., 46: 2601–2604.

McFerran, J.B., 1981. Adenoviruses of vertebrate animals. In: E. Kurstak and C. Kurstak (Editors), Comparative Diagnosis of Viral Diseases Vol. III. Academic Press, New York, London, pp. 101–165.

McFerran, J.B., Nelson, R., McCracken, J.M. and Ross, J.G., 1969. Viruses isolated from sheep. Nature, 221: 194–195.

McFerran, J.B., Nelson, R. and Knox, E.R., 1971. Isolation and characterization of sheep adenoviruses. Arch. Ges. Virusforsch., 35: 232–241.

Müller, H. 1974. Charakterisierung und Typisierung eines aus Schafen isolierten Adenovirus. PhD Thesis, University of Giessen, 119 pp.

Obi, T.V. and Taylor, W.P., 1984. Serological survey of adenovirus antibodies in domestic animals in Nigeria. Comp. Immun. Microbiol. Infect. Dis., 7: 63–68.

Pálfi, V. and Belák, S., 1978. Serologic distribution of antibodies against adenoviruses in sheep of large-scale farms. Acta Microbiol. Acad. Sci. Hung., 25: 213–217.

Pálfi, V. and Belák, S., 1978/1979. Protective effect of vaccination against experimental adenoviral pneumoenteritis in fattening lambs. Vet. Microbiol., 3: 191–198.

Pálfi, V. and Belák, S., 1979. Recall phenomenon induced with ovine adenoviruses. Arch. Virol., 61: 169–173.

Pálfi, V., Belák, S. and Papp, Z., 1980a. Factors influencing the effectiveness of adenovirus vaccination in lambs. Zentralbl. Veterinärmed. B, 27: 345–351.

Pálfi, V., Belák, S. and Palya, V., 1980b. Adenovirus vaccination of pregnant ewes and studies on the colostral immunity of their lambs. Vet. Microbiol., 5: 73–77.

Pálfi, V., Vetési, F., Belák, S. and Papp, L., 1982. Adenovirus infection in lambs. III. Studies on chronic infection. Zentralbl. Veterinärmed. B, 29: 231–241.

Palya, V., Belák, S. and Pálfi, V., 1977. Adenovirus infection in lambs. II. Experimental infection of lambs. Zentralbl. Veterinärmed. B, 24: 529–541.

Peet, R.L., Coackley, W., Purcell, D.A., Robartson, C.W. and Micke, B.M., 1983. Adenoviral inclusions in sheep liver. Aust. Vet. J., 60: 307–308.

Rushton, B. and Sharp, J.M., 1977. Pathology of ovine adenovirus type 4 infection in SPF lambs: pulmonary ond hepatic lesions. J. Pathol., 121: 163–167.

Russo, P., Lambert, M. and Giauffret, A., 1978. Isolement d'un adénovirus chez un agneau atteint d'entérite. Bull. Acad. Vet. Fr., 51: 183–187.

Sharp, J.M., McFerran, J.B. and Rae, A., 1974. A new adenovirus from sheep. Res. Vet. Sci., 17: 268–269.

Sharp, J.M., Rushton, B. and Rimer, R.D., 1976. Experimental infection of specific pathogen free lambs with ovine adenovirus type 4. J. Comp. Path., 86: 621–628.

Sizov, I., Zarkov, I. and Ivanov, J.E., 1988. Serological survey of lambs and sheep for viruses of the respiratory tract. Vet. Shirka, 86: 37–39 (in Bulgarian).

Stipkovits, L., Belák, S., Pálfi, V. and Tury, E., 1975. Isolation of *Mycoplasma ovipneumoniae* from sheep with pneumonia. Acta Vet. Acad. Sci. Hung., 25: 267 273.

Szilágyi, M., Pfeffer, U. and Németh, Gy., 1981. An introduction of the OVIVAC vaccine against pneumoenteritis of lambs. Allategészségügyi és Takarmányozási Közlemények, Phylaxia, Budapest, 3: 128–135 (in Hungarian).

Thurley, D.C., Boyes, B.W., Davies, D.H., Wilkins, M.F., O'Connell, E. and Humphreys, S., 1977. Subclinical pneumonia in lambs. N.Z. Vet. J., 25: 173–176.

Tuboly, S., 1987. Adenovirus-induced immune complexes in ovine fetuses. Vet. Immunol. Immunopathol., 15: 215–222.

Tuboly, S. and Belák, S., 1980. Adenovirus-induced immune response in ovine fetuses. Acta Microbiol. Acad. Sci. Hung., 27: 169–176.

Tury, E., Belák, S. and Pálfi, V., 1975. Experimental infection of lambs with a sheep adenovirus isolate closely related to type 2 bovine adenovirus. Acta Vet. Acad. Sci. Hung., 25: 171–184.

Tury, E., Belák, S., Pálfi, V. and Szekeres, T., 1978. Experimental infection of calves with an adenovirus isolated from sheep and related to bovine adenovirus type 2. II. Pathological and histopathological studies. Zentralbl. Veterinärmed. B, 25: 45–51.

Wigand, R., Bartha, A., Dreizin, R.S., Ische, H., Ginsberg, M.S., Green, M., Hierholzer, J.C., Kalter, S.S., McFerran, J.B., Pettersson, U., Russell, W.C. and Wadell, G., 1982. Adenoviridae: Second Report. Intervirology, 18: 169–176.

Papovaviridae

The term papova was formed by connecting the first two letters from the names of three viruses — papillomavirus, polyomavirus and the vacuolating agent (Latin "papilla" means nipple and "-oma" is a suffix meaning tumor). Polyoma means "many tumors", which appear in mice after polyomavirus infection. The vacuolating agent is a simian virus, also called SV40. The family comprises the two genera *Papillomavirus* and *Polyomavirus*. A "possible bovine polyomavirus" was demonstrated in cultured kidney cells of a newborn calf (Coackley et al., 1980). Antibodies to bovine polyomavirus were found in sera from persons that were in close contact with cattle (e.g. 71% of veterinary practitioners in Great Britain; Parry and Gardner, 1986). Otherwise, only members of the genus *Papillomavirus* occur in ruminants, where they may cause warts.

Papillomaviruses are nonenveloped, icosahedral particles, 50–55 nm in diameter. They are somewhat larger than polyomavirions, which measure about 45 nm across. The capsid is formed of 72 well-discernible capsomers in a skew arrangement. Tubular forms have been seen by EM. The viral core contains a single molecule of infectious circular double-stranded DNA with a mol.wt. of $3-5 \times 10^6$. In papillomas, the virions can be demonstrated only in keratinizing cells of the epidermis.

Buoyant density of papovaviruses is about $1.3 \, g/cm^3$ in CsCl and the sedimentation coefficient is between 240–300 S.

Generally, the papillomaviruses are considered to be species specific, but different types may be associated with various forms of papillomatosis in a single animal species, e.g. in cattle. The subdivision of papillomaviruses into six types is made by serology and, recently, by comparing restriction endonuclease cleavage patterns of their DNAs and the extent of DNA–DNA homology following hybridization. Papillomatosis is also known to occur in sheep, goats, deer and the European elk (for review, see Lancaster and Olson, 1982).

The replication of papillomaviruses occurs in the nucleus, but the viral DNA is not integrated into cellular DNA. A cell type permissive for growth of papillomaviruses in vitro is lacking, but there are established cell lines which respond to infection or transfection with foci of transformation.

REFERENCES

Coackley, W., Maker, D. and Smith, V.W., 1980. A possible bovine polyomavirus. Arch. Virol., 66: 161–166.

Lancaster, W.D. and Olson, C., 1982. Animal papillomaviruses. Microbiol. Rev., 46: 191–207.

Parry, J.V. and Gardner, S.D., 1986. Human exposure to bovine polyomavirus: a zoonosis? Arch. Virol., 87: 287–296.

Chapter 18

Papillomaviruses

C. OLSON

INTRODUCTION

Papillomaviruses, once thought of minor importance in animals and man, are now regarded as highly significant oncogenic factors in some neoplastic entities. Laryngeal papilloma and cancer, as well as carcinomas of the cervix and skin in man, skin cancer in goats, eye cancer in cattle, and equine sarcoid have been associated with papillomavirus infection. These inferences have come about because of application of recent developments in molecular biology and cloning technology (Lancaster and Olson, 1982). By these means at least six types of bovine papillomavirus (BPV) and at about 50 types of human papillomaviruses have been recognized in neoplastic lesions. This progress has been made even though these viruses had not been propagated in cell culture systems. Two fascinating details will be of general interest. First, an internal capsid antigen is common to all known papillomaviruses, so that antisera raised against this antigen can be used to mark the location of various types of papillomavirus in formalinized tissues using immunohistochemistry. The second is that BPV types 1 and 2 can serve as eukaryotic vectors in genetic engineering to insert foreign genetic material (DNA) into cells in culture. A few references (Sarver et al., 1982; Nakabayashi et al., 1983; Brian-Markson et al., 1985; Gorra et al., 1985; Spalholz et al., 1985) will indicate the extent of this work which has stimulated international workshops for coordination of papillomavirus research.

Warts of the skin (cutaneous papillomatosis) are usually self-limiting benign tumors occurring at multiple sites and usually in young animals (Fig. 71). Viruses causing these tumors are similar in morphology, but differ in other characteristics. The bovine papillomavirus (BPV-1 and 2), the ovine papillomavirus (OPV), the deer fibroma virus (DFV), and the European elk papillomavirus (EEOV) will cause a fibroblastic tumor in the hamster. In addition, BPV-1 and 2 will cause a fibroblastic tumor in horses and evidence of the BPV genome is found in naturally occurring equine sarcoids. Otherwise, the known papillomaviruses are oncogenic only for their species of origin (Koller and Olson, 1972; Lancaster and Olson, 1982). Table 19 summarizes the papillomatoses of ruminants.

The virus appears to infect the basal cells of the epithelium, causing some cells to degenerate while others are stimulated to excessive growth and wart formation. New virus particles form in the degenerating cells and may completely replace the nuclear material when the cell reaches the surface; thus, there is much infective virus at the surface of the wart. The virus is quite resistant and may contaminate fences, stanchions and other objects. Skin wounds from such objects frequently lead to infection of susceptible animals.

TABLE 19

Ruminant papillomatoses

Species	Transmission			Lesion
	Natural	Homologous	Heterologous	
Cattle BPV-1, 2	skin, fibropapilloma	skin and brain	horse	fibroma
	skin, fibropapilloma	genital epithelium	hamster	fibrosarcoma
			pika	fibroma
		urinary bladder		fibroma and carcinoma
Cattle BPV-3	skin, fibropapilloma	alimentary tract	0	
	alimentary, fibropapilloma			
Cattle atyp.	skin, papilloma	papilloma	–	
Cattle BPV-4	alimentary, papilloma	squamous papilloma	0	
	alimentary, carcinoma			
Cattle BPV-5	teat, fibropapilloma	papilloma	0	
Cattle BPV-6	teat, papilloma	papilloma	0	
Deer white tail DPV	skin, fibropapilloma	+	hamster	fibroma
Deer mule DPV	skin, fibropapilloma	+	hamster	fibroma
Elk EEPV	skin, fibropapilloma	+	hamster	fibroma
Sheep OPV	skin, fibropapilloma	+	hamster	fibroma
Goat	skin, papilloma	?	0	

+, tumor; –, no response; 0, not determined.

Fig. 71. Natural case of papillomatosis; probably inoculated by scratching back and cheek on boards contaminated with BPV. There had been no response to topical treatment with oils and vaccine therapy.
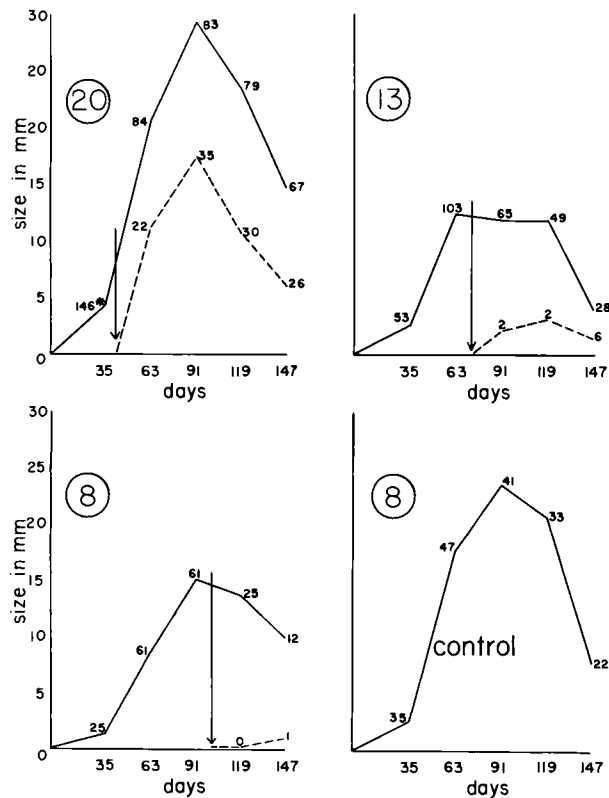
Fig. 72. Growth curve of warts of calves in which 25–50% of warts were removed at about 43, 73 and 100 days after inoculation; recurrence of warts at sites of early removal was common. Numbers in circles indicate number of calves in the experiment; * number of warts measured; (—) average size of intact warts; (---) average size of recurrent warts; arrows indicate the excision date.

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Tattoo instruments and hypodermic needles will transmit the infection in cattle.

Infectious papillomatosis is a self-limiting disease, although the duration of warts on individual animals may vary considerably. A variety of chemicals has been advocated for treatment without agreement on their value. Surgical removal by knife or cryosurgery is recommended if the warts are sufficiently objectionable. Surgical intervention in the early growing stage of a wart may lead to recurrence and stimulation of growth; therefore, warts should be removed when near their maximum size or when regressing (Fig. 72). Affected animals may be isolated from susceptible animals, although with the long incubation period many will have been exposed to the infection before a problem is recognized.

BOVINE PAPILLOMAVIRUS TYPES 1 AND 2

BPV types 1 and 2 cause fibroblastic tumors of the dermis, in addition to the epithelial hyperplasia. These are more properly called fibropapillomas (Fig. 73). Progressively growing fibroblastic tumors of the brain can be experimentally produced in cattle and hamsters (Fig. 74). Polypoid tumors of the urinary bladder can be produced with BPV in calves (Fig. 75). In certain areas of the world, such as northern Japan, parts of Brazil, Scotland, Colombia and Turkey, enzootic bovine hematuria is associated with the bracken fern (*Pteridium aquilinum*) grown on certain soils as well as with BPV infection. A carcinogen from a low-level continuous consumption of bracken fern seems to operate together with the BPV in causing various tumors (Fig. 76). Previously the disease had occurred in parts of the USA, Canada, Europe, Australia, India and New Zealand. When pastures are improved cattle no longer are forced to consume bracken fern and enzootic hematuria ceases to be a problem

Bovine fibropapillomatosis is worldwide in distribution and is more common
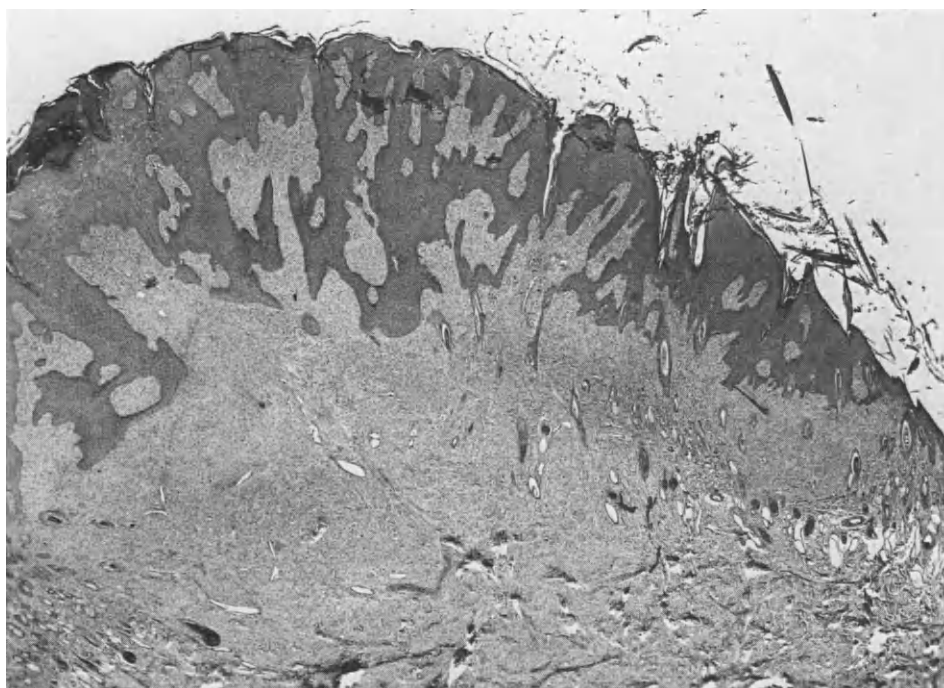
Fig. 73. Fibropapilloma in skin of calf 40 days after inoculation of BPV; note epithelial proliferation over underlying fibroma (× 22).

Fig. 74. A large fibroma almost completely replaces the left hemisphere in the brain of a calf after intracerebral inoculation of BPV 227 days previously.

in cattle less than 2 years of age that are housed in close contact with each other. The skin of the neck, legs, back and abdomen are the more usual sites, since these locations are probably more subject to abrasions and wounds. Cutaneous papillomas in cattle can be of various size — depending on the area infected — have a cauliflower-like appearance and a fibroma base in the dermis. Fibropapillomas of the penis and vulvo-vaginal mucosa have a smooth surface with less epithelial proliferation (Fig. 77). Virus is concentrated in the outer keratinized epithelium of the papilloma and when shed can contaminate fences, stanchions, and boards of the stable. These fomites transmit the disease to susceptible cattle when causing wounds of the skin. Immunity develops in a few weeks after exposure; older animals are more resistant than very young, probably because of prior inapparent exposure. The incubation period for cutaneous warts produced by BPV is approximately 30 days, and the duration of both naturally and experimentally produced fibropapillomas ranges from 1 to 12 months before regression (Fig. 78). Occasionally, cutaneous fibropapillomatosis will persist in a calf and become very extensive. This is probably because of some defect in the immune system. Fibroma regression is not related to serum antibody but probably to CMI (Barthold and Olson, 1974).

Antibodies (detectable by ID, CF and HI) can readily be found after exposure to BPV or to commercial bovine wart vaccines. Formalinized suspension of

Fig. 75. Mucosal aspect of bovine urinary bladder with many 6-cm polyps in the tumor area inoculated 122 days previously with BPV.

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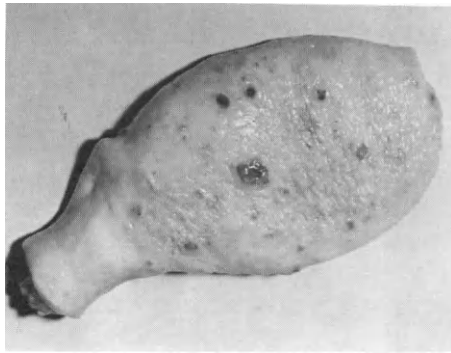
Fig. 76. Mucosal aspect of bovine urinary bladder with numerous distinct hemangiomas; hemorrhage from these and other neoplasias (fibromas, adenomas, carcinomas) gave rise to the clinical term of enzootic hematuria (this case provided by Dr. Bankier of Vancouver, B.C., in 1961).

bovine warts provide a vaccine for prophylactic immunization when cutaneous warts are a problem in a herd (Olson et al., 1962). Such problems can exist in cattle to be used for shows, to be sent to slaughter — where antemortem inspection may cause a reduced price — and particularly in studs, where young bulls can develop fibropapilloma of the penis (Fig. 79). Such penile lesions tend to recur after surgery, with eventual deformation.

Prophylactic vaccination might also be used in bands of young horses where equine sarcoid is common. It may be necessary to begin vaccination as early as at 4–6 weeks of age in calves with about 0.4 ml vaccine intradermally at two sites, repeating the procedure 4–6 weeks later and 1 year of age. Immunity to infection will develop in a few weeks, but this immunity is not related to the (unknown) mechanism involved in regression of the wart. Since exposure to the virus may have occurred prior to vaccination, as e.g. with a contaminated tattoo instrument, the vaccine-induced immunity may develop too late to prevent development of warts. A program of prophylactic vaccination must be in effect for about 3–6 months before its preventive value will be noted (Fig. 80). It should be continued for at least a year after disappearance of the last wart in a herd, since virus may still contaminate the premises. Stalls, stanchions and other inert materials can be disinfected by formaldehyde fumigation, but this must be done at high humidity and temperature to be effective.

Reports of wart tissue vaccines containing formalin-inactivated virus used for treatment indicate variable results. One controlled trial on experimentally produced warts in cattle showed that such a vaccine was of no therapeutic value (Olson and Skidmore, 1959). Since most wart viruses are species specific,
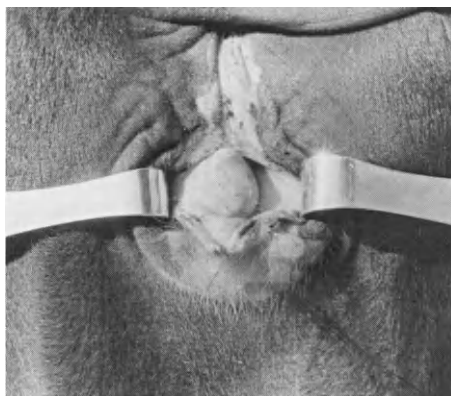
Fig. 77. Fibropapilloma of a young heifer; experimentally produced with BPV inoculated when the animal was 400 days old.
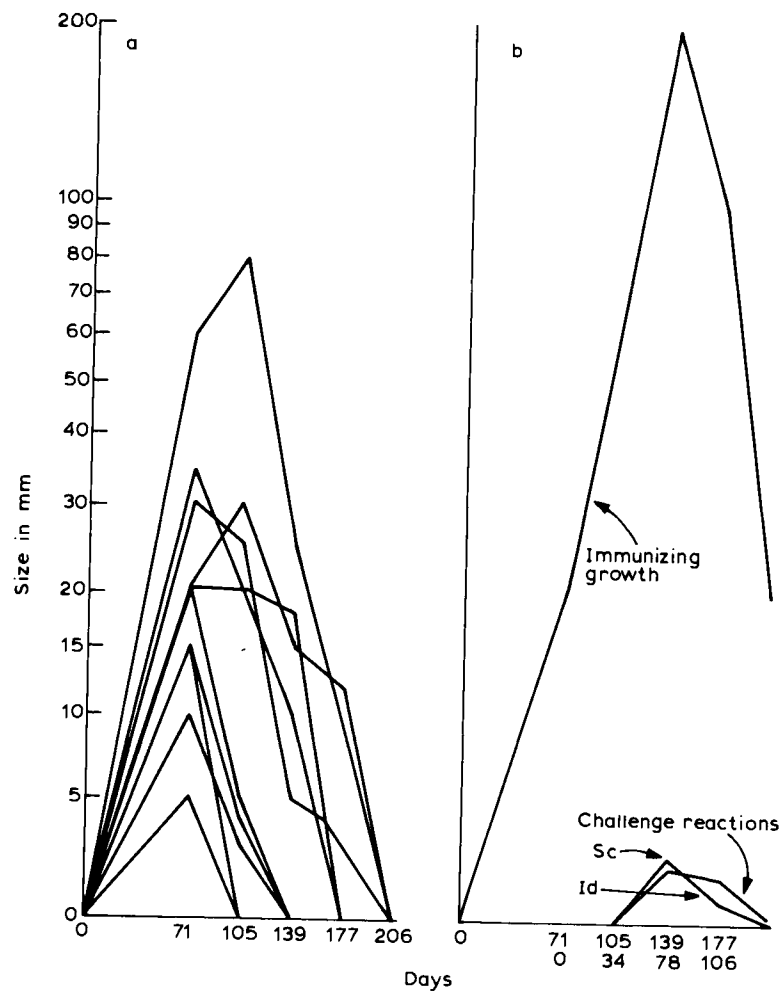
Fig. 78. Growth curves of warts produced with the same bovine papilloma material on scarified skin of ten calves; note the marked variation in size and duration on individual calves. Graph (b) illustrates challenge (Sc, scarified; Id, intradermal) reactions in a calf with a large papilloma induced 71 days previously; such reactions to challenge of immunity were observed in 3 of 60 similarly treated calves.

there is no merit in using a vaccine of wart tissue derived from another species of animal except a bovine vaccine to prevent equine sarcoid.

A fascinating situation has been discovered (Jarrett et al., 1984), in which fibropapillomas of the esophagus and rumen harbor the genome of BPV-2 but neither mature virus could be found by EM nor viral antigen by immunohis-

Fig. 79. Natural case of fibropapilloma of a penis; this can be experimentally produced with BPV.

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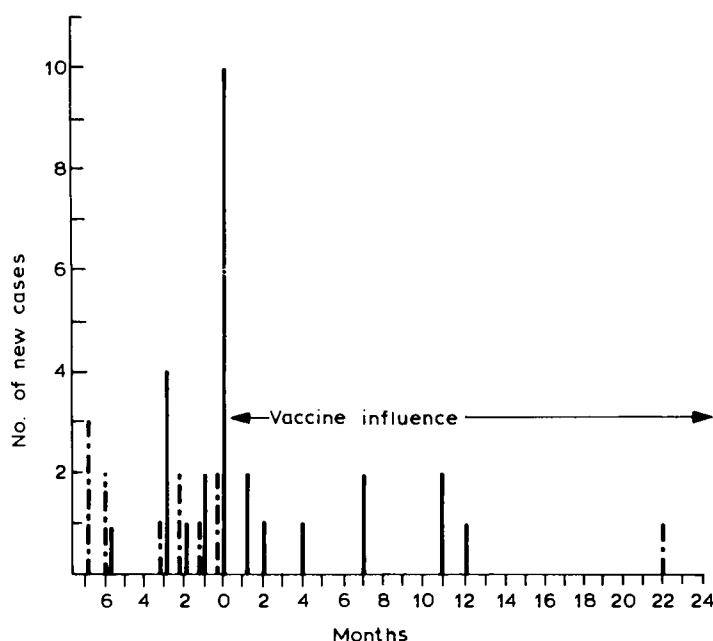
Fig. 80. Fibropapillomas (- ● -, of glans penis; —, of skin) in a population of 40–60 young bull calves during 2½ years of study; prophylactic vaccination with formalin-treated bovine papilloma suspension influences new cases after 0 time.

tochemical methods. This suggests epithelial transformation to neoplasia with no production of infectious virus as in the fibroblasts.

BOVINE PAPILLOMAVIRUS TYPE 3

Pfister et al. (1979) have classified a virus from cutaneous epithelial papillomas of Australian cattle as type 3. The fibropapillomas of Australian cattle have BPV types 1 and 2 (Spradbrow, 1982).

Atypical warts (Fig. 81) in cattle, similar to the Australian type 3 lesions, lack the dermal fibroma component of the BPV-induced cutaneous fibropapilloma (Barthold et al., 1974). The atypical tumors tend to persist rather than regress and involve adult animals as well as young stock. The atypical papillomas contain a virus morphologically identical with BPV; however, the viruses are immunologically distinct. Experimental transmission of the atypical wart virus was unsuccessful. A 3-year herd vaccination program using atypical wart homogenates failed to influence the natural incidence of the disease (Barthold et al., 1974).
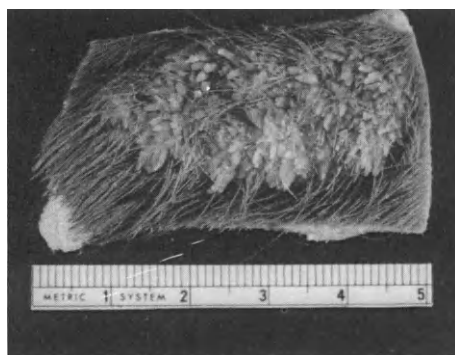
Fig. 81. Naturally occurring atypical bovine wart composed only of epithelial elements and lacking the dermal fibroma (details are given by Barthold et al., 1974).

BOVINE PAPILLOMAVIRUS TYPE 4

On farms of the west coast of Scotland with much growth of bracken fern, papillomas and carcinomas are found on the tongue, pharynx, esophagus,esophageal groove, rumen and intestine of cattle. A virus morphologically similar to BPV, but different in other respects, exists in these lesions; tumor extracts have produced typical papillomas in the esophagus, palate and skin. This virus has been characterized as BPV type 4 (Jarrett et al., 1980). An 80% incidence of alimentary tract papillomatosis has been noted in cattle slaughtered after being pastured in the high cancer area for more than 5 years. Multiple papillomas were found in more than half of the cattle, most of which also had associated squamous carcinoma. While BPV-4 is found in the alimentary papillomas, the alimentary carcinomas had no demonstrable virus or viral antigens (Campo et al., 1985). In 70 cases, only one transforming papilloma of the esophagus and one squamous carcinoma of the tongue contained BPV-4 viral DNA (Jarrett et al., 1984a). Thus, it is believed that BPV-4 DNA is not required for progression from papilloma to and maintenance of the cancer Urinary bladder tumors (enzootic hematuria) were found in about 30% of cattle from the high cancer areas. Similar epidemoid carcinomas of the alimentary tract had been observed in the enzootic hematuria (carcinogenic bracken) area of Brazil (Tokarnia et al., 1969) and Colombia (J. Tobon, personal communication, 1972).

BOVINE PAPILLOMAVIRUS TYPE 5

Papillomas on the teats of cows are not a common herd problem but can cause difficulty in milking individual cows. Meischke (1970a) found papillomas in 36% of cattle processed through a Glasgow abattoir. These were classified as filiform or "rice grain" with no fibroma, as papilloma and as fibropapilloma; papillomavirus particles were seen by EM in all three types.

Meischke (1979b) reported that animals with "rice grain" lesions were susceptible to virus from fibropapillomas (BPV-1 or 2) but were immune to challenge with "rice grain" virus. The "rice grain" virus has been characterized as BPV type 5 (Jarrett et al., 1980).

BOVINE PAPILLOMAVIRUS TYPE 6

Jarrett et al. (1984b) have characterized a BPV-6 in teat "fond" epithelial papillomas from 32 cattle which had concurrent teat "rice grain" fibropapillomas with BPV-5. The DNA of BPV-6 is smaller than and has no sequence homology with BPV-1, BPV-2 or BPV-5. It does share some sequences with BPV-3 and BPV-4.

During a survey of 667 cattle at a Wisconsin abattoir three kinds of teat papillomas were seen in about 25% of the animals. These were classified as atypical filiform, atypical flat and typical fibropapilloma. The bovine papilloma capsid antigen was found in all three kinds. Homogenates of the typical fibropapillomas produced skin lesions in four calves and two ponies. The atypical filiform papillomas produced lesions on skin and teats in two heifers. The atypical flat papillomas produced papillomas on only the teats of two heifers (Olson et al., 1982).

Troublesome papilloma-like lesions not infrequently develop on teats and at the teat meatus of milking cows. They consist of proliferations of epithelium as in a papilloma, but lack the fibroplasia of the underlying dermis as seen in the

typical fibropapilloma of BPV. The etiology of this condition is unknown but a papillomavirus is suspected.

BOVINE PAPILLOMA VIRAL ETIOLOGY SUSPECTED

Virions resembling BPV were found in 8 out of 24 ocular lesions in Australian cattle (conjunctival plaques and papillomas, papillomas of the eyelid and a cutaneous horn of the eyelid; Ford et al., 1982; Spradbrow, 1982). Plaques at the corneal–scleral border could be produced by scarification with BPV, but the lesions were small and soon regressed (C. Olson, unpublished data, 1953).

The histology of bovine vesical fibromatosis of northern Japan (Yoshikawa and Oyamda, 1971), resembles the initial lesions produced experimentally by BPV (Olson et al., 1962; Brobst and Olson, 1965). The various stages of cutaneous papillomas of pasterns (Lindley, 1974) have been described and illustrated by Cheli et al. (1980) and Lindley (1974).

SHEEP PAPILLOMAVIRUS

Fibropapillomas of the muzzle and legs of sheep in England were found by Gibbs et al. (1975) to be associated with a papillomavirus also capable of producing sarcoma in the hamster but not in cattle or goats.

Vanselow et al. (1982) found papilloma virions by EM in squamous carcinoma and papillomas of Merino sheep in Australia. Such squamous carcinomas in non-pigmented areas poorly covered by wool (ear, muzzle, eye and vulva) had been recognized as an economic problem for many years. It is believed that papillomavirus and sunlight are involved in a complex etiology of these tumors. Virions have been demonstrated in papillomas of the eyelid and vulva (Vanselow and Spradbrow, 1983).

DEER FIBROMA VIRUS

A cutaneous fibromatosis occurs in both white-tailed and mule deer and is caused by papillomaviruses which are slightly different in the two deer species (Lancaster and Sundberg, 1982). The virions occur in the moderately hyperplastic epithelium covering the fibromas, and the viral genome can be demonstrated in the fibroma. In nature and experimentally the lesions are usually small and regress (Sundberg et al., 1985). Papillomas and fibropapillomas have been reported in a variety of other deer, in caribou and moose (Sundberg and Nielson, 1981). The deer fibroma viruses produce fibroplastic tumors in hamsters but not in cattle or horses. Deer are not susceptible to bovine papillomavirus.

EUROPEAN ELK PAPILLOMAVIRUS

Cutaneous fibropapillomas have been found to be rather common in the European elk of Sweden. The EEPV produced fibrosarcomas in hamsters and is unrelated to BPV based on molecular hybridization studies (Moreno-Lopez et al., 1982).

GOAT PAPILLOMA

A progression of mammary papillomatosis to carcinoma was reported in goats some years ago (Moulton, 1954). A current study indicates that multiple cutaneous papillomas of the head and neck are squamous in character; mammary and genital warts are fibropapillomas (Theilen et al., 1982). Thus far a virus has not been demonstrated.

PAPILLOMAS IN OTHER ANIMALS

Papilloma viruses no doubt exist in other ruminants, as evidenced, for example, by the presence of papillomavirus antigen in the pronghorn antelope (Sundberg et al., 1983). Such immunohistochemical surveys followed by molecular study of the extracted virus will add new papilloma viruses to the list.

REFERENCES

Barthold, S.W. and Olson, C., 1974. Fibroma regression in relation to antibody and challenge immunity to bovine papillomavirus. Cancer Res. 24: 2426–2431.

Barthold, S.W., Koller, L.D., Olson, C., Studer, E. and Holtan, A., 1974. A typical warts in cattle. J. Am. Vet. Med. Assoc., 165: 276–280.

Brian-Markson, J., Jaudon, C. and Krug, R.M., 1985. Expression of a functional influenza viral cap-recognizing protein by using a bovine papilloma virus vector. Proc. Natl. Acad. Sci. USA, 82: 4326–4330.

Brobst, D.F. and Olson, C., 1965. Histopathology of urinary bladder tumors induced by bovine cutaneous papilloma agent. Cancer Res. 25: 12–19.

Campo, M.S., Moar, M.H., Sartirana, M.L., Kennedy, I.M. and Jarrett, W.F.H., 1985. The presence of BPV type 4 DNA is not required for the progression to, or the maintenance of the malignant state in cancers of the alimentary canal in cattle. EMBO J., 4: 1819–1826.

Cheli, R., Addis, F. and Mortellaro, C.M., 1980. Le Lesioni Digitali del Bovino. Editrice Essegivi, Piacenza, Italy, 181 pp.

Ford, J.N., Jennings, P.A., Spradbrow, P.B. and Francis, J., 1982. Evidence for papillomavirus in ocular lesions in cattle. Res. Vet. Sci., 32: 257–259.

Gibbs, E.P.J., Smale, J. and Lawman, M.J.P., 1975. Warts in sheep. J. Comp. Pathol., 85: 327.

Gorra, J.B., Lancaster, W.P., Kurman, R.J. and Jenson, A.B., 1985. Bovine papillomavirus type 1 monoclonal antibodies. J. Natl. Cancer Inst., 75: 121–126.

Jarrett, W.F.H., McNeil, P.E., Laird, H.M., O'Neil, J., Murphy, J., Campo, M.S. and Moar, M.H., 1980. Papilloma viruses in benign and malignant tumors of cattle. Cold Spring Harbor Conference on Cell Proliferation, 7: 215–222.

Jarrett, W.F.H., Camp, M.S., Blaxter, M.L., O'Neil, B.W. Larid, N.M., Moar, M.H. and Sartirana, M.L., 1984a. Alimentary fibropapilloma in cattle. A spontaneous tumor, nonpermissive for papillomavirus replication. J. Natl. Cancer Inst., 73: 499–504.

Jarrett, W.F.H., Campo, M.S., O'Neil, B.W., Laird, H.M. and Coggins, L.W., 1984b. A novel bovine papilloma virus (BPV-6) causing true epithelial papillomas of the mammary gland skin: A member of a proposed new BPV subgroup. Virology, 136: 244–264.

Koller, L.D. and Olson, C., 1972. Attempted transmission of warts from man, cattle, and horses and of deer fibroma, to selected hosts. J. Invest. Dermatol., 858: 366.

Lancaster, W.D. and Olson, C., 1982. Animal papillomaviruses. Microbiol. Rev., 46: 191–207.

Lancaster, W.D. and Sundberg, J.P., 1982. Characterization of papilloma viruses isolated from cutaneous fibromas of white-tailed deer and mule deer. Virology, 123: 212–216.

Lindley, W.H., 1974. Malignant verrucae of bulls. Vet. Med. — Small Anim. Clin., Dec., 1547–1550.

Meischke, H.R.C., 1979a. A survey of bovine teat papillomatosis. Vet. Rec., 104: 28–31.

Meischke, H.R.C., 1979b. Experimental transmission of bovine papilloma virus (BPV) extracted from morphologically distinct teat and cutaneous lesions and the effects of inoculation of BPV transformed fetal bovine cells. Vet. Res., 104: 360–366.

Moreno-Lopez, J., Sternlund, A., Ahola, H. and Pettersson, U., 1982. The European elk papilloma virus (EEPV): Characterization of the genome, induction of tumors in animals and transformation in vitro. Cold Spring Harbor Conference on Papilloma Viruses, September 14–18, p. 71.

Moulton, J.E., 1954. Cutaneous papillomas on the udders of milk goats. North Am. Vet., 35: 29–33.

Nakabayashi, Y., Chattopadhyay, S.K. and Lowy, D.R., 1983. The transforming function of bovine papillomavirus DNA. Proc. Natl. Acad. Sci. USA, 80: 5832–5836.

Olson, C. and Skidmore, L.V., 1959. Therapy of experimentally produced bovine cutaneous papillomatosis with vaccines and excision. J. Am. Vet. Assoc., 135: 339–343.

Olson, C., Luedke, A.J. and Brobst, D.F., 1962. Induced immunity of skin, vagina and urinary bladder to bovine papillomatosis. Cancer Res., 22: 463–468.

Olson, R.O., Olson, C. and Easterday, B.C., 1982. Papillomatosis of the bovine teat. Am. J. Vet. Res., 43: 2250–2252.

Pfister, H., Linz, U., Gissmann, L., Huchthausen, B., Hoffman, D. and Zur Hausen, H., 1979. Partial characterization of a new type of bovine papilloma virus. Virology, 96: 1–8.

Sarver, N., Byrne, J.C. and Howley, P.M., 1982. Transformation and replication in mouse cells of bovine papillomavirus-pML2 plasmid vector that can be rescued in bacteria. Proc. Natl. Acad. Sci. USA, 79: 7147–7151.

Spalholz, B.A., Yang, Y. and Howley, P.M., 1985. Transactivation of a bovine papilloma virus transcriptional element by the E2 gene product. Cell, 42: 183–191.

Spradbrow, P.B., 1982. Papilloma viruses, papillomas, and ocular and cutaneous carcinomas in ruminant animals. Cold Spring Harbor Conference on Papilloma Viruses, September 14–18, p. 73.

Spradbrow, P.B., Ford, J.N. and Samuel, J.L., 1983. Papillomaviruses with unusual restriction endonuclease profiles from bovine skin warts. Aust. Vet. J., 60: 259.

Sundberg, J.P. and Nielson, S.W., 1981. Deer fibroma: A review. Can. Vet. J., 22: 385–388.

Sundberg, J.P., Williams, E., Thorne, E.T. and Lancaster, W.D., 1983. Cutaneous fibropapilloma in a pronghorn antelope. J. Am. Vet. Med. Assoc., 183: 1333–1334.

Sundberg, J.P., Chiodini, R.J. and Nielson, S.W., 1985. Transmission of the white-tailed deer cutaneous fibroma. Am. J. Vet. Res., 46: 1150–1154.

Theilen, G.H., Wheeldon, E.B., East, N., Madewell, B.R., Lancaster, W.D. and Merrin, R., 1982. Goat papillomatosis. Cold Spring Harbor Conference on Papilloma Viruses, September 14–18, pp. 73a, b.

Tokarnia, C.H., Dobereiner, J. and Canella, C.F.C., 1969. Ocorrencia de hematuria enzootica de carcinomas epidermoides no trato digestivo superior em bovinos no Brasil. Pesq. Agropec. Bras., 4: 209–224.

Vanselow, B.A., Spradbrow, P.B. and Jackson, A.R.B., 1982. Papilloma viruses, papillomas and squamous carcinomas in sheep. Vet. Rec., 110: 561–562.

Vanselow, B.A. and Spradbrow, P.B., 1983. Squamous cell carcinoma of the vulva, hyperkeratosis and papilloma viruses in a ewe. Aust. Vet. J., 60: 194.

Yoshikawa, T. and Oyamda, T., 1971. Primary occurrence of bovine vesical fibromatosis. Kitasato Arch. Exp. Med., 44: 197–204.

Parvoviridae

The particles of parvoviruses measure only 18–26 nm in diameter and are the smallest DNA viruses known so far (Latin "parvus" = small). In addition to the genus *Parvovirus* which contains most of the infectious agents of veterinary importance, the genera *Dependovirus* and *Densovirus* (in insects) belong to this family. Except for minor cross-reactions between rodent representatives, the members of the genus *Parvovirus* show type-specific reactions by hemagglutination-inhibition, neutralization and other serological tests. In contrast, all dependoviruses possess a common antigen, as can be demonstrated by immunofluorescence. Some 20 parvoviruses have been described in vertebrates.

Parvovirions are nonenveloped isometric particles with an icosahedral symmetry and probably 32 capsomeres, 3–4 nm in diameter.

The genome consists of a single molecular of single stranded DNA with a mol.wt. of 1.5–2×10^6. In some genera the single strands of both polarities are encapsidated and form double stranded molecules upon nucleic acid extraction. In mature virions three polypeptides are usually present in the vertebrate parvoviruses, all of which are probably derived from a common sequence.

Infectious virions band at a density of 1.39–$1.42\,\mathrm{g/cm^3}$ in CsCl; particles with still higher densities (precursor particles) have been described. The virion sediments at 110–122 S.

Virus replication is restricted to the nucleus and is dependent either on certain helper functions from the host cell (*Parvovirus*) or from a helper virus (*Dependovirus*). Accumulations of both empty and full progeny virions can be found in the nuclei of infected cells. Parvoviruses are best propagated in young, rapidly dividing cells. Intranuclear inclusion bodies can occasionally be seen in infected cells after staining.

In cattle, representatives of two genera — *Parvovirus* and *Dependovirus* (former Adeno-associated virus) — are found (Siegl. et al., 1985). The first strain (type 1) of bovine parvovirus (BPoV) was isolated in the USA and was called HADEN (HemADsorbing ENteric) virus. Additional strains have been isolated in various countries. A Japanese strain, antigenically different from the HADEN strain, is considered to represent a type 2. Bovine parvoviruses are associated with diarrhea in calves and obstetric problems such as abortion in cattle.

Members of the genus *Dependovirus* are defective but multiply in the presence of a replicating adenovirus or herpesvirus. Bovine and ovine dependoviruses are known to occur. Dependoviruses per se have never been found associated with pathogenic conditions.

REFERENCE

Siegl, G., Bates, R.C., Berns, K.I., Carter, B.J., Kelly, D.C. Kurstak, E. and Tattersall, P., 1985. Characteristics and taxonomy of Parvoviridae. Intervirology, 23: 61–73.

Chapter 19

Bovine Parvoviruses

J. STORZ

INTRODUCTION

In 1959, Abinanti and Warfield isolated from the intestine of calves a virus that was later identified as a parvovirus (Storz and Warren, 1970; Bachmann, 1971). It is now well established that replication of nondefective parvoviruses depends on cellular functions expressed during the S phase of the eukaryotic cell cycle (Margolis and Kilham, 1965; Tennant et al., 1969; Siegl, 1976). Considering the turnover of intestinal cells and their gradient of differentiation, it is not surprising that parvoviruses were cultured frequently from the intestinal tract of calves when specific attempts for isolation were made (Vincent, 1971; Bates et al., 1972; Hinaidy, 1978; Hinaidy et al., 1979; Wosu et al., 1979). Furthermore, evidence for intrauterine infections of bovine fetuses was found when high titers of HI antibodies of bovine parvovirus (BPoV) were detected in commercial bovine fetal serum (Storz et al., 1972).
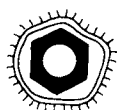
VIRUS PROPERTIES

Physical and chemical characteristics

Parvoviruses are extremely resistant to chemical and physical inactivating factors. The most reliable disinfection is achieved with 0.5% chlorox or ethylene oxide in the form of the nonexplosive mixture of 10% ethylene oxide and 90% carbon dioxide.

Antigenic properties

All the parvoviral isolates studied agglutinate guinea pig and human type O erythrocytes. The isolates from cattle of different countries all are antigenically related or identical to prototype BPoV-1 (Abinanti and Warfield, 1961). One BPoV strain isolated in Japan appears to differ and is separated as BPoV-2 (Inaba et al., 1973a). Bovine parvoviruses differ antigenically from parvoviruses isolated from man, pigs, cats, dogs, rats and rabbits. This is further substantiated by comparisons of genomic nucleotide sequences among parvoviruses, which revealed virtually no homology between rodent parvoviruses, Lu III and BPoV (Banerjee et. al., 1983). A defective parvovirus was found associated with BAV types 1, 2 and 3 (Luchsinger et al., 1970; Myrup et al., 1976; Coria and Lehmkuhl, 1978).

Cultivation

BPoV-1 replicated in all primary bovine fetal cells tested. High levels of hemagglutinin were present in tissue culture fluids following passage in different cell types (Bates and Storz, 1973; Hinaidy et al., 1979; Durham and Johnson, 1985). The virus reached cytopathic and hemadsorption titers of $10^{6.2}$ and $10^{6.8}$ TCID$_{50}$/ml, respectively, on assay after three passages in bovine fetal lung (BFL) cells. Replication occurred to similar titers in bovine fetal testicle (BFT), spleen (BFS), and adrenal (BFA) cells. The least efficient replication of BPoV-1 occurred in bovine fetal kidney (BFK) and bovine fetal intestine (BFI) cells with cytopathic endpoints of $10^{4.2}$ and $10^{3.5}$ TCID$_{50}$/ml, respectively. In all cases, hemadsorption endpoints were approximately 10-fold higher than cytopathic endpoints.

Cell lines from a variety of animal species were tested for their susceptibility to BPoV infection (Bates and Storz, 1973). The line cells examined did not support replication of BPoV-1. Except for decreasing titers observed in the cell strain FB4BN, established from bovine bone marrow, hemagglutinins were not detected in these tissue cultures. Low levels of infectivity and hemagglutinin were detected in BFL cells after three passages of BPoV-1 in MDBK and BHK-21 cells. Plaques resulting from BPoV replication in BFS cells were detected within 5 days after inoculation (Bates and Storz, 1973). The plaque sizes ranged from 1 to 3 mm in diameter at 5 days and increased to 3–5 mm at 7 days p.i. All plaques, irrespective of their size, had fuzzy edges.

The CPE resulting from BPoV replication in actively dividing BFL cells was distinct and reproducible. At 20–24 h after inoculation, infected cells were swollen, refractile, and exhibited a stellate appearance. These cells were scattered over the cell monolayer and ultimately became uniformly round and highly refractile. Soon after rounding, the cells detached from the glass surface. The cytopathic changes continued until all cells were involved, usually by 72 h after inoculation.

Parvovirus-specific fluorescence in infected BFS cells was detected exclusively in the nuclei. Intranuclear inclusions with a unique morphology are formed and can be detected following acridine orange or Giemsa staining (Hinaidy et al., 1979; Leary and Storz, 1980, 1982). Initial changes resulted in a uniformly basophilic nucleoplasm giving the nuclei a glassy appearance. Clumps of dark blue marginated chromatin appeared on the inner nuclear envelope. Along with greater numbers of glassy nuclei, a second nuclear change became apparent, which was characterized by an intensely stained, eosinophilic, finely granular nucleoplasm with a prominent dark blue nucleolus. Thin halos formed around the entire finely granulated nucleoplasm by 18 h after infection. Halo width, intensity of staining, and basophilia of the central inclusion mass increased after 24 h to reach the form of Cowdry type A inclusions (Figs. 82 and 83).

An additional type of inclusion consisting of multiple round, smooth, eosinophilic foci emerged in both glassy and finely granular nuclei at 18–24 h. Halos formed later around individual foci, which remained clearly distinct from nucleoli (Figs. 84 and 85). These foci were often marginally arranged, but they appeared also throughout the nucleoplasm (Hinaidy et al., 1979). All our BPoV strains tested induced multifocal as well as Cowdry type A inclusions (Leary and Storz, 1980, 1982).

Ultrathin sections of many nuclei of infected BFS cells fixed 18–24 h after inoculation contained distinct, fine-grained, electron-dense, circular foci in the nucleoplasm. The foci are local accumulations of viral capsids as revealed by EM (Fig. 86). There appeared to be nuclear compartmentalization of viral replication sites (Bates et al., 1974; Leary and Storz, 1982). Inclusions were not
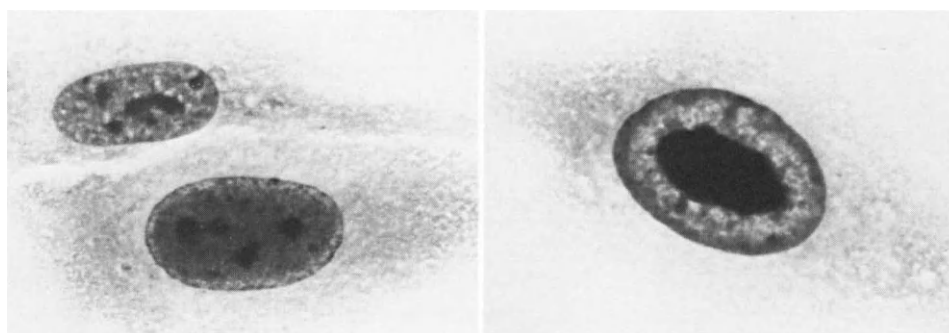
Fig. 82 (left). Early Cowdry type A inclusion within the nucleus of a BFS cell infected with BPoV-1; Giemsa stain (× 1280).

Fig. 83 (right). Mature Cowdry type A inclusion within the nucleus of a BFS cell infected with BPoV-1; Giemsa stain (× 1280).

detectable in infected mitotic cells where the nuclear envelope was dispersed. The presence of characteristic tubular forms in these cells revealed them as infected.

The nucleopathic changes described are characteristic for BPoV and can be used to indicate the infection in cultured cells. Their appearance in infected cells of tissues from animals can be exploited diagnostically.

EPIZOOTIOLOGY

Evidence for parvovirus infection of cattle through detection of antibodies neutralizing infectivity or inhibiting hemagglutination of BPoV was detected in the USA, Algiers, Japan, Brazil, England and Austria (Leary and Storz, 1980). The incidence of cattle with antibodies ranged from 46 to 86% (Table 20). The age of the animals tested was not specified, but the majority of the serum samples involved newborn calves and cattle to 12 months of age. The highest percentage of positive calves was found in February in Austria, where the only attempt was made to analyze seasonal distribution (Hinaidy and Bürki, 1980).
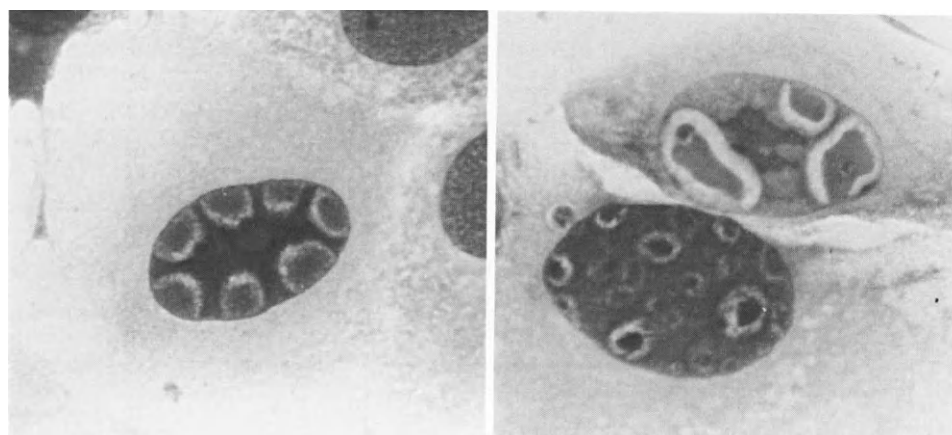
Fig. 84 (left). Multifocal inclusion located peripherally in nucleus of BFS cell infected with BPoV-1; Giemsa stain (× 1280).

Fig. 85 (right). Large and small multifocal inclusions within the nucleus of BFS cell infected with BPoV-1; Giemsa stain (× 1280).

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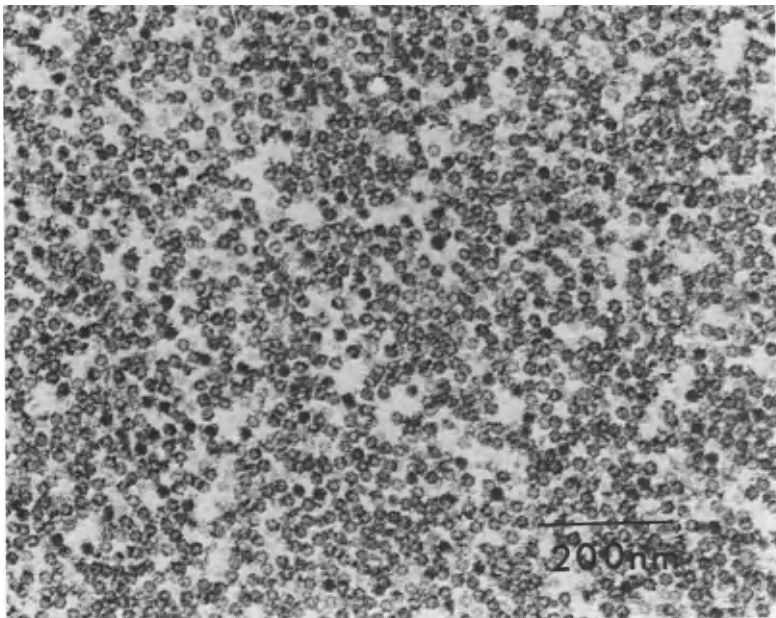
Fig. 86. Ultrastructural detail of section from a nucleus of a BFS cell infected with BPoV-1.

Serum samples from 35 herds of cattle representing 433 animals were tested in the western USA. Antibodies were detected in sera from 243 of 377 animals in 29 herds. Six of the herds did not have cattle with parvovirus antibodies (Storz et al., 1972). Parvoviruses were isolated from cattle of all the countries where serological surveys had been made.

Considering their antigenic properties and minimal homology with parvoviruses of other animal species, one can assume that BPoV antibodies in cattle were not induced through cross-reactions after infection with parvoviruses of other animal species (Bates et al., 1972). In addition, the host cell range of BPoV was found to be virtually restricted to bovine cells. Accordingly, the chain of infection is maintained within the cattle population.

Vertical transmission of BPoV was proven through detection of significant antibody titers in fetal serum and isolation of parvovirus from tissues of a naturally occurring abortion (Storz et al., 1972; Inaba et al., 1973b); it was confirmed through experimental inoculations of pregnant cows or direct fetal inoculations (Storz et al., 1978).

TABLE 20

Parvovirus antibodies in cattle

Country	Positive (%)	Total tested	References
USA, Maryland	86	?	Abinanti and Warfield, 1961
	83	209	Spahn et al., 1966
Colorado	65	377	Storz et al., 1972
Algiers	70	254	Vincent, 1971
Japan	50	48	Inaba et al., 1973b
England	46	114	Huck et al., 1975
Austria	71	148	Hinaidy, 1978
	70	101	Hinaidy and Bürki, 1980

PATHOGENESIS

How do parvoviruses induce enteritis in calves?

The spread of BPoV during infection and its distribution in newborn calves after oral or intravenous inoculations was studied (Storz et al., 1978). Parvoviral antigen was demonstrated by IF test and virus was reisolated.

Calves excreted BPoV 24–48 h after oral as well as intravenous inoculation and continued to do so during the course of the experiment lasting for 11 days. Virus was isolated from mucosal scrapings of the duodenum and lower levels of the intestine for 6 days. The regions most consistently infected were the jejunum, ileum and caecum; the highest titers were measured in the intestinal mucosa of intravenously inoculated calves. Patches of cells with fluorescent nuclei were found in the epithelium of the Lieberkühn crypts, in the transition and intervillous zones of villi, in cells of central lacteals (a site where more fluorescent cells were found after intravenous inoculation) and in cells of the lamina propria. The range and distribution of fluorescent cells was similar to that found in newborn kittens inoculated with feline panleukopenia virus, another parvovirus (Hammon and Enders, 1939; Csiza et al., 1971; Carlson et al., 1977, see volume Virus Infections of Carnivores of this series). It differed from the range of infected cells of adult conventional cats, where the cells of Lieberkühn crypts are the initial sites of intestinal infection.

Viremia associated with leukocytes developed after oral inoculation and reemerged when virus was given intravenously, and more serious diarrhea was then induced. Infections of intestinal cells in crypts, central lacteal and other sites occurred during the systemic phase. Also, infection of cells in the cortex of the adrenal gland (Fig. 87), the thymus, lymph nodes and heart muscle became pronounced during the systemic phase, which is a characteristic feature of the pathogenesis of parvovirus-induced enteric disease.

Fluorescence in intestinal and other lymph nodes was distributed randomly and involved large cells in paracortical areas. The thymus showed fluorescence in large nuclei or reticulum-like cells in the medullar region and cortex. Fluorescent cells were present in the nodular areas of the spleen. The adrenal
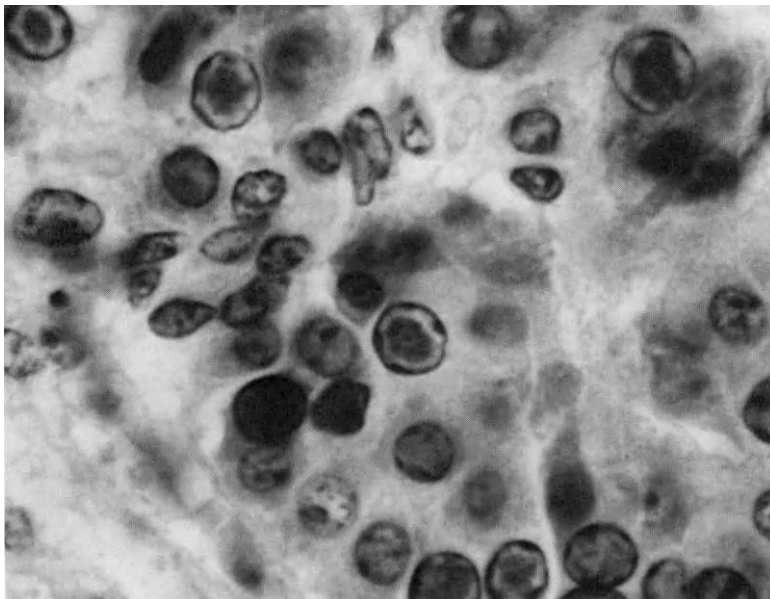
Fig. 87. Section of adrenal gland of a calf infected with BPoV-1; several nuclei have inclusions.

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glands examined had abundant fluorescence in the zona glomerulosa and fasciculata. Net-like pronounced fluorescence was present in heart muscle cells. Fluorescent cells were not found with parvovirus-specific conjugates in the corresponding tissues of one normal control calf or calves inoculated with bovine enteroviruses.

Conventional calves inoculated orally or intravenously with parvovirus propagated in BFS or BFK cells for 8–10 passages developed diarrhea (Storz et al., 1978). Also 2–8-month-old calves became diarrheic 4–7 days after oral or intranasal inoculation with BPoV-1 of an unspecified passage level (Spahn et al., 1966). Calves inoculated intravenously had more severe diarrhea, which was initially watery and then mucoid, than orally inoculated calves. Since parvoviruses were also isolated from conjunctival and tonsillar specimens, it appears that the oropharyngeal and respiratory routes of infections are more common under natural conditions than direct oral intestinal infections. The oropharyngeal and respiratory or parenteral routes of infection are most effective in experimental induction of feline panleukopenia (Csiza et al., 1971).

The most reliable inocula for inducing enteric disease with clinical signs comparable to those observed under natural conditions were suspensions of wild-type virus derived from diarrheic fluid and intestinal mucosal samples in experimental coronaviral and rotaviral enteritis (Storz et al., 1978; Storz and Leary, 1979). Similarly, a reliable inoculum for inducing panleukopenia in cats was a wild-type parvovirus maintained by intestinal infections in pathogen-free cats (Carlson et al., 1977). Cell culture propagated BPoV did not reproducibly cause diarrhea. Evidently, enteropathogenic viruses rapidly become attenuated during passage in cell culture due to mutation and selection of host range mutants with reduced virulence. Although enteritis was induced in our experiments involving parvovirus passed serially in cultured cells at least eight times, we may not have reproduced the naturally occurring infection.

Parvoviruses were detected in mixed intestinal viral infections of calves involving enteroviruses, adenoviruses, pestiviruses and coronaviruses. Because multiple viral infections in enteric diseases are quite common but seldom mentioned and hardly studied, the significance of a parvovirus component should be investigated. The parvoviral infection involves the host cell niche left unoccupied by intestinal coronaviruses or rotaviruses.

Pathogenesis of fetal infections

The pathogenic potential of BPoV to establish infection of the placenta and fetus was investigated by intravenously inoculating pregnant cows or by direct fetal inoculation during laparotomy (Storz et al., 1978). Following intravenous inoculation of pregnant cows, virus was isolated from their blood leukocytes 3 and 6 days later, and placental as well as fetal infection occurred. Fetuses of the first trimester of gestation were highly susceptible. Nuclei of cells of the cotyledonary villi fluoresced in a honeycomb pattern when stained with parvovirus-specific antibodies. The uterine mucosal glands contained some fluorescent cells which were also present in the adrenal glands, lungs, spleen, heart muscle, kidneys and thymus of the fetuses. Virus was isolated from uterine fluid and the fetal organs.

Also fetuses from the second trimester of gestation were susceptible to BPoV infection. When gravid uteri were removed 5–13 days after exposure of the fetuses, parvovirus could be isolated from placental fluids and most organs, including the intestinal tract. The infectivity levels of placental fluids varied from 10^4 to 10^6 PFU per ml. The liver of one fetus contained 7×10^5 PFU/g, and titers of similar levels were detected in adrenal glands, lymph nodes and intestinal tissues. Immunofluorescence findings corresponded with viral isola-

tion results. If fetuses survived and were tested 2 weeks after inoculation or later, virus isolation was irregular because the fetuses had produced antibodies.

Fetuses of the third trimester of gestation became infected, developed antibodies and recovered. These calves were born alive at term, and virus was isolated from them irregularly.

Repeat breeders and embryonic death

A serological survey in 12 commercial dairy herds indicated that BPoV may be involved in other reproductive problems (Barnes et al., 1982). The BPoV seroreactor cows commonly experienced higher rates of embryonic mortality and required more services per conception than did nonreactor cattle. The cows in these herds were adequately protected against the major reproductive diseases. Regular disease prevention programs and vaccination against brucellosis, leptospirosis, BVD, IBR and parainfluenza were applied in these herds. Bluetongue virus infection also was not associated with reproductive problems in these herds.

DISEASE SIGNS

Calves with naturally occurring parvoviral infections proven by isolation had various clinical signs and varied in age from 1 week to 12 months (Abinanti and Warfield, 1961; Vincent, 1971; Bates et al., 1972; Huck et al., 1975; Hinaidy et al., 1979). Diarrhea affected most calves from which we recovered parvoviruses. Calves surviving the diarrheic episode developed circulating antibodies, but parvoviruses could be isolated intermittently from their feces. Most isolates were made from fecal specimens of calves suffering from enteritis, combined occasionally with febrile respiratory illness and conjunctivitis (Table 21). In some instances parvoviruses were isolated from feces of clinically normal young cattle. Holstein calves that had recovered from episodes of parvovirus diarrhea appeared retarded in growth.

Newborn calves deprived of colostrum or given colostrum free of parvoviral (HI) antibodies developed enteritis 24–48 h after oral or intravenous inoculation with cell culture propagated strain 71-1-20W of the eighth–tenth passage in BFS cells (Storz et al., 1978). The diarrhea was mild to moderately severe in orally inoculated calves. Calves given the inoculum intravenously developed a more severe, watery diarrhea and became prostrate. The body temperatures reached 41°C 2 days after exposure. The calves were listless, but they usually drank the offered milk. Others observed diarrhea in 2–8-month-old calves 4–7 days after oral or intranasal inoculation with BPoV-1 of an unspecified passage level in cell cultures (Spahn et al., 1966).

Pregnant cows did not develop clinical signs immediately following inoculation. Their temperature and behavior remained normal. Abortions occurred in the first and early second trimester. The aborted fetuses were edematous, and the placentas were edematous and had necrotic cotyledons (Storz et al., 1978).

PATHOLOGY

Most calves infected with BPoV had a catarrhal enteritis. The levels of the gastrointestinal tract consistently infected were the jejunum, ileum and cecum. Single small patches of fluorescent cells were found in the epithelium of the crypts, in the transition and intervillous zones of villi, the central

TABLE 21

Clinical signs, age, and specimens of calves yielding parvovirus isolates

Country	Specimens yielding isolates	Clinical signs and age	Types and number of isolates	References
USA, Maryland	Feces	Normal, 3 weeks–4 months	BPoV-1 (6)	Abinanti and Warfield, 1961
Colorado	Feces	Diarrhea and normal, 1 week–12 months	BPoV-1 (10)	Bates et al., 1972
Colorado	Lymph Node	Diarrhea, 6 months	BPoV-1(1)	Storz et al., 1976
South Dakota	Feces	Diarrhea (coccidiosis), 6 months	BPoV-1 (1)	Bates et al., 1972
Oregon	Feces Conjunctiva Tonsils	Diarrhea, conjunctivitis Normal, 1–3 months	BPoV-1 (4)	Storz et al., 1978
Algiers	Feces	Normal	BPoV-1 (4)	Vincent, 1971
Japan	Feces	Diarrhea, respiratory signs, 4–12 months	BPoV-1 (4) BPoV-2 (1)	Inaba et al., 1973b
	Fetus	Abortion	BPoV-1 (1)	Inaba et al., 1973a
England	Feces	Diarrhea, 3 months	BPoV-1 (1)	Huck et al., 1975
Austria	Feces	Normal, 6–11 weeks	BPoV-1 (10)	Hinaidy et al., 1979
Australia	Feces	Normal, 6 months	BPoV-1	Wosu et al., 1979

lacteals, and in cells of the lamina propia mucosae. Clusters of fluorescent epithelial cells were also found in the crypts of Lieberkühn. Fluorescence in lymph nodes was distributed randomly and involved single large cells, probably lymphoblasts, in paracortical areas; they were also seen in the germinal centers. Fluorescence in large nuclei of reticulum-like cells in the medullar regions of the thymus and infrequently in cells of the cortex was observed. Similarly, fluorescent cells were found in the nodular areas of the spleen. The adrenal glands examined showed large numbers of fluorescent cells in the zona glomerulosa and fasciolata. The nuclei of numerous heart muscle cells had net-like fluorescence. Histological samples were taken from the experimental calves that were studied virologically. Evaluations of these tissues were not fruitful because of fixation problems. Recognizable nuclear changes (see Fig. 87) were detected in the adrenal glands, which had high titers of infectious virus (Storz and Bates, 1973).

Fetuses aborted during the first and early second trimester of gestation were edematous and had increased amounts of pleural and peritoneal fluid. Intranuclear inclusions were formed in cells of the small intestine, liver, lymph nodes, and the cerebellum. Microscopic lesions consisted of excessive cellular necrosis in the external granule cell layer of the germinative sites of the cerebellum. Lymphoid hyperplasia was also observed in fetuses examined at 10 days p.i. There was no evidence of lymphoid depletion or necrosis in lymph nodes, spleen, or thymus (Storz et al., 1978; Barros, 1980).
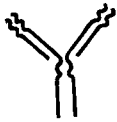
IMMUNE REACTION

Newborn calves and pregnant cows inoculated intravenously had a brisk HI antibody response within 5–7 days. These antibodies also neutralized virus infectivity as tested in the plaque assay. A high percentage of cattle in different herds had antibodies, but there were closed herds found free of this infection (Spahn et al., 1966; Vincent, 1971; Storz et al., 1972; Hinaidy and Bürki, 1980).

The humoral immune response of bovine fetuses to BPoV infection during the second and third trimesters was studied by the single radial immunodiffusion, HI, indirect IF test, micro-neutralization (MN), plaque-neutralization (PN), and double ID tests (Storz et al., 1978; Hayder et al., 1983). Serum samples collected from 23 virus-inoculated and several control fetuses were tested. The IgM concentration reached 355 mg/100 ml at 10 days after fetal inoculation and then decreased. The IgG concentration increased 10 days after inoculation and was maximal 142 days later. A correlation between Ig concentrations and antibodies reacting in the HI, MN, PN, IF, and ID tests existed in all samples. Neutralizing and HI antibodies were detected 10 days after fetal inoculation. The highest MN titer of 4096 was detected in a fetus 10 days after inoculation done during the third trimester. Six sera were positive in the IF test. These samples were collected 10–60 days after the fetuses had been inoculated; they had IgG concentrations of 615 mg/100 ml or higher and titers of 16–64.

Between 5 and 10 days p.i. fetal blood lymphocyte counts tripled, due primarily to an increase in E-rosetting lymphocytes. Peripheral blood lymphocytes of all fetuses reponded well to nonspecific mitogens of phytohemagglutinin, concanavalin A, and pokeweed (Liggitt et al., 1982).

LABORATORY DIAGNOSIS

The BPoV isolates established by investigators in different countries were cultured in BFK or calf testicle cells. Considering the high incidence of cattle

with antibody titers it is apparent that isolation of parvovirus from natural infections was relatively sporadic and inefficient with the various methods used. Optimal replication and pronounced cytopathic changes were observed in BFL and BFS cells (Bates and Storz, 1973). These were used as actively growing cultures in a state of parasynchrony. The cellular requirements of BPoV must be considered before attempting in vitro studies, in viral isolation trials, and in the interpretation of pathogenetic mechanisms. Cultures of BFS cells synthesize DNA in a parasynchronous manner between 18 and 48 hours after passage (Leary and Storz, 1982). Optimum interaction of BPoV with DNA-synthesizing, S-phase cells was insured by infecting cells 18 h after passage. Intracellular infectivity rose 18–24 h p.i., at which time both cytopathic changes and intranuclear inclusions were detected.

Three methods of BPoV isolation from IF pretested samples of experimentally inoculated calves were compared (Storz et al., 1978): (1) parasynchronous BFS cells with freezing and thawing between subpassages to disrupt cells; (2) direct culture of cells from selected organs; and (3) parasynchronous BFS cells and cell-associated virus techniques to maintain cell viability on subpassages.

Cell-free virus techniques with parasynchronous BFS cells detected parvovirus in only 25.5% of the samples that were IF positive. Direct culture of cells from kidneys or testicles produced parvovirus where this technique failed. The cell-associated virus technique employing parasynchronous BFS cells was most reliable and recovered parvovirus from 82.5% of IF-positive tissue specimens. Reports of investigations of parvoviral infections in other animal species, mainly the cat, also infer that it is difficult to isolate virus from infected organs. Method 3 is recommended and should be employed to isolate field strains. Serum free of antibodies against BPoV and of HI inhibitors must be used for successful isolation and virus propagation (Storz et al., 1972).

Direct EM examination of diarrhea fluid is not reliable because of the possible presence of many other small particles, including enteroviruses, caliciviruses, astroviruses, togavirus cores and even small bacteriophages. Recourse to the use of immune EM would be more specific, and enzyme immune assays should be developed.

A unique variety of nucleopathic changes was detected in cultured BFS cells infected with BPoV (Leary and Storz, 1980). They consisted of Cowdry type A as well as multifocal inclusions (see Figs. 82–85). Formation of distinct halos occurred in both instances. The appearance of these nucleopathic changes in infected host tissues has to be analyzed further to establish its diagnostic value.

A most efficient method to detect antibodies against BPoV is the HI test employing guinea pig or human type O red blood cells (Abinanti and Warfield, 1961; Storz et al., 1972). In some serum samples, nonspecific inhibitors of BPoV hemagglutination may be present and must be removed before testing for BPoV antibodies. The HI antibody titers corresponded well with results obtained through infectivity neutralization tests and other methods for the detection of antibodies (Hayder et al., 1983).

Recently, a competitive ELISA was introduced for detection and titration of antibodies to BPoV. The antibodies, if present in the cattle sera, are competing in this assay with two monoclonal antibodies to the parvoviral hemagglutinin. The antibody titers determined in ELISA correlated with the titers in VN tests (N. Juntti, personal communication, 1987).

PROPHYLAXIS AND CONTROL

A high percentage of cattle in the USA and other parts of the world has antibodies resulting from natural infections (Storz and Leary, 1979). However,

there are herds of seronegative cattle and thus without experience with this infection. Attempts to vaccinate cattle have not been described. As we learn more about the infection and the pathogenic potential of BPoV, efforts to develop vaccines may be warranted, particularly for the prevention of reproductive disease problems. This approach would be of benefit in reducing parvovirus-induced disease during the neonatal period of calves.

REFERENCES

Abinanti, F.R. and Warfield, M.S., 1961. Recovery of a hemadsorbing virus (HADEN) from the gastrointestinal tract of calves. Virology, 14: 288–289.

Bachmann, P.A., 1971. Properties of a bovine parvovirus. Zentralbl. Veterinärmed., 18: 80–81.

Bannerjee, P.T., Olson, W.H., Allison, D.P., Bates, R.C., Snyder, C.E. and Mitra, S., 1983. Electron microscopic comparison of the sequences of single-stranded genomes of mammalian parvoviruses by heteroduplex mapping. J. Mol. Biol., 166: 257–272.

Barnes, M.A., Wright, R.E., Bodine, A.B. and Alberty, C.F., 1982. Frequency of bluetongue and bovine parvovirus infection in cattle in South Carolina dairy herds. Am. J. Vet. Res., 43: 1078–1080.

Barros, C.S.L., 1980. Pathology of Experimental Infection of the Bovine Fetus with Bovine Parvovirus. Ph. D. Thesis, Colorado State University, Fort Collins, CO, 199 pp.

Bates, R.C. and Storz, J., 1973. Host cell range and growth characteristics of bovine parvoviruses. Infect. Immun., 7: 398–402.

Bates, R.C., Storz, J. and Reed, D.E., 1972. Isolation and comparison of bovine parvoviruses. J. Infect. Dis., 126: 531–536.

Bates, R.C., Storz, J. and Doughri, A.M., 1974. Morphogenesis of bovine parvoviruses and associated cellular changes. Exp. Mol. Pathol., 20: 208–215.

Carlson, J.H., Scott, F.W. and Duncan, J.R., 1977. Feline panleukopenia. I. Pathogenesis in germfree and specific pathogen-free cats. Vet. Pathol., 14: 79–88.

Coria, M.F. and Lehmkuhl, H.D., 1978. Isolation and identification of a bovine adenovirus type 3 with an adenovirus-associated virus. Am. J. Vet. Res., 39: 1904–1906.

Csiza, C.K., Scott, F.W., De Lahunta, A. and Gillespie, J.H., 1971. Pathogenesis of feline panleukopenia virus in susceptible newborn kittens. I. Clinical signs, hematology, serology, and virology. Infect. Immun., 3: 833–837.

Durham, P.J.K. and Johnson, R.H., 1985. Properties of an Australian isolate of bovine parvovirus type 1. Vet. Microbiol., 10: 335–345.

Hammon, W.D. and Enders, J.F., 1939. A virus disease of cats, principally characterized by aleukocytosis, enteric lesions and the presence of intranuclear inclusions. J. Exp. Med., 69: 327–352.

Hayder, H.A., Storz, J. and Young, S., 1983. Antigenicity of bovine parvovirus in fetal infections. Am. J. Vet. Res., 44: 558–563.

Hinaidy, B., 1978. Isolierung in Zellkulturen, Zytopathologie und Ausbeute boviner Parvoviren und deren serodiagnostische Nachweisverfahren. Dissertation, Universität für Bodenkultur, Vienna, 113 pp.

Hinaidy, B. and Bürki, F., 1980. Serodiagnostische Nachweisverfahren boviner Parvoviren. Zentralbl. Veterinärmed. B, 27: 459–469.

Hinaidy, B., Messner, A. and Bürki, F., 1979. Isolierung in Zellkulturen, Cytopathologie und Kulturausbeute boviner Parvoviren. Wien. Tierärztl. Monatschr., 66: 359–364.

Huck, R.A., Woods, D.W. and Orr, J.P., 1975. Isolation of a bovine parvovirus in the United Kingdom. Vet. Rec., 96: 155–156.

Inaba, Y., Kurogi, H., Omori, T. and Matumoto, M., 1973a. A new serotype of bovine parvovirus. Jap. J. Microbiol., 17: 85–86.

Inaba, Y., Kurogi, H., Takahashi, E., Sato, K., Tanaka, Y., Goto, Y., Omori, T. and Matumoto, M., 1973b. Isolation and properties of bovine parvovirus type 1 from Japanese calves. Arch. Ges. Virusforsch., 42: 54–66.

Leary, J.J. and Storz, J., 1980. Nucleopathic changes in parvovirus-infected cultured cells. Exp. Mol. Pathol., 32: 188–200.

Leary, J.J. and Storz, J., 1982. Kinetics of parvovirus replication, cytopathic effects and mitotic arrest in synchronized bovine fetal spleen cells. Exp. Mol. Pathol., 37: 272–286.

Liggitt, H.D., DeMartini, J.C. and Pearson, L.D., 1982. Immunologic responses of bovine fetus to parvovirus infection. Am. J. Vet. Res., 43: 1355–1359.

Luchsinger, E., Strobbe, R., Wellmans, G., Dekegel, D. and Sprecher-Goldberger, S., 1970. Haemagglutinating adeno-associated virus (aav) in association with bovine adenovirus type 1. Arch. Ges. Virusforsch., 31: 390–392.

Margolis, G. and Kilham, L., 1965. Rat Virus, an agent with an affinity for the dividing cell: In: Slow, Latent, and Temperate Virus Infections. NINDB Monograph No. 2, pp. 361–367.

Myrup, A.C., Mohanty, S.B. and Hetrick, F.M., 1976. Isolation and characterization of adeno-associated viruses from bovine adenovirus types 1 and 2. Am. J. Vet. Res., 37: 907–910.

Siegl, G., 1976. The parvoviruses. In: S. Gard and C. Hallauer (Editors), Virology Monographs 15, Springer, Vienna, New York, 109 pp.

Spahn, G.J., Mohanty, S.B. and Hetrick, F.M., 1966. Experimental infection of calves with hemadsorbing enteric (HADEN) virus. Cornell Vet., 56: 377–386.

Storz, J. and Bates, R.C., 1973. Parvovirus infections in calves. J. Am. Vet. Med. Asoc., 163: 884–886.

Storz, J. and Leary, J.J., 1979. Bovine parvoviruses: Epidemiology and host-virus relationships. International symposium of Viral Enteritis of Humans and Animals, Grignon, France, Inserm Symposia Series, 90: 63–79.

Storz, J. and Warren, G.S., 1970. Effect of antimetabolites and actinomycin D on the replication of HADEN, a bovine parvovirus. Arch. Ges. Virusforsch., 30: 190–194.

Storz, J., Bates, R.C., Warren, G.S. and Howard, T.H., 1972. Distribution of antibodies against bovine parvovirus in cattle and other animal species. Am. J. Vet. Res., 33: 269–272.

Storz, J., Okuna, N., McChesney, A.E. and Pierson, R.E., 1976. Virologic studies on cattle with naturally occurring and experimentally induced malignant catarrhal fever. Am. J. Vet. Res., 37: 875–878.

Storz, J., Leary, J.J., Carlson, J.H. and Bates, R.C., 1978. Parvoviruses associated with diarrhea in calves. Proceedings, Colloquium on Selected Diarrheal Diseases of Young Animals and Humans. J. Am. Vet. Med. Assoc., 173: 624–627.

Tennant, R.W., Layman, L.R. and Hand, R.E., 1969. Effect of cell physiological state on infection by rat virus. J. Virol., 4: 872–878.

Vincent, J., 1971. Isolement en Algérie de quatre souches de parvovirus bovis. Ann. Inst. Pasteur, 121: 811–814.

Wosu, L.O., Johnson, R.H., Goodchild, I. and Bachman, P., 1979. Isolation of a bovine parvovirus type 1 in Australia. Aust. Vet. J., 55: 199–200.

Reoviridae

Reo is an acronym derived from "respiratory enteric orphan". The family comprises the genera *Reovirus, Orbivirus, Rotavirus, Phytoreovirus* and *Fijivirus*, of which only the first three are of veterinary importance (the other two genera occur only in plants). Some 70 viruses have been assigned to the animal genera, most of them being (arthropod-borne) orbiviruses isolated from mosquitos, culicoides, phlebotomines and ticks.

Members of the Reoviridae family are spherical particles with diameters between 60 and 80 nm; they do not possess a lipoprotein envelope, but a double-shelled capsid constructed according to iscosahedral symmetry (Fig. 88). Cores have twelve spikes at the sites of the 5-fold symmetry axes. The three genera can be distinguished morphologically, the most characteristic detail of the orbiviruses being ring-shaped capsomers of the inner capsid (Latin orbis = ring) and of the rotaviruses a wheel-like (Latin rota = wheel) capsid appearance with a wide hub, short spokes and a well-defined rim. The outer capsid layer of rotaviruses (diameter of the complete infectious particle is 69–75 nm) is easily lost, which results in "incomplete" 58–62-nm particles.

The genome of the members of the Reoviridae family is segmented and consists of ten (*Reovirus, Orbivirus*) or eleven (*Rotavirus*) fragments. The nucleic acid molecules are linear and double-stranded and have mol. wts. between 0.2 and 7 $\times$ 10^6. In general, there are six to ten polypeptides in the virions of members of the Reoviridae family, including a transcriptase, nucleo-
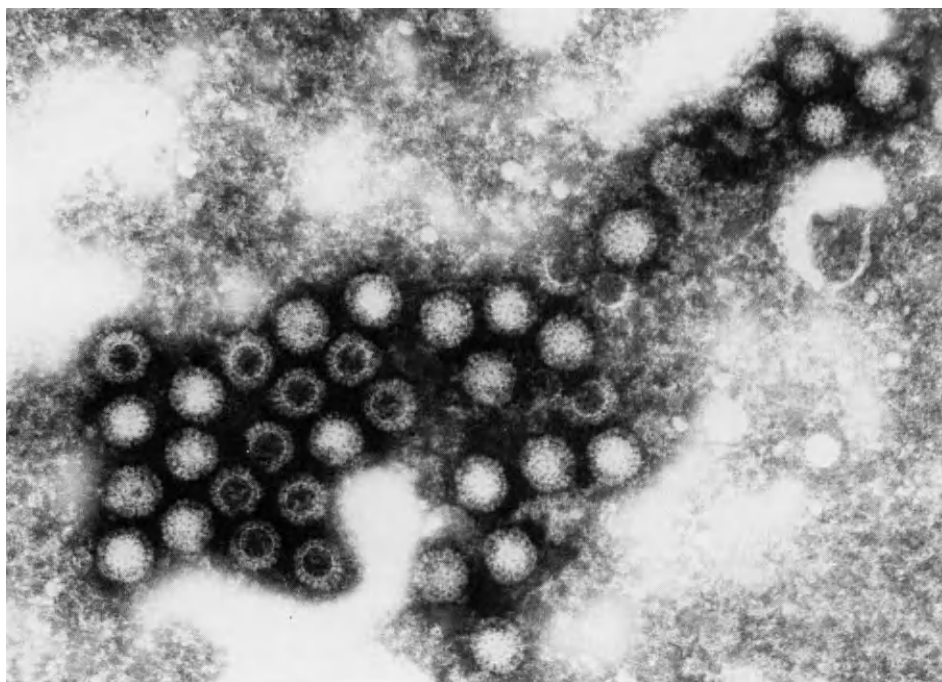
Fig. 88. Reovirions (of avian origin; courtesy J. Moreno-López, Uppsala).

tide phosphohydrolase and capping enzymes. Removal of the outer shell by proteolytic enzymes is required for the activation of the RNA-dependent RNA polymerase.

Virion buoyant densities range between 1.36 and 1.39 g/cm^3 in CsCl; the sedimentation coefficient is about 730 for the reoviruses and 520–540 for the orbi- and rotaviruses. The double-shelled complete virions of porcine rotavirus have a density of 1.36 g/cm^3 and the single-shelled particles of 1.38 g/cm^3.

Replication of reoviruses occurs exclusively in the cytoplasm, where viroplasma zones are visible, sometimes containing virus particles in paracrystalline arrays. Virion transcriptase synthesizes first positive strands and a replicase enzyme makes the negative strands later in the cycle, thereby forming the double-stranded progeny RNA molecules. Morphogenesis of orbiviruses is accompanied by formation of regularly structured filaments and tubules. Unassembled newly synthesized double-stranded RNAs and the excess viral antigens accumulate in large masses in the cytoplasm, forming the characteristic inclusions which give a bright green fluorescence with acridine orange staining (characteristic for double-stranded polynucleotides).

In ruminants, reovirus types 1, 2 and 3 are encountered (amongst others) in acute respiratory/enteric disease in calves. No characteristic syndrome can be attributed to these viruses; rotaviruses, on the other hand, are the causative agents of neonatal calf and lamb diarrhea, and orbiviruses of bluetongue in sheep.

Chapter 20

Acute Respiratory/Enteric Disease in Calves and Sheep

J.H. DARBYSHIRE

INTRODUCTION

Reoviruses in general, including those associated with infections of cattle and sheep, are classified as a genus of the family Reoviridae, and are frequently referred to as the classical reoviruses. The reoviruses of mammalian origin have been divided into three serotypes, although cross-reactions occur. Serological surveys conducted in various parts of the world have indicated that reovirus infections are widely distributed in cattle and sheep populations. Nevertheless, there is still little evidence for the implication of reoviruses as pathogens of the respiratory or alimentary tracts of either species, particularly when compared with the effects produced with other known disease agents.

It has been postulated (Darbyshire and Roberts, 1968; Lamont et al., 1968; Phillip and Darbyshire, 1971) that reoviruses of cattle are of less importance in the etiology of respiratory disease than are a number of other viruses that have been implicated as primary pathogens. Reoviruses are likely to initiate tissue damage in the respiratory tract, thereby enabling secondary agents to invade and replicate to advantage. In the case of the alimentary tract, their role as pathogens is even less well defined.

In sheep, evidence for pathogenic effects of reoviruses is also limited. Reovirus type 1 has been recovered from sheep showing signs of concurrent respiratory and alimentary tract disease, and the virus isolated was then utilized to reproduce the condition experimentally. It seems that the behaviour of reoviruses as pathogens of sheep, as in cattle, depends upon the activities of secondary agents in precipitating disease.
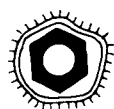
VIRUS PROPERTIES

Physical and chemical characteristics

Virus infectivity is stable between pH 2.2 and 8.0, and resists the effects of ether and chloroform. The virus also resists the effects of 1% phenol for at least 1 h, but is inactivated by 70% ethanol or 3% formalin at 56°C. When heated to 55°C in the presence of magnesium ions, virus infectivity is actually increased; the reverse is true at temperatures below 0°C. Infectivity can also be enhanced by treating the virus with proteolytic enzymes. Virions become cytotoxic following exposure to ultraviolet irradiation.

The efficiencies of various chemical agents for the disinfection of premises do not appear to have been assessed in much detail. The virus is known to survive 1% phenol and 20% lysol for 1 h at room temperature; it is unknown
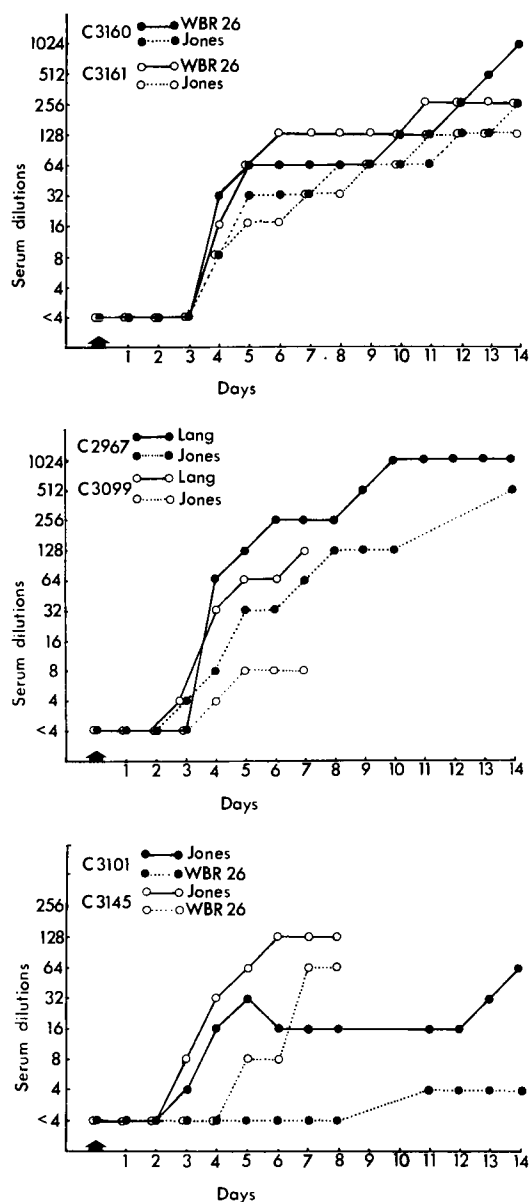
Fig. 89. HI reovirus antibody responses in calves inoculated with (a) bovine strain WBR 26 type 1; (b) human strain Lang type 1; and (c) human strain Jones type 2; note the heterotypic responses to the human strain Jones type 2 (a and b) and bovine strain WBR 26 type 1 (c) (data with permission from Lamont et al., 1968).

whether longer periods in the presence of such chemicals may reduce infectivity. The persistence of reoviruses in animal products has not been reported to date.

Antigenic properties

In respect of their antigenic structure, mammalian reoviruses are comprised of three serotypes. This has been determined both by cross-neutralization and HI tests. Heterotypic reactions can, however, be demonstrated by both techniques (Flammini et al., 1980a, b) as exemplified by Lamont et al. (1968), who used calves infected with reovirus types 1 or 2 and found heterotypic responses in HI tests (Fig. 89).

Isolations of the three reovirus serotypes have been made from animals and

man. All three types occur in cattle and sheep; a report (Kurogi et al., 1974) of the isolation of two further distinct types from cattle was subsequently modified: the viruses were found related to type 2 (Kurogi et al., 1980). The human isolates of all three serotypes are evidently indistinguishable from those recovered from animals, but the five described serotypes of avian origin form a separate group. Strains of reoviruses within the three types recovered from various animal species have usually proved to be indistinguishable, and will apparently cross the species barrier. Human strains of types 1 and 2 have been shown capable of infecting calves (Lamont et al., 1968).

All three reovirus serotypes possess two group-specific antigens in common; each type possesses a type-specific antigen as determined by immunodiffusion. However, immunoelectrophoretic analysis indicates that the reoviral antigens can be further resolved into four group-specific and two type-specific antigens.

EPIZOOTIOLOGY

The host range of the three mammalian reovirus serotypes is very wide indeed: they are found in most animals, including primates, domesticated livestock and wild animals. Reoviruses are also found in birds, including chickens, but these form a separate group. Reservoir hosts may exist but are unknown at the present time; reoviruses have been recovered from culicine mosquitoes, which may act as true vectors or merely as mechanical carriers (Parker et al., 1965). Reoviruses of cattle have been reported in various countries, including North America, Europe, Africa and the Far East. They have been found in sheep in Europe and Australasia.

Reoviruses evidently spread easily among cattle or sheep populations. There have been reports of inapparent infections among cattle in the course of vaccination trials (Blackmer, 1976; Thurber et al., 1977; Morzaria et al., 1979). The economic consequences of reovirus infection are difficult to assess in view of the mild character of the effects of infection. In serological surveys carried out on bovine respiratory disease in the United Kingdom, Phillip et al. (1968) reported that about 26% of cattle associated with outbreaks had a significant increase in reovirus antibody. In Belgium incidence was much lower (Wellemans, 1969) In a later survey in the United Kingdom, Darbyshire and Roberts (1968) recorded an incidence of 22% for reovirus type 2 during a 3-year period of serum monitoring.

Similar seroepidemiological surveys have shown that sheep populations experience infection widely as well (Stanley et al., 1964; Pringle and Cartwright, 1969; McFerran et al., 1973; Munz et al., 1974). Epizootics may occur in sheep (Belák and Palfi, 1974; Belák et al., 1974), and it would appear from the literature that reoviruses initiate a more severe condition of respiratory disease in sheep than in cattle.

PATHOGENESIS

In both cattle and sheep, reoviruses initiate infection in the respiratory and alimentary tracts. The virus enters the cell by phagocytosis and becomes associated with lysosomes. Viral RNA synthesis proceeds in the cytoplasm, and infected cells later display intracytoplasmic inclusions in the Golgi apparatus which accumulate as perinuclear masses within which virus particles may be observed.

After experimental infection of the respiratory and alimentary tracts in cattle there is a latent period of less than 24 h. This is followed by a period of

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viremia which may last for up to one week, depending on the virus strain. Virus can also be recovered from nasal discharge and feces, as well as from the conjunctival sac. Following experimental infection, virus may be recovered for up to 2 weeks.

Once reovirus has initiated infection it spreads to various tissues, probably by means of a viremia. Most virus strains have a tropism for respiratory tract tissue, including the nasal turbinate mucosa, tonsils, trachea, lung and mediastinal lymph nodes. In addition, virus may be demonstrated in the spleen and kidney; it can be found throughout the alimentary tract and in the mesenteric lymph nodes for at least a week after infection.

It has been shown by Phillip et al. (1968) that *Chlamydia* will act synergistically with reoviruses to induce a more severe pneumonopathy than either agent alone. Nonetheless, the clinical response is still mild, although lesions observed at necropsy of such dually infected animals are more severe than those induced by reovirus alone. The conclusion drawn from experimental evidence is that reoviruses are only mild pathogens of the bovine respiratory and alimentary tracts.

The clinical response of sheep to infection, however, would seem to be more pronounced. A strain of reovirus type 1 which had been isolated originally from sheep (Belák and Palfi, 1974a; Belák et al., 1974) in an epidemic of respiratory and enteric disease in a flock was used to inoculate lambs (Belák and Palfi, 1974b, c). After an incubation period of 4–6 days, the lambs showed pyrexia and ocular and nasal discharges; there was clinical evidence of pneumonia accompanied by diarrhea. These clinical signs abated within 3 weeks of infection. Neutralizing antibody titers of 64–128 were demonstrable.

Virus was recovered from the conjunctiva and internal organs of succumbed animals. Such experimental evidence suggests that reoviruses, at least those of serotype 1, are capable of inducing respiratory and intestinal disease in young sheep. There is no evidence for persistence or latency of reoviruses in sheep or cattle.

DISEASE SIGNS

In calves experimentally infected with reovirus types 1 or 2, only rectal temperatures were elevated (Lamount et al., 1968). This accords with a previous report (Abinanti, 1963) that bovine reovirus strains do not produce clinical illness in calves. Later, Phillip et al. (1968) were able to enhance the response by adding *Chlamydia* to reovirus and administering the mixture intratracheally. Diarrhea occurred within 3 days p.i., accompanied by a mucopurulent nasal discharge during the first week.

In lambs, laboratory evidence suggests that reovirus type 1 is capable of producing disease, whereas types 2 and 3 are not (McFerran and Baskerville, 1972; Snodgrass et al., 1976). However, this does not rule out that strains of both types may act as pathogens. Reovirus type 1 administered experimentally produces pyrexia, together with ocular and nasal discharge, sneezing and some dyspnea, all within a period of 4–6 days p.i. In the following week, diarrhea may be evinced, varying in severity and even leading to death before the end of the second week p.i. (Belák and Palfi, 1974c).

In natural outbreaks in sheep attributed to reovirus (Belák et al., 1974), mild respiratory and enteric signs were noted. Lambs with less vitality than the remainder of the flock succumbed, accounting for some 13% of the total number of animals affected. *Pasteurella* organisms were isolated from the lungs, which suggests that the pneumonia resulted from an invasion of secondary organisms following the virus infection.
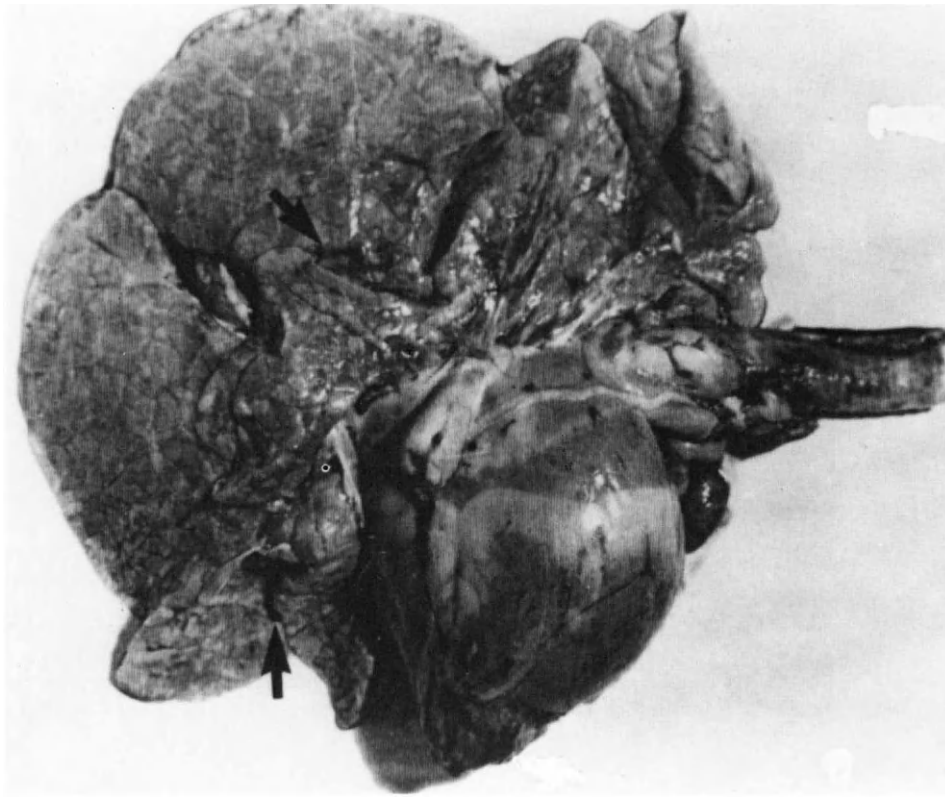
Fig. 90. Ventral surface of the lungs of a calf inoculated with reovirus type 1 (strain WBR 26); arrows indicate the areas of consolidation.

PATHOLOGY

Cattle

The pathology and histopathology of reovirus infections has been deduced from experimental infections with BRD in calves (Lamont et al., 1968). Colostrum-deprived calves inoculated both intranasally and intratracheally with human strains of types 1 and 2, or with a bovine strain of type 1, all produced similar responses.

There were no overt clinical signs apart from pyrexia, but on necropsy lesions of pneumonia were observed (Fig. 90). Histologically, the findings were similar to those noted in other virus infections of the respiratory tract. They include an extensive epithelialization of the lung alveoli, with pseudo-epithelialization of septal cells (Figs. 91, 92). Hepatocytes are often shed into the bloodstream with hemosiderosis.

Alveolar septal cell necrosis has also been observed. No intracytoplasmic inclusions have been described in histological lesions, but the alveolar walls have a marked reticulin content. Small islets of red cells, forming "blood lakes", may be observed.

IMMUNE REACTION

CMI and the development of specific immunoglobulin in bovine and ovine reovirus infections have not been described. In calves, HI antibody can be demonstrated subsequent to infection. For such tests a hemagglutinating

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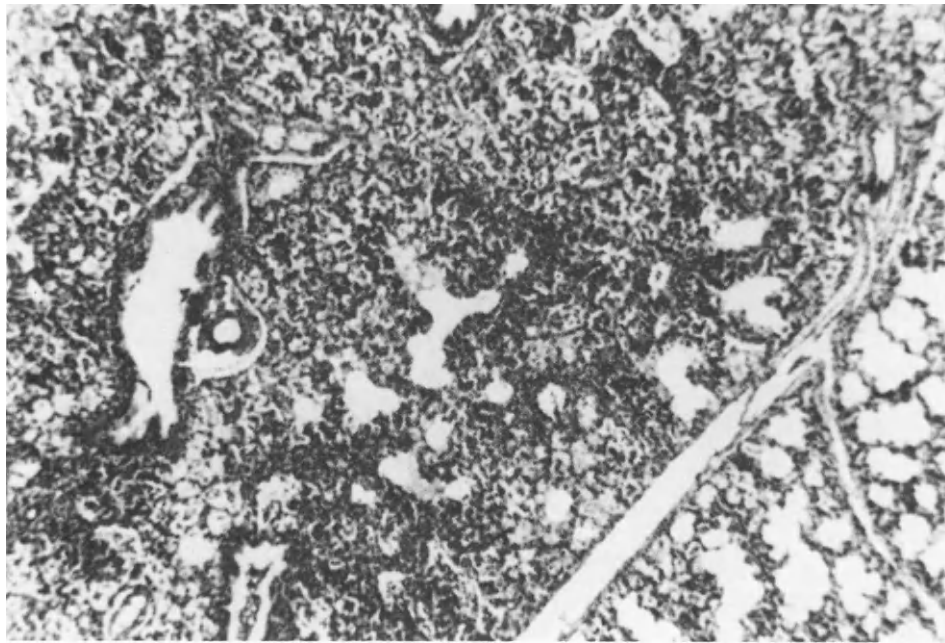
Fig. 91. Section of lung from a calf, 4 days after inoculation with reovirus, showing an early exudative reaction with a peribronchiolar, lymphoreticular hyperplasia and emphysema (data with permission from Lamont et al., 1968)

strain of reovirus is used as antigen. Some bovine strains, originally isolated in pig kidney cell cultures, may require at least one passage in monkey kidney cells to enhance hemagglutinating activity. Antibody becomes demonstrable within 3–4 days p.i. The homotypic response is followed by a heterotypic HI response. A similar heterotypic response had been demonstrated previously in man (Rosen et al., 1963a) as well as in naturally occurring infections in cattle (Rosen et al., 1963b). Such antibody may attain maximum titer within 14 days,
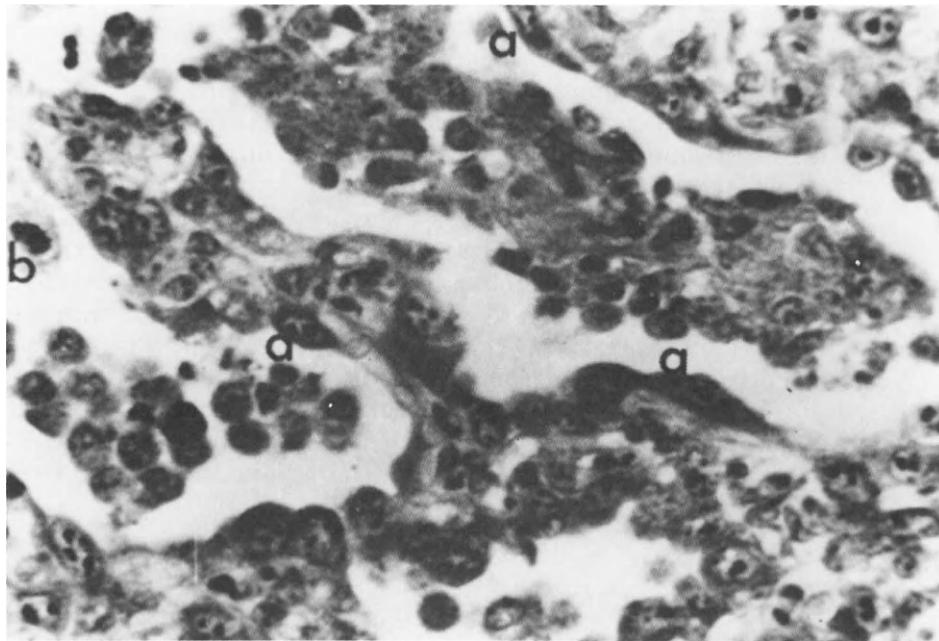
Fig. 92. Section of lung from a calf, 7 days after inoculation with reovirus, showing early epithelization; cell marked "a" shows cytoplasmic attenuation and basophilia; cell marked "b" is in mitosis (data with permission from Lamont et al., 1968).

although occasionally this may take a further 14 days; the levels are maintained for up to a year. Lamont et al. (1968) did not consider HI titers as a true indicator of protection against challenge infection.

Neutralization tests using calf kidney or lamb kidney cultures have also been employed to titrate antibodies in calves or sheep. In the latter, the results from HI and neutralization tests show a good correlation (McFerran et al., 1973).

LABORATORY DIAGNOSIS

Association of reoviruses with a clinical picture must be interpreted with considerable reserve in the first instance. This association may be easier to prove in sheep than in cattle. Nasal swabs should be taken from any discharges and used to infect culture monolayers of pig kidney or lamb kidney cells.

Antigen for HI tests is produced by growing virus in cynomolgus monkey kidney cell monolayers. The fluids are harvested and clarified by centrifugation, the supernatant being used as the antigen without further concentration. The antigen is tested against human erythrocytes group O, to determine the concentration of the hemagglutinin. As mentioned, some bovine strains may require additional passage in monkey kidney cells to produce a hemagglutinating activity. The timing of the collection of samples is probably most important. For the recovery of virus, swabs need to be made within the first few days of onset of illness. The first of paired serum samples should also be collected at this time. The swabs should be placed in a transport medium, such as sterile broth or tissue culture medium containing 0.5% bovine albumin, incorporating antibiotics such as streptomycin, penicillin or polymixin B. The swab fluids are kept at 4°C as long as possible until used to inoculate cultures.

With the collection of second samples from the same animals the sera may be examined for HI and neutralizing antibodies. The neutralization tests are performed in the conventional manner, using a standard virus dose of approximately 100 $TCID_{50}$ against dilutions of serum; 4-fold increases are significant. Neutralizing antibody titers of 1/64 to 1/128 in single serum samples can occur in lambs following either experimental or natural infections. In cattle, similar titers would be significant, whereas HI titers of comparable levels would also be indicative of a recent infection. The latter tests should be conducted against hemagglutinins of all three reovirus types in order to ascertain the highest, and thereby the homotypic titer.

PROPHYLAXIS AND CONTROL

There have been but a limited number of attempts to evaluate vaccines against reoviruses. Phillip et al. (1973) examined the efficacy of 2 commercial vaccines against BRD, both inactivated multifactorial vaccines. One preparation contained PI3 virus, BAV-3 and *Chlamydia*; the other vaccine was directed against PI3 virus, BAV-3, reovirus type 1 and BVD virus. These vaccines were evaluated solely on the basis of antibody responses, and comparisons were made of liveweight gains. Whereas calves were evidently protected on one farm, antibody titers fell sharply on another. The work of Philip et al. (1973) was extended by Morzaria et al. (1979) with a vaccine containing reovirus 1. It was found that whereas humoral responses were obtained in calves, the presence of maternal antibodies interfered with the response to vaccination.

Twiehaus et al. (1975) surveyed the opinions of veterinarians who used oral reovirus vaccines in the field. Performance ratings indicated that such vac-

cines were possibly efficacious in reducing calf diarrhea in vaccinated beef and dairy herds. A combined reovirus and coronavirus vaccine used in calves, however, did not produce any significant difference between vaccinated and unvaccinated animals (Blackmer, 1976). Thurber et al. (1977) also conducted field trials with reovirus vaccines in calves, but considered them of use only when reovirus infections were not very prevalent. In sheep, there are apparently no reports of any vaccine trials or use.

REFERENCES

Abinanti, F.R., 1963. Respiratory disease viruses of cattle and observations on reovirus infections of cattle. Am. Rev. Resp. Dis. Suppl., 88: 290–305.

Belák, S. and Palfi, V., 1974a. Isolation of reovirus type 1 from lambs showing respiratory and intestinal symptoms. Arch. Ges. Virusforsch., 44: 177–183.

Belák, S. and Palfi, V., 1974b. Experimental infection of lambs with a reovirus of sheep origin. Magy. Allatorv. Lap., 30: 329–331 (in Hungarian).

Belák, S. and Palfi, V., 1974c. Experimental infection of lambs with reovirus type 1 isolated from sheep. Acta Vet. Arch. Sci. Hung., 24: 241–247.

Belák, S., Palfi, V. and Huszar, E., 1974. A mass respiratory and intestinal disease of lambs caused by a reovirus. Magy. Allatorv. Lap., 29: 14–16.

Blackmer, P.E., 1976. A practitioner's experience with experimental reo-coronavirus calf diarrhoea vaccine. Vet. Med. Small Anim. Clin., 71: 351–354.

Darbyshire, J.H. and Roberts, D.H., 1968. Some respiratory virus and mycoplasma infections of animals. J. Clin. Pathol., Suppl. 2: 61–87.

Flammini, C.F., Bottarelli, E., Allegri, G., Giulioni, A. and Scatozza, F., 1980a. Serological reactions to reovirus types 1 and 3 in experimentally infected guinea pigs and in intensively reared calves. Microbiologica, 3: 485–489.

Flammini, C.F., Bottarelli, E., Allegri, G. and Giulioni, A., 1980b. Neutralizing antibody to reovirus types 1 and 3 in calves. Microbiologica, 3: 491–496.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Goto, Y., Omori, T. and Matumoto, M., 1974. New serotypes of reoviruses isolated from cattle. Arch. Ges. Virusforsch., 45: 157–160.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K. and Omori, T., 1980. Serological relationship between Japanese strains of reovirus of bovine origin and type 2 reovirus strains. Natl. Inst. Avian Health Q. (Tokyo), 20: 32–33.

Lamont, P.H., Darbyshire, J.H., Dawson, P.S., Omar, A.R. and Jennings, A.R., 1968. Pathogenesis and pathology of infection in calves of strains of reovirus types 1 and 2. J. Comp. Pathol., 78: 23–33.

McFerran, J.B. and Baskerville, A., 1972. Unpublished observations. Cited in: McFerran et al., 1973. Arch. Ges. Virusforsch., 40: 80.

McFerran, J.B., Nelson, R. and Clarke, J.K., 1973. Isolation and characterization of reoviruses isolated from sheep. Arch. Ges. Virusforsch., 40: 72–81.

Morzaria, S.P., Richards, M.S., Harkness, J.W. and Mallard, B.A., 1979. A field trial with a multicomponent inactivated respiratory viral vaccine. Vet. Rec., 105: 410–414.

Munz, E., Reimann, M. and Ackermann, E., 1974. Seroepidemiological studies on reovirus infections of man, domestic and wild animals in Tanzania. Acta Trop., 36: 277–288.

Parker, L., Baker, E. and Stanley, N.F., 1965. The isolation of reovirus type 3 from mosquitoes and a sentinal mouse. Aust. J. Exp. Biol. Med. Sci. 43: 167.

Phillip, J.I.H., Clegg, F.G., Halliday, G.J., Cross, M.H., Hardy, R. and Maund, B.A., 1973. An examination of two bovine respiratory disease vaccines. Vet. Rec., 92: 420–424.

Phillip, J.I.H. and Darbyshire, J.H., 1971. Respiratory viruses of cattle. Adv. Vet. Sci. Comp. Med., 15: 159–199.

Phillip, J.I.H., Omar, A.R., Popovic, V., Lamont, P.H. and Darbyshire, J.H., 1968. Pathogenesis and pathology in calves of infection by *Bedsonia* alone and by *Bedsonia* and reovirus together. J. Comp. Pathol., 78: 89–99.

Pringle, C.R. and Cartwright, L.N., 1969. An inhibitor of reovirus in sheep serum. J. Med. Microbiol., 2: 117–123.

Rosen, C., Evans, H.E. and Spickard, A., 1963a. Reovirus infections in human volunteers. Am. J. Hyg., 77: 29–37.

Rosen, C., Abinanti, F.R. and Hovis, J.F., 1963b. Further observations on the natural infection of cattle with reoviruses. Am. J. Hyg., 71: 250–257.

Snodgrass, D.R., Burrells, C. and Wells, P.W., 1976. Isolation of reovirus type 2 from respiratory tract of sheep. Arch. Virol., 52: 143–149.

Stanley, N.F., Leak, P.M., Grieve, G.M. and Peret, D., 1964. The ecology and epidemiology of reovirus. Aust. J. Exp. Biol. Med. Sci., 42: 373–384.

Thurber, E.T., Bass, E.P. and Beckenhauer, W.H., 1977. Field trial evaluation of a reo-coronavirus calf diarrhoea vaccine. Can. J. Comp. Med., 41: 131–136.

Twiehaus, M.J., Mebus, C.A. and Bass, E.P., 1975. Survey of the field efficacy of reoviral calf diarrhoea vaccine. Vet. Med. Small Anim. Clin., 70: 23–25.

Wellemans, G., 1969. Serological examination of bovine herds affected by respiratory troubles. Ann. Med. Vet., 113: 47–60.

Chapter 21

Bluetongue Virus

B.J. ERASMUS

INTRODUCTION

Bluetongue (BT) is an infectious, noncontagious, insect-borne viral disease of sheep and other dometic and wild ruminants. The disease was first recognized and described in South Africa, and for many decades it was believed to be confined to Africa. In the past 40 years, however, clinical or serological evidence of infection has been found in many countries outside Africa. This led to renewed interest in the condition and, particularly in the past two decades, also to extensive research on different aspects of BT in various research establishments, resulting in major advances in knowledge. A great deal of this information is contained in some excellent reviews (Bowne, 1971; Howell and Verwoerd, 1971; Verwoerd et al., 1979; Sellers, 1981; Gorman et al., 1983; Barber and Jochim, 1985; Campbell and Grubman, 1985) which should be consulted for details and for more complete bibliography sources.

VIRUS PROPERTIES

Bluetongue virus (BTV) is the type species of the *Orbivirus* genus, family Reoviridae. Complete virions have a diameter of about 69 nm and possess a double-layered capsid. The structureless, diffuse outer layer is readily lost during gradient centrifugation in CsCl (or by other treatments), exposing the inner layer, which consists of 32 prominent ring-like structural units from which the genus name was derived (Verwoerd et al., 1972).

The double-stranded RNA genome consists of ten segments. The virion is composed of seven structural proteins, two of which occur in the diffuse outer protein layer (Verwoerd et al., 1972). Type-specific neutralizing antibodies are induced by protein P2, one of the outermost two proteins, whilst group-specific antibodies are induced by protein P7, one of the main components of the core particle (Huismans and Erasmus, 1981).

Unpurified BTV can be regarded as fairly resistant, particularly in the presence of protein. It is stable at 20°C, 4°C and below -70°C but not at -20°C. In the freeze-dried state the virus is also very stable and can be kept almost indefinitely. On account of the double-stranded nature of its RNA, BTV is relatively resistant to ultraviolet and gamma irradiation. It is stable between pH 6.5 and 8.0 and relatively resistant to lipid solvents such as ether and chloroform. The virus is readily inactivated by disinfectants containing acid, alkali, sodium hypochlorite and iodophors (Howell and Verwoerd, 1971).

BT is characterized by the existence of a plurality of antigenically distinct serotypes of virus which can be distinguished by plaque reduction neutraliza-

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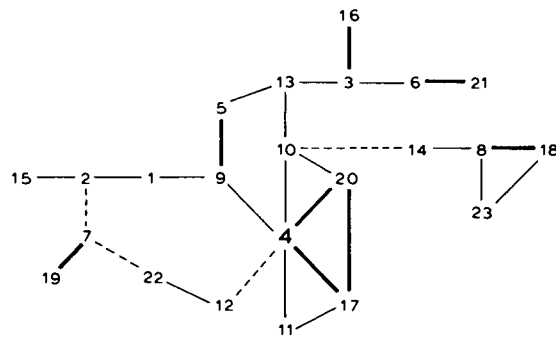
Fig. 93. Diagrammatic scheme to illustrate the interrelationships between BTV serotypes; thick lines represent strong relationships evident in plaque-reduction tests; thin lines represent relationships only evident by cross-protection tests in sheep and by heterotypic antibody responses; dotted lines represent relationships that are very weak or for which insufficient information is available. BTV type 4 could be regarded as the ancestral serotype.

tion tests. At present, 24 serotypes are recognized. However, all of these are to a greater or lesser degree related to one another as evidenced by cross-reactions in in vitro neutralization tests, cross-protection by in vivo challenge infections and the development of heterotypic antibodies in sheep and cattle. A provisional attempt to illustrate this relatedness, which may have an evolutionary basis, is presented in Fig. 93.

EPIZOOTIOLOGY

BT is historically an African disease, and although it has not been reported from all countries it is probably present throughout the entire continent. It presently occurs in the USA, in most (if not all) states of Central and South America, in the Near, Middle and Far East, in Japan, Indonesia and the northern regions of Australia (Luedke, 1985; Ozawa, 1985). Europe appears to be free at present, although the disease has occurred in the Iberian Peninsula, in Cyprus and on Lesbos Island.

BTV has a very wide host range, including sheep, cattle, goats, deer, bighorn sheep, most species of African antelope and various other artiodactyles (Hourrigan and Klingsporn, 1975; Hoff and Hoff, 1976). The outcome of infection may range from fatal (e.g. in sheep and deer) to inapparent, with the latter situation prevailing in most species. It is conceivable that, historically, the primary epizootiological cycle involved species of African antelope and *Culicoides* midges. With the agricultural development of large parts of Africa and the introduction of BTV to countries outside of Africa, the traditional epizootiological role of wild game animals has been taken over largely by cattle. Sheep are apparently involved in a secondary cycle. The infection seems to progress in the cattle–midge cycle and once a certain level of infection is attained it spills over to sheep. This generally occurs in late summer or in autumn and is often characterized by simultaneous outbreaks in sheep in various regions of a country.

Midges of the genus *Culicoides* act as biological vectors of BTV. The most significant vector species are *C. variipennis* and *C. insignis* in the USA, *C. fulvus*, *C. wadai*, *C. actoni* and *C. brevitarsis* in Australia and *C. imicola* in Africa and the Middle East (Standfast et al., 1985; Wirth and Dyce, 1985). Midges become infected by feeding on overtly or latently infected animals and virus replicates in the salivary glands, where it reaches a maximum concentration in about 6–8 days. Infected midges remain infective for life, but there is

to date no evidence of transovarial transmission. Overwintering of BTV results from survival of infected midges in areas with very mild and short winters as well as from latently infected cattle which circulate virus for long periods, perhaps even for years.

The prevalence of BT is governed largely by ecological factors such as high rainfall, temperature and humidity which favor the insect vector life cycle. In most countries, therefore, it has a distinct seasonal occurrence.

BT is not contagious, and effective transmission occurs only when infective blood or tissue suspensions are inoculated parenterally. Excretions and secretions from infected animals contain minimal concentrations of virus and susceptible animals are quite refractory to per os infection. Animal products such as meat, milk and wool from infected farms, and even from infected animals can, therefore, be regarded as harmless from an epidemiological point of view.

Semen from BTV-infected bulls collected during the viremic phase of the disease may be infective, and cows inseminated with such semen may also become infected (Bowen et al., 1985a). Shipment of semen from BTV-infected countries to BTV-free countries is therefore not without hazard.

In contrast, embryo transfer in cattle appears to be without risk of transmitting BTV, even when the embryo donors are viremic at the time of transfer (Bowen et al., 1985b). In sheep, however, embryos collected from infected ewes may occasionally give rise to BTV infection in recipient ewes (Gilbert, 1985).

PATHOGENESIS

Following natural infection, replication of BTV first takes place in regional lymph nodes, thereafter in other lymph nodes and lymphoreticular tissues as well as in endothelium, periendothelial cells and pericytes of capillaries, small arterioles and venules (Stair 1968; Pini, 1976; Lawman, 1979). This may lead to cytoplasmic vesiculation, nuclear and cytoplasmic hypertrophy, pycnosis and karyorrhexis. The resultant necrosis as well as the subsequent regenerative hyperplasia and hypertrophy of endothelium lead to vascular occlusion, stasis and exudation. This in turn results in hypoxia of overlying tissues with secondary development of lesions in the epithelium. The severity of secondary lesions, particularly in stratified squamous epithelium, is greatly influenced by pressure and mechanical abrasion (Stair, 1968).

There is mounting evidence that involvement of endothelial cells is highly selective. This means that there is not necessarily generalized involvement of endothelial cells throughout the body. Certain organs and certain blood vessels (and even particular branches of blood vessels) are more commonly involved than others. The basis for this selectivity is not understood but it could depend on differences in endothelial cells or the specific tropism of particular virus strains.

Once replication in the target cells has taken place, virus appears in the blood stream and spreads through the entire body, and hence most organs and tissues will contain a certain background of virus.

Virus is first detected in the blood stream from about day 3–6 p.i., depending upon the infecting dose and the route of infection. The viremia reaches its peak on about day 7 and 8, whereafter it declines fairly rapidly. In sheep, virus can rarely be demonstrated in the blood after day 14, but in cattle viremia can apparently persist for much longer.

BTV is largely cell associated, involving the erythrocytes and the leukocytes, while only a very small fraction of virus is found free in the plasma (Lawman, 1979; Collisson and Barber, 1983). It has not been established if virus

occurs inside the red blood cell and whether a particular white cell type is involved.

A panleukopenia which somewhat precedes the appearance of viremia and the febrile reaction is a consistent feature of BTV infection in sheep, goats and cattle. The cause of the leukopenia has not been established, but it could be due to the replication of BTV in the leukocytes or in the stem cells of the hemopoietic system (Lawman, 1979).

In cattle, unlike sheep, there is mounting evidence that expression of clinical disease is due to an IgE-mediated hypersensitivity reaction induced by previous exposure to BTV or related viruses. The appearance of symptoms and lesions is paralleled by a rapid elevation of serum-IgE-specific BTV antibodies, which in turn is associated with a release of endogenous mediators such as histamine, prostaglandins and thromboxane A_2 (Emau et al., 1984; Anderson et al., 1985).

DISEASE SIGNS

Although BTV may infect many species of ruminants, clinical signs of disease are generally associated with sheep and consequently most descriptions of the disease apply to sheep (Erasmus, 1975).

All breeds of sheep are susceptible to BTV infection, although the clinical outcome may vary remarkably. Indigenous African breeds are resistant and generally show little more than a mild febrile reaction, whereas the Merino and the European mutton breeds are highly susceptible and frequently die. A very marked variation in susceptibility is also encountered between different individuals of the same breed and even the same flock. This individual variation is entirely independent of previous exposure to homologous or related viruses and cannot be explained at present.

Furthermore, the severity of infection depends to a marked extent upon environmental conditions, most notably exposure to sunlight. This fact has been known for at least 40 years but has frequently been ignored. It is practically impossible to reproduce the disease with regard to severity and range of clinical signs in sheep kept in stables where they are not exposed to sunlight or UV light. In fact, it appears as if sheep infected with BTV develop some form of photosensitivity which accounts for some of the severe signs seen under field conditions.

The average incubation period following artificial infection of sheep is 4–6 days but it may be as short as 2 days and occasionally as long as 15 days. The first sign of infection is almost invariably a rise in body temperature, which reaches an acme on the 7th or 8th day. In many instances the fever exceeds 41°C and occasionally even 42°C. The average duration of the febrile reaction is 6 days, although it may vary from 2 to 11 days. In the majority of instances there is a fair correlation between the height and particularly the duration of the febrile reaction and the severity of the clinical signs.

Within 1–2 days after the onset of fever other clinical signs may become evident. The skin of the muzzle and lips as well as the oral mucosa become hyperemic. Edema of the lips, face, eyelids and ears develops, and some of this fluid may accumulate in the intermandibular space. This submandibular edema may be very extensive and occasionally extend down the neck and rarely to the axillary region. Petechial hemorrhages may be observed on the muzzle and in the mucous membranes of the mouth and eyes. Shallow erosions develop on the muzzle and the nostrils (Fig. 94) as well as in the mouth, particularly opposite the corner incisors. Very extensive erosions can also develop in the cheeks and on the tongue opposite the molar teeth. Such erosions are normally difficult to
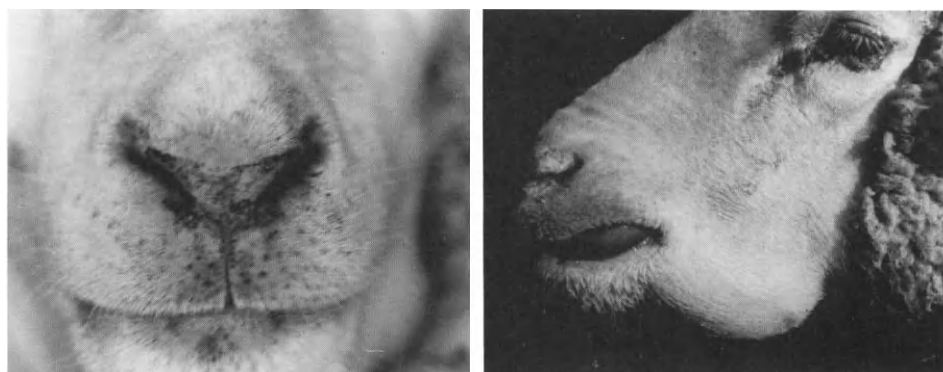
Fig. 94. Hyperemia of the lips and erosions of the muzzle and nostrils.

Fig. 95. Edema of the tongue, lips and submandibular space.

see but a fetid breath is noticed. Pain is evidenced by smacking of the lips and frequent movement of the tongue, resulting in slight frothing, and also immersion of the mouth and lips in the drinking water for long periods. Affected sheep obviously also resent handling and examination of their mouths and find it difficult to eat.

The tongue is frequently involved and initially may show intense hyperemia with petechial hemorrhages of the papillae near the tip. It may later become grossly swollen and edematous and may even protrude from the mouth (Fig. 95). Occasionally, in very severe cases, it may become cyanotic, hence the name "bluetongue".

A watery nasal discharge is frequently present which later becomes mucopurulent and eventually dries to form crusts, causing severe dyspnea. The respiratory rate may increase and panting is frequently observed. In peracute cases alveolar edema develops which leads to marked dyspnea and, terminally, froth may exude from the nostrils.

The hyperemia of the skin of the lips may extent to the entire face, ears and the rest of the body. It is usually most pronounced in the groin, axilla, perineum and the lower limbs. The slightest abrasion or handling may result in extensive subcutaneous hemorrhages, particularly in the groin and axilla. The extensive dermatitis causes abnormal wool growth, which is evidenced about 3–6 weeks later by a "break" in the wool that can lead to casting of the entire fleece.

Foot lesions usually develop with the subsidence of fever but occasionally during the peak of the febrile response. Initially, hyperemia of the coronary band is observed. Soon petechial hemorrhages appear under the periople, which later become streaky in appearance (Fig. 96). The hind feet are more frequently affected, and the lesions are more pronounced on the bulbs of the feet, particularly on the lateral hooves. As a result of the pain, affected sheep are reluctant to walk, are mostly recumbent or stand with an arched back and may even attempt to walk on their knees. Within 7–14 days the horny laminae may start to separate from the sensitive laminae and a clear "break" in the hoof develops. The old hoof gradually grows out and is sloughed completely about 3–4 months after infection.

In some instances BTV-infected sheep show rapid and extreme emaciation and weakness. Although this state can partially be ascribed to fasting and dehydration, it results mostly from very severe muscle degeneration and necrosis. Some sheep develop torticollis, which can have serious consequences as the animals find it difficult to maintain their balance.

The course of the disease depends largely on the severity of the condition. In mild cases recovery is generally rapid and uneventful. In more severe cases

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Fig. 96. Coronitis with linear hemorrhages in horny tubules of hooves.

peracute death may take place about 7–9 days p.i. Mouth lesions normally heal quickly, and extensive ulcerations may only leave a small scar after 7 days. Similarly, lesions on the muzzle and nostrils disappear in about 5 days. Sheep with extensive muscle involvement may become weak and debilitated, and recovery could be protracted.

The mortality rate obviously depends on various factors but under normal field conditions varies from 2 to 30%. However, it can be much higher if infected sheep are exposed to cold, wet conditions, as often happens in late autumn when the morbidity rate may be near its peak.

Although the frequency of infection of cattle with BTV is generally higher than in sheep, disease in cattle is rare. Similarly, attempts to experimentally produce disease in cattle with virulent virus rarely succeed. Clinical infection is actually a hypersensitivity reaction. Infected animals may show a transient febrile response, ranging from 40 to 41°C. Stiffness or lameness is common, the respiratory rate may be increased and there may be lacrimation and increased salivation. The lips and tongue are sometimes swollen, and ulcers may occur in the oral mucosa. The skin of the muzzle is often inflamed and later cracks and peels. Similarly, the skin of the neck, flanks, perineum and teats may be affected (Anderson et al., 1985; Jochim, 1986).

Hydranencephaly and congenital deformities may develop in bovine and sheep fetuses of BTV-infected dams. The severity of lesions depends on the gestational age of the fetus at infection. Fetuses seem to be most susceptible during the period of active brain development.

PATHOLOGY

The severity and distribution of the lesions found in BTV-infected sheep are obviously related to the severity of the clinical disease as well as to the particular stage of the disease at which death occurs. The following description largely applies to sheep dying within 14 days of infection.

Fig. 97. Sharply delineated hyperemia of anterior third of omasum with focal necrosis at base of folds.

The oral mucosa is hyperemic, edematous and possibly cyanotic, and petechiae or ecchymoses may be present. Excoriations are evident on the inner surface of the lips (particularly opposite the corner incisors), dental pad, the sides, tip and anterior dorsum of the tongue, and particularly on the internal surface of the cheeks opposite the molar teeth. Hyperemia of the ruminal papillae, the rumen pillars, reticular folds and the anterior third of the omasal mucosa is commonly observed. The sharp demarcation of the omasal hyperemia is striking (Fig. 97) and is often associated with corresponding subserosal hemorrhage. Hemorrhage may also be found in the mucosa around the esophageal groove and in the subserosa encircling the pylorus.

The lungs may show severe alveolar and interstitial edema and the entire bronchial tree may be filled with froth. In addition the lungs may be severely hyperemic and show extensive subpleural hemorrhages. Alternatively, the thoracic cavity may contain up to 2 l of plasma-like fluid. Lung edema and hydrothorax are probably the main causes of acute death in sheep.

The pericardial sac may show numerous petechial hemorrhages and contains a variable amount of fluid. The vast majority of cases shows a distinctive hemorrhage in the tunica media near the base of the pulmonary artery. This highly pathognomonic lesion may vary from a few pin-point hemorrhages (which are only visible when the artery is stretched and held against the light) to a bulging hemorrhage up to 15 mm in diameter (Fig. 98). Subepicardial and subendocardial hemorrhages, particularly involving the left ventricle, are commonly found. Occasionally focal necrosis and even calcification of the papillary muscle of the left ventricle is observed. Generalized damage to the cardiovascular system is evidenced by widespread hyperemia, edema and hemorrhage.

The spleen may be slightly enlarged with subcapsular hemorrhages. The lymph nodes are commonly enlarged and edematous and have a pale washed-out appearance.

The skin often shows intense hyperemia, particularly in the areas not covered by wool. The vascular network on the inside of the skin is generally

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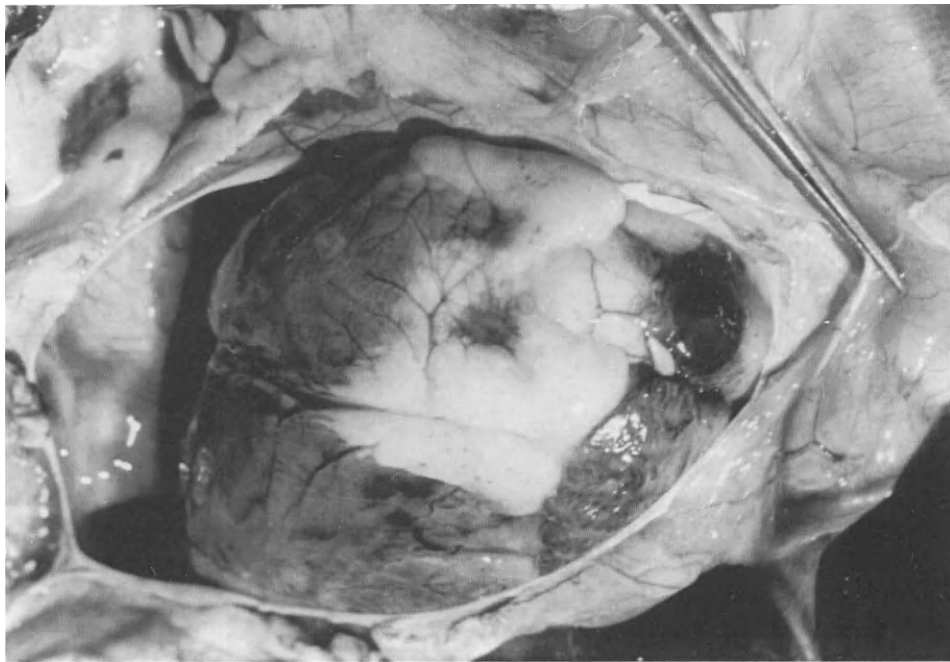
Fig. 98. Hemorrhage in wall of pulmonary artery and of ventricles as well as evidence of hydropericardium.

markedly congested and even the smallest blood vessels become very conspicuous.

Animals that die later than 14 days p.i. often show dramatic degeneration and necrosis of the skeletal musculature (Fig. 99), resulting in severe wasting of the carcass. Individual muscle fibers or entire muscles lose their pigmentation and the intermuscular fasciae are infiltrated with a clear fluid, resulting in a gelatinous appearance. The cause of death in such animals is not clear but it could be due to general exhaustion.

Fig. 99. Focal areas of muscle necrosis.

Histopathological examination does not reveal any pathognomonic changes and generally confirms the gross pathological finding. A detailed description has been given by Stair (1968).
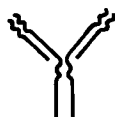
IMMUNE REACTION

Infection with BTV in immunocompetent animals is followed within 7–10 days by the formation of group-specific and type-specific antibodies. The group-specific antibodies persist for about 6–18 months, whereas type-specific neutralizing antibodies can often still be demonstrated after 3 years.

Protective immunity is generally associated with neturalizing antibodies, but occasionally animals with no demonstrable neutralizing antibodies resist infection with virulent BTV. It is becoming apparent that CMI responses to BTV infection in sheep are also protective and may in fact be less type-specific than humoral responses (Jeggo and Wardley, 1985; Jochim, 1985). Sheep that recover from virulent BTV infection will show partial protection when challenged with a heterologous virulent serotype and complete protection when challenged once more, despite the complete absence of demonstrable neutralizing antibodies against the second and third challenge viruses (Jeggo et al., 1983; B.J. Erasmus, unpublished data).

Lambs born to BTV-immune ewes obtain passive immunity; duration of this colostral immunity depends on the initial level and persists for a maximum period of 6 months. Lambs that possess passive immunity cannot be effectively vaccinated with live attenuated vaccine until it has waned sufficiently.

LABORATORY DIAGNOSIS

Although a presumptive diagnosis of BT, based on the clinical and pathological signs, can frequently be made, laboratory confirmation may be required.

Blood should be collected from febrile animals as early as possible in anti-coagulants such as heparin, EDTA or citrate. In fatal cases pieces of spleen and lymph nodes should be collected as soon as possible after death and kept at 4°C during shipment to the diagnostic laboratory.

BTV can most readily be isolated by intravascular inoculation of 10–12-day-old embryonated eggs. Occasionally, isolations can also be made by intracerebral inoculation of new-born mice or by inoculation of cell cultures (L929, BHK21, Vero or *Aedes albopictus*). Initial identification of virus isolates can be made by group-specific tests such as IF or CF. For serotyping, best results are achieved with plaque reduction or plaque inhibition tests.

Detection of BTV antibodies can be achieved by DF, ID, ELISA and hemolysis-in-gel tests. Type-specific antibodies can be demonstrated by plaque-reduction and by HI tests (Luedke, 1985). For diagnostic purposes it is imperative to use acute and convalescent phase serum samples. In animals that have suffered multiple BTV infections, results of neutralization tests may be difficult to interpret in view of heterotypic antibody responses.

PROPHYLAXIS AND CONTROL

Once BT has established itself in a country it may be impossible to eradicate and even difficult to control. On account of the wide host range and the possible existence of latently infected carrier animals, elimination of the source of virus may not be possible. It is equally impossible or impractical, in the light of

present knowledge, to attempt the eradication or even an effective reduction of insect vectors. Although integrated insect control should be encouraged, infected midges could still be blown in on the wind from other farms, districts or states. Stabling of animals and the strategic use of insect repellants should reduce the prevalence of infection but may not always be possible. Prophylactic immunization of sheep therefore remains the most practical and effective control measure.

Live attenuated BTV vaccine has been in use for 40 years and is known to induce a very effective and lasting immunity. However, in a country such as South Africa, where 21 of the 24 known serotypes occur, polyvalent vaccines present unique problems. In view of the interference between vaccine strains, the number as well as the composition of the serotypes in a vaccine must be carefully considered. At present three pentavalent vaccines, administered at intervals of at least 3 weeks, are used in South Africa (Erasmus, 1980). Lambs possessing colostral immunity cannot be effectively vaccinated before 6 months of age. The vaccine is safe except if used in ewes during the first half of pregnancy, in which case it may cause serious damage to the fetal brain.

Promising experimental results are being obtained with inactivated BTV vaccines. Furthermore, the identification of the structural protein (P2) responsible for the induction of protective neutralizing antibodies has paved the way for the development of novel cloned or even synthetic BTV vaccines (Huismans et al., 1985).

REFERENCES

Anderson, G.A., Stott, J.L., Gershwin, L.J. and Osburn, B.I., 1985. Subclinical and clinical bluetongue disease in cattle: Clinical, pathological and pathogenic considerations. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 103–107

Barber, T.L. and Jochim, M.M., 1985. Bluetongue and Related Orbiviruses (Progress in Clinical and Biological Research, Vol. 178). Alan R. Liss, New York, 746 pp.

Bowen, R.A., Howard, T.H. and Pickett, B.W., 1985a. Seminal shedding of bluetongue virus in experimentally infected bulls. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 91–96.

Bowen, R.A., Howard, T.H., Elsden, R.P. and Seidel, G.E., 1985b. Bluetongue virus and embryo transfer in cattle. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 85–89.

Bowne, J.G., 1971. Bluetongue disease. In: C.A. Brandly and C.E. Cornelius (Editors), Advances in Veterinary Science and Comparative Medicine, 15: 1–46.

Campbell, C.H. and Grubman, M.J., 1985. Current knowledge on the biochemistry and immunology of bluetongue. In: R. Pandey (Editor), Progress in Veterinary Microbiology and Immunology, 1: 58–79.

Collisson, E.W. and Barber, T.L., 1983. Blood cells associated with bluetongue virus infection in cattle. Proc. 26th Annu. Meet. Am. Assoc. Vet. Lab. Diagn., pp. 287–300.

Emau, P., Giri, S.N., Anderson, G.A., Stott, J.L. and Osburn, B.I., 1984. Function of prostaglandins, thromboxane A_2, and histamine in hypersensitivity reaction to experimental bluetongue disease in calves. Am. J. Vet. Res., 45: 1852–1857.

Erasmus, B.J., 1975. Bluetongue in sheep and goats. Aust. Vet. J., 51: 165–170.

Erasmus, B.J., 1980. The epidemiology and control of bluetongue in South Africa. Bull. Off. Int. Epizoot., 92: 461–467.

Gilbert, R.O., 1985. The transfer of bluetongue virus by embryo transfer in sheep. Thesis, University of Pretoria.

Gorman, B.M., Taylor, J. and Walker, P.J., 1983. Orbiviruses. In: W.K. Joklik (Editor), The Reoviridae. Plenum, New York, pp. 287–357.

Hoff, G.L. and Hoff, D.M., 1976. Bluetongue and epizootic hemorrhagic disease: A review of these diseases in non-domestic artiodactyles. J. Zoo. Anim. Med., 7: 26–30.

Hourrigan, J.L. and Klingsporn, A.L., 1975. Bluetongue: The disease in cattle. Aust. Vet. J., 51: 170–174.

Howell, P.G. and Verwoerd, D.W., 1971. Bluetongue virus. In: S. Gard, C. Hallauer and K.F. Meyer (Editors), Virology Monographs, 9: 35–74.

Huismans, H. and Erasmus, B.J., 1981. Identification of the serotype-specific and group-specific antigens of bluetongue virus. Onderstepoort J. Vet. Res., 48: 51–58.

Huismans, H., Van der Walt, N.T. and Erasmus, B.J., 1985. Immune response against the purified serotype specific antigen of bluetongue virus and initial attempts to clone the gene that codes for the synthesis of this protein. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 347–353.

Jeggo, M.H. and Wardley, R.C., 1985. Bluetongue vaccine: cells and/or antibodies. Vaccine, 3: 57–58.

Jeggo, M.H., Gumm, I.D. and Jaylor, W.P., 1983. Clinical and serological response of sheep to serial challenge with different bluetongue virus types. Res. Vet. Sci., 34: 205–211.

Jochim, M.M., 1985. Bluetongue: A review of the immune response of sheep and cattle to bluetongue virus infection. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 455–460.

Jochim, M.M., 1986. Bluetongue in cattle. In: J.L. Howard (Editor), Current Veterinary Therapy: Food Animal Practice 2. W.B. Saunders, Philadelphia, PA, pp. 488–489.

Lawman, M.J.P., 1979. Observations on the pathogenesis of bluetongue virus infections in sheep. Thesis, University of Surrey.

Luedke, A., 1985. WHO/FAO Working Team Report: Virology. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 665–688.

Ozawa, Y., 1985. Bluetongue and related orbiviruses: Overview of the world situation. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 13–20.

Pini, A., 1976. A study on the pathogenesis of bluetongue: Replication of the virus in the organs of infected sheep. Onderstepoort J. Vet. Res., 43: 159–164.

Sellers, R.F., 1981. Bluetongue and related diseases. In: E.P.J. Gibbs (Editor), Virus Diseases of Food Animals: A World Geography of Epidemiology and Control. Academic Press, London, pp. 567–584

Stair, E.L., 1968. The pathogenesis of bluetongue in sheep: A study by immunofluorescence and histopathology. Dissertation, Texas A & M University, College Station, TX.

Standfast, H.A., Dyce, A.L. and Muller, M.J., 1985. Vectors of bluetongue virus in Australia. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 177–186.

Verwoerd, D.W., Els, H.J., De Villiers, E.M. and Huismans, H., 1972. Structure of the bluetongue virus capsid. J. Virol., 10: 783–794.

Verwoerd, D.W., Huismans, H. and Erasmus, B.J., 1979. Orbiviruses. In: H. Fraenkel-Conrat and R.R. Wagner (Editors), Comprehensive Virology, 14: 285–345.

Wirth, W.W. and Dyce, A.L., 1985. The current taxonomic status of the *Culicoides* vectors of bluetongue viruses. In: T.L. Barber and M.M. Jochim (Editors), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 151–164.

Chapter 22

Bovine and Ovine Rotavirus

C.A. MEBUS
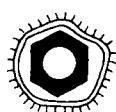
VIRUS PROPERTIES

Rotavirus particles are approximately 70 nm in diameter and are characterized by an icosahedral core containing eleven segments of doubled-stranded RNA surrounded by an inner and outer capsid layer. All known rotaviruses have a common (group) antigen associated with the inner capsid layer. Type-specific antigens are associated with the outer capsid layer. Gel electrophoresis of the genome segments has revealed differences in RNA patterns for rotaviruses obtained from different species as well as for different isolates from the same species (Rodger et al., 1981). In contrast to the numerous electrophoretic types for a species, the number of serotypes was more limited.

Calf rotavirus has been shown to remain viable for 6 months in fecal material at room temperature (Flewett et al., 1975). A 10-s exposure to iodophore (4%, 1% and 0.31%), sodium hypochlorite solution (3%), lysol (5%), or formaldehyde–saline (10%) had little effect on the titer of rotavirus in the intestinal contents from infected lambs. Lysol (5%) and formaldehyde–saline (10%) were effective when the intestinal contents were exposed for 2 h (Snodgrass and Herring, 1977).

At least two different serotypes were found when isolates from calves in Japan and the United Kingdom were examined by serum cross-neutralization (Murakami et al., 1983b; Ojeh et al., 1984). The majority of the isolates was related to the Lincoln strain from Nebraska, which in the future should represent serotype 1 of the serogroup A. Ovine rotaviruses isolated in Japan were different from the Lincoln strain (Makabe et al., 1985). There are obviously isolates from both calves and lambs belonging to a different serogroup (B) and showing "atypical" electropherograms of their eleven RNA segments (Snodgrass et al., 1984). At present, and to the best of our knowledge, a classification of rotaviruses is still missing.

EPIZOOTIOLOGY

Rotavirus infections have been recorded in many species of domestic and zoo ruminants (Eugster et al., 1978), monogastric animals and man. The importance of cross-species infection is not known. The USA calf rotavirus isolate did not infect piglets, while the British calf isolate caused diarrhea in piglets (Hall et al., 1976). The mouse rotavirus did not infect calves. Human infant rotavirus caused diarrhea in calves, but the severity of illness and amount of virus shed was less than that caused by infection of calves with the USA calf rotavirus (Mebus et al., 1977). In spite of these experimental cross-species infections there is no evidence that, under field conditions, cross-species infection is important.

Calf rotavirus and most likely lamb rotavirus infection has a worldwide distribution. In different surveys, rotavirus has been found to be associated with 41–48% of the cases of neonatal calf diarrhea (England et al., 1976; Morin et al., 1976; Martel and Perrin, 1981). The estimated economic loss in the USA (1970–1976) from all causes of bovine neonatal gastroenteritis was $95 500 000 per year (House, 1978).

Animals confined in sheds and barns frequently have a high incidence of neonatal diarrhea because of exposure to accumulated viruses and bacteria. This exposure is not materially reduced if the cows or ewes and their young are placed in large pastures, because of their gregarious nature. Feeding hay or supplement on pasture increases congregation and exposure. An animal in the early stage of diarrhea can have up to 10^8–10^9 virions per ml of feces.

The highly contagious nature of rotavirus diarrhea should be considered when caring for neonatal animals. Attendants should be advised to care for healthy animals before sick animals and to sanitize hands, gloves, boots and clothing after working with sick animals.

In the established larger herds, rotavirus diarrhea frequently develops a pattern — at a certain age there will be a high probability that the animals will develop diarrhea; in one herd the age may be 3–5 days, in another herd 14 days. There is apparently no age resistance, for rotavirus infection has been reported in a yearling calf in the USA (Fernelius et al., 1972) and in two cows in England (Woode and Bridger, 1975).

Cattlemen over the years have associated adverse weather with an increased incidence of diarrhea. In the colder climates this is rain, sleet or snowstorm, while in warm areas it can be heat, wind or blowing sand. A draft in a barn can have the same effect. How these stress conditions cause an increased incidence in neonatal diarrhea is not known.

Mortality from rotavirus diarrhea depends to a large extent on secondary infection. In uncomplicated rotavirus infections, animals develop diarrhea but mostly recover without treatment. However, when complicating infections with e.g. *E. coli*, *Salmonella*, *Clostridia* or *Cryptosporidium* occur, particularly in the very young animals, mortality can be high.

PATHOGENESIS

Portal of entry for rotavirus is the mouth. The primary site of replication is the mature villous epithelial cells of the small intestine (Mebus et al., 1971; Mebus and Newman, 1977). In the lamb, limited replication has also been found to occur in the epithelium of the cecum and colon (Snodgrass et al., 1977). Viremia has not been detected, but calf rotavirus has been isolated from mesenteric lymph nodes and lung. Viral replication outside the intestinal tract is minimal and is believed not to contribute to the clinical disease. Rotavirus is shed in the feces; titers of 10^8–10^9/ml are not unusual in the first few hours of diarrhea; thereafter, the titer drops. Circumstantial evidence suggests that healthy calves can be carriers, for diarrhea has occurred in herds within a few days after introducing a new calf. Also cows may be carriers: when only adult animals from a herd having a history of rotavirus diarrhea were moved to a ranch that had not had a problem, rotavirus diarrhea has occurred during the subsequent calving. Persistent infection has also been suggested (Schlafer and Scott, 1979).

DISEASE SIGNS

The most reliable information on the clinical course of rotavirus infections has been obtained from experimentally infected gnotobiotic calves (Mebus et al., 1971) and lambs (Snodgrass et al., 1977). The incubation period in experimental animals after oral inoculation can be as short as $12\frac{1}{2}$ h and as long as 36 h; inocula containing large amounts of virus caused shorter incubation periods. Incubation time in natural infection can also be short, for calves less than 24 h old can have rotavirus diarrhea.

Clinical signs usually progress in the following order: anorexia, depression, drooling a few strings of thick saliva and then diarrhea. Onset of illness is rapid; within 2 h a calf can become so depressed that it cannot stand and will then develop diarrhea. Diarrhea in gnotobiotic calves lasts for 5–6 h, during which time the animal will pass 300–400 ml of liquid yellow feces. The amount of feces is somewhat dependent on the amount of milk consumed after inoculation. Twenty-four hours after the onset of diarrhea the calf will suckle and then pass an increased amount of pulpy yellow feces. In field cases, the diarrhea lasts for longer periods; this variation is thought to be due to secondary infection. In contrast to calves, diarrhea in gnotobiotic lambs was present throughout the 6-day observation period.

PATHOLOGY

Uncomplicated cases of rotavirus enteritis have no gross lesion in the intestinal tract. Secondary infection can cause congestion of the mucosa and in severe cases hemorrhage.

Light, IF and EM studies of rotavirus infected calves (Mebus et al., 1977) suggest the following pathogenesis. After oral inoculation, the columnar epithelial cells over the small intestinal villi become infected. When the animal becomes depressed and diarrhea begins, the villous epithelial cells in the lower part of the small intestine appear normal by light microscopy but contain a large quantity of viral antigen. It is postulated that the viral infection results in the redirection of cell function from absorption to virus production, causing digestive fluids and partially digested milk to accumulate in the intestinal lumen. As the infection proceeds, there is accelerated migration of infected epithelial cells up the villi; these cells are shed and replaced by cubodial (immature) epithelial cells. After the mature epithelial cells have been lost, the villi appear shortened with an irregular surface. During recovery, the villious epithelial cells gradually become columnar and villi become longer.
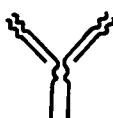
IMMUNE REACTION

In both calves and lambs circulating rotavirus antibody, particularly IgG, appears to have little effect in preventing rotavirus enteritis. Passive protection results from the presence of rotavirus immunoglobulin in the lumen of the intestinal tract (Snodgrass and Wells, 1978; Soulebot et al., 1981). Susceptible calves inoculated with an attenuated rotavirus are resistant to challenge inoculation 48 h after vaccination (Mebus et al., 1973). The mechanism of this protection has not been elucidated.

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LABORATORY DIAGNOSIS

Diagnosis of rotavirus enteritis requires the demonstration of rotavirus antigen or virions in diarrheic feces. Serology is of little value, for most lambs and calves fed colostrum have circulating antibodies.

Routine isolation of bovine or ovine rotaviruses was made possible following introduction of the MA-104 line of fetal rhesus monkey kidney cells and treatment of the virus with trypsin or pancreatin (Matsuno et al., 1977; Murakami et al., 1983a; Ojeh et al., 1984; Makabe et al., 1985). CPE was noted after prolonged incubation or after several blind passages in MA-104 cells. Even plaque-assays could be performed using MA-104 or BSC-1 cells in the presence of trypsin and DEAE-dextran in the overlay medium (Matsuno et al., 1977; Archambault et al., 1984). In infected cultures not showing a cytopathic effect, foci of immunofluorescence might be seen after fluorescent-antibody staining.

Isolation of rotaviruses in cell culture is not a rapid method; therefore, EM, IF, CF, immunoelectrophoresis, solid-phase radioimmunoassay and ELISA are used for diagnosis (Ellens, 1981). Since the viral titer in feces drops off quickly after onset of diarrhea, fecal material should be collected as soon as possible. At least 10 ml of diarrheic feces should be collected into an appropriate container directly from the animal. If possible, specimens should be collected from several calves. Specimens can be kept frozen until use.

In some laboratories a rapid diagnosis is made by PAGE of viral RNA extracted from infected fecal samples. A combination of PAGE and silver-staining is recommended; it can be applied following extraction from 0.01 ml of a fecal sample (Herring et al., 1982).

PROPHYLAXIS AND CONTROL

Circulating antibody does not protect against rotavirus infection (Snodgrass and Wells, 1978; Woode et al., 1975). Passive protection against disease results from the presence of rotavirus antibody from colostrum or milk in the lumen of the intestinal tract (Bridger and Woode, 1975; Snodgrass and Wells, 1978).

The frequent occurrence of rotavirus diarrhea in animals less than a week old is believed to be caused by the rapid fall of antibody titer in milk 3–7 days after parturition (Snodgrass et al., 1980). The administration of rotavirus vaccine to pregnant cows or ewes will increase and prolong the secretion of antibody in milk and decrease the incidence of diarrhea (Mebus et al., 1973). Two disadvantages of passive protection are: (1) animals must continuously be fed milk from an immunized dam, and (2) animals must become infected, hopefully subclinically, in order to develop active immunity.

Oral administration of attenuated rotavirus as soon as possible after birth has successfully reduced morbidity and mortality (Mebus et al., 1972). Efficacy of the oral vaccine is affected by the rotavirus antibody in the colostrum and the timing of vaccination in relation to the first feeding.

Theoretically, calf vaccination with an attenuated rotavirus is the desirable approach, for it induces an active immunization. The problem of colostral antibody neutralizing the vaccine virus can be overcome by placing the vaccine into the amniotic fluid at 7–8 months of gestation. The fetus ingests the virus and becomes immunized. Calves vaccinated in this manner possess circulating antibody when delivered by hysterotomy and are resistant to challenge inoculation at 1 day of age. Development of this method of vaccination may offer a novel and more consistent way of actively immunizing animals against enteric infections (Wyatt et al., 1979).

REFERENCES

Archambault, D., Roy, R.S., Dea, S. and Elazary, M.A.S.Y., 1984. Comparative study of bovine rotavirus isolates by plaque assay. Can. J. Comp. Med., 48: 286–289.

Bridger, J.C. and Woode, G.N., 1975. Neonatal calf diarrhea: Identification of a reovirus like agent (rotavirus) in feces by immunofluorescence and immune electron microscopy. Br. Vet. J., 131: 528–535.

Ellens, D.J., 1981. Diagnosis by enzyme-linked immunosorbent assay. Curr. Top. Vet. Med. Anim. Sci., 13: 22–32.

England, J.J., Frye, C.S. and Enright, E.A., 1976. Negative contrast electron microscopic diagnosis of viruses of neonatal calf diarrhea. Cornell Vet., 66: 172–182.

Eugster, A.K., Strother, J. and Hartfiel, D.A., 1978. Rotavirus (reovirus-like) infection of neonatal ruminants in a zoo nursery. J. Wildl. Dis., 14: 351–354.

Fernelius, A.L., Ritchie, A.E., Classick, L.B., Norman, J.O. and Mebus, C.A., 1972. Cell culture adaptation and propagation of a reovirus like agent of calf diarrhea from a field outbreak in Nebraska. Arch. Ges. Virusforsch., 37: 114–130.

Flewett, T.H., Bryden, A.S., Davies, H. and Morris, C.A., 1975. Epidemic viral enteritis in a long-stay children's ward. Lancet, 1: 4–5.

Hall, G.A., Bridger, J.C., Chandler, R.L. and Woode, G.N., 1976. Gnotobiotic piglets experimentally infected with neonatal calf diarrhea reovirus-like agent (rotavirus). Vet. Pathol., 13: 197–210.

Herring, A.J., Inglis, N.F., Ojeh, C.K., Snodgrass, D.R. and Menzies, J.D., 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamid gels. J. Clin. Microbiol., 16, 473–477.

House, J.A., 1978. Economic impact of rotavirus and other neonatal disease agents of animals. J. Am. Vet. Med. Assoc., 173: 573–576.

Makabe, T., Komaniwa, H., Kishi, Y., Yataya, K., Imagawa, H., Sato, K. and Inaba, Y., 1985. Isolation of ovine rotavirus in cell cultures. Arch. Virol., 83: 123–127.

Martel, J.L. and Perrin, B., 1981. Infectious aetiology of diarrhea in newborn calves. Prevalence in France of *E. coli* K99+ and rotavirus. Practical diagnosis techniques. Bull. Group Tech. Vet., 4: 45 54.

Matsuno, S., Inouye, S. and Kono, R., 1977. Plaque assay of neonatal calf diarrhea virus and the neutralizing antibody in human sera. J. Clin. Microbiol., 5, 1–4.

Mebus, C.A. and Newman, L.E., 1977. Scanning electron, light, and immunofluorescent microscopy of intestine of gnotobiotic calf infeted with reovirus-like agent. Am. J. Vet. Res., 38: 553–558.

Mebus, C.A., Stair, E.L., Underdahl, N.R. and Twiehaus, M.J., 1971. Pathology of neonatal calf diarrhea induced by a reo-like virus. Vet. Pathol., 8: 490–505.

Mebus, C.A., White, R.G., Stair, E.L., Rhodes, M.B. and Twiehaus, M.J., 1972. Neonatal calf diarrhea: Results of a field trial using a reo-like virus vaccine. Vet. Med. Small Anim. Clin., 67: 173–178.

Mebus, C.A., White, R.G., Bass, E.B. and Twiehaus, M.J., 1973. Immunity to neonatal calf diarrhea virus. J. Am. Vet. Med. Assoc., 163: 880–883.

Mebus, C.A., Wyatt, R.G. and Kapikian, A.Z., 1977. Intestinal lesions induced in gnotobiotic calves by the virus of human infantile gastroenteritis. Vet. Pathol., 14: 273–282.

Morin, M., Lariviere, S. and Lallier, R., 1976. Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhea. Can. J. Comp. Med., 40: 228–240.

Murakami, Y., Nishioka, N., Hashiguchi, Y. and Kuniyasu, C., 1983a. Primary isolation of cytopathic bovine rotaviruses on fetal rhesus monkey kidney cells. Vet. Microbiol., 8: 135–139.

Murakami, Y., Nishioka, N., Hashiguchi, Y. and Kuniyasu, C., 1983b. Serotypes of bovine rotaviruses distinguished by serum neutralization. Infect. Immun., 40: 851–855.

Ojeh, C.K., Snodgrass, D.R. and Herring, A.J., 1984. Evidence for serotypic variation among bovine rotaviruses. Arch. Virol., 79: 161–171.

Rodger, S.M., Bishop, F.R., Birch, C., McLean, B. and Holmes, I.H., 1981. Molecular epidemiology of human rotavirus in Melbourne, Australia, from 1973 to 1979, as determined by electrophoresis of genome ribonucleic acid. J. Clin. Microbiol., 13: 272–278.

Snodgrass, D.R. and Herring, J.A., 1977. The action of disinfectants on lamb rotavirus. Vet. Rec., 101: 81.

Snodgrass, D.R. and Wells, P.W., 1978. The immunoprophylaxis of rotavirus infections in lambs. Vet. Rec., 102: 146–148.

Snodgrass, D.R., Anguss, K.W. and Gray, E.W., 1977. Rotavirus infection in lambs: pathogenesis and pathology. Arch. Virol., 55: 263–274.

Snodgrass, D.R., Fahey, K.J., Wells, P.W., Campbell, I. and Whitelaw, A., 1980. Passive immunity in calf rotavirus infections: Maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. Infect. Immun., 28: 344–349.

Snodgrass, D.R., Herring, A.J., Campbell, I., Inglis, J.M. and Hargreaves, F.D., 1984. Comparison of atypical rotaviruses from calves, piglets, lambs and man. J. Gen. Virol., 65: 909–914.

Soulebot, J.P., Dauvergne, M., Brun, A. and Espinass, J., 1981. Current immunology information regarding intestinal rotavirus in calves. Journ. Vet. Fr. Egypt. Carire, Novembre, 9–11: 1–18.

Woode, G.N. and Bridger, J.C., 1975. Viral enteritis of calves. Vet. Rec., 96: 85–88.

Woode, G.N., Jones, J.M. and Bridger, J.C., 1975. Levels of colostral antibodies against neonatal calf diarrhea virus. Vet. Rec., 97: 148–149.

Wyatt, R.G., Mebus, C.A., Yolken, R.H., Kalica, A.R., James, H.D., Kapikian, A.Z. and Chanock, R.M., 1979. Rotaviral immunity in gnotobiotic calves: Heterologous resistance to human induced by bovine virus. Science, 203: 548–550.

Togaviridae

Togaviruses owe their name to the envelope (Latin toga = cloak) which surrounds the isometric nucleocapsid core. The Togaviridae family at present consists of the four genera *Alphavirus*, *Rubivirus*, *Pestivirus* and *Arterivirus*; the flaviviruses have recently been assigned an independent family status. There are some 25 members in the *Alphavirus* genus, whereas the *Rubivirus* and *Arterivirus* genera contain one virus only (rubella virus and equine arteritis virus, respectively). The *Pestivirus* genus is represented by mucosal disease/bovine diarrhea virus, the very closely related border disease virus of sheep and hog cholera virus.

Togaviruses are small enveloped animal RNA viruses (40–70 nm). The envelope tightly surrounds a spherical nucleocapsid 25–37 nm in diameter whose icosahedral symmetry has been proven only for alphaviruses. Surface projections are demonstrable in most togaviruses.

The nucleic acid is a single molecule of positive-sense, single-stranded RNA with a mol. wt. of about 4×10^6. Genomic nucleotide sequence data of alphaviruses and recently also of pestiviruses have been obtained. The virion contains three or four polypeptides, one or more of which are glycosylated.

The virion buoyant density is about $1.25 \, g/cm^3$ in CsCl and varies between 1.13 and $1.24 \, g/cm^3$ in sucrose. Sedimentation coefficients between 150 (pestiviruses) and 300 S have been reported.

Togaviruses replicate in the cytoplasm and mature by budding of either pre-assembled (alphaviruses) or assembling nucleocapsids through the plasma membrane. During the replication of alphaviruses and rubiviruses a subgenomic 26 S RNA is synthesized which contains the information for the virion structural proteins. Arteriviruses produce five subgenomic mRNAs as a nested set.

With recent data on the genomic organization and transcription strategies acumulating, inclusion of the pestiviruses and arteriviruses into the Togaviridae family becomes untenable. In contrast to alphaviruses, pestiviruses lack polyadenylation of the genomic RNA as well as a subgenomic mRNA species, and have their virion protein coding sequences near the 5′ end of the genome. From these data, it would appear that pestiviruses are more related to flaviviruses than to togaviruses. The coronavirus-like transcription strategy of arteriviruses should be a reason to eliminate them from their present taxonomic cluster.

In ruminants, the non-arthropod-borne togaviruses are bovine viral diarrhea virus (BVDV) and border disease virus (BDV). It appears as if BDV is a BVDV adapted to sheep; both are antigenically closely related to hog cholera (swine fever) virus. These three viruses from the genus *Pestivirus*.

BVDV is the causative agent of bovine viral diarrhea (BVD) and mucosal disease (MD) but may also participate in the etiology of acute respiratory/enteric disease in calves. Both BVDV and BDV are teratogenic and show a trend to viral persistence. The teratogenicity of BVDV and BDV is expressed by congenital conditions of the newborn, first described in lambs from the borders of Wales. Later it became evident that intrauterine infection mostly

after transplacental transfer may result in "late onset" disease of cattle, with the majority aged 6 months to 2 years. In a few cases BVDV may persist for life without obvious clinical signs. A late onset MD-like syndrome has also been described in sheep recovered from clinical border disease .

FLAVIVIRIDAE

Yellow fever virus (Latin flavus = yellow) is the type species of this recently established monogeneric family. Most of the 50 viruses are either mosquito-borne or tick-borne, but there are also flaviviruses without arthropod transmission.

The enveloped spherical virions measure 40–50 nm in diameter and consist of a core particle surrounded by a projection-bearing membrane. Fuzzy surface projections have been visualized.

The virus particle contains a single molecule of a single-stranded RNA with positive polarity and a mol. wt. of 4–4.6 $\times$ 10^6. The nucleocapsid protein (mol. wt. 14 k) and a small additional polypeptide (mol. wt. 8 k) as well as one glycosylated envelope protein (mol. wt. 53–63 k) constitute the structural polypeptides.

The buoyant density of flaviviruses is 1.22–1.24 g/cm^3 in CsCl and 1.19 g/cm^3 in sucrose; sedimentation coefficients of around 200 S have been determined.

Flaviviruses replicate in the cytoplasm; maturation is presumed to occur by budding through intracytoplasmic membranes (mostly endoplasmic reticulum) but has only incidentally been visualized. Subgenomic mRNAs do not occur, and posttranslational processing of one large precursor molecule is the mechanism involved.

The International Catalogue of Arboviruses 1985, published by the American Society of Tropical Medicine and Hygiene, San Antonio, TX, can be recommended as a source of detailed information on alpha- and flaviviruses; tables giving the numbers of viruses from different taxonomic clusters isolated from naturally infected vertebrates are especially valuable for the epidemiologist.

There are two flaviviruses which have recognized associations with ruminants: the louping-ill and the Wesselsbron disease virus. Louping-ill virus is transmitted by ticks and can cause disease in most categories of domestic animals as well as man but is most frequently associated with sheep and is thought to be unique to the British Isles. Wesselsbron disease virus (WBV) is transmitted by *Aedes* mosquitos and causes abortion and death of young lambs in South Africa.

Chapter 23

Bovine Viral Diarrhea Virus

B. LIESS

VIRUS PROPERTIES

Physical and chemical characteristics

The lipid-containing membrane enveloping the nucleocapsid of BVDV accounts for a low buoyant density of the virion; it is easily broken by organic solvents such as ether and chloroform and infectivity is thus readily inactivated (Hermodsson and Dinter, 1962; Dinter, 1963). Considerable decrease of infectivity is achieved by treatment of BVDV suspensions with trypsin (0.5 mg/ml, 37°C, 60 min). The effect of pH in the range between 5.7 and 9.3 revealed relative stability, but outside this range the infectivity decreased rapidly (Hafez and Liess, 1972b).

The lack of information on inactivation of BVDV by various agents and disinfectants necessitates comparison within the genus *Pestivirus*. The effects of physical and chemical treatment on hog cholera virus (HCV) might also apply to BVDV (Liess, 1981).

Antigenic properties

BVDV applied by natural routes (oral, intranasal) induces antibodies upon multiplication in the natural host (bovine, small ruminants, pigs). Such immune sera can neutralize BVDV as shown by experiments in calves (Baker et al., 1954) and other host systems, e.g. cell cultures of bovine origin (Noice and Schipper, 1959; Gillespie et al., 1960). Neutralizing antibodies specifically bind to surface antigens of the BVD virion. Antibodies develop also against internal antigen(s) of the viral particle, as has been demonstrated by other serological methods. However, these methods (e.g. agargel precipitation) are less suitable for the identification of BVDV strains and their differentiation from the closely related HCV (Darbyshire, 1962). Work on monoclonal antibodies has lead to the identification of viral proteins that are type- or strain-specific. Within the nonarbo togavirus group, no cross-reaction occurs between rubella virus, equine arteritis virus, lactic dehydrogenase virus and the pestiviruses (Horzinek, 1981). The only serologically related viruses are HCV and BVDV. This relationship is expressed not only by common soluble antigens detected by, for example, agar double diffusion test (Gutekunst and Malmquist, 1963), but also by the presence of neutralizing antibodies against BVDV in pig sera (Snowdon and French, 1968).

Therefore it has been questioned whether HCV, BVDV and the closely related virus found in cases of Border Disease (BD) in sheep are separate viruses or not (Horzinek, 1981). In this concept HCV and BVDV would be
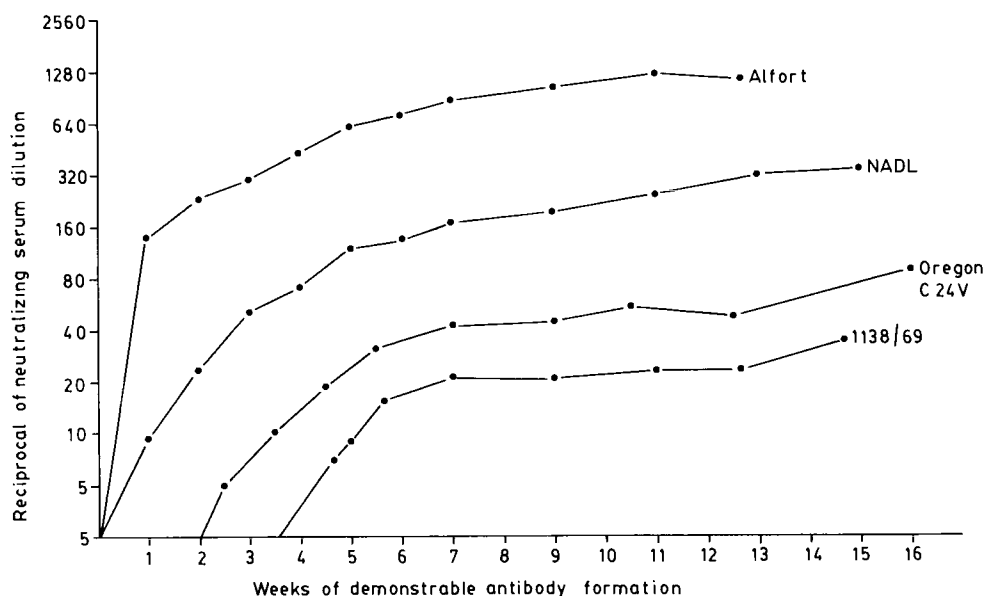
Fig. 100. Means of heterologous neutralizing antibody titers against HCV strain Alfort and BVDV strains NADL, Oregon C24V and A1138/69 after intranasal inoculation of six pigs with HCV strain GLENTORF.

separate "species" due to subgroup-specific antigens that are located on the membrane of the virions. But even neutralization would not differentiate between the "species" unless homologous and heterologous titers are compared. The quantitative differences have their basis in the existence of either (i) a common and a type-specific antigen in the membrane, or (ii) a variable type-specific antigen, which would also explain the antigenic variation amongst strains of BVDV. The latter has been well established, mainly by neutralization using immune sera prepared against different BVDV strains in calves (Gillespie et al., 1961; Castrucci et al., 1975). In order to exclude individual differences, groups of pigs have been inoculated with low virulent strains of HCV and mean antibody titers plotted against heterologous HCV as well as BVDV strains. Distinct profiles have been obtained, reflecting the antigenic variation of BVDV and the relationship amongst the strains tested (Fig. 100).

EPIZOOTIOLOGY

The host range for BVDV seems to be restricted to domestic and wild ruminants and pigs. These species can be infected in the field as judged from the detection of antibodies specific for BVDV reported by many investigators. Adaptation of BVDV by serial passages to rabbits has been reported, but this species must not be regarded as a natural host (Baker et al., 1954).

No reservoir hosts are known except persistently infected cattle; calves are infected in utero by transplacental virus transmission (Malmquist, 1968).

Vectors amongst invertebrates have not been reported; however, resemblance of BVDV to the "arbo" togaviruses demands attention.

The lack of antibody and the absence of clinical symptoms indicate that BVDV is not transmitted to man; a justified conclusion, considering the wide distribution of BVDV in the cattle population.

BVDV is widespread not only in North America and Europe but has a global distribution. It is therefore astonishing that there are apparently countries

where BVDV does not occur, as judged from the absence of neutralizing antibodies in the cattle population. This was reported from China where most cattle are kept in small holdings (J. Chen, personal communication, 1982).

Serological surveys revealed a high prevalence of neutralizing antibodies in the cattle population of many countries; an intensive cattle industry with frequent movements of animals within the country as well as imports favor the spread of BVDV. The incidence of BVDV infections with clinical disease or with a lethal course confirmed by laboratory investigation seems to be low as compared with the figures obtained by serosurveys. However, without intensive recording, notification of disease and detailed information on diagnostic methods, the economic consequences of BVD can hardly be estimated.

Spread of BVDV, its pattern and speed cannot be properly determined; in most cases it will occur unnoticed. Over 90% of the infections are inapparent.

PATHOGENESIS

Under field conditions BVDV enters the host organism by the oral route or, depending on the circumstances, by the nasal route. In tonsils and lymphoid tissues lining the oropharynx it finds cells for primary multiplication, particularly the epithelial cells in the crypts of tonsils, where viral antigen can be demonstrated by IF. BVDV or virus-infected cells (debris) may be taken up by phagocytic cells and transported to the draining lymphoid tissues (Bielefeldt Ohmann, 1983).

Virus could be detected in the blood 2-4 days after oral and nasal BVDV inoculation of calves (Mills and Luginbuhl, 1968; Rohde and Liess, 1970). Infectivity was mainly found in leukocytes (buffy coat) for up to 11 days after onset of demonstrable antibody formation. This suggests that the virus is associated with leukocytes in such a way that it is not accessible for neutralizing antibodies. In blood plasma infectivity disappeared as soon as neutralizing antibodies could be detected.

Further spread of the virus in the organism from the site of primary multiplication occurs through viremia, which eventually ceases so that the infected organism seems to have eliminated the virus. Before that, BVDV is excreted via secretions and excretions, with large quantities of virus found in saliva, nasal discharge and, to a lesser extent, in urine and feces (if at all, in the absence of diarrhea).

Only in rare experimental cases did BVDV infections run a more serious or even fatal course (Baker et al., 1954) allowing investigation of organ manifestations after viremia. The more or less severe clinical symptoms and pathological changes relate to the terms that were introduced when the condition was first described: Bovine Virus Diarrhea (Olafson et al., 1946; Olafson and Rickard, 1947) and Mucosal Disease (Ramsey and Chivers, 1953).

The epizootiological impact of viral latency and persistence are of great interest. While BVDV acks properties that would allow the genome to become integrated into the cell genome, persistence in BVDV infections is well established. It plays an important role not only in the epizootiology but also in the pathogenesis of the disease. Diagnostic procedure as well as control strategies that include immune prophylaxis should take persistence into account.

Recovery of BVDV from lungs and bronchial lymphnodes for up to 56 days p.i. (intranasal virus application by atomizer) as reported by Mills and Luginbuhl (1968) appears insufficient to claim viral persistence. In the present context it is meant that BVDV persists lifelong (e.g. in buffy coat cells) without causing clinical signs of BVD. This state was attributed to specific immune tolerance by Liess (1973) and Coria and McClurkin (1978). Specific unrespon-

siveness of the humoral immune system to BVDV in a highly contaminated milieu was suggested by Holling (1971). Johnson and Muscoplat (1973) investigated immunologic abnormalities in calves with the chronic BVD syndrome and offered two theories for the unresponsiveness of lymphocytes to phytohemagglutinin stimulation as well as a deficient humoral response: (i) immunosuppression as an effect of the disease, and (ii) immunologic unresponsiveness as a factor in the cause of the disease. Neither of these theories could be verified by the available data.

Shope et al. (1976) studied the role of cellular immunity and passive antibody in protecting neonatal calves against primary BVDV infection using administration of corticosteroids (0.5 mg/kg/day for 10 days). They observed that after intratracheal infection calves without antibody died while others with high levels of passively transferred antibodies, under otherwise similar conditions, did not. In contrast, administration of 0.5–1 mg/kg/day starting 3 days before oral and nasal inoculation of a field strain of BVDV resulted in only mild clinical symptoms (Rohde and Liess, 1970). Shope et al. (1976) concluded that "severe fatal viremia in immunosuppressed calves apparently resulted in more extensive dissemination of BVD virus to target organs, particularly lymphoid tissue".

Once BVD viral persistence was considered a precondition for the development of severe clinical disease, total protein and immunoglobulins in serum of persistently infected animals became of interest. The fact that immunoglobulins G_1, G_2 and M could not be detected in precolostral sera of eleven persistently BVD-infected calves was attributed to insensitivity of the test and to complexing of Ig with BVDV, since virus was isolated from all sera (Coria et al., 1983). Using a more sensitive test procedure, Coulibaly (1984) was able to demonstrate a significantly decreased IgG_2 concentration in sera of 20 healthy, persistently BVD-infected cattle in the range of 7 months to 7 years of age. This confirmed results obtained by Steck et al. (1980) from cattle fatally affected by the BVD–MD syndrome. Depletion of circulating B and T lymphocytes — the latter in particular — (Bolin et al., 1985a), as well as a depressed response to mitogen stimulation of B and T lymphocytes (Muscoplat et al., 1973) reflects the influence of BVDV on the immune system. Whether immunosuppression plays a role in the fatal outcome of BVDV infections still needs to be shown. Corticosteroid treatment may have a suppressive effect on local antibody formation only. It appears somewhat unlikely that neutralizing antibody controls viremia, as suggested by Shope et al. (1976), since BVDV infectivity is intimately associated with leukocytes in the peripheral blood and persisted up to 40 days p.i. (Mills and Luginbuhl, 1968); in the presence of neutralizing antibodies virus could be recovered for at least 10 days (Rohde and Liess, 1970).

A factor to be taken into account in explaining the pathogenetic mechanism by which BVDV infections can run a severe course is that fatally infected cattle frequently do not produce detectable antibodies against BVDV. It has been presumed that this phenomenon is due to immunologic tolerance as a consequence of antigenic experience at or before birth (Malmquist, 1968). Although after exposure to low-passage BVDV some neonatal calves died (Liess, 1967; Lambert et al., 1969), the cause of fatal BVDV infections and the role of immunologic tolerance remained obscure. Nevertheless, cases of fatal BVDV infections have to be expected among the seronegative animals in herds where a high percentage of heifers and cows is seropositive to BVDV. On the other hand, selection of seronegative animals in a highly contaminated environment offers the chance of finding persistently infected animals that will die sooner or later (Liess et al., 1974). This hypothesis resulted in the detection of persistently BVDV-infected calves, heifers, young bulls and pregnant cows

which were followed up for longer periods of time (McClurkin et al., 1979; Liess et al., 1983). Most of these persistently infected animals were probably exposed to BVDV during intrauterine life; Bürki and Germann (1964) and Romvary (1965) had reported BVDV infections in newborn and day-old calves.

The outcome of transplacental virus transfer to the bovine fetus depends on the gestational stage of the seronegative dam. The earliest date of maternal infection at which neutralizing antibody formation in the fetus was stimulated appeared to range between 79 (Scott et al., 1973) and 93 days of gestation (Kendrick, 1971). The length of period for the transplacental transfer is unknown as yet but probably ranges from 10 days onwards. Transplacental infection also interferes with fertilization, this being the principal manifestation of the adverse effects of BVDV infection (Grahn et al., 1984).

As a result of investigations on the ontogenesis of the bovine immune system, Schultz (1973a, b) concluded that the bovine fetus develops immunologic responsiveness to BVDV antigen between the 90th and 120th day of gestation. Attempts to isolate BVDV from fetuses after they had produced neutralizing antibodies do not promise to be successful (Kahrs, 1973; Done et al., 1980; Orban et al., 1983).

For the establishment of persistent viremia as shown by lifelong detection of BVDV in buffy coat cells (Coria and McClurkin, 1978), transplacental transfer of BVDV within the first trimester of gestation is most important. Only recently could it be shown that seronegative cows, 50–120 days pregnant when vaccinated with a commercial BVD modified live virus vaccine, delivered viremic calves — some of them persistently viremic. After some months these animals showed growth retardation and runting, or they remained healthy for up to 2 years (Fig. 101); at the same time they were shedding (vaccine?) virus by various routes (Liess et al., 1984). None of the persistently infected calves showed precolostral neutralizing antibodies to the infecting virus.

The finding that live modified BVDV vaccines and BVDV-contaminated vaccines (Lohr et al., 1983) can establish persistent infections must also be considered in the light of reports on the detection of BVDV in commercial fetal calf sera used in cell culture (Kniazeff et al., 1967; Malmquist, 1968; King and Harkness, 1975; Nuttall et al., 1977; B. Liess et al., unpublished data, 1981).

Experimentally, cattle with a persistent noncytopathogenic (NC) BVDV viremia may develop MD after infection with a cytopathogenic (C) strain of BVDV (Brownlie et al., 1984; Bolin et al., 1985b, c). MD may also be a consequence of a mutation of the persistently infecting NC-BVDV to a C-BVDV (Brownlie et al., 1986; Howard et al., 1987). The latter hypothesis is supported by results of cross-neutralization tests. A C- and a NC-strain, being a pair of strains derived from a certain case of MD, were indistinguishable from each other but different from pairs of strains isolated from other cases of MD (Howard et al., 1987).

Larsson (1987) studied the activities of mononuclear cells (MNC) in peripheral blood during MD and persistent infection with BVDV. Cattle with MD commonly showed normal MNC counts but with an altered composition of subpopulations. The mean proportion of Ig-bearing cells (B cells) was increased, while cells being neither Ig^+ nor attaching the T cell marker *Helix pomatia* A hemagglutinin (HP) were decreased. The proportion of mononuclear phagocytes and their phagocytic capacity varied within the normal range. Persistently infected healthy cattle did not show the altered composition of MNC as seen in cattle with MD. During MD and subclinical persistent infection, the proliferative response of MNC to mitogens was suppressed, which was partly due to an increased suppressor activity of cells with receptors for the Fc part of IgG (Fc_γ^+ cells), but not by an increased number of
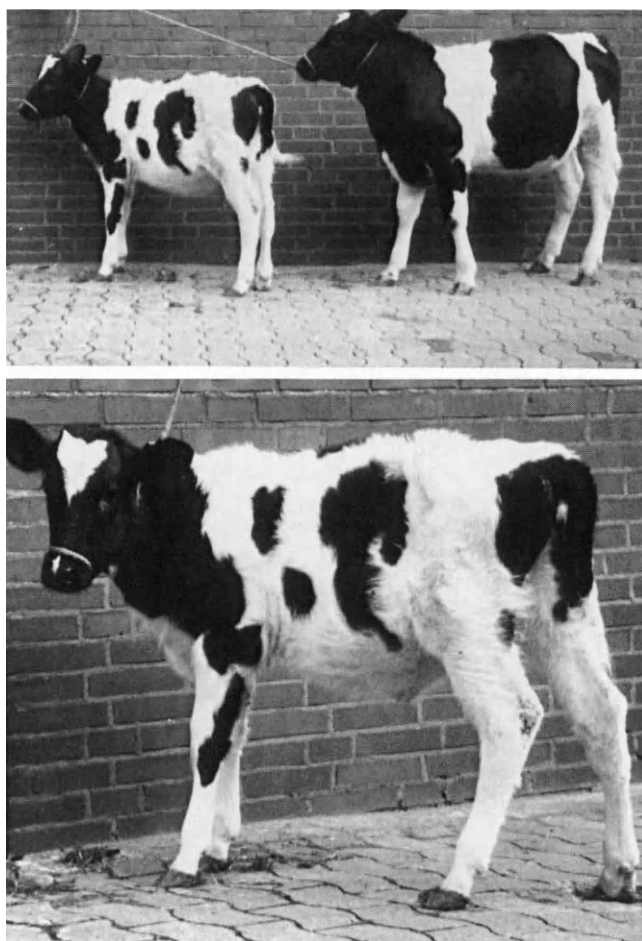
Fig. 101. (Top) Bull calf 4 months old (right) and female calf 5½ months old (left) persistently infected with BVDV following vaccination of their dams with commercial attenuated live BVDV vaccine on gestational days 76 (right) and 118 (left); note unthriftiness of female calf compared with 1½ months younger and normal appearing bull calf.

(Bottom) Female calf (see top) exhibiting chronic disease signs of persistently BVDV infected animals.

these cells. Almost 70% of te Fc_γ^+ cells attached to HP, indicating that these cells with suppressor activity are a subset of T cells.

DISEASE SIGNS

Olafson and coworkers (Olafson et al., 1946; Olafson and Rickard, 1947) described "Bovine Virus Diarrhea" as a febrile disease characterized by leukopenia, salivation, nasal discharge, profuse scouring, coughing, depression, anorexia and development of ulcers. Mild cases with no clinical symptoms detectable were called "sleeper" or subclinical cases; these animals were observed to abort 10 days to 3 months later.

Transmission experiments by feeding animals fecal material, subcutaneous injection of blood from febrile animals and other modes of inoculation resulted in variable symptoms and courses of disease, comparable to observations in naturally infected animals. Only occasionally did experimental animals die after infection with BVDV. The first reports by Olafson and coworkers described most of the clinical lesions which were later confirmed by other investigators. Unfortunately this has led to the introduction of new terms for the

same infection, notably "Mucosal Disease" (Ramsey and Chivers, 1953), and has contributed to the confusion which arose over clinically similar but etiologically different diseases. The situation became even more complicated when a group of syndromes, some of which were later recognized as being etiologically different from BVD, was referred to as "mucosal diseases of cattle" (FMD, rinderpest, IBR, etc.). Consequently, names like "mucosal disease complex" (Malmquist, 1968) or "a mucosal disease" (Ramsey, 1956; Huck, 1957) should be avoided.

Because of a "marked resemblance of mucosal disease to rinderpest", Ramsey (1956) considered the possibility that an "incorrect diagnosis of mucosal disease could be made in the early stages of an outbreak of rinderpest with potentially serious consequences"; earlier, Walker and Olafson (1947) had shown that cattle reconvalescent of BVD were not immune to rinderpest and had no detectable antibodies to rinderpest virus.

It is now accepted that BVD and MD represent two different clinical pictures with a common etiologic agent (Gillespie et al., 1961), and that MD can be considered as a late onset, immunologically mediated condition that can occur in cattle persistently infected with BVDV (Liess, 1973; Roeder, 1982) and displays criteria of a "slow virus infection" (Porter, 1971; Liess et al., 1974).

Apart from BVD initially described as an acute gastroenteritis with severe diarrhea (Olafson et al., 1946) and MD reported to occur with low morbidity and high lethality, a third course was identified: chronic infections of several weeks' duration in calves up to 1 year of age (Johnson and Muscoplat, 1973). This chronic syndrome was characterized by interdigital hyperkeratosis and ulceration, diarrhea, dehydration, weakness and failure to thrive.

Malmquist (1968) had mentioned that calves with MD had persistent viremia and failed to develop neutralizing antibodies over extended periods, the longest being approximately 4 months. The animals remained unthrifty, but it was not the "slow-gainer or poor-doer that became sick, because frequently it had been sick for some time". Coria and McClurkin (1978) related unthriftiness to congenital infection with BVDV; calves surviving for more than a few months were usually persistently infected and chronically ill.

All these reports compare with details on "chronic outbreaks" with prolonged coursed of up to several months, which however, mainly affected adult animals (Huck, 1957).

Finally, mild outbreaks in herds characterized by subclinical or transient symptoms have been reported by Huck (1957); mild courses in individual animals are known since the first report by Olafson et al. (1946) as cases without prominent symptoms ("sleeper" or subclinical cases). This is in agreement with transmission experiments to calves, which in general did not reproduce the complete clinical picture of the disease (Malmquist, 1968). Cases where calves died from enteritis within 24–48 h after birth must be seen in the light of present knowledge on viruses causing neonatal diarrhea (e.g. rotaviruses, coronaviruses); they are rare events in BVDV infections, except in those established in utero.

From the literature it is obvious that transmission experiments so far failed to fulfill the second Henle–Koch postulate, which requires that the virus material used for inoculation is not contaminated with other viruses (Liess, 1967). Usually BVDV serially passaged in bovine cell cultures served as an inoculum; reports on any selection procedure (e.g. limiting dilution, plaque isolation) are lacking. This point needs consideration in view of the frequent occurrence of NC-BVDV in bovine material. Primary bovine cell cultures and fetal calf sera used for the propagation of the BVDV isolate must be checked before use, e.g. by fluorescent antibody or interference tests

PATHOLOGY

The macroscopic lesions first described by Olafson and coworkers (Olafson et al., 1946; Olafson and Rickard, 1947) varied considerably according to the course of the infection (severe acute, mild, chronic). In severe cases extensive ulceration or diffuse necrosis of the mucous membranes of the upper digestive and respiratory tract occurred. Occasionally calves died without developing ulcers, or only very few were present in the stomach mucosa. Reddening, petechiae and hemorrhages in the omasum, small intestine and cecum appeared variably, as did hemorrhages in subcutaneous tissue, epicardium and the vaginal mucosa.

Ramsey (1956) described the variation in gross lesions noted in one affected herd with so-called mucosal disease. Erosive, ulcerative and cystic lesions were confined to the epithelia and the mucosa of the alimentary canal. Hyperemia and hemorrhages were not common findings. In the oral cavity necrosis of the epithelium produced lesions irregular in outline. Loss of the necrotic surface epithelium left erosions and ulcers with a hyperemic base. Necrosis and erosions appeared at varying degrees in other locations, e.g. esophagus, forestomach and abomasum. Severe catarrhal enteritis was noticed mainly in the jejunum, but inflammation in all other parts of the intestine wsa common.

Histopathologic examination revealed thrombosis of vessels in the submucosal areas of ulceration. Small arterioles of the abomasum and gut were described containing hyaline emboli. Other lesions of the circulatory system consisted of increased capillary permeability and diffuse periarteritis as shown by circumferential necrosis with leukocytic infiltration. In lymph nodes, marked decrease in mononuclear cells exposed the basal tissue of the lymphoid organ. Marked decrease of lymphocytes was also found in the spleen, indicating lymphoid exhaustion (Carlson et al., 1957; Tyler and Ramsey, 1965). Liver, pancreas and salivary gland generally appeared normal (Ramsey, 1963).

In the respiratory system diffuse lesions consisted of petechial and ecchymotic hemorrhages with no evidence of inflammation in the subvisceral pleura and tracheal mucosa. However, trachea and lungs appeared normal in most animals (Ramsey, 1956), and edema of larynx and trachea occurred in only 10% of the cases (Carlson et al., 1957). Epithelial hyaline droplets in the renal glomeruli and tubules were common findings (Ramsey, 1956).

While gross and microscopic alterations of the central nervous system were not found by Ramsey (1956; 1963), encephalitis was reported by others (Carlson et al., 1957; Khater et al., 1964).

Lesions in the skin consist of focal proliferative dermatitis (Ramsey, 1956); they are noted in protracted cases only (Ramsey, 1963).

According to Carlson et al. (1957), gross and microscopic changes are most prominent in animals with subacute, chronic and recovering cases and least prominent in early and experimental cases. In MD, pathological changes were described as much more severe than in BVD. They consist mainly of erosions, ulcerations and destruction of lymphatic tissue in the alimentary tract and are similar to those in BVD in distribution and character. In a comparative study on pathologic responses to selected isolates from cases of BVD and MD, Tyler and Ramsey (1965) concluded that it was impossible to clarify the variability of syndromes seen in the field. The experimental syndrome was always mild and resembled virus diarrhea rather than MD.

The respiratory tract is the portal of entry of BVDV. Bielefeldt Ohmann (1983) concluded that BVDV initially replicates in the epithelia of tonsils and bronchioli from where virus and infected cells are transported to the draining tissues by phagocytic cells. Involvement of blood vessels is accompanied by degenerative changes. However, according to Bielefeldt Ohmann (1983) BVDV-

antigen-containing migrating mononuclear cells were not related to degenerative changes in the vicinity. Cutlip et al. (1980), investigating clinically healthy cattle persistently infected with BVDV, found viral antigen in endothelial cells by direct IF. Some blood vessels in the brains were cuffed with mononuclear cells.

The beginning of the lesions is in the submucosa, which shows degenerative changes of the basal layers without surface necrosis, although often the necrosis extends to the deeper layers of the epithelium. The degenerative process is characterized by vacuolization of the cytoplasm mainly of the stratum spinosum with the initial disappearance of intercellular bridges. Cell enlargement due to increased cytoplasmic volume, pycnosis of the nuclei and formation of small vesicles leads to detachment of the surface epithelium leaving erosions (Ramsey, 1956; Hansen et al., 1962). According to Seibold (1956) circulatory failure in the lamina propria of the gastrointestinal mucosa results in hyperemia and hemorrhages (petechiae and ecchymoses).

BVDV is known to cross the placenta, giving rise to abortion and antibody formation in the fetus (Romvary, 1965; Kahrs, 1968). Pathologic changes can be initiated by experimental intrauterine infection at any time of fetal development. After intravenous inoculation of eleven cows 150–217 days pregnant, only three neonatal calves showed signs of disease. The newborn to a cow inoculated when the fetus was 150 days of age was ataxic, blind and had a low presuckle titer of neutralizing antibodies to BVDV as compared with all the other calves. In the two other calves weakness, buccal erythema and erosions were reported, while the majority of calves appeared normal (Ward et al., 1969).

While BVDV has apparently a mild or no pathological effect on older fetuses, more severe reactions can be expected to occur in embryos or young fetuses after natural infection; there should be an age below which the virus kills the conceptus.

Nine cows inoculated with BVDV during the first trimester of pregnancy delivered three normal calves, one calf with partial alopecia, one mummified fetus, three abortions and one stillbirth (Kendrick, 1971). In another study the fetus, amniotic vesicle or placentome in 26 cows between 51 and 256 days of gestation were inoculated with BVDV irrespective of the presence of neutralizing antibody. Again severity of lesions decreased as the age of the fetuses increased (Casaro et al., 1971). Congenital anomalies consisted of inflammation with moderate necrosis of the bronchial epithelium, nonsuppurative dermatitis with necrosis of the basal cells of the epidermis, and nonsuppurative meningitis with destruction of the external granular layer of the cerebellum. These lesions occurred in the period of organogenesis.

Lesions resulting from maternal infection during the first trimester are represented mainly by malformations and degeneration of the cerebellum and eyes (cataract, retinal degeneration) while later the fetuses acquire the ability to react with inflammations (Scott et al., 1973). Inoculation of cattle on gestation day 100 or later with field strains of BVDV resulted in intrauterine death (with or without abortion), mummification, growth retardation (with or without malformations) and/or dysmyelination of the central nervous system (Done et al., 1980; Bielefeldt Ohmann, 1984; Trautwein et al., 1986).
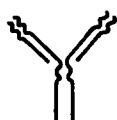
IMMUNE REACTION

BVDV usually produces lifelong immunity (Kahrs, 1968); the immunologic basis for this phenomenon is unknown. It can be stated that the presence of neutralizing antibody in blood serum reflects immunity (Robson et al., 1960). Immune competent cattle actively immunized following field infections or

application of BVD live viral vaccines can be considered as immune beyond the life expectation of a dairy cow (Kahrs et al., 1966).

Since transplacental transmission of BVDV occurs frequently, a review on the immune reaction to BVDV must include the fetus. The stage when the bovine fetus acquires immune competence to BVDV has been estimated by Casaro et al. (1971) to be around day 180 of gestation.

Brown et al. (1979) studied the immune reactions in 24 fetuses at a gestational age of about 150 days and quantitated the serum immunoglobulins between 4 and 140 days after intravenous inoculation of serologically negative pregnant heifers. IgM appeared first in the fetus 14 days after inoculation of the dam, followed by IgG_1 and IgG_2. BVDV-specific antibodies were not detected in the fetus before 56 days after maternal inoculation. It was assumed that immunoglobulin production had occurred in response to the infection of the fetus, since maternal immunoglobulins normally do not cross the placenta.

More recently, Bielefeldt Ohmann et al. (1982) inoculated four 120–165-day-old fetuses with BVDV in utero. Three weeks later, after caesarian section, they were tested for immunoglobulins and neutralizing antibodies against the infecting BVDV strain and another laboratory strain. Again all fetuses responded with marked IgM and IgG production; immunoglobulin was absent from abbattoir fetuses of the same age. Appearance of IgA was related to the development of Peyer's patches and the presence of plasma cells in the intestinal submucosa — a major source of IgA in ruminants. Two fetuses showed neutralizing antibodies against the homologous strain, but none against the heterologous strain. BVDV antigen was detected in the fetal tissues. These two fetuses represent the youngest individuals so far reported of having mounted a specific response to experimental BVDV infection within 3 weeks; other investigators reported 20–30 days (Casaro et al., 1971; Schultz, 1973a, b).

As outlined above, viral persistence plays a major role in BVD. It may be the result of specific immune tolerance and is apparently related to the development of chronic BVD and MD. Steck et al. (1980) studied cattle fatally infected with BVDV; in 24 out of 25 cattle they found a significant reduction of IgG_2 levels while the other immunoglobulins were normal; the same observation was made in clinically healthy cattle persistently infected with BVDV (Coulibaly, 1984).

According to Steck et al. (1980) the sera were devoid of BVDV antibodies, but neutralized other viruses to the same extent and degree as sera of healthy cattle when reacted with rotavirus, PI3 or bovine parvovirus.

A lack of IgG_2 had been observed by Nansen (1972) with high frequency among animals affected by pyogenic bacteria and was believed to be associated with a reduced resistence to infections.

Immune-competent susceptible cattle respond to BVDV with the production of usually high titering neutralizing antibodies which are highly specific as to the antigenic variant strain used. This has been convincingly shown by Steck et al. (1980) in vaccination experiments using viremic animals. They were sero-negative to the homologous isolate as well as to the vaccine strain before vaccination and remained so when tested against the homologous BVDV strain but developed high neutralizing titers against the vaccine strain. Recently this finding has been confirmed when testing persistently viremic cattle in the field (Liess et al., 1983). However, immune-competent animals infected with one strain are obviously immune to heterologous strains and also develop neutralizing antibodies to these strains. Variant strains are differentiated by measuring neutralizing antibody titers, which show higher values against the homologous than against heterologous strains. Each strain is recognized specifically by immune-competent cattle and pigs as shown in Fig. 100 (Malmquist, 1968; Castrucci et al., 1974; Liess et al., 1977). In summary, the BVDV strain

which induces immune tolerance will not be recognized immunologically even after the fetal organism has acquired immune competence, while other strains are recognized resulting in strain specific antibody formation.

Calves born to infected dams take up maternal BVDV antibodies by ingesting colostrum. Within 24–48 h all calves in a naturally infected herd possess antibodies. The serum titers decline exponentially to undetectable levels between 105 and 230 days, depending on the level of the initial titers (Malmquist, 1968; Kendrick and Franti, 1974). For high, medium, and low initial values Kendrick and Franti (1974) expected the titer to approach zero in approximately 230, 165, and 105 days, respectively. The half-life of neutralizing antibodies in sera of colostrum-fed calves was calculated to be 19–20 days (Landelius, 1977).

CMI responses have been studied by only a few investigators, although the significance of small lymphocyte depletion for the defense mechanism in relation to secondary bacterial or viral infections was emphasized by Malmquist (1968). Such studies were hampered by the impossibility of consistently producing clinical cases of MD. Therefore Steck et al. (1980) selected cattle fatally infected with BVDV for their studies on in vitro responsiveness of lymphocytes to stimulation by phytohemagglutinin or Concanavalin A. Differences between sick animals and controls were not seen, except that the sera from animals in later stages of fatal BVD–MD showed a suppressive effect on the stimulation. Johnson and Muscoplat (1973) had used lymphocytes from calves with chronic BVDV infection and reported inhibition of the mitogenic effect of phytohemagglutinin as compared with lymphocyte responses in healthy control calves.

In vitro studies on the stimulatory effect of pokeweed mitogen on bovine splenic lymphoid cells infected with BVDV supported the hypothesis that BVDV can alter B-cell responses, plasma cell development and subsequent synthesis of IgG and IgM (Atlurn et al., 1979). However, these observations do not explain the development of severe, fatal courses of BVDV infections. The decrease of polymorphonuclear leukocytes and the impairment of their function may, in part, explain the increased susceptibility of cattle to secondary bacterial infection during BVDV infection (Roth et al., 1981). It remains open whether inhibition of in vitro responsiveness of lymphocytes to mitogens and decrease in B-cell function in connection with impaired IgG and IgM synthesis are an effect or a cause of fatal "late onset" forms of BVDV infections.

Considering the various disease pictures of BVD one is reminded of immune pathogenetic phenomena, especially of the immunologic tolerance in persistently infected animals to a particular antigenic variant of BVDV. In calves, failure to make antibodies has been attributed by Coria and McClurkin (1978) to immune tolerance, immune paralysis or immune suppression and unresponsiveness. Later Cutlip et al. (1980) described microscopic lesions in the kidneys of persistently infected cattle and BVDV antigen in mesangial and endothelial cells of glomeruli. This suggests an immune-mediated reaction with deposition of small amounts of antibody (incomplete immune tolerance) complexed with viral antigen in the renal glomeruli along the basement membrane, similar to the situation in lymphocytic choriomeningitis of mice (Oldstone and Dixon, 1969). Immune complexes have indeed been demonstrated in renal glomeruli of persistently BVDV-infected cattle in exactly the same locations as reported by others (Prager and Liess, 1976).

Sera from infected, seropositive cattle did not unequivocally discriminate infections with NC strains from infections with C-BVDV biotypes in radioimmune precipitations of cell lysates. This is because the viral protein of 80 k, present in lysates of cells infected with a C-BVDV strain, shares epitopes with the precursor protein of 118 k, present in cells infected with C and NC strains (Donis and Dubovi, 1987). The absence or low prevalence of protein 80 k in cells

infected with NC strains, i.e. the absence of 118 k protein processing was
described by Akkina (1982) and confirmed by Pocock et al. (1987) and others.

LABORATORY DIAGNOSIS

Clinical cases suspect of BVDV infection must be confirmed by laboratory
testing; this is also true for specimens from animals submitted for post mortem
examination, even without an anamnestic suspicion of such an infection. In
addition it may be necessary to perform tests on the prevalence of antibodies
against BVDV in cattle populations.

Inapparent infections

Detection of antibody by means of virus neutralization is the most frequent-
ly used test; the variable serum–constant virus procedure is routinely employed
in the calculation of neutralization titers. An initial serum dilution of 1/2 to 1/5
is used; positive titers arise soon after infection, reaching values far beyond the
initial dilution (usually 1/100 to 1/10 000), depending on the strain and amount
of BVDV used. Neutralization may be measured in the presence of C and NC
strains of BVDV. In the latter case, fluorescent antibodies (Fernelius, 1964) or
peroxidase-linked antibodies (Holm Jensen, 1981) may be used. The microtiter
system can be used with highly cytopathogenic strains, e.g. Oregon C24V
(Gillespie et al., 1960). The cytopathic effect may be increased by seeding
culture cells together with the virus–serum mixture (Frey and Liess, 1971). In
only few instances determinatin of the neutralization index or reduction of
plaque numbers were used for antibody quantitation (Gillespie et al., 1961;
Hafez and Liess, 1972a).

For the detection of animals persistently infected with BVDV negative
neutralization (titers < 1/5) gives an indication (Malmquist, 1968); it can be used
to identify carriers and to prevent losses which might occur among such
animals sooner or later (Holling, 1971). Seronegative and persistently infected
cattle may appear healthy, and BVDV can be isolated from their blood (Coria
and McClurkin, 1978; Liess et al., 1983). After vaccination they may develop
neutralizing antibodies to the BVD vaccine strain; vaccination does not affect
the state of viral persistence, as further circulation of the persistent virus even
in the plasma is found.

Chronic infections

Persistently BVDV infected cattle may develop signs of chronic disease. In
these cases the virus can be cultured from the buffy coat of peripheral blood
even in the presence of demonstrable neutralizing antibody (Coria and McClur-
kin, 1978). The antibodies may have been transferred by colostrum, or they
were actively produced by the persistently infected animal against heterolo-
gous BVDV, e.g. vaccine virus (Steck et al., 1980).

For virus isolation blood samples are suitable that contain heparin or EDTA
to prevent coagulation and to facilitate separation and collection of the leuko-
cyte fraction after low-speed centrifugation. After washing and resuspension,
the buffy coat leukocytes are inoculated into cultures of susceptible bovine
cells. After incubation for some days at 37°C the cell-culture fluids are collected
for further passages, preferably in established monolayer cell cultures. These
are examined for the presence of BVDV by fluorescent antibody, enzyme-linked
antibody or interference, especially if it concerns a NC strain. C-BVDV strains
are detected in only few instances; NC strains are frequently isolated from

persistently infected healthy animals (Coria and McClurkin, 1978) and even more so from chronically affected cattle (Liess et al., 1983).

Chronic infections may not be very impressive to begin with but become more apparent as the animals age. Therefore the search for persistently infected and virus excreting animals amongst those not showing clinical signs is of utmost importance. Characteristically, they are found among seronegative animals older than 9 months. Young animals may be viremic in spite of possessing maternal antibodies.

BVD/MD (severe acute form)

In cases of acute disease blood samples may be collected from the sick animals for virus isolation as well as for determination of neutralizing antibody. This is especially valuable if animals are at an age in which maternal antibodies are still present (6-9 months). Antibody may interfere with diagnostic procedures if the animals are allowed to die. Homogenization of tissues for virus isolation and the usual treatment with antibiotics may result in neutralization of BVDV during incubation by antibodies present in the lymph (Fernelius and Lambert, 1969).

Cells containing BVDV antigen over long periods in the presence of antibodies were considered by Fernelius and Lambert (1969) as evidence for a low-grade "carrier state". The results by Fernelius and Lambert found support by Bielefeldt Ohmann et al. (1982), who were not able to isolate BVDV from fetuses inoculated at 4-6 months of age and examined 3 weeks later. Instead, viral antigen was demonstrated in sections of several fetal organs, primarily lymphoid tissues (Bielefeldt Ohmann et al., 1981, 1982); the hyperimmune serum for these experiments had been raised in pigs by repeated intravenous injections of BVDV.

BVDV antigen detection by fluorescein- or enzyme-linked antibody is a widely used laboratory diagnostic procedure; tissue sections from various organs, preferably tonsil, parotis, spleen, lymph nodes and mucosa of the gastrointestinal tract are suitable. The main limitations of this method come from the presence of maternal antibodies, or from autolysis in field material because of inadequate transport conditions. Moreover, the specificity of the conjugate is very important. It is usually prepared from bovine serum which may contain antibodies against many other viruses. High-titer BVDV antiserum was raised in pigs by taking advantage of the anamnestic response when challenged with HCV (Overby, 1973; Liess et al., 1977; Hyera et al., 1987).

Fetal infections

From the fact that fetal infections with BVDV occur to an extent higher than commonly expected, diagnostic procedures must be applied to fetuses in cases of abortion, stillbirth or perinatal death. This is supported by the finding that amongst 57 aborted fetuses in Denmark 25% were positive for BVDV by virus isolation or/and specific antibody detection in peritoneal fluid (L. Ronsholt, personal communication, 1982).

Since blood samples can only incidentally be obtained in the above cases, laboratory diagnosis has to be based on detection of viral antigen by IF or immunoperoxidase techniques and on virus isolation in primary calf kidney cells or bovine cell lines, as outlined by Carbrey (1971).

Peritoneal fluid from fetuses or young calves, from which blood samples are not available, should be included in the diagnostic procedures. Since in many cases NC strains of BVDV are involved, proper techniques for the isolation and identification are of utmost importance. This includes three to four sub-

passages whenever virus cannot be found after one passage and subsequent inoculation on coverslip cell cultures or interference testing.

Considering diagnosis in congenitally infected calves fed on colostrum, maternal antibodies could interfere with the above diagnostic procedures. Antibodies found in, for example, peritoneal fluid of aborted fetuses older than 135 days of gestational age are probably actively produced (Bielefeldt Ohmann et al., 1982), as are those detectable in precolostral blood samples of newborn calves (Orban et al., 1983).

PROPHYLAXIS AND CONTROL

In the past, prophylaxis against BVDV infections has been the subject of many controversial publications. After what has been said before, it seems plausible that transplacental transmission of BVDV can be prevented by immunizing cattle before pregnancy (Duffell et al., 1984; Liess, 1984).

The first vaccine against BVD consisted of an attenuated virus (strain Oregon C24V) which after 32 passages did not produce illness in calves and was concluded to be safe and efficacious (Gillespie et al., 1960; Coggins et al., 1961). After extensive use of this vaccine, a postvaccinal condition was observed with characteristics similar to MD; it was thought to be due to a failure of the immune mechanism in a few animals (Peter et al., 1967).

Postvaccinal complications have been discussed in the USA in the years 1968–1973. Administration of attenuated BVDV was considered to be more harmful than beneficial, particularly for animals in a "carrier state" (Gutekunst, 1968). Apart from further possible postvaccinal conditions (Bittle, 1968), no disagreement was noticed as to the efficacy of live attenuated BVDV vaccines. Duration of immunity was estimated to be longlasting if not lifelong (as in BVDV field infections) provided the vaccine was administered at a time when colostral immunity did not interfere with immunization (Kahrs et al., 1966).

The problem of safety resulted in attempts to develop inactivated vaccines in order to prevent effects following passage of BVDV via the placenta to the fetus (Bognár, 1970, 1973). Abortion, cerebellar hypoplasia, ocular lesions, stillbirth, weakness and diarrhea were suspected to occur, as in natural BVDV infections during the first half of gestation (Ward, 1971; McClurkin et al., 1975). In a report describing resistance to acriflavine as a marker of an attenuated BVDV strain (ACR), Rockborn et al. (1974) called for further studies to ascertain whether the ACR strain is sufficiently attenuated and immunogenic for use as vaccine strain. In addition the need for tests on the pathogenicity for pregnant cows and the growing fetus was stressed. Thus the demand for studies on the pathogenesis of fetal BVDV infections and the effect of BVDV infection on fetal development was repeated (Kahrs, 1968).

Although McClurkin et al. (1975) reported that killed BVDV vaccines may be safe in pregnant cattle and also produce "adequate antibody to protect the fetus from an intrauterine infection", live attenuated BVDV vaccines continued to be manufactured on a large scale and used widely. This stimulated field studies on the transmissibility and the possible fetopathogenic effects of BVDV after administration of a commercial live virus vaccine to dams during the first half of gestation, commencing on gestational day 51. The vaccine was applied to seronegative pregnant cattle in herds where less than 50% of the animals had neutralizing antibodies. Five abortions occurred between 8 and 60 days after vaccination, but BVDV infection was confirmed in only one of three cases submitted to the laboratory. Five calves with torticollis and opisthotonus, ataxia or astasia were born at term (their dams had been vaccinated
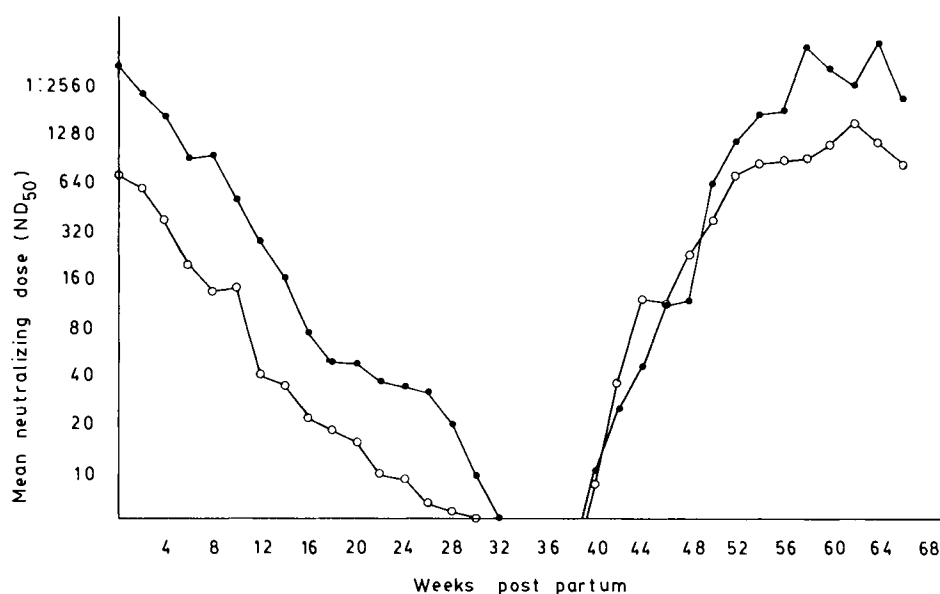
Fig. 102. Kinetics of maternal antibody decay in calves (3 days to 32 weeks post partum) and antibody development (40–66 weeks post partum) after field infection with two strains of BVDV; each point represents the mean of neutralizing doses measured at one time in eleven animals up to the 58th week; (●—●) strain NADL; (○—○) strain A1138/69.

between 90 and 118 days of gestation); nine calves had precolostral viremia which persisted until the animals were sold or sacrificed because of chronic disease (Fig. 101). One calf became 2 years old and showed growth retardation but appeared otherwise normal. Calves born with persistent viremia usually had no precolostral antibody except three with very low titers (Liess et al., 1984).

This demonstrates the hazard caused by vaccines which have not been properly tested. It cannot be decided whether it was the vaccine virus or a BVD field virus which had been picked up and caused the effects. Bovine cell cultures used for the propagation of any vaccine virus need proper pretesting in order to rule out dissemination of BVD field virus by vaccination.

Control of BVD by use of live attenuated vaccine in calves requires determination of the susceptible age for vaccination because of colostral immunity and its interference with immunization. According to Kahrs et al. (1966) this immunity lasts for about 9 months; it correlates with the presence of maternally derived neutralizing antibody (Robson et al., 1960). The rates of antibody decline are similar in calves from vaccinated and naturally immune dams with a calculated half-life of 19–20 days (Landelius, 1977). A shorter duration resulted from lower initial titers (Kahrs et al., 1966) or was due to the use of antigenically different strains of BVDV, as shown in Fig. 102. Since frequent revaccination is not feasible, one or two vaccinations might assure successful immunization of the greatest number of calves at the earliest possible age.

The early possibility of a BVD field infection became visible in a group of calves (Fig. 103) which developed active immunity when the titer of antibodies was still high (Lackmann-Pavenstedt, 1978). Since usually BVDV enters the bovine organism by the oronasal route, even high antibody titers in the blood do not prevent infection, resulting in active immunization (Liess et al., 1982).

Little is known about local immunity in BVD, but it must be expected to play an important role in protection. Therefore, live BVDV vaccines should probably be applied locally, for example by intranasal instillation. Although there are no reports on pertinent studies available it should be remembered that the intranasal route has been used successfully for challenge with BVDV in

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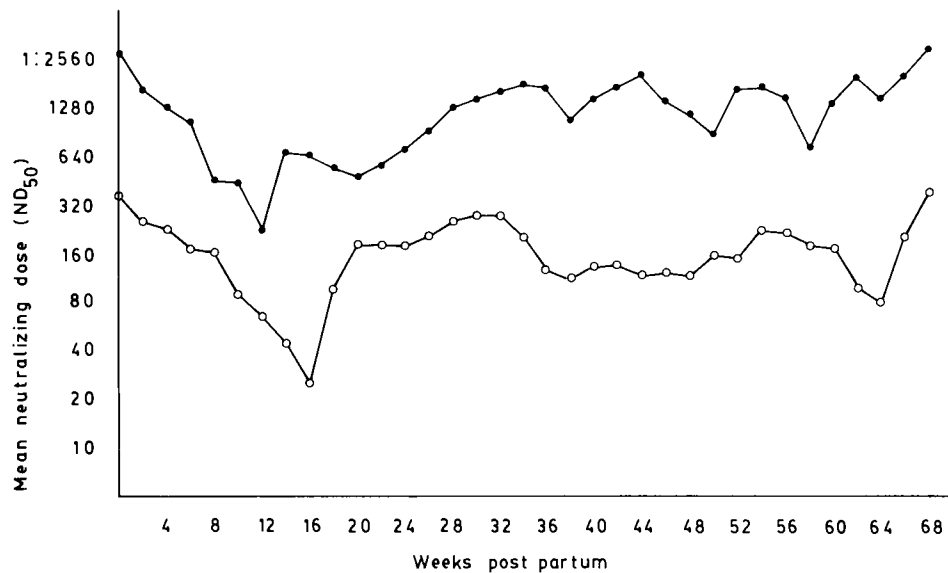
Fig. 103. Kinetics of neutralizing antibody passively acquired on day of birth; in spite of high titer of colostrally transferred antibody, field infection with BVDV initiated antibody development as demonstrable for the first time 14 weeks post partum; each point represents the mean of neutralizing dose measured at one time in twelve animals until the 60th week post partum; (●—●) strain NADL; (○—○) strain A1138/69.

several investigations. Immunity and neutralizing antibodies were efficiently induced.

Veterinary control measures rely on diagnostic tools to detect persistently infected cattle and on vaccination using safe vaccines. While there is no question that persistently BVDV infected animals should be eliminated from a herd, the application of a live virus vaccine to the seronegative group should be considered under various aspects. The vaccine should be applied well before breeding age and prior to situations involving a high risk of exposure. However, vaccination becomes a gamble as soon as antibody prevalence within the parent population approaches 50%; then also 50% of the newborn calves would receive maternal antibodies.

Immunization failure would result from too early vaccination while late vaccination would retain susceptibility during early calfhood (Kahrs et al., 1966). Control measures depend on management conditions and may have to be adapted accordingly (Malmquist, 1968; Kahrs, 1972).

REFERENCES

Akkina, R.K., 1982. Analysis of immunogenic determinants of bovine viral diarrhea virus. PhD, Thesis, University of Minnesota, 109 pp.

Atlurn, D, Notowidjoja, W., Johnson, D.W. and Muscoplat, C.C., 1979. Suppression of in vitro immunoglobulin biosynthesis in bovine spleen cells by bovine viral diarrhea virus. Clin. Immunol. Immunopathol., 13: 254–260.

Baker, J.A., York, C.J. Gillespie, J.H. and Mitchel, G.B., 1954. Virus diarrhea in cattle. Am. J. Vet. Res., 15: 525–531.

Bielefeldt Ohmann, H., 1982. Experimental fetal infection with bovine viral diarrhea virus. II. Morphological reactions and distribution of viral antigen. Can. J. Comp. Med., 46: 363–369.

Bielefeldt Ohmann, H., 1983. Pathogenesis of bovine viral diarrhoea-mucosal disease: distribution and significance of BVDV antigen in diseased calves. Res. Vet. Sci., 34: 5–10.

Bielefeldt Ohmann, 1984. An ocular–cerebellar syndrome caused by congenital bovine viral diarrhoea virus infection. Acta Vet. Scand., 25: 36–49.

Bielefeldt Ohmann, H. and Dalsgaard, K., 1980. Indirect immunofluorescence using F(ab')₂· immunoreagents for the demonstration of bovine viral diarrhea virus (BVDV) antigen in

lymphoid tissue. Acta Vet. Scand., 21: 705-707.

Bielefeldt Ohmann, H., Jensen, M.H., Sorensen, K.J. and Dalsgaard, K., 1981. Demonstration of bovine viral diarrhea virus antigen in cryostat- and paraffin-sections of bovine tissues by the immunoperoxidase technique. Acta Pathol. Microbiol. Scand., C, 89: 281-285.

Bielefeldt Ohmann, H., Jensen, M.H., Sorensen, K.J. and Dalsgaard, K., 1982. Experimental fetal infection with bovine viral diarrhea virus. I. Virological and serological studies. Can. J. Comp. Med., 46: 357-362.

Bittle, J.L., 1968. Vaccination for bovine viral diarrhea-mucosal disease. J. Am. Vet. Med. Assoc., 152: 861-865.

Bognár, K., 1970. Findings indicative of prenatal active immunization after vaccination of pregnant cows with live virus. Acta Vet. Hung., 20: 109-111.

Bognár, K., 1973. Foetal active immunization of calves following inoculation of the dam with a bovine viral diarrhoea vaccine (vedevac). Acta Vet. Hung., 23: 1-11.

Bolin, S.R., McClurkin, A.W. and Coria, M.F., 1985a. Effects of bovine viral diarrhea virus on the percentages and absolute numbers of circulating B and T lymphocytes in cattle. Am. J. Vet. Res., 46: 884-886.

Bolin, S.R., McClurkin, A.W., Cutlip, R.C. and Coria, M.F., 1985b. Severe clinical disease induced in cattle persistently infected with non-cytopathic bovine viral diarrhea virus by superinfection with cytopathic bovine viral diarrhea virus. Am. J. Vet. Res. 46: 573-576.

Bolin, S.R., McClurkin, A.W., Cutlip, R.C. and Coria, M.F., 1985c. Response of cattle persistently infected with non-cytopathic bovine viral diarrhea virus to vaccination for bovine viral diarrhea and to subsequent challenge exposure with cytopathic bovine viral diarrhea virus. Am. J. Vet. Res., 46: 2467-2470.

Brown, T.T., Schultz, R.D., Duncan, J.R. and Bistner, S.T., 1979. Serological response of the bovine fetus to bovine viral diarrhea virus. Infect. Immun., 25: 93-97.

Brownlie, J., Clarke, M.C. and Howard, C.J., 1984. Experimental production of fatal mucosal disease in cattle. Vet. Rec., 114: 535-536.

Brownlie, J., Clarke, M.C., Howard, C.J. and Pocock, D.H., 1986. Mucosal disease: The dilemma of experimental disease. I. In: P.J. Hartogan and M.L. Monaghan (Editors), Proceedings of the 14th World Congress on Diseases of Cattle. World Association for Buiatrics. Dublin, Ireland, pp. 199-203.

Bürki, F. and Germann, E., 1964. Letale Pneumoenteritiden bei Kälbern, verursacht durch den Erreger der bovinen Virusdiarrhoe. Berl. Münch. Tierärztl. Wochenschr. 16: 324-326; 17: 333-335.

Carbrey, E.A., 1971. Recommended standard laboratory techniques for diagnosing infectious bovine rhinotracheitis, bovine virus diarrhea, and shipping fever (Parainfluenza-3). Proc. 75th Annu. Meet. U.S. Anim. Health Assoc., pp. 629-648.

Carlson, R.G., Pritchard, W.R. and Doyle, L.P., 1957. The pathology of virus diarrhea of cattle in Indiana. Am. J. Vet. Res., 18: 560-568.

Casaro, A.P.E., Kendrick, J.W. and Kennedy, P.C., 1971. Response of the bovine fetus to bovine viral diarrhea-mucosal disease virus. Am. J. Vet. Res., 32: 1543-1562.

Castrucci, G., Titoli, F., Ranucci, S., Portugal, F.L.C., Cilli, V. and Pedini, B., 1974. Study of the experimental infection of pigs with bovine viral diarrhea (BVD) virus. Boll. Ist. Sieroter. Milan, 53: 585-591.

Castrucci, G., Avellini, G., Cilli, V., Pedini, B., McKercher, D.G. and Valente, C., 1975. A study of immunologic relationships among serologically heterologous strains of bovine viral diarrhea virus by cross immunity tests. Cornell Vet., 65: 65-72.

Coggins, L., Gillespie, J.H., Robson, D.S., Thompson, J.D., Phillips, W.V., Wagner, W.C. and Baker, J.A., 1961. Attenuation of virus diarrhea virus (strain Oregon C24V) for vaccine purposes. Cornell Vet., 51: 539-545.

Coria, M.F. and McClurkin, A.W., 1978. Specific immune tolerance in an apparently healthy bull persistently infected with bovine viral diarrhea virus. J. Am. Vet. Med. Assoc., 172: 449-451.

Coria, M.F., McClurkin, A.W. and Bolin, S.R., 1983. Total protein and immunoglobulins G_1, G_2, and M in serum of calves persistently infected with bovine viral diarrhea virus. Am. J. Vet. Res., 44: 1938-1939.

Coulibaly, C.O.Z., 1984. Quantitative Bestimung der IgG-Subklassen mittels radialer Immunodiffusion im Blutserum persistierend mit BVD-Virus infizierter Rinder. Thesis, Hannover Veterinary School, Hannover, 143 pp.

Cutlip, R.C., McClurkin, A.W. and Coria, M.F., 1980. Lesions in clinically healthy cattle persistently infected with the virus of bovine viral diarrhea-glomerulonephritis and encephalitis. Am. J. Vet. Res., 41: 1938-1941.

Darbyshire, J.H., 1962. Agar gel diffusion studies with a mucosal disease of cattle. II. A serological relationship between mucosal disease and swine fever. Res. Vet. Sci., 3: 125-128.

Dinter, Z., 1963. Relationship between bovine virus diarrhoea virus and hog cholera virus. Zentralbl. Bakteriol. Parasitenkd. Infektionskr., Hyg. Abt. 1, Orig., 188: 475-486.

Done, J.T., Terlecki, S., Richardson, C., Harkness, J.W., Sands, J.J., Patterson, D.S.P., Sweasey, D.,

Shaw I.G., Winkler, C.E. and Duffel, S.J., 1980. Bovine virus diarrhoea–mucosal disease virus: Pathogenicity for the fetal calf following material infection. Vet. Rec., 106: 473–479.

Donis, R.O. and Dubovi, E.J., 1987. Molecular specificity of the antibody response of cattle naturally and experimentally infected with cytopathic and non-cytopathic bovine viral diarrhea virus biotypes. Am. J. Vet. Res., 48: 1549–1554

Duffel, S.J., Sharp, M.W., Winkler, C.E., Terlecki, S., Richardson, C., Done, J.T., Roeder, P.L. and Hebert, C.N., 1984. Bovine virus diarrhoea–mucosal disease virus-induced fetopathy in cattle: Efficacy of prophylactic maternal pre-exposure. Vet. Rec., 114: 558–561.

Fernelius, A.L., 1964. Noncytopathogenic bovine viral diarrhea viruses detected and titrated by immunofluorescence. Can. J. Comp. Med. Vet. Sci., 28: 121–126.

Fernelius, A.L. and Lambert, G., 1969. Detection of bovine viral diarrhea virus and antigen in tissues of experimental infected calves by cell inoculation and fluorescent antibody techniques. Am. J. Vet. Res., 30: 1551–1559.

Frey, H.-R. and Liess, B., 1971. Vermehrungskinetik und Verwendbarkeit eines stark zytopath-ogenen VD–MD-Virusstammes für diagnostische Untersuchungen mit der Mikrotiter-Methode. Zentralbl. Veterinärmed. B., 18: 61–71.

Gillespie, J.H., Baker, J.A. and McEntee, K., 1960. A cytopathogenic strain of virus diarrhea virus. Cornell Vet., 50: 73–79.

Gillespie, J.H., Coggins, L., Thompson, J. and Baker, J.A., 1961. Comparison by neutralization tests of strains of virus isolated from virus diarrhea and mucosal disease. Cornell Vet., 51: 155–159.

Grahn, T.C., Fahning, M.L. and Zemjanis, R., 1984. Nature of early reproductive failure caused by bovine viral diarrhea virus. J. Am. Vet. Med. Assoc., 185: 429–432.

Gutekunst, D.E., 1968. Comments on vaccination for bovine viral diarrhea–mucosal disease. J. Am. Vet. Med. Assoc., 152: 865–866.

Gutekunst, D.E. and Malmquist, W.A., 1963. Separation of a soluble antigen and infectious particles of bovine viral diarrhea viruses and their relationship to hog cholera. Can. J. Comp. Med., 27: 121–123.

Hafez, S.M. and Liess, B., 1972a. Studies on bovine viral diarrhea-mucosal disease virus. I. Cultural behaviour and antigenic relationship of some strains. Acta Virol., 16: 388–398.

Hafez, S.M. and Liess, B., 1972b. Studies on bovine viral diarrhea–mucosal disease virus. II. Stability and some physico-chemical properties. Acta Virol., 16: 399–408.

Hansen, H.-J., Ronéus, O. and Dinter, Z., 1962. Untersuchungen über Mucosal Disease. II. Pathologische Anatomie der Krankheit im Vergleich mit dem cytopathogenen Effekt des Virus der Virusdiarrhoe auf die Gewebekultur. Zentralb. Veterinärmed. 9: 854–864.

Hermodsson, S. and Dinter, Z., 1962. Properties of bovine virus diarrhoea virus. Nature, 194: 893–894.

Holling, F., 1971. Kulturelle und serologische Untersuchungen zum Nachweis von VD-MD-Infektionen unter natürlichen Infektionsbedingungen. Thesis, Hannover Veterinary School, Hannover, 90 pp.

Horzinek, M.C., 1981. Nonarbo togavirus infections of animals: Comparative aspects of diagnosis. In: E. Kurstak and C. Kurstak (Editors), Comparative Diagnosis of Viral Diseases. Academic Press, New York, Vol. IV, pp. 441–476.

Howard, C.J., Brownlie, J. and Clarke, M.C., 1987. Comparison by the neutralization assay of pairs of non-cytopathogenic and cytopathogenic strains of bovine viral diarrhea virus isolated from cases of mucosal disease. Vet. Microbiol., 13: 361–369.

Huck, R.A., 1957. A mucosal disease of cattle. Vet. Rec., 69: 1207–1213.

Hyera, J.M.K., Dahle, J., Liess, B., Moennig, V. and Frey, H.R., 1987. Production of potent antisera raised in pigs by anamnestic response and use for direct immunofluorescent and immunoperoxidase techniques. In: J.W. Harkness (Editor), Pestivirus Infections of Ruminants. ECSC-EEC-EAEC, Brussels/Luxembourg, pp. 870–102.

Holm Jensen, M.H., 1981. Detection of antibodies against hog cholera virus and bovine viral diarrhea virus in porcine serum. A comparative examination using CF, PLA and NPLA assays. Acta Vet. Scand., 22: 85–98.

Johnson, D.W. and Muscoplat, C.C., 1973. Immunologic abnormalities in calves with chronic bovine viral diarrhea. Am. J. Vet. Res., 34: 1139–1141.

Kahrs, R.F., 1968. The relationship of bovine viral diarrhea–mucosal disease to abortion in cattle. J. Am. Vet. Med. Assoc., 153: 1652–1655.

Kahrs, R.F., 1972. Bovine viral diarrhea–mucosal disease. Proc. Oklahoma Vet. Med. Assoc., 24: 10–12.

Kahrs, R.F., 1973. Effects of bovine viral diarrhea on the developing fetus. J. Am. Vet. Med. Assoc., 163: 877–878.

Kahrs, R.F., Robson, D.S. and Baker, J.A., 1966. Epidemiological considerations for the control of bovine virus diarrhea. Proc. U.S. Livest. Sanit. Assoc., 70: 145–153.

Kendrick, J.W., 1971. Bovine viral diarrhea–mucosal disease virus infection in pregnant cows. Am. J. Vet. Res., 32: 533 544.

Kendrick, J.W. and Franti, C.E., 1974. Bovine viral diarrhea: Decay of colostrum-conferred antibody in the calf. Am. J. Vet. Res., 35: 589–591.

Khater, A.R., Messow, C. and Stoeber, M., 1964. Der histologische Gehirnbefund als differential-diagnostisches Hilfsmittel zur Abgrenzung von Mucosal Disease, bösartigem Katarrhalfieber und Rinderpest. Dtsch. Tierärztl. Wochenschr., 71: 127–131.

King, A.A. and Harkness, J.W., 1975. Viral contamination of foetal bovine serum. Vet. Rec., 97: 16.

Kniazeff, A.J., Rimer, V. and Gaeta, L., 1967. γ-Globulin in foetal bovine sera: significance in virology. Nature, 214: 805–806.

Lackmann-Pavenstedt, B., 1978. Kinetik neutralisierender Antikörper gegen BVD-Virusstämme bei Kälbern und Aufnahme von Kolostrum serologisch BVD-positiver Mütter. Beitrag II: 34–70. Lebenswoche. Thesis, Hannover Veterinary School, Hannover, 40 pp.

Lambert, G., Fernelius, A.L. and Cheville, N.F., 1969. Experimental bovine viral diarrhea in neonatal calves. J. Am. Vet. Med. Assoc., 154: 181–189.

Landelius, L., 1977. Kinetik neutralisierender Antikörper gegen BVD-Virusstämme bei Kälbern nach Aufnahme von Kolostrum serologisch BVD-positiver Mütter. Beitrag I: Erste bis 32. Lebenswoche. Thesis, Hannover Veterinary School, Hannover, 37 pp.

Larsson, B., 1987. Mucosal disease and persistent infection with bovine viral diarrhea virus: Studies on mononuclear cells in relation to their activities. Ph.D. thesis. University of Agricultural Sciences, Uppsala.

Liess, B., 1967. Die ätiologische Abgrenzung selbständiger Virusinfektionen, insbesondere der Virusdiarrhoe–Mucosal Disease im sogenannten "Mucosal-Disease-Komplex" bei Rindern. Dtsch. Tierärztl. Wochenschr., 74: 46–49.

Liess, B., 1973. Pathogenese virusbedingter Darminfektionen der Tiere. Dtsch. Tierärztl. Wochenschr., 80: 341–368.

Liess, B., 1981. Hog Cholera. In: E.P.J. Gibbs (Editor), Virus Diseases of Food Animals. A World Geography of Epidemiology and Control. Academic Press, London, Vol. II, pp. 627–650.

Liess, B., 1984: Feldimpfung gegen Bovine Virusdiarrhoe. Prakt. Tierarzt, 64: 926–927.

Liess, B., Frey, H.-R., Kittsteiner, H., Baumann, F. and Neumann, W., 1974. Beobachtungen und Untersuchungen über die "Mucosal Disease" des Rindes — einer immunbiologisch erklärbaren Spätform der BVD–MD-Virusinfektion mit Kriterien einer "slow virus infection"? Dtsch. Tierärztl. Wochenschr., 81: 481–487.

Liess, B., Frey, H.-R. and Prager, D., 1977. Antibody response of pigs following experimental infections with strains of hog cholera and bovine viral diarrhea virus. In: Hog Cholera/Classical Swine Fever and African Swine Fever. Comm. Europ. Commun., Brussels-Luxembourg, Publ. Nr. EUR 5904, pp.200–213.

Liess, B., Landelius, L. and Lackmann-Pavenstedt, B., 1982. Bovine Virusdiarrhoe (BVD) — Ergebnisse einer serologischen Langzeitstudie unter enzootischen Infektionsbedingungen in einem mittelgroßen Rinderzuchtbestand. Prakt. Tierarzt, 63: 118–121.

Liess, B., Frey, H.-R., Orban, S. and Hafez, S.M., 1983. Bovine Virusdiarrhoe (BVD) — "Mucosal Disease": Persistente BVD-Feldvirusinfektionen bei serologisch selektierten Rindern. Dtsch. Tierärztl. Wochenschr., 90: 261–266.

Liess, B., Orban, S., Frey, H.-R., Trautwein, G., Wiefel, W. and Blindow, H., 1984. Studies on transplacental transmissibility of a Bovine Virus Diarrhoea (BVD) vaccine virus in cattle. II. Inoculation of pregnant cows without detectable neutralizing antibodies to BVD virus 90–229 days before parturition (51st to 190th day of gestation). Zentralbl. Veterinärmed. B, 31: 669–681.

Lohr, C.H., Evermann, J.F. and Ward, A.C., 1983. Investigation of dams and their offspring inoculated with a vaccine contaminated by bovine viral diarrhea virus. Vet. Med. Small Anim. Clinic., 78: 1263–1266.

Malmquist, M.A., 1968. Bovine viral diarrhea–mucosal disease: etiology, pathogenesis and applied immunology. J. Am. Vet. Med. Assoc., 152: 763–768.

McClurkin, A.W., Coria, M.F. and Smith, R.L., 1975. Evaluation of acetylethyleneimine-killed bovine viral diarrhea–mucosal disease virus (BVD) vaccine for prevention of BVD infection of the fetus. Proc. U.S. Anim. Health Assoc., 79: 114–123.

McClurkin, A.W., Coria, M.F. and Cutlip, R.C., 1979. Reproductive performance of apparently healthy cattle persistently infected with bovine viral diarrhea virus. J. Am. Vet. Med. Assoc., 174: 1116–1119.

Mills, J.H.L. and Luginbuhl, R.E., 1968. Distribution and persistence of mucosal disease virus in experimentally exposed calves. Am. J. Vet. Res., 29: 1367–1375.

Muscoplat, C.C., Johnson, D.W. and Stevens, J.B., 1973. Abnormalities of in vitro lymphocyte responses during bovine viral diarrhea virus infection. Am. J. Vet. Res., 34: 753–755. der Togavirusgruppe. Thesis, Verterinary School, Hannover.

Nansen, P., 1972. Selective immunoglobulin deficiency in cattle and susceptibility to infection. Acta Pathol. Microbiol. Scand. B, 80: 49–54.

Noice, F. and Schipper, I.A., 1959. Isolation of mucosal disease virus by tissue culture in mixture 199, Morgan, Morton and Parker. Proc. Soc. Exp. Biol. Med., 100: 84–86.

Nuttall, P.A., Luther, P.D. and Stott, E.J., 1977. Viral contamination of bovine foetal serum and cell cultures. Nature, 266: 835–837.

Olafson, P. and Rickard, C.G., 1947. Further observations on the virus diarrhea (new transmissible disease) of cattle. Cornell Vet., 37: 104–106.

Olafson, P., MacCallum, A.D. and Fox, F.H., 1946. An apparently new transmissible disease of cattle. Cornell Vet. 36: 205–213.

Oldstone, M.B.A. and Dixon, F.J., 1969. Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice. J. Exp. Med., 129.: 483–505.

Orban, S., Liess, B., Hafez, S.M., Frey, H.-R., Blindow, H. and Sasse-Patzer, B., 1983. Studies on transplacental transmissibility of a bovine virus diarrhea (BVD) vaccine virus. I. Inoculation of pregnant cows 90 to 15 days before parturition (190th to 265th day of gestation). Zentralbl. Veterinärmed. B 30: 619–634.

Overby, G., 1973. Immunization against swine fever with a Danish bovine viral diarrhoea virus stain UG 59. Nord. Vet. Med. 25: 497–503.

Peter, C.P., Tyler, D.E. and Ramsey, F.K., 1967. Characteristics of a condition following vaccination with bovine virus diarrhea vaccine. J. Am. Vet. Med. Assoc., 150: 46–52.

Pocock, D.H., Howard, C.J., Clarke, M.C. and Brownlie, J., 1987. Variation in the intracellular polypeptide profiles from different isolates of bovine viral diarrhea virus. Arch. Virol., 94: 43–53.

Porter, D.D., 1971. A quantitative view of the slow virus landscape. In: J.L. Melnick (Editor), Progress in Medical Virology. S. Karger, Basel, Vol. 13, pp. 339–372.

Prager, D. and Liess, B., 1976. Nachweis von Immunkomplex-Komponenten in Nierenglomerula bei der Bovinen Virusdiarrhea–Mucosal Disease. Zentralbl. Veterinärmed. B, 23: 458–469.

Ramsey, F.K., 1956. Pathology of a mucosal disease of cattle. Thesis, Iowa State College, Ames, IA. 32 pp.

Ramsey, F.K., 1963. The pathology of bovine mucosal disease–virus diarrhea complex. Proc. XVIIth World Vet. Congr., Vol. 1, pp. 399–403.

.Ramsey, F.K. and Chivers, W.H., 1953. Mucosal disease of cattle. North Am. Vet., 34: 629–633.

Robson, D.S., Gillespie, J.H. and Baker, J.A., 1960. The neutralization test as an indicator of immunity to virus diarrhea. Cornell Vet., 50: 503–509.

Rockborn, G., Diderholm, H. and Dinter, Z., 1974. Bovine viral diarrhea virus: Acquired resistance to Acriflavine as markers of an attenuated strain. Arch. Ges. Virus-forsch. 45: 128–134.

Roeder, P.L., 1982. Bovine mucosal disease — A persistent viral infection. Proc. Int. Symp. World Assoc. Vet. Microbiol. Immunol., Barcelona.

Rohde, G. and Liess, B., 1970. Untersuchungen zur Pathogenese der Virusdiarrhoe–Mucosal-Disease des Rindes unter Verwendung einer heterotypischen Immunofluoreszenztechnik. Zentralbl. Veterinärmed. B, 17: 686–700.

Romvary, J., 1965. Incidence of virus diarrhoea among newborn calves. Acta Vet. Acad. Sci. Hung., 15: 341 347.

Roth, J.A., Kaeberle, M.L. and Griffith, R.W., 1981. Effects of bovine viral diarrhea virus infection on bovine polymorphonuclear leukocyte function. Am. J. Vet. Res., 42: 244–250.

Schultz, R.D., 1973a. Developmental aspects of the fetal bovine immune response: a review. Cornell Vet., 63: 507–535.

Schultz, R.D., 1973b. Comments on the immune response of the fetus and neonate. J. Am. Vet. Med. Assoc., 163: 804–806.

Scott, F.W., Kahrs, R.F., Delahunta, A., Brown, T.T., McEntee, K. and Gillespie, J.H., 1973. Virus induced congenital anomalies of the bovine fetus. I. Cerebellar degeneration (hypoplasia), ocular lesions and fetal mummification following experimental infection with bovine viral diarrhea–mucosal disease virus. Cornell Vet., 63: 536–560.

Seibold, H.R., 1956. The pathology of mucosal disease in Alabama. J. Am. Vet. Med. Assoc., 128: 21–26.

Shope, R.E., jr., Muscoplat C.C., Chen, A.W. and Johnson, D.W., 1976. Mechanism of protection from bovine viral diarrhea virus infection. I. The effects of dexamethasone. Can. J. Comp. Med., 40: 355–359.

Snowdon, W.A. and French, E.L., 1968. The bovine mucosal disease–swine fever virus complex in pigs. Aust. Vet. J., 44: 179–184.

Steck, F., Lazary, S., Fey, H., Wandeler, A., Huggler, C., Oppliger, G., Baumberger, H., Kaderli, R. and Martig, J, 1980. Immune responsiveness in cattle fatally affected by bovine virus diarrhea–mucosal disease. Zentralbl. Veterinärmed., B, 27: 429–445.

Trautwein, G., Hewicker, M., Liess, B., Orban, S. and Grunert, E., 1986. Studies on transplacental transmissibility of a bovine virus diarrhoea (BVD) vaccine virus in cattle. III. Occurrence of central nervous system malformations in calves born from vaccinated cows. J. Vet. Med., B, 33: 260–268.

Tyler, D.E. and Ramsey, F.K., 1965. Comparative pathologic, immunologic, and clinical responses produced by selected agents of the bovine mucosal disease–virus diarrhea complex. Am. J. Vet. Res., 26: 903–913.

Walker, R. and Olafson, P., 1947. Failure of virus diarrhea of cattle to immunize against rinderpest. Cornell Vet., 37: 107–111.

Ward, G.M., 1971. Bovine viral diarrhea–mucosal disease implicated in a calf with cerebellar hypoplasia and ocular disease. A case report. Cornell Vet., 61: 224–228.

Ward, G.M., Roberts, S.J., McEntee, K. and Gillespie, J.H., 1969. A study of experimentally induced bovine viral diarrhea–mucosal disease in pregnant cows and their progeny. Cornell Vet., 59: 525–538.

Chapter 24

Border Disease Virus

R.M. BARLOW
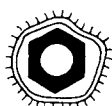
VIRUS PROPERTIES

The virus of border disease (BD) or hairy shaker disease of lambs, of which both cytopathic and noncytopathic variants are known, has been grown in cell structures of fetal lamb kidney, sheep choroid plexus, porcine kidney and bovine testis. It is inactivated by moderate heat (56°C for 30 min), lipid solvents, common disinfectants, UV light and desiccation. It has limited ability to survive outside the host even in animal products and under moist conditions.

BDV has been shown to pass a filter membrane of 50 nm average pore diameter but be retained by one of 35 nm (Harkness and Vantsis, 1982). Thus in size it is comparable with the closely related BVDV, reported as being a 45–54 nm particle with a core of about 30 nm (Chasey and Roeder, 1981; Bielefeldt-Ohmann and Bloch, 1982).

The buoyant density values of BDV are also similar (1.09–1.15 gm/ml) to those obtained with the NADL strain of BVDV under similar conditions. In sucrose density gradients the sedimentation coefficients of BDV, BVDV and HCV grown in a sheep cell line were found to be identical ($S_w^{20} = 139 \pm 12$). Although the physical similarities illustrate the closeness of the relationship between pestiviruses of different animals, strain differences within BDV, in terms of pathogenicity for different breeds of sheep, have been recognized. Moreover, the varying ability of BDV antibodies to neutralize or protect against different strains of virus in vitro and in vivo is an indication of appreciable antigenic differences. Despite this, there are no routine methods available for distinguishing between BDV and BVDV.

EPIZOOTIOLOGY

As a clinicopathological entity BD is essentially a disease of sheep, though experimentally it has been shown that goats, cattle and swine are susceptible to infection. There is also serological evidence of BDV infection in several species of wild deer, and wild ungulates may serve as a reservoir and source of infection for domestic ruminants.

BD has now been reported from most sheep-rearing countries, many of which also have substantial cattle populations. Experimentally it has been shown that contact spread of BDV from sheep to cattle and of BVDV from cattle to sheep readily occurs. However, serological surveys indicate that whereas about 70% of adult cattle have experienced infection with BVDV or BDV, the proportion of seropositive sheep rarely exceeds 20%, even in areas in which BD is endemic (Barlow, 1982). The reasons for this difference are not clear, but

some strains of virus may spread more readily in cattle than in sheep and vice versa. Also, as will become evident later, animals infected in utero may become persistent excretors of virus; it may be that bovine persistent excretors survive better than their ovine counterparts and thus provide more durable reservoirs of infection in cattle populations.

Infection of immunologically naive adults with these viruses is usually subclinical and shortlived. If pregnant dams are infected, however, the fetus also becomes infected and may be expelled or born alive at full term, either immune or as a persistently infected virus excretor. The breeding season therefore represents the period of greatest risk from infection with BVDV or BDV. The virus is present in the aborted fetus and its membranes and in the secretions and excretions of persistently infected animals. Infection is probably acquired by inhalation or ingestion. Breed differences in susceptibility to BD infection have not been observed, though there is evidence that the manifestations of disease in the newborn, e.g. fleece abnormalities and neuropathological changes may be influenced by host genetic factors.

Outbreaks of BD characterized by clinically obvious "hairy shaker" lambs (Fig. 104) vary greatly in severity (from < 10% rising in exceptional circumstances to 80% of the lamb crop). However, it has been shown that infection is more widespread than clinical disease suggests; subclinical infection may result in early embryonic death, late returns to service, low lambing percentages and ill-thrift among surviving lambs; these lambs require greater management/feed inputs and time to reach marketable weight. All these features can seriously affect gross margins.

PATHOGENESIS

In nonpregnant sheep, infection with BDV is invariably subclinical, though mild pyrexia and transient leukopenia have sometimes been encountered 6–11 days p.i., during which time viremia probably occurs. Serum neutralizing antibodies quickly appear. They have been detected as early as 1 week after inoculation and within 2 weeks in control sheep housed in contact with inoculated animals (Hamilton and Timony, 1972). Thus in the preimplantation phase of pregnancy, viremia could be terminated before a firm villous union has formed between the caruncle and the trophoblast, and the fetus may escape

Fig. 104. BD-affected "hairy shaker" and normal Dorset Horn lambs, 2 days old.
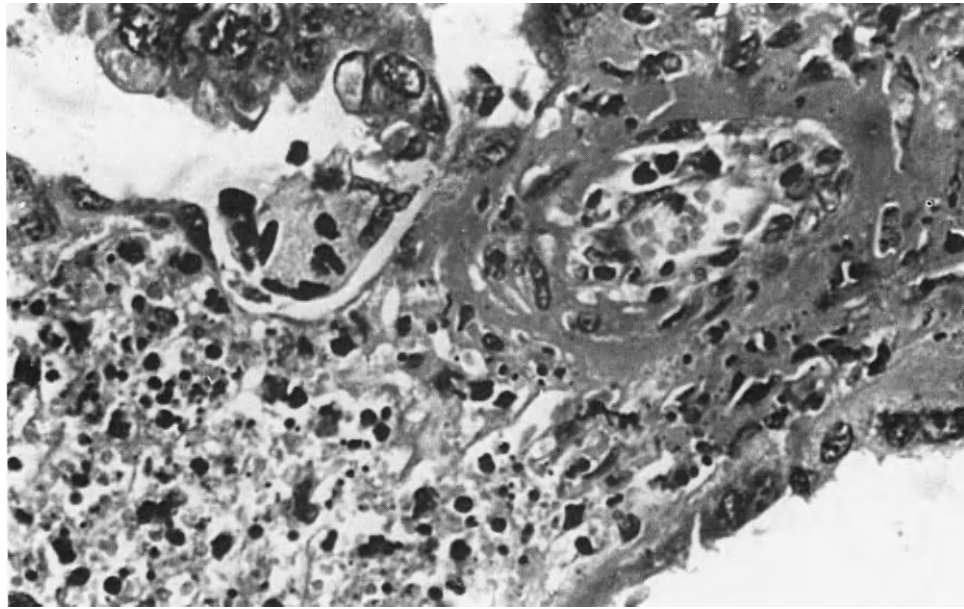
Fig. 105. Caruncular septum showing perivascular exudate and necrosis of the crypt wall; early lesion, 10 days p.i.; H&E, × 500.
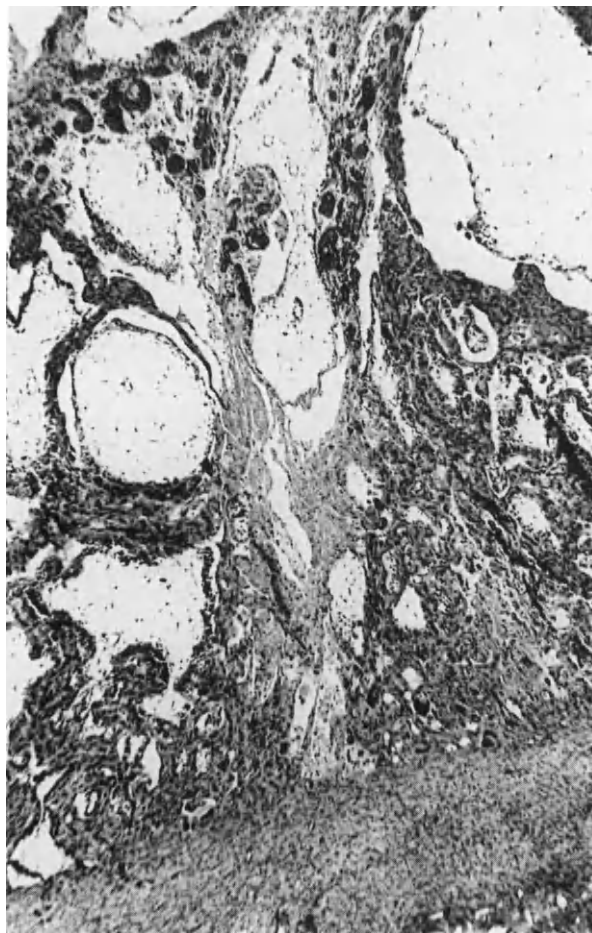
Fig. 106. Local necrosis of caruncular septum with adjacent villus atrophy; established lesion, 12 days p.i.; H&E, × 50.

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infection. At all postimplantation stages of pregnancy, however, the virus readily crosses the placenta, which remains impermeable to maternal neutralizing antibody.

The effects upon the fetus depend upon the developmental stage reached at infection and vary according to the degree of fetal immune responsiveness. The virus is distributed widely in fetal tissues, but the morphological effects of infection are most obvious as developmental abnormalities in the central nervous system, skeleton and skin. Placentitis occurs (Figs. 105, 106) and may contribute to the death and expulsion of the fetus, a not infrequent consequence of BD infection. The abortus is a rich source of infection for susceptible flockmates. Infections initiated at less than about 80 days of gestation may also result in the birth of viable lambs which excrete virus persistently but develop no neutralizing antibody, or only at very low fluctuating levels. Such animals are important disseminators of virus, usually for life.

DISEASE SIGNS

The signs of BDV infection are manifold. On a flock basis infection may be manifested by an increase in the number of ewes barren at lambing time, or by evidence of abortion. Abortion may occur at any stage of pregnancy but is common up to about 90 days of gestation. The aborting ewe shows little discomfort. There is rarely retention of placenta or postpartum metritis, and as the fetus is usually small and mummified, abortion may pass unnoticed.

Badly deformed fetuses may be carried to term but cause dystocia because of kyphoscoliosis or arthrogryposis.

The most characteristic sign of BDV infection is the birth of small weakly lambs, some of which may show varying degrees of tremor and, in smooth-coated breeds of sheep, have abnormally coarse, hairy birth coats. These lambs bleat piteously; if they can stand they will seek the ewe's udder but be unable to feed because of the violence of the tremor. However, most will suck if held to the teat.

Some BD-affected lambs are neither hairy nor show significant tremor but have disproportionally long metacarpals, metatarsals and first phalanges—so-called "camel-legged" lambs (Fig. 107). If they can get to their feet they show

Fig. 107. "Camel-legged" lamb.

little teat-seeking drive but tend to wander aimlessly and appear blind. Other BD-affected lambs show only skeletal abnormalities such as a small narrow head with brachygnathia, fine long bones and incomplete carpal extension, and yet others appear physically normal but fail to thrive.

Under field conditions many BD-affected lambs die in the first few weeks of life. In surviving "hairy shakers" the tremor gradually regresses and has usually disappeared by about 20 weeks of age. Deaths may continue to occur throughout the suckling period and following the stress of weaning. Many of these later deaths are associated with severe episodes of diarrhea or respiratory distress. These conditions have been attributed to secondary infections, but it has been shown that they may also be part of a mucosal disease-like manifestation of BD (Barlow et al., 1983; Gardiner et al., 1983).

PATHOLOGY

The mild or subclinical disease which follows infection in the nonpregnant adult may be associated with slight pyrexia and transient leukopenia. Morbid anatomical changes have not been described. Tiny focal infiltrations of small mononuclear cells have been observed histologically in some tissues (Barlow et al., 1983). Though nonspecific in character, such infiltrations were not seen in controls.

In the pregnant female at all postimplantation stages of gestation a focal necrotizing carunculitis develops in the basal third of the placentome. Such lesions may resolve in 25–30 days, but in some cases they expand and coalesce to form diffuse bands of necrosis in the deeper parts of the placentome; this will obviously affect fetal viability and contribute significantly to fetal death and abortion. The aborted fetus is usually mummified, but if fresh shows serosanguinous hydrops foetalis and hydrops amnii.

The pathology in the full term lamb depends to a considerable extent on the gestational age at infection. The characteristic "hairy shaker" lamb is the product of infection before about the 80th day. The gross morphological changes resemble those of lambs subjected to severe nutritional stress in utero—a shallow narrow body with short thin bones containing growth arrest lines but fewer ossified centers than normal (Richardson, 1982).

In normally smooth-coated breeds of sheep the hairiness of the "hairy shaker" fleece is due to an increase in size of the primary wool follicles with concomitant increase in the proportion of medullated fibers and a reduction in number of secondary follicles, i.e. the secondary:primary (S/P) follicle ratio is reduced (Orr, 1982).

The nervous system of such lambs appears macroscopically normal; histologically there is a diffuse or patchy deficiency of myelin in the brain and spinal cord. Axons are frequently naked or clad with abnormally thin myelin sheaths with few lamellae and abnormal, irregular profiles in cross section: hypomyelinogenesis congenita. The peripheral nerves are unaffected. This form of BD is essentially noninflammatory (Fig. 108).

In the "camel-legged" lambs which experimentally have been shown to arise from infection before 80 days of gestation and also in those with arthrogryposis and kyphoscoliosis, the skeletal defect has not been investigated. The S/P wool follicle ratio is normal and hypomyelinogenesis congenita is not obvious. However, there are intracranial malformations which are frequently of a gross nature. These comprise porencephaly or hydranencephaly, cerebellar hypo- or dysplasia, hydrocephalus and persistent cavum septum pellucidum (Fig. 109). These lesions usually contain numerous plump macrophages. Glial nodules and perivenous lymphoid cuffs are present in the substance of the brain and

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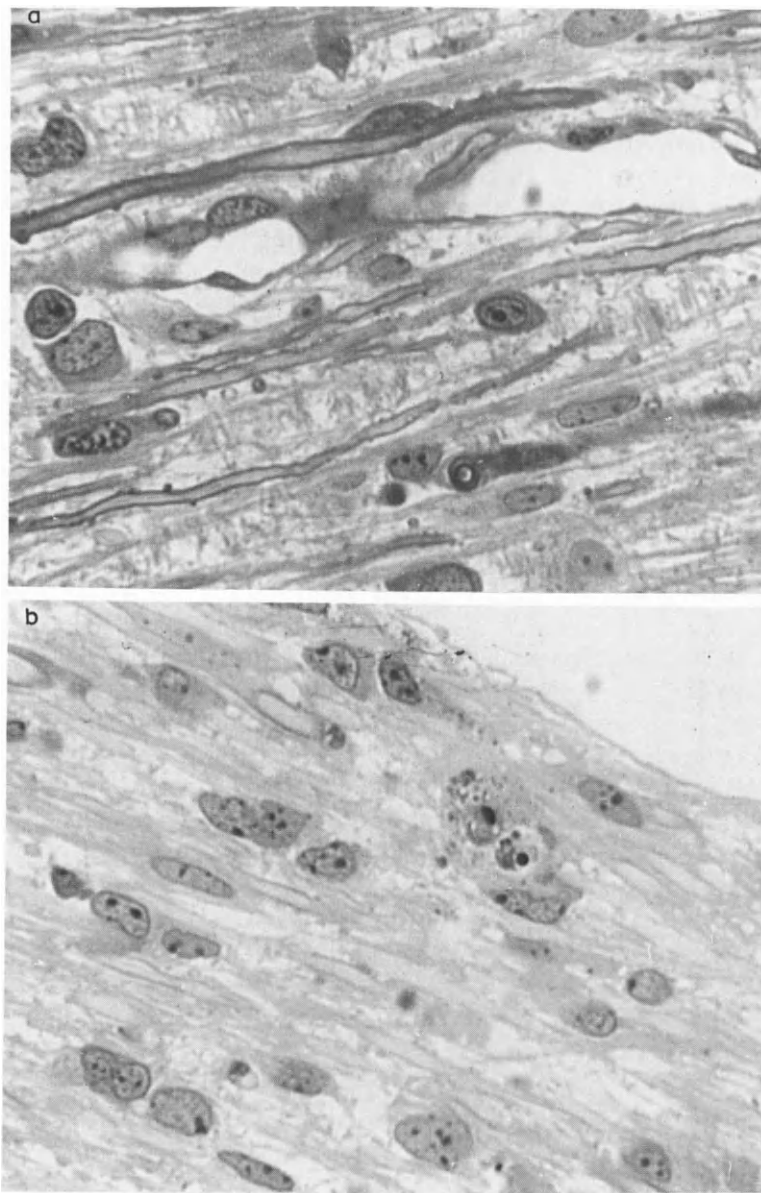
Fig. 108. Longitudinal sections of (a) normal and (b) BD-affected spinal cord; note the paucity of myelin in the affected animal, the swollen glial nuclei and the intracellular dense lipoidal droplets; both specimens are from fetuses at 80 days of gestation; perfused with glutaraldehyde, embedded in Araldite and stained with Giemsa, × 800.

spinal cord; thus this form of BD clearly has an inflammatory component. Virus is not recovered from such lambs, most of which have serum neutralizing antibody.

No macroscopic changes are present in lambs born after infections initiated later than about 80 days of gestation; histologically there is a nodular periarteritis which is disseminated throughout most tissues but is most frequently encountered in the CNS and meninges (Fig. 110). The nodules consist mainly of lymphoid cells and macrophages. The lesions are remarkably uniform in their cellular characteristics; they are also durable, as they have been found for up to one year after birth. Viral antigen can be demonstrated in the lesions for about 6 months and neutralizing antibody has been detected in the serum of some such lambs.
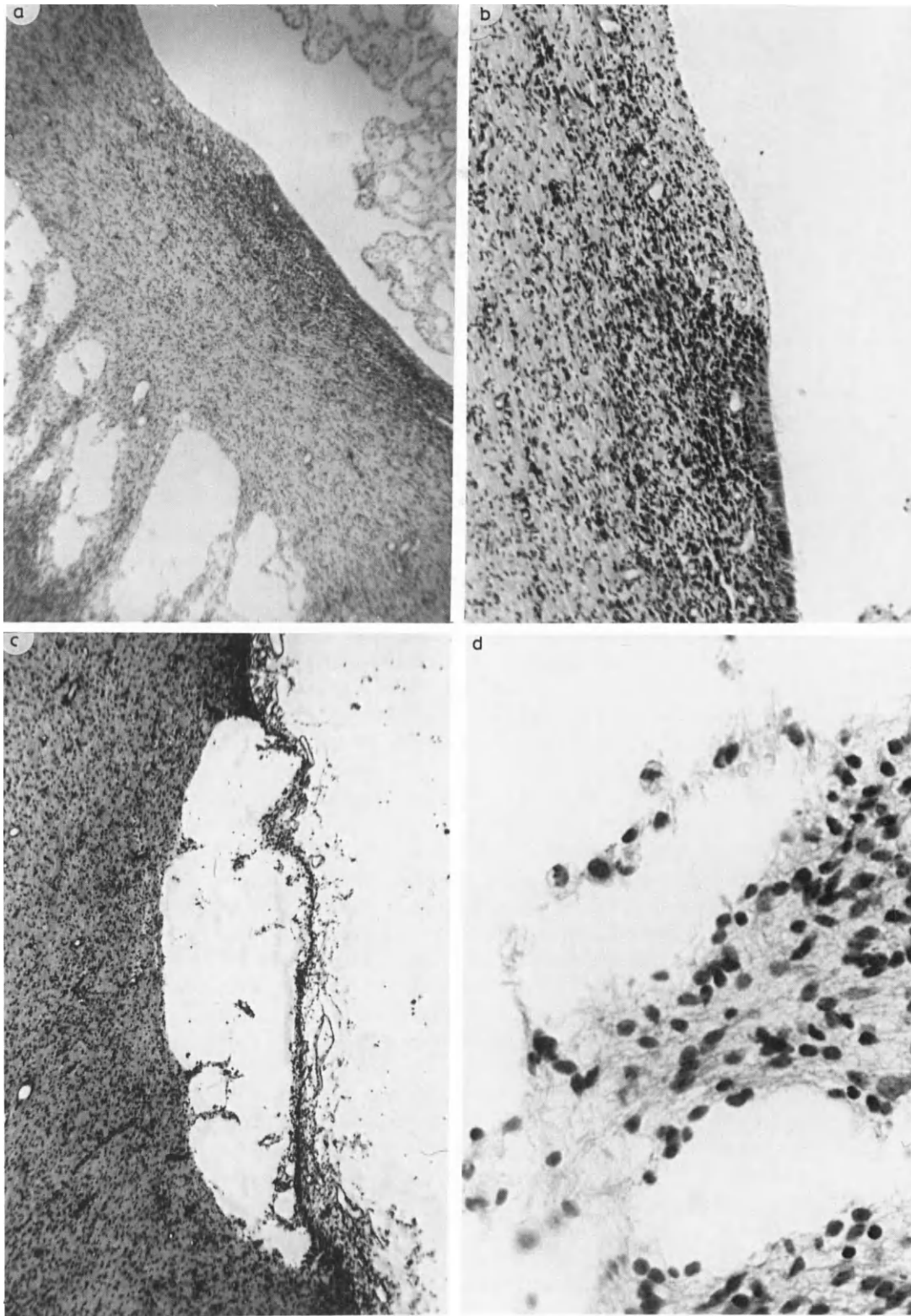
Fig. 109. (a) Developing porencephalic lesion in BD-affected fetus at 84 days of gestation; H&E, × 33. (b) High-power picture of the lining of the ventricle of 109a; note the discontinuity of the ependyma and partial loss of the adjacent subependymal mantle layer; H&E, × 133. (c) Cerebellar cortex of BD-affected fetus at 84 days of gestation, showing focal loss of the external granular (granulo-prival) layer, the forerunner of a hypoplastic cerebellum; H&E, × 133. (d) Edge of cavity shown in 109a with infiltration of phagocytic cells; H&E, × 500.

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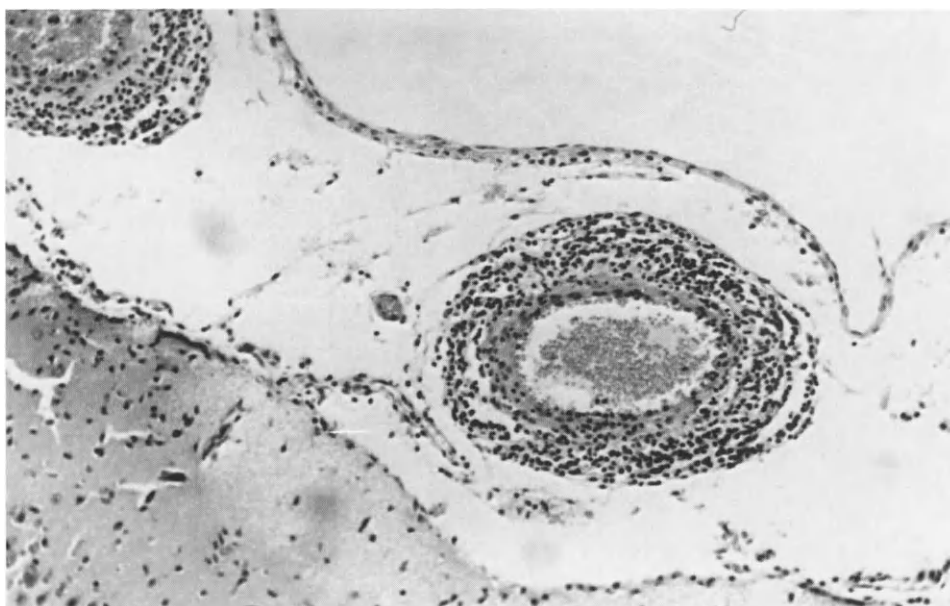
Fig. 110. Periarteritis; cerebral meninges of newborn lamb from a ewe infected with BDV at 120 days of gestation; the infiltrate is composed mainly of lymphocytes and macrophages; the lesion is usually nodular and persistent; H&E, × 130.

Intercurrent infections are frequently implicated in the mortality amongst older recovering "hairy shaker" lambs and may complicate assessment of the pathological changes. However, characteristic changes have been recognized (Barlow et al., 1983), e.g. hyperplastic ulcerative typhlocolitis with heavy lymphoid infiltrations of the mucosa and submucosa (Fig. 111), periventricular encephalitis (Fig. 112) and focal lymphoid infiltrations in visceral organs (Figs. 113, 114). These lymphoproliferative changes are associated with intractable scour and/or respiratory disease. Concurrently cytopathogenic virus is isolated from blood, whereas previous isolates are invariably noncytopathogenic (Gardiner et al., 1983). The mechanisms involved in the apparent disturbance in the equilibrium between virus and tolerant host are as yet unclear.

In the fetus, the immune response varies according to the gestational age at infection and the developmental state of the immune system at that time. Fetuses are usually immunologically unresponsive to BDV when the ewe is infected at less than 70–80 days of gestation, and the surviving fetuses become tolerant persistent excretors of virus. However, under some circumstances, fetuses at this early stage of development have responded with intense inflammatory reactions in the CNS, resulting in the intracranial malformations described. Of these the majority that survive to term have virus neutralizing antibody in their serum prior to sucking, and no virus is isolated from them. The conditions that give rise to this altered response are poorly understood; however, it has been reproduced experimentally by injecting susceptible pregnant ewes with BDV that had been preincubated with antibody to BVDV, or by injecting pregnant ewes with BDV following infections with BVDV or another strain of BDV. This has led to the conclusion that virus combined with related but heterologous antibody may in some way stimulate a precocious immune response in the fetus.

Fetuses that become infected after 80 days of gestation are usually immunologically responsive to BDV. Serum neutralizing antibody was found in 19 out of 40 progeny of ewes infected at 90 days of gestation. A cellular response to viral antigen in the adventitia of small and medium-sized arteries has also
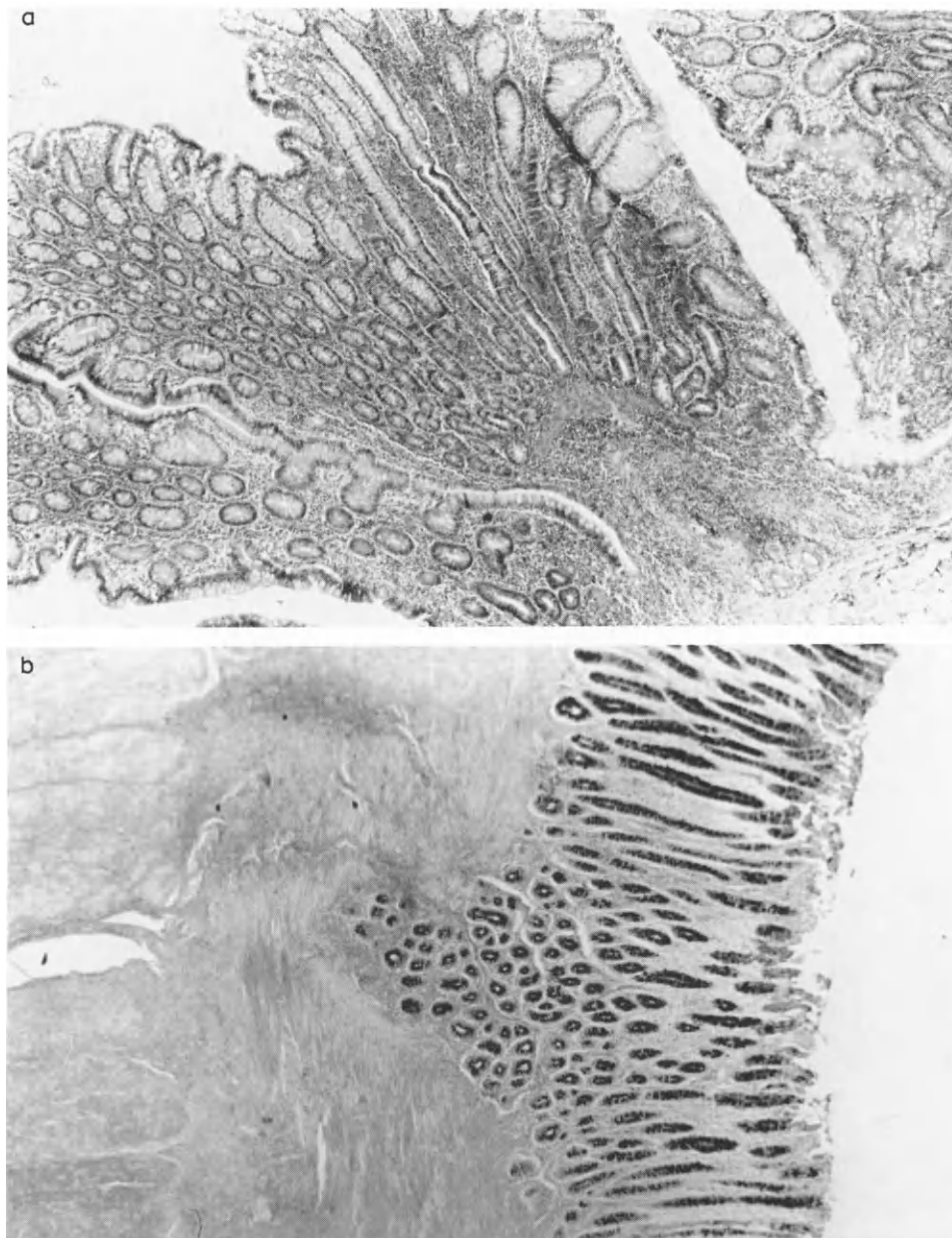
Fig. 111. (a) Polypoid hyperplasia of mucosa of the cecum in a scouring "recovered" hairy shaker lamb; H&E, × 35. (b) Hyperplastic mucosa displacing the muscle coats of the cecum; Alcian blue/PAS, × 25.

been demonstrated within 17 days of infection in late pregnancy. This has been confirmed as a cell-mediated allergic reaction by specific cutaneous hypersensitivity to BD viral antigens in the newborn lamb.

LABORATORY DIAGNOSIS

The best materials for specific diagnosis of BD are of course the newborn affected lamb, alive and unsuckled, if possible together with clotted blood samples from the mother and a number of her flockmates. These should be sent to a laboratory which has facilities for virus isolation, IF examination of cryostat sections, neuropathology and serology. The most rapid and accurate
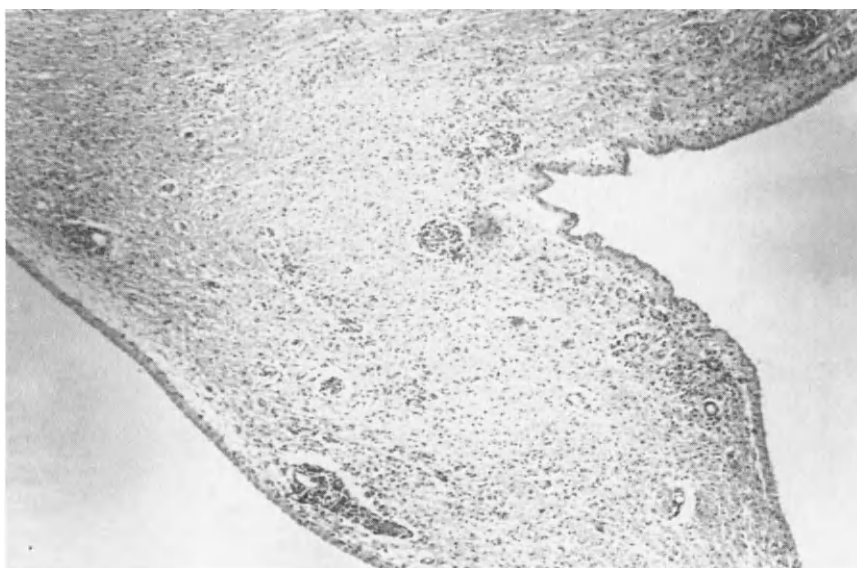
Fig. 112. Periventricular encephalitis in "recovered" hairy shaker lamb; the walls of the ventricles and the interventricular septum show perivascular cuffing by lymphocytes and a diffuse gliosis; H&E, × 100.

diagnostic method is IF microscopy on cryostat sections of small pieces of tissue, e.g. kidney, gonad or cerebellum, which have been quenched in liquid nitrogen, or a "dry-ice"/isopentane freezing mixture.

These tissues together with blood clots are valuable for virus isolation in tissue culture and if taken in the field should be sent to the laboratory in transport medium as soon as possible.

For neuropathological examination the entire CNS should be removed carefully and fixed in Baker's calcium formol (10% formalin + 1% anhydrous calcium chloride). Phosphate-buffered formalin should be avoided, as it may form soluble soaps with some components of myelin and result in the loss of diagnostically valuable lipid droplets from histological preparations.

Serological examination for BDV antibodies, on its own, is the least valuable diagnostic method even when paired samples are obtained. This is because

Fig. 113. Nonsuppurative myocarditis in a scouring "recovered" hairy shaker lamb; H&E, × 100.
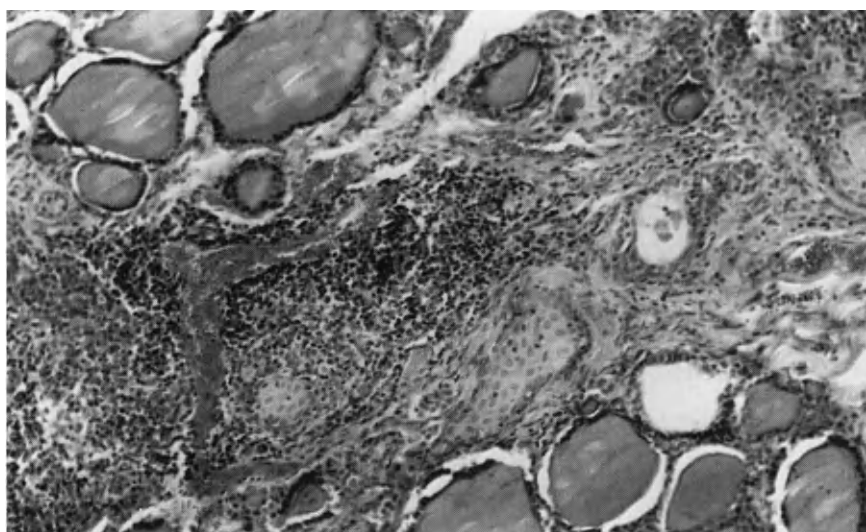
Fig. 114. Thyroiditis in a scouring "recovered" hairy shaker lamb; H&E, × 100.

even in a new disease outbreak maternal and in-contact ewe titers will probably have reached a plateau by the time samples are collected and will only indicate prior exposure to the virus. Titers in suckled lambs are also of little value, as they simply reflect maternal antibody status. The presence of antibody in precolostral lamb blood is of course diagnostic, but it must be remembered that the typical "hairy shaker" lamb is usually immunologically tolerant to BDV. Isolation of virus from the lamb and absence of antibody in its mother would indicate that the latter is a tolerant carrier and possibly responsible for introducing infection to the flock.

PROPHYLAXIS AND CONTROL

It has been shown that infection with BDV confers immunity to challenge with the same strain during subsequent pregnancies. Experimentally both live and killed vaccines have been prepared and have afforded protection to the fetus in the face of maternal challenge with virus of the same strain. However, ewes with immunity to one BDV strain may be susceptible to another strain and give birth to "camel-legged" lambs with gross intracranial malformation or to "hairy shaker" lambs (Gardiner, 1982).

Most new outbreaks of BD result from livestock movements at the beginning of the breeding season. The introduction of acutely infected or carrier sheep (and cattle) is the most likely source of infection (Terlecki, 1977). Direct evidence implicating wild animals in the introduction of BD is not available, though there is serological evidence for infection in several species of deer. In the prevention of BD it would be prudent to allow close cohabitation of the breeding flock with introduced ruminant stock well before the breeding season commences so that all animals have the opportunity to become immune before becoming pregnant.

In the event of BD appearing early in the lambing season complete segregation of later lambing groups should be undertaken; this measure may not be effective in controlling disease, as infection already may be widespread. After lambing, affected lambs and breeding stock, including new additions, should be closely mixed to allow maximum opportunity for spread of infection and development of immunity before the next breeding season. All surviving BD

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lambs should be disposed of to slaughter before the next breeding season. If flock size permits, breeding stock can be examined at this time for virus neutralizing serum antibody, and blood clots from seronegative animals can be cultured in order to identify carrier animals.

These methods are time consuming and expensive and will not protect against the introduction of another strain of BDV. In small flocks or in flocks where small outbreaks have occurred and segregation procedures have been effective it may be satisfactory simply to slaughter all members of affected families.

REFERENCES

Barlow, R.M., 1982. Epidemiology. In: R.M. Barlow and D.S.P. Patterson (Editors), Border Disease of Sheep: A Virus Induced Teratogenic Disorder (Fortschritte der Veterinärmedizin, Heft 36). Paul Parey, Berlin, Hamburg, pp. 70–71.

Barlow, R.M., Gardiner, A.C. and Nettleton, P.F., 1983. The pathology of a spontaneous and experimental mucosal disease-like syndrome in sheep recovered from clinical border disease. J. Comp. Pathol., 93: 451 461.

Bielefeldt-Ohmann, H. and Bloch, B., 1982. Electron microscopic studies of bovine virus diarrhoea virus in tissues of diseased calves and in cell cultures. Arch. Virol., 71: 57–74.

Chasey, D. and Roeder, P.L., 1981. Virus-like particles in bovine turbinate cell infected with bovine virus diarrhoea-mucosal disease virus. Arch. Virol., 67: 325–332.

Gardiner, A.C., 1982. Immunity and protection. In: R.M. Barlow and D.S.P. Patterson (Editors), Border Disease of Sheep: A Virus Induced Teratogenic Disorder (Fortschritte der Veterinärmedizin, Heft 36). Paul Parey, Berlin, Hamburg, pp. 65–69.

Gardiner, A.C., Nettleton, P.F. and Barlow, R.M., 1983. Virology and immunology of a spontaneous and experimental mucosal disease-like syndrome in sheep recovered from clinical border disease. J. Comp. Pathol., 93: 463–469.

Hamilton, A. and Timoney, P.J., 1972. BVD virus and "Border disease". Vet. Rec., 91: 468.

Harkness, J.W. and Vantsis, J.T., 1982. Virology. In: R.M. Barlow and D.S.P. Patterson (Editors), Border Disease of Sheep: A Virus Induced Teratogenic Disorder (Fortschritte der Veterinärmedizin, Heft 36). Paul Parey, Berlin, Hamburg, pp. 56–64.

Orr, M.B., 1982. Pathology of the skin and fleece. In: R.M. Barlow and D.S.P. Patterson (Editors), Border Disease of Sheep: A Virus Induced Teratogenic Disorder (Fortschritte der Veterinärmedizin, Heft 36). Paul Parey, Berlin, Hamburg, pp. 13–21.

Richardson, C., 1982. Morphology. In: R.M. Barlow and D.S.P. Patterson (Editors), Border Disease of Sheep: A Virus Induced Teratogenic Disorder (Fortschritte der Veterinärmedizin, Heft 36). Paul Parey, Berlin, Hamburg, pp. 46–52.

Terlecki, S., 1977. Border disease: a viral teratogen of farm animals. Vet. Annu., 17: 74 79.

Chapter 25

Louping-ill Virus

H.W. REID

INTRODUCTION

Louping-ill virus is a tick-transmitted *Flavivirus* that causes acute meningoencephalomyelitis in sheep and other species. Until recently the disease was assumed to be restricted to the British Isles, but it has now been diagnosed in Norway, Spain, Bulgaria and Turkey as well (Reid, in press). The name is derived from the Scottish vernacular term "to loup" meaning "to leap".

Louping-ill virus belongs to a group of eight antigenically closely related viruses known collectively as the tick-borne encephalitis (TBE) complex. These viruses are distributed throughout the northern temperate latitudes and comprise the Central European TBE, Far Eastern Russian TBE, Omsk hemorrhagic fever, Kyasanur Forest disease, Langat, Powassan, Negishi, and louping-ill viruses (Clarke, 1964). Differentiation of the TBE complex by antigenic analysis alone is not easy, but glycoprotein and peptide mapping clearly identify louping-ill as a distinct entity within the group (Heinz and Kunz, 1981, 1982).

There is evidence that 32 vertebrate species can be infected with louping-ill virus. Probably all species may be regarded as susceptible to infection, and likewise all tick species tested could transmit virus suggesting an equally catholic vector potential. Most infections are accompanied by negligible clinical responses; natural disease following infection has been described in sheep (Stevenson, 1807; M'Fadzean, 1894), cattle (Dunn, 1952), horse (Fletcher and Galloway, 1937), dog (MacKenzie et al., 1973), pig (Bannatyne et al., 1980), red deer (*Cervus elaphus*) (Reid et al., 1978a), roe deer (*Capreolus capreolus*) (Reid et al., 1976), red grouse (*Lagopus scoticus*) (Reid et al., 1978b) and man (Brewis et al., 1949; Williams and Thorburn, 1962). Clinical disease is most frequent in sheep; despite the potential of other tick species to transmit the virus, *Ixodes ricinus* would appear to be the sole natural vector.

VIRUS PROPERTIES

The morphology of louping-ill virus is typical of a *Flavivirus*. Replication occurs entirely in the cytoplasm within compartments of the endoplasmic reticulum or Golgi apparatus (Doherty et al., 1971a). The virus is thermolabile, sensitive to organic solvents, detergents and acidic conditions, but is stable at alkaline pH values and at low temperatures (little infectivity being lost at −80°C over extended periods of storage) and withstands lyophilization.

The virus will agglutinate gander, rooster and pigeon red cells (Clarke and Casals, 1958). Hemagglutinin may be prepared from infected suckling mouse brains or from infected tissue culture (BHK-21) supernates. Although hemagglutination can be demonstrated in suitable preparations without extraction,

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activity is greatly augmented following cold acetone extraction. Antigens suitable for CF, ID and ELISA tests can also be easily prepared from infected brains or tissue culture.

Virus may be propagated in a wide variety of cultured cells, including those of chick embryo (Wilson, 1945), pig kidney (Williams, 1958; Madrid and Porterfield, 1969), sheep kidney, baby hamster kidney (BHK-21) (Brotherston and Boyce, 1969; Brotherston et al., 1971). and a number of tick cell lines (Pudney et al., 1978). For detecting infectivity and neutralizing antibody, a plaque test using the pig kidney cell line IB/RS2 (clone 60) under an overlay containing sodium carboxymethyl cellulose has proved to be of greatest utility (Reid and Doherty, 1971b), while infected BHK-21 cell cultures have been found to produce high titers of hemagglutinin. Virus may also be propagated by intracerebral inoculation of suckling or 3-week-old mice (Alston and Gibson, 1931).

On evidence obtained by analyzing isolates from a variety of species and from different geographical locations by plaque morphology, neutralization, HI and pathogenesis studies it was concluded that the virus is homogenous throughout its distribution (Reid, 1984); reports of variation between isolates (Williams et al., 1963; Timoney, 1971) have probably arisen through failure to standardize the methodology employed. However, recent studies with a panel of monoclonal antibodies have indicated the presence of several biotypes (H.W. Reid, unpublished observations, 1988).

The virus is also very stable in vitro and no attenuation for sheep occurred after 170 passages in chick embryo cultures (Wilson, 1945).

EPIZOOTIOLOGY

The epizootiology of arthropod-transmitted viruses is determined by the interaction of vector and vertebrate characteristics. Detailed analysis of the biology of the vector is beyond the scope of this chapter, but further information is contained in specialist reviews (Campbell, 1952; Reid, 1984).

The distribution and seasonal occurrence of louping-ill will be dictated by the prevalence of the vector. The only identified vector, *I. ricinus*, is a three-host tick, requiring a single blood meal at each stage and completing its life cycle in 3 years. Active feeding is restricted to approximately 3 weeks of this 3-year life cycle, the remainder of the time being spent in the vegetational mat close to the soil where the relative humidity is close to saturation. The requirement for such microclimatic conditions restricts the distribution of *I. ricinus* to the rough grazings of the UK. The activity of ticks follows a distinct seasonal pattern, there being few questing ticks through the winter months, but when the threshold temperature for activity is exceeded in spring the numbers of questing ticks rapidly increase, reaching peaks in April or May. Those ticks that fail to gain access to a host will desiccate and die, while those that have fed fall back into the vegetation, where the blood meal is digested. Molting and egg laying are completed over the summer months, during which time there are no active ticks. The resultant flat ticks will not generally feed again until the following spring. The period of tick activity in the autumn that occurs in some parts of the country is due to a separate population of ticks that overwinters as replete ticks, molts through the summer and quests in the autumn.

The host range of *I. ricinus* is wide and the immature stages will feed on any available vertebrate. Thus all vertebrates present in the ticks' habitat may be parasitized and infected with louping-ill virus. Ticks acquire virus when they engorge on a viremic host; high blood titers of virus are required before it can establish infection in the cells of the tick and translocate to the salivary gland of the subsequent stage. Threshold titers for larvae were found to be in the

order of 10^4 plaque-forming units per 0.2 ml of blood and approximately 10-fold less for nymphs (Beasley et al., 1978). As transovarial transmission does not occur, infection of the adult stages is irrelevant.

It is therefore apparent that the intensity and duration of the host's viremia will determine its epizootiological relevance. While all vertebrates may be infected, only those species that develop viremias in excess of threshold titers are involved in the maintenance of louping-ill virus. To assess the potential role of candidate hosts in the epizootiology of louping-ill the course of experimental infection was examined in a variety of mammalian and avian species (Table 22; Reid, 1975, 1978, 1984, Reid et al., 1983). Virus was injected subcutaneously and the intensity and duration of the viremia was determined. The possible impact that any species might have on the epizootiology can be assessed from the number of days when threshold titers to larvae and nymphs were exceeded. From these data it is apparent that threshold titers generally were not achieved and that infection of most species has little or no biological relevance. Of the mammalian species only the sheep regularly developed viremias in excess of threshold titers and therefore can be regarded as a potential maintenance host. While three grouse species (ptarmigan, willow grouse and red grouse) also developed substantial viremias, only red grouse inhabit areas infested with *I. ricinus*; thus ptarmigan and willow grouse are unlikely to become infected under natural circumstances. In contrast, infection does occur in red grouse (Williams et al., 1963), a species peculiar to the British Isles, inhabiting the heather (*Calluna* spp.) dominated upland grazings where ticks can be prevalent.

However, of the experimentally infected red grouse 80% died with evidence of acute virus encephalitis; when wild populations in louping-ill enzootic areas were examined, virus infection was found to account for a mortality in excess of the reproductive capacity of the grouse. It was therefore concluded that infection of red grouse could have only a temporary, local amplifying effect on virus circulation and that in enzootic areas grouse died out.

Thus the only vertebrate species identified in these studies as capable of regularly transmitting virus to the tick was the sheep. No evidence of a cycle involving wild native vertebrates and the tick was forthcoming, and it was concluded that the maintenance of louping-ill virus is essentially dependent on a tick ↔ sheep cycle. This conclusion is consistent with the marked susceptibility of the red grouse, the habitat of which was free of louping-ill prior to the introduction of sheep during the 19th century. Before that time red grouse had not encountered infection and they represent a naive population in which no innate resistance was evolved.

Further confirmation of the importance of the sheep ↔ tick maintenance cycle has emerged from two studies on islands off the west coast of Scotland from which louping-ill appears to have been eradicated by eliminating susceptible sheep. On one island all sheep were removed, and while ticks have remained prevalent on resident deer, the absence of antibody to louping-ill virus in their serum suggests that the virus has been eliminated (Adam et al., 1977). On the other island all sheep were systematically vaccinated over a period of 3 years, at the end of which time the absence of seroconversion in sentinel cattle and of clinical disease in sheep indicated that louping-ill has been eliminated.

Thus there is no evidence to support the assumption that louping-ill is maintained in a cycle between the vector ticks and native wild vertebrates as proposed by Williams et al. (1963) and Smith et al. (1964b).

Following challenge with virus only a few sheep develop fatal encephalitis; surviving animals acquire a life-long immunity which can be transferred in colostrum to lambs, resulting in solid protection (Reid and Boyce, 1976). At the

TABLE 22

Viremia in experimental louping-ill infection of some vertebrates

Species	No. examined	No. days when infective[a]		Mean maximum titer[b]
		Larvae	Nymphs	
Red fox (*Vulpes vulpes*)	8	0	0	1.6 (1.4–2.4)
Wood mouse (*Apodemus sylvaticus*)	28	0	0	2.2 (1.6–2.9)
Bank vole (*Clethrionomys glareolus*)	25	0	0	1.6 (0.0–2.8)
Field vole (*Microtus agrestis*)	59	0.07	0.46	2.2 (0.0–4.4)
Brown rat (*Rattus norvegicus*)	3	0	0	0.43 (0.0–1.3)
Blue hare (*Lepus timidus*)	3	0	0	0.43 (0.0–1.3)
Cattle (*Bos taurus*)	6	0	0.17	1.3 (0.1–3.0)
Goat (*Capra aegagrus*)	7	0.14	0.42	2.6 (1.6–4.0)
Sheep (*Ovis aries*)	33	2.25	3.09	5.6 (3.2–7.1)
Red deer (*Cervus elaphus*)	4	0	0	1.2 (0.8–1.8)
Roe deer (*Capreolus capreolus*)	3	0	0.33	2.6 (2.3–3.2)
Red grouse (*Lagopus lagopus scoticus*)	27	3.48	5.04	5.3 (3.7–6.9)
Ptarmigan (*Lagopus mutus*)	7	5.71	7.43	6.2 (5.7–7.4)
Willow grouse (*Lagopus lagopus*)	5	4.20	5.60	4.8 (4.3–5.7)
Black grouse (*Tetrao tetrix*)	4	0	0	2.5 (2.4–2.6)
Capercaillie (*Tetrao uragallus*)	7	0	0.29	2.2 (0.7–3.5)
Pheasant (*Phasianus colchicus*)	5	0	0	1.5 (0.0–2.7)

[a]Total number of days when viremia exceeded threshold titers for larvae and nymphs, respectively.
[b]pfu/0.2 ml of whole blood or plasma; range given in parentheses.

start of the spring period of tick activity the sheep flock consists of breeding ewes with lambs and 1-year-old ewe lambs to be used as replacement breeding stock. The efficiency with which colostrum-derived antibody protects lambs ensures that there will be an equal proportion of susceptible ewes and lambs. However, because adult sheep can host more ticks, infection in the older animals is epizootiologically more significant than in lambs. Where the majority of the breeding stock is immune, most of the lambs will also be immune by passively acquired antibody. While this antibody will provide solid protection through the first season of exposure, such animals will be fully susceptible the following year. Thus the minimum number of susceptible sheep in a flock is represented by the replacement breeding stock, which is unlikely to consist of less than 1/5 of the sheep. Infections in this number of sheep are adequate to ensure perpetuation of the virus. Tick activity declines during the summer months to reappear in some parts of the country in the autumn, when most of the lambs will no longer be protected by colostral antibody. However, as lambs tend to be weaned and removed from the flock by the time of this recrudescence, maintenance of virus in this population of ticks is dependent on the availability of susceptible adult sheep. Virus survives in replete and molting ticks during interepizootic periods.

Although infection is normally transferred by inoculation of virus by infected ticks it can also be established by ingestion. An outbreak of disease in pigs occurred after they had been fed lambs that had died from louping-ill (Bannatyne et al., 1980); goat kids became infected and died after sucking virus-infected nanny goats which were excreting virus in their milk (Reid et al., 1984). However, lambs that had ingested milk from louping-ill virus infected ewes did not become infected despite the presence of high virus titers (Reid and Pow, 1985).

PATHOGENESIS

Louping-ill virus causes an acute meningoencephalomyelitis which has been studied in detail in sheep (Doherty and Reid, 1971a, b; Reid and Doherty, 1971a, b; Doherty et al., 1971b, 1972; Reid et al., 1971; Doherty and Vantsis, 1973). Following peripheral inoculation, initial viral replication occurs in the drainage lymph nodes and viremia can be determined within 24 h thereafter, rising exponentially for 2–4 days (Fig. 115). Viremia then declines precipitously and is associated with the appearance of neutralizing and HI antibody.

Invasion of the CNS invariably occurs early in infection, and while titers in the periphery continue to decline, titers in the CNS reach maxima later and decline more slowly. Elimination of virus from the CNS is also dependent on immune mechanisms. High titers of viral antibody can be found in the cerebrospinal fluid of acutely affected and recovered animals. This antibody, which is responsible for eliminating virus from the brain, is synthesized within the CNS and almost certainly originates from cells with the ultrastructural and immunohistological characteristics of antibody-secreting B-cells that have been recruited into the perivascular spaces. Immunosuppressed sheep tend to survive longer (Reid et al., 1981) but the proportion that develops fatal encephalitis is greater. Thus, as with other acute viral encephalitides, the immune system may precipitate death of virus-infected cells, causing earlier mortality; but without an effective immune response to restrict virus spread more neurons become infected and death is the inevitable outcome due to extensive, virus-induced cytolysis of neurons.

Before it was realized that viral invasion of the CNS is probably an inevitable consequence of infection it was considered that virus had gained access to the brains of only those animals that succumbed with acute encephalitis.

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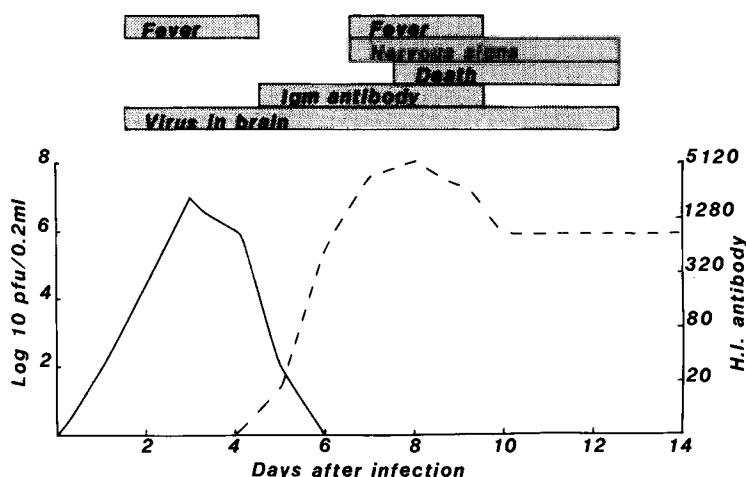
Fig. 115. Schematic diagram of the course of louping-ill virus infection of sheep; (———) virus in blood; (— — —) serum antibody.

Great emphasis was therefore placed on factors that might facilitate access of virus to the CNS, and concurrent tick-borne-fever (*Cytoecetes phagocytophila*) was considered to be a predisposing factor (Gordon et al., 1962; Smith et al., 1964a). It was suggested that this normally benign infection affects the permeability of the vasculoendothelium of the blood-brain barrier. Experimental infection of sheep with both organisms produced a much higher mortality in the dually infected animals, but this was attributed to a profound immunosuppression induced by the combined infection with death resulting from systemic mycotic invasion (Reid et al., 1986).

DISEASE SIGNS

The clinical signs are typical of an acute virus-induced meningoencephalomyelitis primarily affecting locomotor functions (see Fig. 115). During the initial visceral phase of infection few clinical signs are detected—the rectal temperature may be raised and the animal may appear dull and inappetent, but frequently no abnormalities are detected. The subsequent clinical course is very variable: the majority of infected sheep exhibit no further symptoms and make an uncomplicated recovery while a variable proportion develop signs of neurological dysfunction, which may vary from transient ataxia and spasmodic muscular contractions to sudden death. The first signs are nervous nibbling and smacking of the lips accompanied by ataxia, particularly of the hind limbs, progressing to recumbency. Terminally affected animals paddle wildly before becoming comatose, death occurring within 24–48 h of initial signs being apparent. In a proportion of recovered animals residual paresis affecting the limbs or neck may last for weeks or months following the acute phase of the disease. The clinical signs in other ruminants are similar, though in cattle the course is more protracted, with affected animals becoming recumbent but continuing to eat and drink for several days. Animals may recover if attentively nursed.
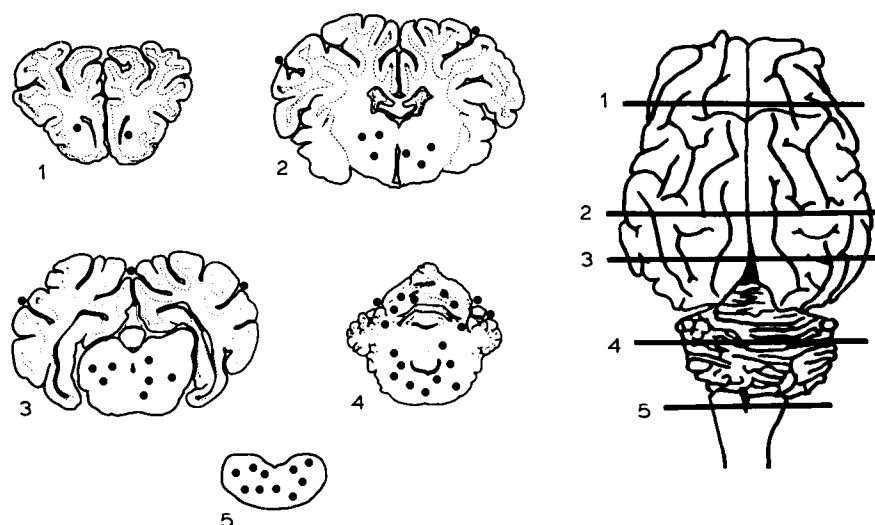
Fig. 116. Distribution of neuropathological changes in the brain of a sheep with louping-ill.

PATHOLOGY

Specific lesions in sheep affected with louping-ill are restricted to the CNS, although not infrequently a terminal bacterial pneumonia may develop. Lesions which consist of a nonsuppurative encephalomyelitis are most pronounced in the brain stem and ventral horns of the spinal cord, while the forebrain is relatively unaffected (Fig. 116). The lesions consist of neuronal necrosis, neuronophagia, focal gliosis and perivascular cuffing by lymphoid cells. The distribution of viral antigen and infective virus within the brain correlates with the distribution of neuronal necrosis, and it has been concluded that cell death is a direct consequence of viral cytolysis. The perivascular accumulations of inflammatory cells contain globulin-producing (IgM and IgG) cells with the ultrastructural characteristics of plasmacytes (Doherty et al., 1971). These are apparently responsible for the viral antibody within the CNS which is believed to limit virus spread and neuronal destruction. In surviving animals, irrespective of the clinical severity of infection, neuropathological lesions persist for several weeks. They consist of foci of inflammation, gliosis and neuronophagia distributed throughout the CNS but most apparent in the brain stem and spinal cord.

Earlier reports of the pathology of louping-ill in sheep tended to emphasize the involvement of Purkinje cells (Brownlee and Wilson, 1932). However, this is now recognized to be a feature of the pathology following intracerebral inoculation of virus and not of the changes following peripheral infection.

LABORATORY DIAGNOSIS

Before contemplating any investigation of louping-ill it should be borne in mind that the virus can cause severe and sometimes fatal encephalitis in man. The first obligation of the diagnostician is therefore to ensure that suitable equipment and facilities are available to minimize the risk of infecting laboratory personnel. In addition, it is recommended that those anticipating regular involvement in the diagnosis of louping-ill should be vaccinated against TBE using a commercially available vaccine (Immuno AG., Industriestrasse 72, A-1200 Wien, Austria).

When the clinical features and epidemiological circumstances suggest louping-ill, laboratory confirmation should be sought.

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Serology

Antibody to louping-ill virus may be detected using gel diffusion (Thorburn and Williams, 1966), CF (Casals et al., 1951), HI (Clarke and Casals, 1958), neutralization (Reid et al., 1971), and IF tests (Doherty and Reid, 1971b). The HI test has proved of greatest utility and is regularly employed in diagnosis. Virus antigen will agglutinate trypsinized human O, rooster and pigeon red blood cells, but for large-scale testing gander cells have proved most practical. Male bird red blood cells only must be employed, as cells from females are less sensitive, particularly during egg-laying periods. Antigen may be prepared from either infected suckling mouse brain or from BHK-21 culture supernatant; to obtain maximum activity it should be extracted with cold acetone (Clarke and Casals, 1958). Before testing sera, nonspecific inhibitors must be removed using either cold acetone or acid-washed kaolin, the latter method being the most convenient, economic and best suited for routine use.

The presence of IgM class antiviral antibody in serum can readily be detected by comparing the HI antibody in whole serum and in serum that has been heated to 62.5°C for 30 min. A 4-fold or greater reduction in the titer of the heated sample implies the presence of IgM antibody and hence recent virus infection. Unfortunately, most of the antibody present from about day 10 p.i. is IgG; hence the detection of IgM is of diagnostic value only in early cases, and failure to detect IgM antibody does not exclude louping-ill. In cases that recover examination of paired serum samples is of value.

Virus isolation

Definitive diagnosis relies on the isolation of virus from the CNS. Highest virus titers are present in the brain stem and cord, which therefore are the tissues of choice for virus isolation attempts. As the virus is thermolabile and susceptible to acid pH, 50% glycerol saline should be used as a transport medium, and precautions should be taken to ensure that the material is kept cool. Clarified 10% w/v homogenates of the material are inoculated either directly onto monolayer cultures of the pig kidney cell line IB/RS2 (clone 60) or intracerebrally into 3-week-old-mice. By incubating infected monolayers under an overlay containing carboxymethyl cellulose, plaques will develop within 3–4 days. If duplicate plates containing antiserum to louping-ill virus are also inoculated, virus will be neutralized and no plaques will develop allowing a specific diagnosis.

Within 6–14 days p.i. mice become excited, then hunched, develop posterior paralysis and die after approximately 24 days. If suckling mice are employed death occurs earlier but may be confused with nonspecific effects, which makes isolation by this method cumbersome. In either case the presence of virus in brains of mice should be confirmed by preparing hemagglutinin for HI tests or by plaque neutralization in tissue culture.

Histopathology

The identification of typical neuropathological lesions can also be useful in reaching a diagnosis of louping-ill. That portion of the brain that is not used for virus isolation should be fixed in 10% formol saline. A series of blocks is then prepared from samples of brain taken from at least five levels and sections cut and stained with H & E. The detection of typical lesions distributed in the brain as described above permits a presumptive diagnosis in areas where louping-ill is the only agent recognized to cause nonsuppurative meningoencephalomyelitis.

PROPHYLAXIS AND CONTROL

Shortly after the isolation of louping-ill virus in 1931 a vaccine consisting of formalinized infected sheep brain, cord and spleen was developed (Gordon, 1934). This vaccine did not cause seroconversion but was suggested to prime animals to viral antigen, which ensured that on subsequent infection serum antibody developed rapidly in the absence of clinical signs.

Such tissue-derived vaccines suffer from the risk of contamination by adventitious agents, and in 1937 a disastrous outbreak of scrapie occurred in sheep that had received one batch of the vaccine which had been prepared from the brains of eight lambs whose dams subsequently developed scrapie (Gordon, 1946). A further disadvantage of this vaccine was that its preparation was hazardous for the laboratory personnel, and after an incident in which three technicians became seriously ill production was terminated.

A vaccine using tissue-culture-propagated virus was developed and tested in the field (Brotherston et al., 1971). This vaccine consists of formalin-inactivated virus from BHK-21 cell cultures concentrated by ultrafiltration and incorporated in an emulsion with an oil adjuvant. This vaccine stimulates the production of serum antibody, and a single inoculation is sufficient to provide lifelong immunity. Normally only the sheep stock to be retained for breeding or that have been introduced from tick-free properties are vaccinated, colostrum-derived antibody being relied on to protect lambs. Vaccine should be given at least 14 days before introducing animals to tick-infested pasture to ensure that they are immune prior to exposure.

Systematic vaccination of sheep flocks should interrupt the circulation of louping-ill virus by eliminating susceptible hosts in that area and, after 2–3 years, result in its eradication. However, unless the spread of ticks on straying domestic or wild vertebrates from contiguous properties can be prevented it would be unwise to terminate a vaccination regime.

Methods designed to control the vector of louping-ill virus are beyond the scope of this book and have been considered by Reid (1984).

REFERENCES

Adam, K.M.G., Beasley, S.J. and Blewett, D.A., 1977. The occurrence of antibody to *Babesia* and to the virus of louping-ill in deer in Scotland. Res. Vet. Sci., 23: 133–138.

Alston, J.M. and Gibson, H.J., 1931. A note on the experimental transmission of louping-ill to mice. Br. J. Exp. Pathol., 12: 82–88.

Bannatyne, C.C., Wilson, R.L., Reid, H.W., Buxton, D. and Pow, I., 1980. Louping-ill virus infection in pigs. Vet. Rec., 106: 13.

Beasley, S.J., Campbell, J.A. and Reid, H.W., 1978. Threshold problems in infection of *Ixodes ricinus* with the virus of louping-ill. In: J.K.H. Wild (Editor), Tick-borne Diseases and Their Vectors. Edinburgh University Press, Edinburgh, pp. 497–499.

Brewis, E.G., Neubauer, C. and Hurst, E.W., 1949. Another case of louping-ill in man, isolation of the virus. Lancet, 1: 689–691.

Brotherston, J.G. and Boyce, J.B., 1969. A new vaccine against louping-ill. Vet. Rec., 84: 514–515.

Brotherston, J.G., Bannatyne, C.C., Mathieson, A.O. and Nicolson, T.B., 1971. Field trials of an inactivated oil-adjuvant vaccine against louping-ill (*Arbovirus* group B). J. Hyg. (Camb)., 69: 479–489.

Brownlee, A. and Wilson, D.R., 1932. Studies in the histopathology of louping-ill. J. Comp. Pathol. Therap., 45: 67–92.

Campbell, J.A., 1952. Recent work on the ecology of the pasture tick *Ixodes ricinus* L. in Britain. Rep. 14th Int. Vet. Congr. Lond., 1949, 2: 113–119.

Casals, J., Olitsky, P.K. and Anslow, R.O., 1951. A specific complement-fixation test for infection with poliomyelitis virus. J. Exp. Med., 94: 123–137.

Clarke, D.H., 1964. Further studies on antigenic relationships among the viruses of the group B tick-borne complex. Bull. World Health Org., 32: 45–56.

Clarke, D.H. and Casals, J., 1958. Techniques for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg., 7: 561–573.

Doherty, P.C. and Reid, H.W., 1971a. Experimental louping-ill in sheep and lambs. II. Neuropathology. J. Comp. Pathol., 81: 331–337.

Doherty, P.C. and Reid, H.W., 1971b. Louping-ill encephalomyelitis in the sheep. II. Distribution of virus and lesions in nervous tissue. J. Comp. Pathol., 81: 531–536.

Doherty, P.C., Smith, W. and Gray, E.W., 1971a. Necrosis of infant hamster cerebellum due to a tick-borne encephalitis virus. J. Neurol. Sci., 14: 215–224.

Doherty, P.C., Reid, H.W. and Smith, W., 1971b. Louping-ill encephalomyelitis in the sheep. IV. Nature of the perivascular inflammatory reaction. J. Comp. Pathol., 81: 545–549.

Doherty, P.C., Smith, W. and Reid, H.W., 1972. Louping-ill encephalomyelitis in the sheep. V. Histopathogenesis of the fatal disease. J. Comp. Pathol., 82: 337–344.

Doherty, P.C. and Vantsis, J.T., 1973. Louping-ill encephalomyelitis in the sheep. VII. Influence of immune status on neuropathology. J. Comp. Pathol., 83: 481–491.

Dunn, A.M., 1952. Louping-ill. A study of the disease in cattle. PhD. Thesis, Edinburgh University, Edinburgh, 136 pp.

Fetcher, J.M. and Galloway, I.A., 1937. Louping-ill in the horse. Vet. Rec., 49: 17–19.

Gordon, W.S., 1934. The control of certain diseases of sheep. Vet. Rec., 14: 1–8.

Gordon, W.S., 1946. Advances in veterinary research. Vet. Rec., 58: 516–525.

Gordon, W.S., Brownlee, A., Wilson, D.R. and MacLeod, J., 1962. The epizootiology of louping-ill and tick-borne fever with observations on the control of these sheep diseases. Symp. Zool. Soc. Lond., 6: 1–27.

Heinz, F.X. and Kunz, C., 1981. Homogenicity of the structural glycoprotein from European isolates of tick-borne encephalitis virus: comparison with other flaviviruses. J. Gen. Virol., 57: 263–274.

Heinz, F.X. and Kunz, C., 1982. Molecular epidemiology of tick-borne encephalitis virus: Peptide mapping of large non-structural proteins of European isolates and comparison with other flaviviruses. J. Gen. Virol., 62: 271–285.

M'Fadzean, J., 1894. Louping-ill in sheep. J. Comp. Pathol. Therap., 13: 145–154.

MacKenzie, C.P., Smith, S.T. and Muir, R.W., 1973. Louping-ill in a working collie. Vet. Rec., 92: 354–356.

Madrid, A.T. de, and Porterfield, J.S., 1969. A simple microculture method for the study of group B arboviruses. Bull. World Health Org., 40: 113–121.

Pudney, M., Varma, M.G.R. and Leake, C.J., 1978. The growth of some arboviruses in tick cell lines. In: J.K.H. Wild (Editor), Tick-borne Diseases and Their Vectors. Edinburgh University Press, Edinburgh, pp. 490–496.

Reid, H.W., 1975. Experimental infection of red grouse with louping-ill virus (*Flavivirus* group). 1. The viraemia and antibody response. J. Comp. Pathol., 85: 223–229.

Reid, H.W., 1978. The epidemiology of louping-ill. In: J.K.H. Wild (Editor), Tick-borne Diseases and Their Vectors. Edinburgh University Press, Edinburgh, pp. 501–507.

Reid, H.W., 1984. Epidemiology of louping-ill. In: M.A. Mayo and K.A. Harrup (Editors), Vectors in Virus Biology. Academic Press, London, pp. 161–178.

Reid, H.W. (in press). Louping-ill. In: W.B. Martin and I.D. Aitken (Editors), Diseases of Sheep. Blackwell Scientific Publications, Oxford.

Reid, H.W. and Boyce, J.B., 1976. The effect of colostrum-derived antibody on louping-ill virus infection in lambs. J. Hyg. (Camb)., 77: 349–354.

Reid, H.W. and Doherty, P.C., 1971a. Experimental louping-ill in sheep and lambs. I. Viraemia and antibody response. J. Comp. Pathol., 81: 291–298.

Reid, H.W. and Doherty P.C., 1971b. Louping-ill encephalomyelitis in the sheep. 1. The relationship of viraemia and the antibody response to susceptibility. J. Comp. Pathol., 81: 521–529.

Reid, H.W. and Pow, I., 1985. Excretion of louping-ill virus in ewe's milk. Vet. Rec., 177: 470.

Reid, H.W., Doherty, P.C. and Dawson, A.M., 1971. Louping-ill encephalomyelitis in the sheep. III. Immunoglobulins in the cerebrospinal fluid. J. Comp. Pathol., 81: 537–543.

Reid, H.W., Barlow, R.M., Boyce, J.B. and Inglis, D.M., 1976. Isolation of louping-ill virus from a roe deer. (*Capreolus capreolus*). Vet. Rec., 98: 116.

Reid, H.W., Barlow, R.M., Pow, I. and Maddox, J., 1978a. Isolation of louping-ill virus from red deer (*Cervus elaphus*). Vet. Rec., 102: 463–464.

Reid, H.W., Duncan, J.S., Philips, J.D.B., Moss, R. and Watson, A., 1978b. Studies on louping-ill virus (*Flavivirus* group) in wild red grouse (*Lagopus lagopus scoticus*). J. Hyg. (Camb)., 81: 321–330.

Reid, H.W., Buxton, D., Gardiner, A.C., Pow, I., Finlayson, J. and MacLean, M.J., 1981. Immunosuppression in toxoplasmosis: studies in lambs and sheep infected with louping-ill virus. J. Comp. Pathol., 92: 181–190.

Reid, H.W., Buxton, D., Pow, I. and Moss, R., 1983. Experimental louping ill virus infection of black grouse (*Tetrao tetrix*). Arch. Virol., 78: 299–302.

Reid, H.W., Buxton, D., Pow, I. and Finlayson, J., 1984. Transmission of louping-ill virus in goat milk. Vet. Rec., 114: 582–584.

Reid, H.W., Buxton, D., Pow, I., Brodie, T.A., Holmes, P.H. and Urquhart, G.M., 1986. Response of sheep to experimental concurrent infection with tick-borne fever (*Cytoecetes phagocytophyla*) and louping-ill virus. Res. Vet. Sci., 41: 56–62.

Smith, C.E.G., McMahon, D.A., O'Reilley, K.J., Wilson, A.L. and Robertson, J.M., 1964a. The epidemiology of louping-ill in Ayrshire the first year of studies in sheep. J. Hyg. (Camb)., 62: 52–68.

Smith, C.E.G., Varma, M.G.R. and McMahon, D., 1964b. Isolation of louping-ill virus from small mammals in Ayrshire, Scotland. Nature (Lond.), 203: 992–993.

Stevenson, R., 1807. Cases of sickness. Trans. Highland Agric. Soc. Scotl., 3: 380–420.

Thorburn, H. and Williams, H., 1966. A serological examination of Scottish strains of louping-ill and their relation to other members of the complex. Arch. Ges. Virusforsch., 19: 155–160.

Timoney, P.J., 1971. Variation in the haemagglutinin content of crude mouse brain antigens containing louping-ill virus. Res. Vet. Sci., 12: 490–491.

Williams, H.E., 1956. Growth and titration of louping-ill virus in monolayer tissue culture of pig kidney. Nature (Lond.), 181: 497–498.

Williams, H. and Thorburn, H., 1962. Serum antibodies to louping-ill virus. Scott. Med. J., 7: 353–355.

Williams, H., Thorburn, H. and Ziffo, G.S., 1963. Isolation of louping-ill from the red grouse. Nature (Lond.), 200: 193–194.

Wilson, D.R., 1945. Studies in louping-ill. I. Cultivation of louping-ill in vitro. II. Pathogenic properties of culture virus. J. Comp. Pathol., 55: 250–267.

Chapter 26

Wesselsbron Disease Virus

B.J.H. BARNARD

VIRUS PROPERTIES

Wesselsbron virus (WBV) shares basic physical and chemical properties with other members of the *Flavivirus* genus. It has a particle diameter of 45 nm and an electron-dense core of 25 nm. It is inactivated by ordinary disinfectants.

Although only one serotype exists, it cross-reacts with other members of the *Flavivirus* genus in serological tests (Theiler and Casals, 1958). It can best be distinguished from other flaviviruses with virus neutralization tests. Primary infections are recognized by specific and relatively high titers of antibody, while secondary infections are marked by high heterologous antibody titers (Blackburn and Swanepoel, 1980). VERO and CER (chicken embryo related) cell cultures infected with WBV and other members of the flavivirus group produce more acid than noninfected cultures. This phenomenon can be utilized for virus titration (Barnard, 1984).

EPIZOOTIOLOGY

WBV has a wide host range including cattle, sheep, goats, pigs, horses, donkeys, camels, guinea-pigs, rabbits, dogs, wild birds, wild mammals and man. Serological evidence indicates widespread occurrence of WBV in African countries, including South Africa, Zambia, Zimbabwe, Malawi, Mozambique, Kenya, Chad, Nigeria and Madagascar. Despite the widespread occurrence and wide host range of WBV, the disease has a very low incidence and appears to exist in an enzootic form over the greater part of Africa. Only three outbreaks and some sporadic cases have been recorded in South Africa since 1956.

The disease is encountered almost exclusively in sheep (Weiss, 1957). In cattle it is usually limited to a mild febrile reaction in a few animals only, and in man infection may result in an influenza-like syndrome. Recovered animals are immune to reinfection, and animals previously infected by other members of the flavivirus group seem to be more resistant against experimental infection with WBV (Fagbami and Ojeh, 1981).

The disease usually manifests itself after abundant rains and a consequent increase in the arthropod vector population. *Aedes caballus* (Kokernot et al., 1958) and *A. circumluteolus* (Muspratt et al., 1957) are known vectors of the virus. Contact and/or airborne transmission to man and sheep have been described.

PATHOGENESIS

Experimental infection of lambs resulted in viremia after approximately 27 h (Coetzer et al., 1978). The viremia lasted for an average of 50 h; in adult sheep it is of shorter duration (Theodoridis and Coetzer, 1980). Although pathological lesions are restricted to the liver and lymphatic tissue, the virus could be reisolated from all organs, with the highest titers in liver, blood, spleen and brain. In cattle, viremia is not always present and, when detected, is low and of short duration.

The virus may pass the placenta in both sheep and cattle, causing abortion, congenital abnormalities or weak animals that die shortly after birth.

DISEASE SIGNS

WB disease is characterized in sheep by abortion, neonatal death and a low incidence of congenital abnormalities. However, a rise in body temperature is sometimes the only symptom. In both lambs and adult sheep a biphasic febrile reaction occurs (Coetzer et al., 1979). The first peak in temperature is of short duration, but the increase in body temperature during the second peak may last for 3–6 days. In fatal cases in lambs the symptoms are more severe and include anorexia, staring coat, lethargy, general weakness, sunken flanks and an increased respiratory rate. The perineum may be soiled with bright yellow to orange feces.

In cattle a rise in body temperature may occur and an occasional abortion with abnormal or weak calves may be encountered.

In both cattle and sheep, aborted fetuses may show arthrogryposis, porencephaly, hydranencephaly and cerebellar hypoplasia (Coetzer and Barnard, 1977).

PATHOLOGY

Macroscopical changes include mild to severe icterus, slight to moderate hepatomegaly, generalized lymphadenopathy and petechial and ecchymotic hemorrhages in the mucosa of the abomasum (Coetzer and Theodoridis, 1982). Microscopy on the liver may show a mild to extensive necrosis of the parenchyma diffusely scattered throughout the liver. Active regeneration of parenchymal cells is indicated by hepatocytes with large nuclei and mitotic figures. Other changes, for example Kupffer cell proliferation, sinusoidal leucostasis, bile duct proliferation and infiltration of mononuclear cells in the portal triads are frequently encountered. Moderate to severe cholestasis, intranuclear inclusions and intracytoplasmic acidophilic or Councilman-like bodies are frequently present.
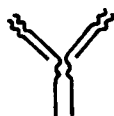
IMMUNE REACTION

Infection in sheep, cattle or goats, whether asymptomatic or accompanied by clinical signs, results in the development of antibodies (Blackburn and Swanepoel, 1980). HI antibody titers increase 2–8-fold within 1 week after infection and reach a peak at about 3 weeks, after which they decline slowly over a period of months. Peak VN titers are reached at 2–3 weeks, and these are detained much longer. Cattle develop antibodies between epizootics and at any given time more than 50% may possess detectable antibodies.

The immunity that develops after infection is generaly of lifelong duration, but antibodies may wane to undetectable levels within 2–3 years after infection.

LABORATORY DIAGNOSIS

WBV can be isolated by intracerebral inoculation of infant mice with spleen, liver, and brain suspensions prepared from dead animals or with blood collected during the febrile reaction. The mice usually die within 5–10 days. The virus can be identified by means of specific SN tests. All animals and humans that become infected develop antibodies demonstrable in their sera. Complement fixation, HI and VN tests can be used to determine antibody titers. However, cross-reactions with other flaviviruses must be borne in mind. In general, sera are broadly cross-reactive in HI, less cross-reactive in CF and virtually monospecific in VN tests. Antibodies are least cross-reactive early in infection and become progressively more cross-reactive later. It is recommended that all known flaviviruses of a region should be included in tests intended to determine the occurrence of antibody to a member of the group (Theiler and Casals, 1958). CF antibodies in serum are too transient to be used in surveys; HI titers wane more slowly. Therefore, the use in surveys of the three different tests in conjunction would allow an estimate to be made of how recently infection took place.

PROPHYLAXIS AND CONTROL

Control measures are purely prophylactic. An attenuated virus strain is used for the production of a live-virus vaccine in VERO cells. Susceptible animals can be safely vaccinated at any age, but the progeny of immune animals must not be vaccinated before they are at least 6 months old. As the vaccine may cause abortion, pregnant animals should not be immunized. A good lifelong immunity develops about 3 weeks after vaccination. Since no vaccine is completely effective, other preventive measures such as mosquito control, avoidance of low lying areas and stabling of valuable animals should also be undertaken.

REFERENCES

Barnard, B.J.H., 1984. Acid production by flavivirus infected VERO and CER cell cultures. Onderstepoort J. Vet. Res., 51: 89–90.

Blackburn, N.K. and Swanepoel, R., 1980. An investigation of flavivirus of cattle in Zimbabwe with particular reference to Wesselsbron virus. J. Hyg., 85: 1–33.

Coetzer, J.A.W. and Barnard, B.J.H., 1977. *Hydrops amnii* in sheep associated with hydranencephaly and arthrogryposis with Wesselsbron disease and Rift Valley fever viruses as aetiological agents. Onderstepoort J. Vet. Res., 44: 119–126.

Coetzer, J.A.W. and Theodoridis, A., 1982. Clinical and pathological studies in adult sheep and goats experimentally infected with Wesselsbron disease virus. Onderstepoort J. Vet. Res., 49: 19–22.

Coetzer, J.A.W., Theodoridis, A. and Van Heerden, A., 1978. Wesselsbron disease. Pathological, haematological and clinical studies in natural cases and experimentally infected new-born lambs. Onderstepoort J. Vet. Res., 45: 93–106.

Coetzer, J.A.W., Theodoridis, A., Herr, S. and Kritzinger, L., 1979. Wesselsbron disease: A cause of congenital porencephaly and cerebellar hypoplasia in calves. Onderstepoort J. Vet. Res., 46: 165–169.

Fagbami, A.H. and Ojeh, C., 1981. Wesselsbron virus infection in West African Dwarf Sheep: Effect of preinfection serum flavivirus antibodies on severity of disease. Vet. Microbiol.. 6: 287–293.

Kokernot, R.H., Paterson, H.E. and De Meillon, B., 1958. Studies on the transmission of Wessels-
bron virus by *Aedes (Ochlerotatus) caballus* (Theo.). S. Afr. Med. J., 32: 546–548.

Muspratt, J., Smithburn, K.C., Paterson, H.E. and Kokernot, R.H., 1957. Studies on arthropod-
borne viruses of Tongoland. X. The laboratory transmission of Wesselbron virus by bite of
Aedes (Banksinella) circumluteolus (Theo.). S. Afr. J. Med. Sci., 22, 121–126.

Theiler, M. and Casals, J., 1958. The serological reactions in yellow fever. Am. J. Trop. Med. Hyg.,
7: 585–594.

Theodoridis, A. and Coetzer, J.A.W., 1980. Wesselsbron disease: Virological and serological studies
in experimentally infected sheep and goats. Onderstepoort J. Vet. Res., 47: 221–229.

Weiss, K.E., 1957. Wesselsbron virus disease. Bull. Epizoot. Dis. Afr., 5: 431–458.

Coronaviridae

The characteristic club-shaped surface projections studding the virion envelope (from Latin corona = crown, wreath) have given the family its name. Serological relationships suggest two avian and two mammalian groups. Some twelve viruses are established members of this family, and several morphologically similar particles, which are referred to as coronavirus-like, have been found by electron microscopy and await detailed analysis.

Coronaviruses are pleomorphic enveloped viruses averaging 100 nm in diameter which bear widely spaced projections of about 20 nm length. The capsid is of helical symmetry.

The genome is one molecule of infectious RNA of about 7×10^6 mol.wt. Virions characteristically contain three major classes of structural proteins: the nucleocapsid protein (50–60 k), the matrix or envelope protein (20–35 k) and the surface (peplomer) protein (90–200 k). The capsid protein is phosphorylated and both the matrix and the peplomer proteins are glycosylated.

The average buoyant density is $1.18 \, g/cm^3$ in sucrose and $1.23-1.24 \, g/cm^3$ in CsCl. Coronavirions sediment at between 330 and 490 S.

Coronaviruses replicate in the cytoplasm; characteristic is the production of 3' coterminal subgenomic RNAs which form a nested set with unique sequences extending in the 5' direction. Virions bud into cisternae acquiring their lipid membranes from the cell. The virions are subsequently transported to and accumulated in Golgi vesicles. No budding from the peripheral plasma membrane occurs.

There are several coronaviruses of importance to veterinary medicine, such as avian infectious bronchitis virus, porcine transmissible gastroenteritis and hemagglutinating encephalitis viruses, feline infectious peritonitis virus, etc.; there is only one coronavirus so far known to occur in ruminants—the bovine coronavirus, initially named neonatal calf diarrhea coronavirus.

The bovine coronavirus is one of the agents of diarrhea during calfhood, the "second one" after rotaviruses. Occasionally it also participates in respiratory tract infections of calves. In adult cattle the bovine coronavirus may be the agent of a disease called winter dysentery. All the isolates of bovine coronavirus belong to a single serotype.

Chapter 27

Neonatal Calf Diarrhea Virus

C.A. MEBUS
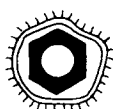
VIRUS PROPERTIES

Calf diarrhea coronavirus particles in negatively stained preparations have a mean diameter of 120 nm and are covered by petal-shaped projections about 20 nm long. Viral replication occurs in the cytoplasm. The virus is sensitive to ether, chloroform, deoxycholate and exposure to 50°C for 1 h. Thermosensitivity is stabilized in the presence of 1 M $MgCl_2$. The virus is stable at pH 3. Hemadsorption and hemagglutination occur with erythrocytes of hamsters, mice and rats. Formalin treatment (0.02% at 37°C for 24 h) completely inactivates the infectivity in cell culture fluid (Sharpee et al., 1976). Stability of the virus in the environment is not known. To date, one serotype has been identified.

EPIZOOTIOLOGY

Calf diarrhea coronavirus infects only the bovine species. Under natural conditions, calves 1 day to 3 or more weeks old are affected. Gnotobiotic piglets inoculated with the calf coronavirus developed no clinical signs. No recognized illness has occurred in people working with the virus. Circumstantial evidence suggests that recovered calves introduced into a healthy herd can be responsible for an outbreak of diarrhea, and that there may be carrier cows. Disease patterns and the effect of adverse conditions are the same for coronavirus as described for rotavirus infections.

Morbidity in an outbreak of diarrhea is usually high, but mortality is influenced by the age of the calf when infected, management and type of secondary infection.

PATHOGENESIS

Portal of entry is the mouth through contact with teats, feed or fomites contaminated with infected feces. Primary sites of viral replication are the mature epithelial cells on the small intestinal villi and surface epithelial cells of the colon. During the first few hours of diarrhea the feces can contain up to 10^{10} virions/ml.

DISEASE SIGNS

Uncomplicated coronavirus infection has been studied in gnotobiotic calves. The incubation period in experimental calves varies from 20 to about 36 h. In

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contrast to rotavirus infections, gnotobiotic calves infected with coronavirus become anorectic but do not become as depressed. The appearance of the initial diarrheic feces is the same as for rotavirus; the feces are liquid, yellow, and the volume is somewhat dependent on the amount of milk fed after inoculation. Within 12–24 h after the onset of diarrhea, coronavirus-infected calves will be hungry, but if fed milk will continue to have liquid feces which contain some curd and mucus; the volume of feces will depend on the amount of milk fed. When normal milk feedings are continued, diarrhea in gnotobiotic calves will persist for 5–6 days. Bacteria-free calves fed normal quantities of milk may die of severe dehydration 48–62 h after onset of diarrhea. If an oral electrolyte solution is fed instead of milk, the calves recover.

Although the bovine coronavirus is much more known as one of the agents of enteritis, this virus was also isolated (as a member of mixed infections) from the trachea and lungs of calves with a respiratory disease (McNulty et al., 1984). These authors showed that in newborn calves the respiratory isolates, inoculated nasally and tracheally, caused a mild respiratory disease (cough) accompanied by diarrhea. The isolates are thus probably neither genuine respiratory, nor genuine enteric strains; also it was shown by cross-serology and cross-immunization that both belong to a single serotype (McNulty et al., 1984; Reynolds et al., 1985).

PATHOLOGY

Calves necropsied during the acute or later stages of coronavirus diarrhea have no gross lesion in the small or large intestine other than an increased amount of liquid contents. Calves that die show signs of dehydration. Congestion, hemorrhage or other gross lesions, if seen in coronavirus infection are due to secondary infection.

Light microscopic, IF and EM examination of intestine from calves killed at different intervals after the onset of diarrhea revealed the following changes. At the onset of diarrhea, the small intestinal villous epithelial cells and surface colonic epithelial cells have a normal morphology by light microscopy, but are full of viral antigen and virus. Thus the initial diarrhea is believed to result from a redirection of epithelial function from absorption to virus production. As the disease progresses, the infected epithelial cells of the small intestine and colon are lost. The villi become truncated, covered by squamous to cuboidal epithelial cells, and adjacent villi particularly in the lower ileum may fuse. In the colon, the lost epithelial cells on the colonic ridges are replaced by cuboidal cells, and the ridge structure is lost. The persistence of immature epithelial cells for several days is believed to be responsible for the continued diarrhea and dehydration; the immature epithelial cells cannot complete the digestion of milk, and thus there is decreased absorption of milk and digestive fluids (Mebus et al., 1973b, 1975a).
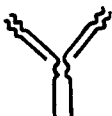
IMMUNE REACTION

Circulating coronavirus antibody does not prevent infection (Mebus et al., 1973b), but may cause an attenuation of the infection through resecretion of antibody into the lumen of the intestine (Mebus et al., 1975b). Regular ingestion of colostrum and milk from an immune dam and the consequential presence of antibodies in intestinal lumen can apparently be protective.

Oral administration of an attenuated coronavirus to newborn calves causes no adverse reaction, and the calves are resistant to challenge inoculation when

96 h old (Mebus et al., 1975b). Isolated intestinal loops (Thiry-Vella loops) have been used to study the immune response. Virus was isolated from loop washings for 6–7 days after inoculation. Beginning at 6–7 days after inoculation, neutralizing antibody identified as IgM and IgA appeared in the loop washings, rose to a respectable titer by 10 days post-inoculation, and persisted. Circulating antibody appeared at 7–8 days post-inoculation. Thus it appears that resistance to coronavirus infection is primarily due to the presence of colostral and/or milk antibody or actively produced IgM and/or IgA in the intestinal lumen (Mebus et al., 1975b).

LABORATORY DIAGNOSIS

Coronavirus diagnosis can be made by EM examination of feces collected during the early stages of diarrhea. The specimens should be collected into a suitable container directly from an animal. When possible, specimens should be collected from several animals; specimens can be frozen. Another method of diagnosis is IF staining of frozen sections of spiral colon collected from a calf killed 1–4 days after the onset of diarrhea. Pieces of colon should be shipped frozen to the laboratory.

In fecal samples the coronaviral antigens can be detected by immunoelectroosmophoresis (Dea et al., 1979), reversed passive HA (Sato et al., 1984), and ELISA (Reynolds et al., 1985). A modified ELISA was used to detect antigen–antibody complexes in fecal samples during chronic shedding of bovine coronavirus by clinically normal cows (Crouch et al., 1985).

The bovine coronavirus has been cultivated with cytopathic effect in primary bovine embryonic kidney cells (Mebus et al., 1973a) and in a continuous line of bovine embryonic kidney (BEK-1) cells (Inaba et al., 1976). The replication of isolates could be enhanced by addition of trypsin to cells of a line of bovine embryonic lung (BEL) cells (Toth, 1982) or to cells of a line of human rectal tumor (HRT-18) (Reynolds et al., 1985). Isolates adapted to growth in HRT-18 cells could be titrated on these cells by a plaque assay (Vautherot, 1981). Bovine coronavirus isolates did replicate in organ cultures of bovine fetal trachea and intestine as was shown by EM, indirect IF and specific agglutination of rat erythrocytes (Bridger et al., 1978; McNulty et al., 1984).

PROPHYLAXIS AND CONTROL

Vaccination of cows prior to parturition will apparently increase the amount of antibody in the colostrum and milk and thus reduce the incidence and/or severity of diarrhea. However, with passive protection, the calf has to become infected to develop an active immunity. Ideally, this will be a subclinical infection.

Oral vaccination of newborn calves will induce an active immunity. However, the problem with an oral vaccine is the timing of vaccination in relation to the ingestion of colostrum and amount of antibody in the colostrum. Vaccination seems to be more successful in herds in which coronavirus diarrhea is occurring in the very young calf.

Interference by colostral antibody with an oral attenuated virus vaccine can be overcome by vaccination of the calf in utero. Attenuated coronavirus inoculated into the amnionic fluid of 7–8-month-old fetuses caused no adverse reaction, and the animals had circulating antibody when delivered by hysterotomy. These calves were not challenge inoculated, but based on the results of rotavirus challenge results of in utero vaccinated calves, one would expect them to be protected.

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Equally important to vaccination are good management practices to mini-
mize introduction of the infection into the herd: new animals should not be
introduced into the herd immediately before or during the calving season, and
hygiene measures should be followed when people move between herds.

REFERENCES

Bridger, J.C., Caul, E.O. and Egglestone, S.I., 1978. Replication of an enteric bovine coronavirus
   in intestinal organ cultures. Arch. Virol., 57: 43–51.
Crouch, C.F., Bielefeldt Ohmann, H., Watts, T.C. and Babiuk, L.A., 1985. Chronic shedding of
   bovine enteric coronavirus antigen–antibody complexes by clinically normal cows. J. Gen.
   Virol., 66: 1489–1500.
Dea, S., Roy, R.S. and Betin, M.E., 1979. Counterimmunoelectroosmophoresis for detection of
   neonatal calf diarrhea coronavirus: methodology and comparison with electron microscopy. J.
   Clin. Microbiol., 10: 240–244.
Inaba, Y., Sato, K., Kurogi, H., Takahashi, E., Ito, Y., Omori, T., Goto, Y. and Matumoto, M., 1976.
   Replication of bovine coronavirus in cell line BEK-1 culture. Arch. Virol., 50: 339–342.
McNulty, M.S., Bryson, D.G., Allan, G.M. and Logan, E.F., 1984. Coronavirus infection of the
   bovine respiratory tract. Vet. Microbiol., 9: 425–434.
Mebus, C.A., Stair, E.L., Rhodes, M.B. and Twiehaus, M.J., 1973a. Neonatal calf diarrhea: Propaga-
   tion, attenuation, and characteristics of a coronavirus-like agent. Am. J. Vet. Res., 34: 145–150.
Mebus, C.A., Stair, E.L., Rhodes, M.B. and Twiehaus, M.J., 1973b. Pathology of neonatal calf
   diarrhea induced by a coronavirus-like agent. Vet. Pathol., 10: 45–64.
Mebus, C.A., Newman, L.E. and Stair, E.L., 1975a. Scanning electron, light, immunofluorescent
   microscopy of intestines of gnotobiotic calf infected with calf diarrheal coronavirus. Am. J. Vet.
   Res., 36: 1719–1725.
Mebus, C.A., Torres-Medina, A., Twiehaus, M.J. and Bass, E.P., 1975b. Immune responses to orally
   administered calf reovirus-like and coronavirus vaccine. Dev. Biol. Stand., 33: 396–403.
Reynolds, D.J., Debney, T.G., Hall, G.A., Thomas, L.H. and Parsons, K.R., 1985. Studies on the
   relationship between coronaviruses from the intestinal and respiratory tracts of calves. Arch.
   Virol., 85: 71–83.
Sato, K., Inaba, Y., Tokuhisa, S., Miura, Y., Kaneko, N., Asagi, M. and Matumoto, M., 1984.
   Detection of bovine coronavirus in feces by reversed passive hemagglutination. Arch. Virol., 80:
   23–31.
Sharpee, R.L., Mebus, C.A. and Bass, E.P., 1976. Characterization of a calf diarrheal coronavirus.
   Am. J. Vet. Res., 37: 1031–1041.
Toth, T.E., 1982. Trypsin-enhanced replication of neonatal calf diarrhea coronavirus in bovine
   embryonic lung cells. Am. J. Vet. Res., 43: 967–972.
Vautherot, J.-F., 1981. Plaque assay for titration of bovine enteric coronavirus. J. Gen. Virol., 56:
   451–455.

Chapter 28

Winter Dysentery of Adult Cattle Virus

J. ESPINASSE, M. SAVEY and M. VISO

INTRODUCTION

During the 1930s, the bacterium *Vibrio jejuni* (*Campylobacter jejuni*) was considered the etiological agent of winter dysentery in the USA (Jones and Little, 1931; Jones et al., 1931). Recently, Al Mashat and Taylor (1980b) and Firehammer and Myers (1981) have shown that it can induce fever, diarrhea —sometimes with dysentery—and lesions similar to those described by Jones and Little. In addition, Garcia et al. (1983) suspect that *C. jejuni* is one of the many etiological agents of winter dysentery. However, *C. jejuni* was isolated also from the feces of normal calves (Allsup et al., 1972), and in Great Britain *Campylobacter fetus* subspecies *fetus* was identified in cases of winter dysentery (Al Mashat and Taylor, 1980a).

In view of the difficulties encountered during the microbiological study as well as the experimental reproduction of the condition, the participation of a virus was suggested after transmission of material from feces passed through a Seitz filter. However, a specific agent was not found to be associated with the illness (MacPherson, 1957). Afterwards, the following viruses were eliminated: IBRV, BVDV-MDV, BAV-3 and BAV-7, reovirus type 3, PI3 virus, bovine rotavirus, enterovirus, syncytial virus, BPoV and *Chlamydia psittaci* (Scott et al., 1973; Takahashi et al., 1980; Espinasse et al., 1981, 1982). In Israel, Komarov et al. (1959) isolated a virus by inoculation of egg embryos and mouse brain. In France, Charton et al. (1963a,b) found that the disease was related to an enterovirus able to induce hemorrhagic diarrhea in two 12–13-month-old heifers following intravenous inoculation. Andersen and Scott (1976) found this virus to be different from the agent involved in New York state.
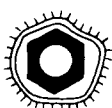
VIRUS PROPERTIES

Based on experiments by Horner et al. (1976) and Durham et al. (1979) in New Zealand and especially by Takahashi et al. (1980) and Akashi et al. (1980, 1981) in Japan, a virus with morphological, physical, chemical and antigenic properties similar to those of coronaviruses was identified in the feces of calves affected with diarrhea; it was associated specifically with winter dysentery. In France, our observations were similar (Espinasse et al., 1981, 1982; Fig. 117), as were those in Belgium (Broes et al., 1984).

The Kalegawa strain isolated in Japan and grown in BEK-1 cells (Inaba et al., 1976) replicated in the presence of 5-iodo-2′-deoxyuridine, indicating that its viral nucleic acid was RNA. It was highly sensitive to ether and chloroform and moderately sensitive to trypsin and heat. It was stabilized by cations to treat-
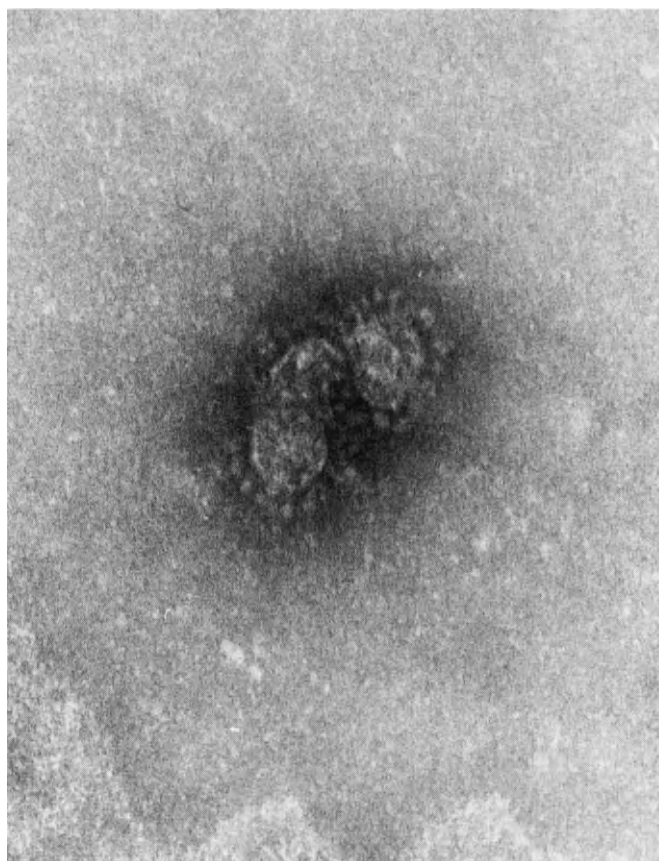
Fig. 117. Typical corona-like viral forms in a feces sample (× 265000).

ment at 50°C for 1 h. Its infectivity was slightly reduced at pH 3. The virus passed through a membrane filter of 200 nm pore size but not through 100-nm pores.

The peak of infectivity and hemagglutinating activity was found at a sucrose density of 1.182 g/ml, which is in agreement with results obtained with the USA strain of calf coronavirus (Pensaert and Callebaut, 1978; Sharpee et al., 1976) and other coronaviruses (McIntosh, 1974). Neutralization and HI tests showed a close relationship between the Kalegawa and the USA strains of calf coronavirus.

The Kalegawa strain could be propagated in suckling mice. Infected animals died with neurologic symptoms, and serial passage was readily accomplished by intracerebral inoculation with brain suspensions. The third-passage viral material from infected mice evoked the same disease in suckling mice, rats and hamsters inoculated by the intracerebral or by the subcutaneous route. Virus recovered from mice, rats and hamsters could be clearly differentiated from mouse hepatitis virus by neutralization test (Akashi et al., 1981).

Virus particles were observed by EM in and outside of the degenerating neurons. They had a core 70 nm in diameter and an envelope with spikes (Kubo et al., 1982).

EPIZOOTIOLOGY

The disease has been reported in the USA, Canada, Great Britain, Sweden (Hedstrom and Isaksson, 1951), Germany (Rolle et al., 1955), France, Belgium, Israel, Japan, Australia (Hutchins et al., 1958) and New Zealand.

First and most frequently, young mature (2–3 years old) dairy cattle are infected during the winter in crowded barns, especially during the postpartum period. The young calves (under 4 months) generally resist the disease or are affected with a milder form.

The disease has a distinct epizootic character within an area and generally prevails in winter. The disorders literally explode in susceptible herds and initially affect 10–20% of the population. The morbidity can reach 100% in 1 or 2 weeks, depending on the size of the herd. The death rate usually does not exceed 10%. MacPherson (1957) recorded a 1–2% death rate following secondary bacterial infection.

The economic consequences of winter dysentery mainly concern milk production. It is reduced by 25–95% (50% on average) for 1 or 2 weeks. Afterwards, lactation rarely regains the estimated performance; however, the general tendency is to overestimate the losses (Kahrs et al., 1973; Jactel et al., 1986).

The feces of affected animals and probably those of healthy adult cattle (Crouch et al., 1985) are the source of contagion. Virus is transmitted from one animal to another by oral infection (contaminated food), from one farm to another within a small region by the equipment used by, for example, cattle dealers, breeders, technicians, inseminators and veterinarians, and perhaps by animal vehicles (dogs, wild birds).

Susceptibility of the animals is an essential element in the emergence of disorders and their experimental reproduction. Besides the breed and age of the animals as well as their state of immunity, disease expression mainly seems to depend on external factors. The role of sudden temperature changes (cooling) and atmospheric pressure (fall) during the cold season appears to be a determining factor. Consumption of poor quality (moldy) food is usually considered destabilizing for the gastrointestinal ecosystem.

PATHOGENESIS

The disease can be transmitted by oral or oronasal administration of fresh feces within 3–5 days, or of material kept at − 60°C with 10% dimethylsulfoxide within 7–11 days (Scott et al., 1973).

In the absence of more precise data on the pathogenesis of winter dysentery, the following hypotheses may be formulated:

—the coronavirus isolated by the Japanese workers may be a single serotype which is capable of causing diarrhea in cattle regardless of their age; it acts on the apical cells of the villus (enterocytes) of the small intestine and colon, inducing a malabsorption syndrome;

—the campylobacter(s), in particular *C. jejuni*, act as opportunistic agents due to their invading property and colonize the crypts in the same regions;

—lipopolysaccharides or endotoxins of the bacteria induce a phenomenon of localized and/or disseminated intravascular coagulation, the source of dysentery.

DISEASE SIGNS

The incubation period is between 3 and 7 days.

There is a sudden onset of symptoms in the herd after (in some cases) a brief period of hyperthermia (39.5–40.5°C), anorexia and apathy (24–48 h). Oculonasal discharge and coughing are also frequently noted. The cough has been variously characterized as dry, harsh, hacking and moist.

In affected animals, the feces are sometimes expelled as a spurt of diarrhea during a coughing fit. The color varies from dark green to black. The feces from 5–10% of the animals contain blood or blood clots. The consistency is liquid or slightly thicker, like that of a dilute vegetable soup. The diarrhea falls to the ground in unbroken lines and may contain large "soap" bubbles. The feces often have a characteristic odor. Descriptions range from fetid, to musty, to sweet–nasty. This dung attract dogs who avidly consume it.

Milk production is reduced before, during and above all after the phase of diarrhea, especially in animals that have just calved. In an individual animal the diarrhea may resolve within several hours, but most often it lasts for several days (less than a week). In a herd, the diarrheic episode lasts for 3–4 weeks, 2 weeks on the average. Convalescence is shorter, with milder symptoms.

Death rarely occurs with winter dysentery and results from hemorrhagic anemia following intense asthenia or respiratory or intestinal complications (like peritonitis) (Campbell and Cookingham, 1978).

PATHOLOGY

The lesions are those of a catarrhal inflammation: injected serosal blood vessels, edematous intestinal walls and swollen, wrinkled and congested mucosa. Ecchymosis and petechiation are commonly found. The intestinal contents have been observed to be dark and fluid, sometimes mixed with mucus or blood (Fig. 118).

Lesions of the respiratory mucosa can extend from the posterior nasal cavity down the trachea to the large bronchi; they appear as congestion, swelling, petechiation and serofibrinous exudation.
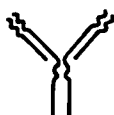
IMMUNE REACTION

In general, immunity is found after a herd epizootic, protection ranging from 6 months to 2 or 3 years. It seems that the period of protection is directly correlated with the number of infected animals (Roberts, 1957).

MacPherson (1957) claims that greater susceptibility in young adults is due to the loss of colostral antibodies and increasing stress.

LABORATORY DIAGNOSIS

The diagnosis of winter dysentery is not based on laboratory methods. This is due to the lack of knowledge concerning the etiological agent(s); the epidemiological and clinical characteristics of the illness are necessary to provide a positive diagnosis. For a differential diagnosis BVD–MD, rinderpest, salmonellosis, paratuberculosis, coccidiosis, gastrointestinal strongylosis, liver fluke and food poisoning should be considered.

In feces, coronavirus can be found in conjunction with winter dysentery by EM, as demonstrated by the authors. Recently, Sato et al. (1984) have recommended reverse passive hemagglutination for the detection of bovine coronavirus in feces (bovine coronavirus antibodies raised in rabbits bound to sheep erythrocytes). This method seems to be specific and sensitive. The test detected bovine coronavirus in 13 out of 22 fecal samples (59%) from natural cases of diarrhea, while the positive rates were 14% (3/22) and 22% (5/22) for IF staining (primary cultures of calf kidney cells infected with the specimens) and IEM, respectively.

Fig. 118. Lesions of hemorrhagic enterocolitis.

Coronavirus can be isolated from feces in bovine fetal kidney cells and identified by direct IF using a rabbit antiserum conjugated with fluorescein isothyocyanate (Takahashi et al., 1980).

The test for seroconversion of bovine coronavirus by SN or HI is another means of approaching the etiological diagnosis of winter dysentery (Takahashi et al., 1980).

PROPHYLAXIS AND CONTROL

No vaccine has been developed due to the lack of knowledge concerning the source of winter dysentery. Moreover, a number of specific vaccines, e.g. against leptospirosis, IBR and BVD–MD (Kahrs et al., 1973) are not effective. American practitioners use inactivated vaccine preparations containing different bacteria (*Pasteurella*), with generally satisfactory results (Williams, 1980).

Prevention of the disease depends on the standard measures of isolation and disinfection used in all epizootics, in and around all centers of infection: immediate isolation of affected animals, traffic restriction, both human and animal, and disinfection of footwear and equipment; however, even when all precautions have been taken, disease outbreaks occur.

Treatment of winter dysentery aims at controlling diarrhea and its consequences. Intestinal sulfonamides and astringents (clay) or a combination of both in pellet form probably only have symbolic value, as there tends to be spontaneous recovery. However, therapy is indicated in animals presenting the most pronounced symptoms, as it makes possible more rapid recovery and avoids complications. In subjects with severe signs of dehydration and/or anemia, oral and/or general fluid and electrolyte replacement and blood transfusions are recommended.

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REFERENCES

Akashi, H., Inaba, Y., Miura, Y., Tokuhisa, S., Sato, K. and Satoda, K., 1980. Properties of a coronavirus isolated from a cow with epizootic diarrhea. Vet. Microbiol., 5: 265–276.

Akashi, H., Inaba, Y., Miura, Y., Sato, K., Tokuhisa, S., Asagi, M. and Hayashi, Y., 1981. Propagation of the Kalegawa strain of bovine coronavirus in suckling mice, rats and hamsters. Arch. Virol., 67: 367–370.

Allsup, T.N., Matthews, K.P. and Hogg, S.D., 1972. Vibrios in diseased and healthy calves. Vet. Rec., 90: 14–15.

Al Mashat, R.R. and Taylor, D.J., 1980a. *Campylobacter* spp. in enteric lesions in cattle. Vet. Rec., 107: 31–34.

Al Mashat, R.r. and Taylor, D.J., 1980b. Production of diarrhoea and dysentery in experimental calves by feeding pure culture of *Campylobacter fetus* subspecies *jejuni*. Vet. Rec., 107: 459–464.

Andersen, A.A. and Scott, F.W., 1976. Serological comparison of french WD 42 enterovirus isolate with bovine winter dysentery in New-York State. Cornell Vet., 2: 232–239.

Broes, A., Van Opdenbosch, E. and Wellemans, G., 1984. Isolement d'un coronavirus chez des bovins atteints d'entérite hémorragique hivernale (Winter Dysentery) en Belgique. Ann. Méd. Vét., 128: 289–303.

Campbell, S.G. and Cookingham, C.A., 1978. The enigma of winter dysentery. Cornell Vet., 4: 423–441.

Charton, A., Faye, P. and Lecoanet, J., 1963a. Entérite hémorragique infectieuse hivernale des bovins (winter dysentery). Rec. Méd. Vét., 139: 803–808.

Charton, A., Faye, P., Lecoanet, J., Desbrosse, H. and Le Layec, Cl., 1963b. Etude clinique expérimentale d'une entérite hémorragique hivernale des bovins associée a la présence dans le tube digestif d'un ultra-virus pathogène. Rec. Méd. Vét., 139: 897–908.

Crouch, C.D., Bielefeldt Ohmann, H., Watts, T.C. and Babiuk, L.A., 1985. Chronic shedding of bovine enteric coronavirus antigen–antibody complexes by clinically normal cows. J. Gen. Virol., 66: 1489–1500.

Durham, P.J., Stevenson, D.J. and Farquharson, B.C., 1979. Rotavirus and coronavirus associated diarrhea in domestic animals. N.Z. Vet. J., 27: 30–32.

Espinasse, J., L'Haridon, R., Viso, M., Savey, M., Laval, A., Le Layec, Cl., Blot, J.P. and Cohen, J., 1981. Mise en évidence d'un agent du type coronavirus dans les feces des bovins atteints d'entérite hémorragique d'hiver (winter dysentery). Bull. Acad. Vét. Fr., 54: 465–472.

Espinasse, J., Viso, M., Laval, A., Savey, M., Le Layec, Cl., Blot, J.P., L'Haridon, R. and Cohen, J., 1982. Winter dysentery: a coronavirus-like agent in the faeces of beef and dairy cattle with diarrhea. Vet. Rec., 110: 385.

Firehammer, B.D. and Myers, L.L., 1981. *Campylobacter fetus* subsp. *jejuni*: its possible significance in enteric disease of calves and lambs. am. J. Vet. Res., 6: 918–922.

Garcia, M.M., Eaglesome, M.D. and Rigby, C., 1983. Campylobacters important in veterinary medicine. Vet. Bull., 9: 793–815.

Hedstrom, H. and Isaksson, A., 1951. Epizootic enteritis in cattle in Sweden. Cornell Vet., 41: 251–253.

Horner, G.W., Hunter, R. and Kirkbridge, C.A., 1976. A coronavirus like agent present in faeces of cows with diarrhea. N.Z. Vet. J., 23: 48.

Hutchins, D.R., Hyne, R.H.J., Studdert, M.J., Littlejohns, I.R. and Johnston, K.G., 1958. Epizootic diarrhoea of cattle. Aust. Vet. J., 34: 300.

Inaba, Y., Sato, K., Kurogi, H., Takahashi, E., Ito, Y., Omori, T., Goto, Y. and Matumoto, M., 1976. Replication of bovine coronavirus in cell line BEKI culture. Arch. Virol., 50: 339–342.

Jactel, B., Espinasse, J., Viso, M. and Valiergwe, H., 1986. L'enterite hémorragique hivernale en France. XIVth World Congress on Diseases of Cattle, Dublin, 1: 350–355.

Jones, F.S. and Little, R.B., 1931. The etiology of infectious diarrhea (winter scours) in cattle. J. Exp. Med., 53: 835–843.

Jones, F.S., Orcutt, M. and Little, R.B., 1931. Vibrios (*Vibrio jejuni*) associated with intestinal disorders of cows and calves. J. Exp. Med., 53: 853–863.

Kahrs, R.F., Scott, F.N. and Hillman, R.B., 1973. Epidemiologic observation on bovine winter dysentery. Bovine Pract., 8: 36–39.

Komarov, A., Goldsmit, L., Kalmar, E., Adler, J.H. and Egyed, M., 1959. Isolation of a viral agent from winter dysentery of cattle. Refu. Vet., 16: 152–159.

Kubo, M., Akashi, H., Inaba, Y., Osada, M. and Konno, S., 1982. Pathological studies on encephalitis in mice experimentally inoculated with bovine coronavirus. Natl. Inst. Anim. Health Q. (Jap.), 22: 45–53.

MacIntosh, K., 1974. Coronaviruses: a comparative review. Curr. Top. Microbiol. Immunol., 63: 85–129.

Macpherson, L.W., 1957. Bovine virus enteritis (winter dysentery). Can. J. Comp. Med., 21: 184–192.

Pensaert, M. and Callebaut, P., 1978. The coronaviruses. Clinical and structural aspects with some

practical implications. Ann. Med. Vet., 122: 301–322.

Roberts, S.J., 1957. Winter dysentery in dairy cattle. Cornell Vet., 47: 372–388.

Rolle, M., Mundt, N. and Zintz, G., 1955. *Vibrio jejuni* als Enteritiserreger beim Rind. Tierärztl. Umschau, 10: 391–392.

Sato, K., Inaba, Y., Tokuhisa, S., Miura, Y., Kaneko, N., Asagi, M. and Matumoto, M., 1984. Detection of bovine coronavirus in feces by reversed passive hemagglutination. Arch. Virol., 80: 23–31.

Scott, F.W., Kahrs, R.F., Campbell, S.G. and Hillman, R.B., 1973. Etiologic studies on bovine winter dysentery. Bovine Pract., 40–43.

Sharpee, R.L., Mebus, C.A. and Bass, E.P., 1976. Characterization of a calf diarrheal coronavirus. Am. J. Vet. Res., 37: 1031–1041.

Takahashi, E., Inaba, Y., Sato, K., Ito, Y., Kurogi, H., Akashi, H., Satoda, K. and Omori, T., 1980. Epizootic diarrhea of adult cattle associated with a coronavirus-like agent. Vet. Microbiol., 5: 151–154.

Williams, E.J., 1980. Winter dysentery. In: H.E. Amstutz (Editor), Bovine Medicine and Surgery. American Veterinary Publications, Drawer KK, Santa Barbara, CA, pp. 679–681.

Toroviridae

The term Toroviridae has been proposed as a family name for a group of antigenically related viruses demonstrated in horses, cattle and man (Latin torus = a doughnut-shaped ring). Most studies have been done with Berne virus of horses and Breda virus of cattle. Evidence of infection has been obtained in other ungulates (pig, sheep, goat), in lagomorphs and in rodents.

Toroviruses are enveloped RNA viruses consisting of a peplomer-bearing envelope and an elongated tubular nucleocapsid of helical symmetry. The nucleocapsid may be bent into an open torus, thereby conferring a kidney- or disk-shaped morphology to the virion (average diameter 120–140 nm), or it may be straight, resulting in a rod-shaped particle (diameter 35 nm, length approaching 180 nm).

Berne virus, which has been proposed as the prototype, possesses a single-stranded genome of positive polarity; its mol. wt. has been estimated as about 8×10^6. Two major proteins occur in the virion, a phosphorylated 20 k nucleocapsid polypeptide and a 22 k protein representing the main envelope constituent. Additional species of 37 k and in the 75–100 k range are present in the virus particle, the former being a membrane constituent, the latter a peplomer subunit.

Buoyant density of Berne virus is 1.17 g/ml in sucrose; a sedimentation coefficient of about 400 S has been determined.

Toroviruses replicate in the cytoplasm, where four subgenomic mRNAs are formed; as in coronaviruses, these probably form a 3'-coterminal nested set. Replication is dependent on some nuclear function of the host cell. Tubular structures resembling virion nucleocapsids have been found in the nucleoplasm and cytoplasm of infected cells. Budding of preformed Berne virus nucleocapsids through intracytoplasmic membranes was demonstrated.

Chapter 29

Breda Virus

G.N. WOODE

INTRODUCTION

The Breda virus of calves was isolated from a calf in acute neonatal diarrhea (scours). This virus had a superficial resemblance to bovine coronavirus but was antigenically different to bovine coronavirus and to other bovine viruses, did not elute from erythrocytes and, in the opinion of the authors, was morphologically distinct (Woode et al., 1982). For these reasons and to avoid confusion, the term Breda virus (named from the township in Iowa in which the farm was situated) is now used.
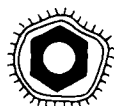
VIRUS PROPERTIES

This virus is antigenically related and morphologically similar to an equine virus (Berne virus). Berne virus has been cultured in vitro, purified and characterized as an enveloped RNA virus. It has morphological and molecular biological properties which distinguish it from the coronavirus group, and it has been proposed that the Berne/Breda group of viruses be classified in a new family of viruses, the Toroviridae (Horzinek et al., 1987; Weiss and Horzinek, 1987). Viruses that are morphologically similar to Breda virus and are agglutinated by antiserum to Breda virus serotype 2 have been isolated from humans with diarrhea (Beards et al., 1986).

In negatively stained preparations of intestinal contents, feces or tissue culture fluids, virus particles are either elongated and frequently curved into a kidney shape, with dimensions 105–140 × 12–40 nm, or spherical, with mean diameter of 82 nm, with a peplomer-bearing envelope. Peplomers on most particles are short, 7–9 nm (Breda virus serotype 1) or long, 20 nm (Berne virus and Breda virus serotype 2). Many particles of Breda virus have short stubby peplomers, making the virus readily distinguishable from coronavirus (Fig. 119). If Breda virus is pelletted at 80–100 000 × g, most of the long projections are lost, leaving the short peplomers. This is not observed with bovine coronavirus. The integrity of the Breda virus peplomers is maintained better at 30 000 × g.

Thin sections of jejunal epithelial cells infected with Breda virus 1 or 2 show brick-shaped or elongated virus particles with rounded ends, with dimensions of 80–100 × 35–42 nm. The particles are pleomorphic and vary considerably in length. The outer membrane bears short projections and there is an internal core measuring 23–25 nm in diameter. Virus particles are observed most frequently in autophagolysosomes and in the gut lumen closely associated with the epithelial cell membrane and damaged microvilli (Pohlenz et al., 1984).
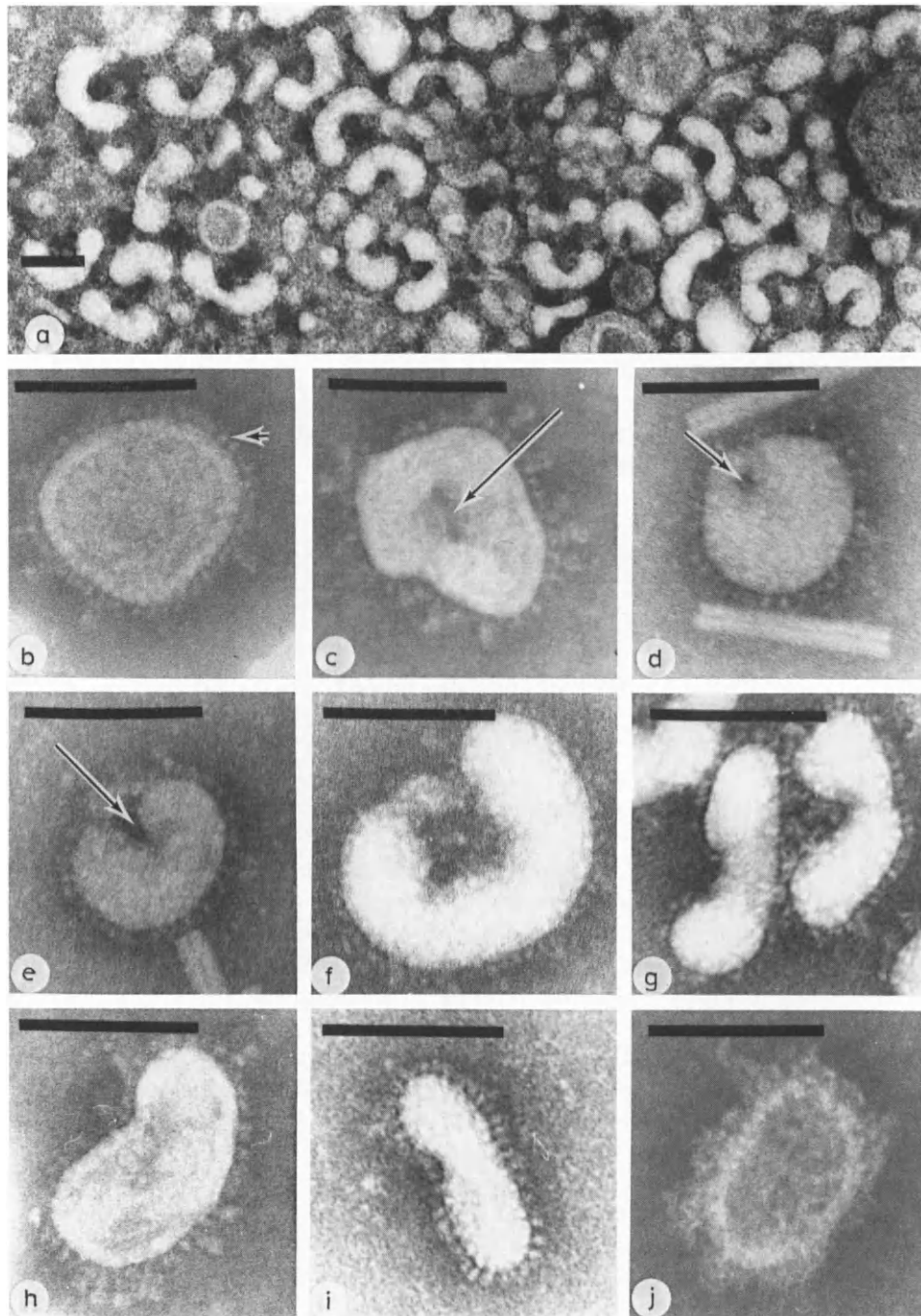
Fig. 119. Ultrastructural morphology of the Breda virus in potassium phosphotungstate negative stain preparations, from calf fecal specimens; bar = 100 nm. (a) Original calf sample of Breda agent; numerous kidney-shaped particles are evident. (b–h) Various round and kidney-shaped forms of the Breda virus; (b) arrow indicates short projections (7.6–9.5 nm); longer projections are occasionally seen and are thought to represent adherent tissue debris; (c–h) the relationship of the two particle types; arrows indicate the cleft in which stain has penetrated in spherical particles (c, d and e) and larger clefts of kidney-shaped particles (f, g and h); rods in c, d and e are portions of TMV. (i) Similar particle observed in the feces of one diarrheic calf from South Dakota. (j) IEM of the Breda virus with convalescent calf serum.

Breda virus serotype 2 infection has been studied in gnotobiotic calves, which were killed at different time intervals following infection. From this data the morphogenetic pathway for the virus was proposed. The virus enters the intestinal epithelial cells by receptor-mediated endocytosis, through coated pits situated between microvilli. It does not attach to the microvilli of goblet cells nor to the microfolds of M cells associated with Peyer's patches. Viral uncoating and release is related to lysosomal or other degradation of the virus-containing vesicles. Virus replication is associated with the Golgi system. Tubules with the same dimensions as the viral nucleocapsid are seen both in the cytoplasm associated with the Golgi apparatus and in the nucleus. Thus the virus appears to have a nuclear phase in its replication, as does Berne virus (Horzinek et al., 1985, 1987). Virus nucleocapsid passes through the rough endoplasmic reticulum, at which site the envelope proteins are taken up by the virus. Virions are most frequently seen in swollen vesicles of the Golgi apparatus. Viral release probably occurs following fusion of the vesicles with the plasma membrane (reverse pinocytosis). The replicative cycle and release of the virus occurs before cytopathic changes are observed in the cell. Morphologically, Breda virions in infected cells most closely resemble the "bacillary" forms of rhabdoviruses in which both ends are rounded. The virion core (nucleocapsid) is highly electron-dense with an electron-lucent central channel (Fagerland et al., 1986; Pohlenz et al., 1984).

Breda virus may also have a nuclear phase of replication, as in thin sections infected cells show tubules in the nucleoplasm (Pohlenz et al., 1984). Breda virus isolates possess an hemagglutinin for rat and mice erythrocytes. Serotype 1 does not elute from erythrocytes but the serotype 2 does.

Breda viruses carry common antigens measurable by IF test and ELISA (Woode et al., 1982, 1983, 1985). HI tests have shown distinct differences between two isolates from Iowa and one from Ohio. It is proposed that there are two distinct serotypes, Breda virus serotype 1 (first Iowa isolate) and Breda virus serotype 2 (Ohio and the second Iowa isolate) (Woode et al., 1985). By these tests Breda virus is not related antigenically to other bovine hemagglutinating viruses, including bovine coronavirus, parvovirus, rotavirus, PI3 virus and reovirus 3, and there is no cross-reaction with bovine pestivirus (BVD) (Woode et al., 1982, 1983). A bovine isolate from France, Lyon-4 virus (Moussa et al., 1983), is neutralized by antisera to both serotypes.

Berne virus shows cross-neutralization with Breda virus and the Lyon-4 virus. There is wide distribution of the Berne virus in the Swiss horse population and antibodies also occur in sera from cattle, sheep, goats, pigs, laboratory rabbits and two species of wild mice. Antisera against the three coronavirus clusters (represented by mouse hepatitis, transmissible gastroenteritis and avian infectious bronchitis viruses) do not cross-react with Berne virus by IF (Weiss et al., 1983, 1984).

Studies have not been reported on the effect of disinfection or heat sterilization. Infectivity of Breda virus can be lost after 2–3 weeks storage of feces at 4°C (G.N. Woode, unpublished data, 1983), and it is probable that this virus group is readily destroyed by disinfectants and heat.

EPIZOOTIOLOGY

Breda virus was isolated from acute enteritis (scours) of a calf aged 5 days, from a herd in which 56% of the calves born developed diarrhea sometime during the first 20 days of life, with most calves becoming diarrheic during the first 3–5 days. Fifteen percent of the diarrheic calves died. The affected calves were housed and had direct and indirect contact with each other. Experimental

data of transmission of infection confirms the conclusion that natural spread under these conditions follows the fecal/oral route (Woode et al., 1982). However, Breda virus 2 was isolated from a calf produced by caesarean section under strict aseptic conditions and housed in a disinfected experimental calf unit, in which previous animals had not been infected with this virus. It is possible that the dam was excreting this virus subclinically and contamination occurred during or after surgery. Subclinical excretion of rotavirus by sows at parturition has been recorded by Benfield et al. (1982), and bovine dams are possibly a common source of neonatal viral infections.

There are no reports of reservoir hosts or vectors. The virus has been isolated in the USA, in Ohio and South Dakota, in addition to Iowa (Woode et al., 1982, 1985), and is related antigenically to viruses isolated in France (Moussa et al., 1983; Lamouliatte et al., 1987).

The virus spreads rapidly through a group of susceptible young calves with an incubation period as short as 24 h under experimental conditions. Infection occurs both in calves housed and in those born and reared at pasture, as well as in yearlings and adult cows. Insufficient outbreaks have been studied to comment further on morbidity and mortality. However, studies by ELISA have shown that 88.5% of calf and cow sera obtained from states in the midwest of the USA and from Florida, and 94% from The Netherlands and the Federal Republic of Germany, have antibodies to Breda virus (Woode et al., 1985; Koopmans et al., 1989).

PATHOGENESIS

Virus fed, or inoculated intranasally, into colostrum-deprived or gnotobiotic calves aged 1 h to 21 days rapidly infects the small intestine and reaches the feces within 24–72 h, the incubation period depending on the dose of inoculum. Diarrhea or change in appearance of the feces coincides with virus excretion. The virus is detectable in the feces for 2–4 days after the onset of diarrhea (Woode et al., 1982). In naturally infected calves virus shedding can precede clinical signs and subclinical infections occur. After recovery, virus can be excreted repeatedly for at least 4 months. In calves fed sufficient amounts of immune colostrum, infection is not prevented, but clinical signs usually are mild (Koopmans et al., in press).

Viremia has not been reported, nor has the virus been observed outside the small and large intestine.

DISEASE SIGNS

Calves first develop depression and anorexia followed within a few hours by greenish-yellow soft feces. This soon changes to a greenish-yellow or yellow fluid stool. Dehydration may develop rapidly, within 24–48 h, and death can occur within 48–72 h after the onset of diarrhea. Some calves show an uncontrollable continuous muscular shivering at 12–24 h after infection. Immediately prior to and at the onset of diarrhea there is a rise in body temperature to 39.4–40.0°C.

PATHOLOGY

Apart from the signs of dehydration, reddening and loss of tone of the thin-walled small intestine, no macroscopic signs are observed. Infection has

been recorded in the epithelium from mid-jejunum through ileum and in all regions of the large intestine. Villus atrophy is the consequence of virus infection of both the crypt and villus epithelial cells. Virus infection and lesions are seen from the surface deep into the folds of the large intestine. At all sites there is focal necrosis in association with mild to moderate inflammatory response in the small intestine. Cytopathic infection occurs of dome epithelium over Peyer's patches. Fusion of regions of the villi or folds occurs, with the "bridge" frequently composed of virus-infected cells. Ultrastructurally, the epithelial cells are rounded, vacuolated and separating from each other. There are no pathological signs which might distinguish this infection from coronavirus, except possibly the lesions in the crypts (Woode et al., 1982; Pohlenz et al., 1984).
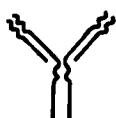
IMMUNE REACTION

Following primary infection nonimmune animals develop Breda-virus specific IgM and IgG antibodies, which reach peak serumtiters 1–2 and 3–4 weeks after infection. After reinfection, circulating IgA antibodies can be detected within 2 weeks.

Passively derived antibodies do not necessarily protect calves from infection; IgM is produced in these calves relatively late (3–5 weeks after infection). No IgG seroconversion occurs (Koopmans et al., in press).

LABORATORY DIAGNOSIS

Fecal samples should be taken during the first 3 days of diarrhea. Breda virus is stable morphologically in fecal samples for at least 10 days at 18–25°C, and the hemagglutinin retains its specificity under these same conditions. Infectivity is probably lost within a day or two at temperatures above 4°C, and is not stable unless frozen at − 70°C. EM detection of the virus using 3–4% potassium phosphotungstate, pH 7.0, can be used to identify infected animals, but few calves excrete highly characteristic particles in sufficient numbers for diagnostic purposes. Breda virions can be distinguished from coronavirus particles by IEM. As the virus has not been grown in cell culture, the best diagnostic methods at present available are the HI test, ELISA and IEM. Viral hemagglutin titers of fecal samples vary from 20 000 to > 500 000 in different calves. Normal, virus-negative feces have titers of 16–32. Specific Breda virus antisera will block the hemagglutinin, and this test distinguishes between the two Breda virus serotypes which do not share common HA antigens. The HI test will not identify other serotypes of Breda virus, as this is a serotype-specific test. As all Breda virus isolates share common antigens in IF, frozen sections of the large intestine (particularly spiral colon) can be examined, and the antigen persists in large intestinal epithelial cells for 3–4 days p.i. ELISA has been used to identify Breda virus purified from feces, and again this test demonstrates antigens shared amongst all Breda virus isolates. This test can also be used for a survey of serum antibodies, in contrast to the HI test, which is not satisfactory because of the relatively low HI response of convalescent animals. These various diagnostic methods have been described in detail by Woode et al. (1982, 1983, 1985).

As this infection is primarily one which can occur despite the presence of passively derived antibodies, the paired serum response is not a useful test. ELISAs for detection of Breda-virus-specific IgM antibodies can be used to test paired serum samples of colostrum-fed calves, bearing in mind that the interval

can vary from 3 to 5 weeks and that not all calves develop detectable amounts of IgM. In animals older than 4 months paired serum response is a useful test.

PROPHYLAXIS AND CONTROL

There are no specific methods available for control of this infection. As the virus is probably easily destroyed by heat, disinfection or desiccation, good hygiene practices should be adopted. Avoidance of contact between uninfected and infected calves is recommended. Prolonged feeding of small amounts (500 ml/day) of antibody-containing colostrum is advisable in view of the presence of antibodies in the gut lumen.

REFERENCES

Beards, G.M., Brown, D.W.G., Green, J. and Flewett, T.H., 1986. Preliminary characterization of Torovirus-like particles of human: comparison with Berne virus of horses and Breda virus of calves. J. Med. Virol., 20: 67–78.

Benfield, D.A., Stotz, I., Moore, R. and McAdaragh, J.P., 1982. Shedding of rotavirus in feces of sows before and after farrowing. J. Clin. Microbiol., 16: 186–190.

Fagerland, J.A., Pohlenz, J.F.L. and Woode, G.N., 1986. A morphologic study of the replication of Breda virus (proposed family Toroviridae) in bovine intestinal cells. J. Gen. Virol., 67: 1293–1304.

Horzinek, M.C., Ederveen, J. and Weiss, M., 1985. The nucleocapsid of Berne virus. J. Gen. Virol., 66: 1287–1296.

Horzinek, M.C., Flewett, T.H., Saif, L.J., Spaan, J.M., Weiss, M. and Woode, G.N., 1987. A new family of vertebrate viruses: Toroviridae. Intervirology, 27: 17–24.

Koopmans, M., Van den Boom, U., Woode, G.N. and Horzinek, M.C., 1989. Seroepidemiology of Breda virus in cattle using ELISA. Vet. Microbiol., 19: 233–243.

Koopmans, M., Cremers, H., Woode, G.N. and Horzinek, M.C., (in press). Bredavirus (Toroviridae) infection and systemic antibody response in sentinel calves. Am. J. Vet. Res.

Lamouliatte, F., Du Pasquier, P., Rossi, F., Laporte, J. and Loze, J.P., 1987. Studies on bovine Breda virus. Vet. Microbiol., 15: 261–278.

Moussa, A., Dannacher, G. and Fedida, M., 1983. Nouveaux virus intervenant dans l'étiologie des entérites néonatales des bovins. Rec. Méd. Vét., 159: 185–190.

Pohlenz, J.F.L., Cheville, N.F., Woode, G.N. and Mokresh, A.H., 1984. Cellular lesions in intestinal mucosa of gnotobiotic calves experimentally infected with a new unclassified bovine virus (Breda virus). Vet. Pathol., 21: 407–417.

Weiss, M. and Horzinek, M.C., 1987. The proposed family Toroviridae: Agents of enteric infections. Arch. Virol., 92: 1–15.

Weiss, M., Steck, F. and Horzinek, M.C., 1983. Purification and partial characterization of a new enveloped RNA virus (Berne virus). J. Gen. Virol., 64: 1849–1858.

Weiss, M., Steck, F. and Kaderli, R., 1984. Antibodies to Berne virus in horses and other animals. Vet. Microbiol., 9: 523–531.

Woode, G.N., Reed, D.E., Runnels, P.L., Herrig, M.A. and Hill, J.T., 1982. Studies with an unclassified virus isolated from diarrheic calves. Vet. Microbiol., 7: 221–240.

Woode, G.N., Mohammed, K.A., Saif, L.J., Winand, N.J., Quesada, M., Kelso, N.E. and Pohlenz, J.F., 1983. Diagnostic methods for the newly discovered "Breda" group of calf enteritis inducing viruses. In: Proceedings of the Third Symposium, World Association of Veterinary Laboratory Diagnosticians, pp. 533–538.

Woode, G.N., Saif, L.J., Quesada, M., Winand, N.J., Pohlenz, J.F. and Gourley, N.K., 1985. Comparative studies on three isolates of Breda virus of calves. Am. J. Vet. Res., 46: 1003–1010.

Paramyxoviridae

Viruses possessing a hemagglutinin (H) and neuraminidase (N) were originally called myxoviruses since mucoproteins (Greek myxa = mucus) carry receptor sites for the hemagglutinin and neuraminidase action. Formerly the influenza viruses, Newcastle disease virus and mumps virus formed the myxovirus group. Later studies on the structure and replication revealed substantial differences, which led to the subdivision into orthomyxoviruses (Greek ortho = straight) and the paramyxoviruses (Greek para = next to). The Paramyxoviridae family contains the genera *Paramyxovirus, Morbillivirus* (formerly the measles/rinderpest/distemper group) and *Pneumovirus* (encompassing the respiratory syncytial viruses of man, cattle and mice).

Paramyxovirions are pleomorphic, but usually roughly spherical, 150 nm or more in diameter. The envelope is derived from cellular lipids and contains viral glycoproteins at its outer surface and a nonglycosylated inner protein. In the *Paramyxovirus* genus the virion envelope bears two types of projections, the larger one (HN) being responsible for both the hemagglutinating and neuraminidase activities whereas the smaller type (F) causes cell fusion and hemolysis. Morbilliviruses (Latin morbillus is a diminutive form of morbus = disease) lack the neuraminidase activity. Canine distemper virus, rinderpest virus and the virus of the peste-des-petits-ruminants (PPR) lack both the hemagglutinin and neuraminidase activities, which is also true for the mem-
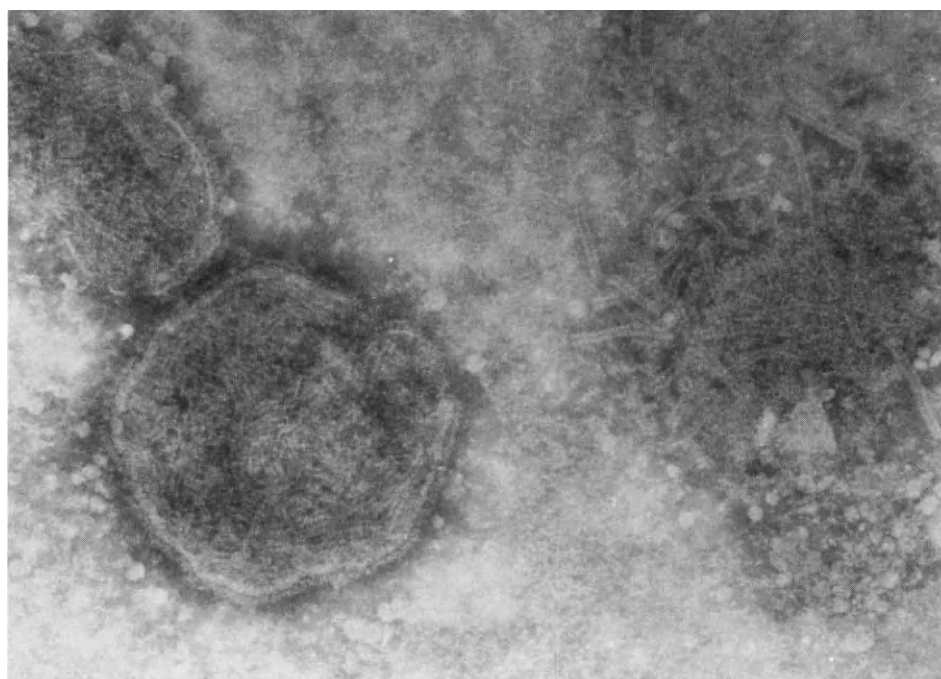
Fig. 120. Bovine P13 virus negatively stained. To the left, two virus particles are visible—note the virus envelope with its projecting spikes enclosing filamentous nucleocapsids; to the right nucleocapsids are observed lying free; × 90 000 (courtesy Dr. D.G. Boyson).

bers of the genus *Pneumovirus* (Greek pneuma = breath). Nucleocapsids are in one piece, have a diameter of 12-17 nm, depending on the genus, and may reach a length of 1 μm in some genera (Fig. 120).

Virions contain one molecule of single-stranded RNA, which is of negative polarity in most virions (some may contain positive-sense strands, which leads to self-annealing during isolation). The mol. wt. of the genome ranges between 5 and 7 $\times$ 10^6. Five to seven structural polypeptides have been identified, with mol.wts. ranging between 35 k and 200 k; in addition to the proteins mentioned above, transcriptase, polyadenylate transferase and mRNA methyl transferase have been identified.

In sucrose, virions possess a buoyant density of 1.18–1.20 g/cm^3; sedimentation coefficients over 1000 S have been determined.

Paramyxoviruses enter the cell by fusion of their envelopes with the cell surface membrane. The nucleocapsid is the functional template for transcription of complementary viral mRNAs and for RNA replication. Independently assembled nucleocapsids are enveloped at the cell surface at sites at which the viral surface (envelope) proteins had been inserted. Virions leave the cell by budding.

Paramyxovirus

Parainfluenza-type 3 (PI3) virus is commonly encountered in cattle and sheep, and is found amongst various other viruses in acute respiratory/enteric disease in calves and lambs.

Morbillivirus

The prototype is measles virus. The envelope of this virus contains a glycoprotein H- and a nonglycosylated F-protein. Related antigenically to measles virus as well as to each other are canine distemper virus, rinderpest virus, and PPR virus. Only measles virus has a well-developed hemagglutinin activity. All morbilliviruses produce both cytoplasmic and intranuclear inclusions which contain viral ribonucleoprotein. In cattle rinderpestvirus causes devastating enzootics which have been known to occur since ancient times. PPR is a disease similar to rinderpest but restricted to goats and sheep.

Pneumovirus

The envelope of pneumoviruses bears projections that possess cell-fusing activity, since the virus gives rise to syncytium formation. In cattle this genus is represented by the bovine respiratory syncytial virus (BRSV), which causes an acute respiratory disease. Recently, a related caprine respiratory syncytial virus has been described. A review on respiratory syncytial virus is given by Stott and Taylor (1985).

REFERENCE

Stott, E.J. and Taylor, G., 1985. Respiratory syncytial virus. Arch. Virol. 84: 1–52.

Chapter 30

Parainfluenza-3 Virus in Cattle

D.G. BRYSON
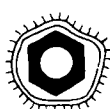
VIRUS PROPERTIES

The structure, physical and chemical properties of a bovine parainfluenza type 3 virus (PI3 virus), a member of the family Paramyxoviridae, have been described by many workers, including Dawson (1964), Kahn et al. (1969), McLean and Deane (1971), Shibuta et al. (1979; 1986), Sakai et al. (1987) and Shioda et al. (1988). Important viral proteins are the hemagglutinin-neuraminidase protein and the glycosylated fusion (F) protein. Paramyxoviruses attach to receptors on the cell surface with the hemagglutinin and penetrate cells by fusion with the cell membrane, which requires the active F protein. Syncytium formation is also mediated by the F protein (Shibuta et al., 1986). Drzeniek et al. (1967) have shown that various PI3 virus strains differ in neuraminidase activity with some being neuraminidase strong and some neuraminidase weak.

Strains of bovine PI3 virus grow in cells of bovine, porcine and human origin, producing a CPE characterized by plaque formation, syncytia and eosinophilic inclusion bodies. Maturation occurs at the plasma membrane and virus is released from infected cells by budding. Variations in cytopathogenicity, plaque morphology and hemagglutinating and syncytium-inducing activity have been described between strains of bovine PI3 virus (Dinter et al., 1960; Hermodsson et al., 1961; Burroughs and Sulerman, 1971; Shibuta et al., 1986).

No major antigenic differences have been described between strains of PI3 virus recovered from dairy and beef cattle and from cattle in different countries (McKercher, 1963; Burroughs and Sulerman, 1971). However, with monoclonal antibodies variations in the structure of viral envelope proteins have been detected in some bovine PI3 virus strains and it has been suggested that these differences might be related to viral pathogenicity (Shibuta et al., 1986). Using monoclonal antibodies, antigenic differences have been detected between bovine and human PI3 viruses (Ray and Compans, 1986; Rydbeck et al., 1987).

EPIZOOTIOLOGY

PI3 virus was first isolated in the USA from the nasal mucus of cattle showing clinical signs of shipping fever (Reisinger et al., 1959). In the following years PI3 virus was isolated from cattle in many European countries and subsequently its distribution in the cattle population has been found to be worldwide. Surveys in the USA, most European countries, Japan, UAR, Australia and New Zealand have revealed infection with PI3 virus to be endemic in both beef and dairy cattle, with the majority of animals having antibody. An increase in the percentage of serum samples positive for PI3 virus antibody and

the geometric mean titer was noted with increasing age. Most reports of bovine PI3 virus activity have been in groups of young cattle with respiratory diseases such as enzootic calf pneumonia and shipping fever. Reports of PI3 virus-associated respiratory disease in adult cattle have been much less commonly described. However, bovine PI3 virus infections are not invariably associated with disease, and subclinical infections often occur (Frank and Marshall, 1973).

Infections with bovine PI3 virus have been observed to be most common in the autumn and winter months. In studies on dairy farms in the Netherlands, Van Nieuwstadt et al. (1982) noted that virus circulation occurred nearly every year in groups of calves. In a longitudinal survey of respiratory virus infections of young cattle in a large beef rearing unit in England, Stott et al. (1980) found that infections with PI3 virus were common, were significantly associated with disease, and mostly occurred during the months from October to March. PI3 virus infection may be accompanied by concurrent infection of the respiratory tract by other viruses such as respiratory syncytial virus, adenovirus or mucosal disease virus (Rosenquist and Dobson, 1974; Bryson et al., 1979a).

Many calves are infected by the respiratory route; a common method of infecting susceptible animals is to introduce them into an air space already occupied by animals harbouring the virus. Such a situation frequently occurs on dairy farms where accommodation is often limited and where calf houses may never be empty. Also in saleyards or transport vehicles, where calves from a variety of sources are mixed together often under conditions of severe stress this situation arises. PI3 virus multiplies to high titer in the upper and lower respiratory tract, and large quantities of virus are excreted in nasal mucus, ocular secretions and in droplets. PI3 virus is very stable in aerosols of nasal secretion, particularly where temperatures are low (Elhazary and Darbyshire, 1979); assays of nasal mucus from infected cattle have revealed titers as high as 10^6–10^7 $TCID_{50}$/ml (Frank and Marshall, 1973).

In intensive calf-rearing units PI3 virus-associated respiratory illness may be encountered in closed groups of calves assembled at a few days of age and reared in houses which had been empty, thoroughly disinfected and might never have housed calves previously (Bryson et al., 1978a). In such situations a number of calves may have been harbouring PI3 virus at the time of their introduction. Furthermore, infections with PI3 virus are occasionally demonstrable in the respiratory tract of calves only a few days of age, removed from their dams shortly after birth and reared under isolation conditions (McNulty and Bryson, unpublished results, 1988). The source of this early calfhood infection is not clear but infection may have occurred in utero or shortly after birth. PI3 virus has on occasions been isolated from aborted bovine fetuses (Sattar et al., 1965) and antibody has been demonstrable in fluids from bovine fetuses (Dunne et al., 1973). It has been possible to establish PI3 virus infection of the bovine fetus by direct intrafetal inoculation and pneumonic lesions have been observed in both the acute and chronic stages of experimental infection (Swift and Kennedy, 1972).

The possibility of cross-infections between species must also be considered. Experimentally calves have been successfully infected with ovine (Stevenson and Hore, 1970) and human (Woods, 1968) strains of PI3 virus. In lambs, bovine strains of PI3 virus produced severe clinical respiratory disease and pneumonia (Stevenson and Hore, 1970). The possibility therefore exists that cross-infections with PI3 virus particularly between cattle and sheep occur naturally. Although PI3 virus infection is not considered an important zoonosis, isolated reports have described infections with respiratory illness in children by bovine strains of PI3 virus (Ben-Ishai et al., 1980).

Whatever the initial source of infection, PI3 virus can spread quickly in

susceptible young cattle, particularly when these are confined in close contact in poorly ventilated buildings. Such conditions are encountered frequently in modern intensive calf rearing systems (Bryson et al., 1978a).

PATHOGENESIS

Bovine PI3 virus usually enters into the animal body via the respiratory tract, and most experimental studies of the pathogenesis of the virus have utilized aerosol, intranasal or combined intranasal and intratracheal routes of inoculation. Experimentally cattle of all ages have been infected with PI3 virus, although Bergman et al. (1978) found that calves less than 4 weeks old could not be infected with neuraminidase-weak strains. However, such calves can be successfully infected with neuraminidase-strong strains. Once PI3 virus is established in the respiratory tract viremia may occur (Dawson et al., 1965; Woods et al., 1965) with usually transient location of PI3 virus in a variety of sites outside the respiratory tract (Van der Maaten, 1969). Occasionally PI3 virus infection has been associated with systemic lesions such as acute splenitis (Woods et al., 1965) or enteritis (Hamdy, 1966). However, bovine PI3 virus is primarily a respiratory tract pathogen, exerting its most severe effect on respiratory tract epithelial cells (Bryson et al., 1979b, 1983a).

Before infecting cells of the respiratory tract, PI3 virus has first to penetrate the mucus layer, which consists of a gel phase and a lower sol phase. The glycoproteins of the mucus secretions are rich in N-acetyl-neuraminic acid (NANA), a molecule for which paramyxoviruses have a great affinity. The mucus glycoproteins thus act as "receptors" for bovine PI3 virus. After viral adsorption to the "receptor site", viral neuraminidase splits off the NANA from the gel glycoproteins and the virus becomes free to attach to the next receptor-bound NANA. This process appears to be the basic mechanism by which bovine PI3 virus penetrates the mucus barrier to reach epithelial cells (Morein and Dinter, 1975). The virus replicates in epithelial cells of the upper and lower respiratory tract and in alveolar macrophages. Replication is predominantly within the cytoplasm, and aggregations of viral nucleocapsids have been observed in ciliated cells of the upper respiratory tract, ciliated and nonciliated bronchiolar cells, in type I and II pneumonocytes and in a fraction of alveolar macrophages (Tsai, 1977; Bryson et al., 1983a). Virus particles are released from infected cells by "budding" from cell membranes. Nucleocapsids and budding virus particles are observed most commonly in the first 7 days post-infection (Fig. 121).

Replication of PI3 virus in the bovine respiratory tract can cause epithelial changes, including hyperplasia and necrosis. Important consequences in the acute stage of infection are destruction and loss of cilia and of ciliated cells (Fig. 122) and hyperplasia of type II pneumonocytes, leading to thickening of the respiratory barrier by cuboidal epithelialization of alveolar walls (Fig. 123; Bryson et al., 1983a). In an in vitro study, PI3 virus infection of calf alveolar macrophages has been shown to decrease their cytotoxicity for virus-infected target cells (Probert et al., 1976). In vitro and in vivo studies have also indicated that PI3 virus infections can depress phagocytosis and bacterial killing by calf alveolar macrophages (Liggitt et al., 1985; Slauson et al., 1987; Brown and Ananaba, 1988). PI3 virus-induced defects in bovine neutrophil function have also been suggested (Briggs et al., 1988). The important role of the mucociliary apparatus and alveolar macrophages in pulmonary defence mechanisms is well known, and the destruction of cilia and ciliated cells by PI3 virus together with interference with alveolar macrophage function is likely to lower the defence of the respiratory tract to subsequent infectious challenge.

Fig. 121. Nucleocapsid aggregate (arrow) in cytoplasm of a Type II pneumonocyte from a calf 2 days post-experimental infection with PI3 virus; × 8000.

Primary infection of seronegative calves with PI3 virus will result in virus shedding from the respiratory tract. Calves with maternal antibody to PI3 virus will also shed virus (Marshall and Frank, 1975; Bergman et al., 1978). In most experimental studies of virus excretion a single exposure has been used, and the duration of shedding was usually 8–10 days (Frank and Marshall, 1973). Bryson et al. (1979b) after repeatedly exposing calves to PI3 virus over a period of several days (a model which is more likely to reflect the field situation), were unable to detect virus excretion after 12 days post initial infection. Reinfection of seropositive calves several weeks after primary exposure has also been studied experimentally (Frank and Marshall, 1971). Reinfected calves may or may not excrete virus, but where virus excretion does occur it is usually of
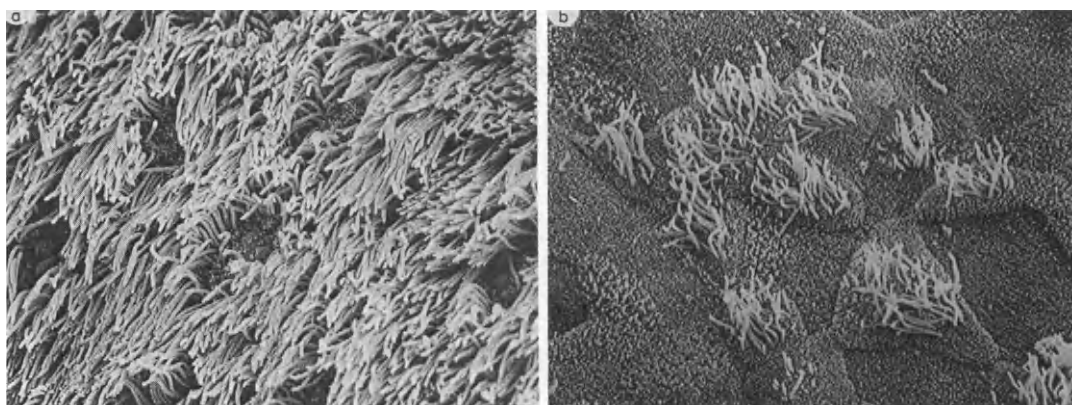
Fig. 122. (a) Scanning electron micrograph of bronchial epithelium of a control calf showing a "carpet" of cilia periodically interrupted by the apices of adjacent nonciliated cells; × 3500. (b) Scanning electron micrograph of an equivalent area of bronchial epithelium 10 days post initial infection with PI3 virus; cilia have been lost and there is exposure of microvilli; × 4500.
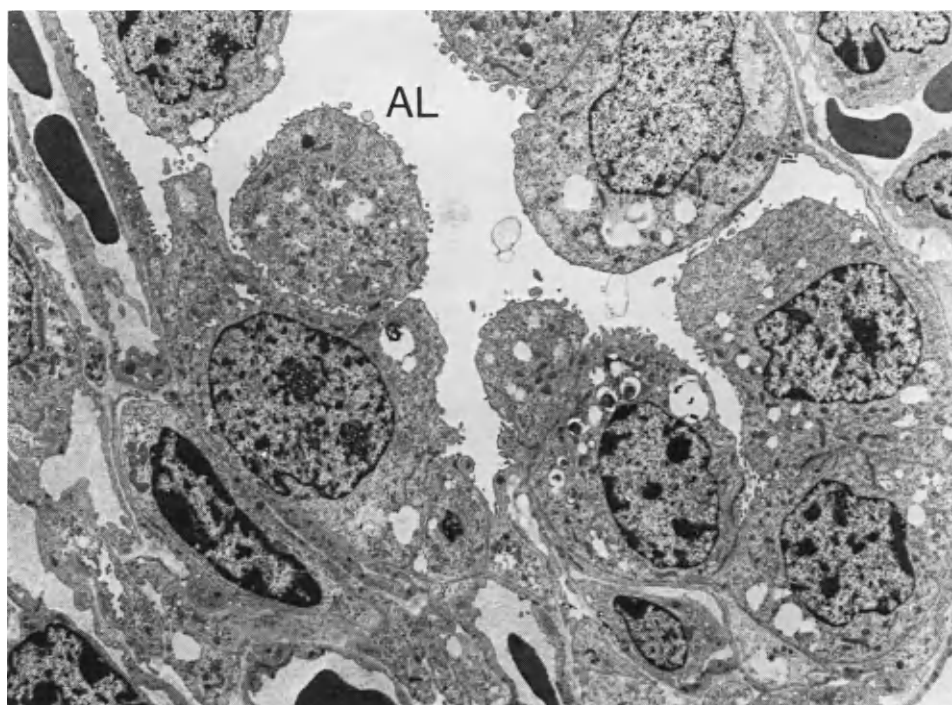
Fig. 123. Alveolus lined by cuboidal epithelial cells 11 days post-experimental infection with PI3 virus; AL = alveolar lumen; × 3000.

shorter duration than in primary infections. In naturally occurring PI3 virus infection, virus has been found in the respiratory tract of individual calves over a period of months (Allan et al., 1978; Stott et al, 1980). Whether this was due to a persistent primary infection or to reinfection was unclear, but it does indicate that under field conditions an infection focus may remain active for a considerable length of time.

The amount of damage inflicted on the respiratory tract by PI3 virus depends on the virulence of the infecting strain, the magnitude and duration of challenge, the immune status of the animal and on environmental stress factors. Experimentally, most severe respiratory tract damage was produced when young seronegative calves were infected with certain strains of PI3 virus, including te J121 strain (Omar et al., 1966), T1 strain (Dawson et al., 1965) Canadian (Tsai and Thompson, 1975) and Northern Ireland strains (Bryson et al., 1979b). Observations on naturally occurring outbreaks by Bryson et al. (1978a) indicated that environmental stress factors and in particular bad ventilation will maximize the severity of the respiratory disease problem. These findings are supported by Mihajlovic et al. (1972), who reported that calves exposed to environments with high levels of carbon dioxide and ammonia (such as occur under conditions of poor ventilation) and inoculated with PI3 virus developed respiratory disease of greater severity than calves inoculated but not exposed.

In natural outbreaks there is rarely an opportunity to study an uncomplicated PI3 virus infection. Usually it is accompanied by other microorganisms, including other respiratory viruses, mycoplasmas and bacteria. The most important respiratory diseases in which PI3 virus activity has been described are enzootic pneumonia of calves and shipping fever.

Enzootic calf pneumonia is a general term incorporating a range of respiratory tract lesions occurring in young calves in their first period indoors. A number of viruses, including PI3 virus, mycoplasmas and bacteria, are involved

in the production of respiratory tract damage. PI3 virus has been reported frequently in outbreaks of enzootic pneumonia in many countries (Ide, 1970), with viral activity occurring mainly in the early stages of pneumonia outbreaks.

"Shipping fever pneumonia" is a problem in many countries, but particularly in the USA, where it is the most common, serious and costly disease of feedlot cattle. Yates (1982) has defined shipping fever as "a pneumonic condition of cattle which is of undetermined though multifactorial etiology and is usually associated with *Pasteurella haemolytica* or less commonly *Pasteurella multocida*." The activity of PI3 virus along with *Pasteurella* species has frequently been demonstrated in outbreaks of shipping fever. Synergism between PI3 virus and *Pasteurella haemolytica* has been shown experimentally when the interval between viral and bacterial exposure was 3–10 days (Jericho et al., 1982). However, despite a variety of reports of combined infections of calves with PI3 virus and *Pasteurella* species (reviewed by Yates, 1982) the pathogenesis of shipping fever and the role of PI3 virus remains poorly understood.

DISEASE SIGNS

In experimental reports where it has been possible to study uncomplicated infection of the bovine respiratory tract with PI3 virus, the severity of clinical signs has varied considerably; sometimes the infection has been asymptomatic. In the majority of cases a mild clinical illness characterized by coughing, fever, nasal and sometimes ocular discharge has been produced (Frank and Marshall, 1973). A moderate to severe respiratory illness with marked tachypnea and hyperpnea and evidence of pneumonia on auscultation has occasionally been described (Omar et al., 1966; Bryson et al., 1979b, 1989).

Enzootic calf pneumonia is a high-morbidity disease and the clinical picture can vary from mild outbreaks characterized mainly by coughing to severe pneumonia outbreaks with the majority of calves developing tachypnea, hyperpnea and losing condition. Mortalities approaching 10% have been recorded; survivors may be left with permanent lung damage, leading to depressed food conversion efficiency and liveweight gain (Ide, 1970; Thomas, 1973; Thomas et al., 1978; Bryson et al., 1978a).

In recent years there have been reports of viral infections of the bovine respiratory tract where the clinical signs were particularly acute and severe and where lung damage has been caused by virus activity alone. Of particular importance is the distinctive rapid-onset respiratory distress syndrome of young cattle seen mainly in autumn. Up to 10% of calves in an affected group may develop dyspnea, and on clinical examination there is evidence of severe pneumonia in the cranial lung lobes with pulmonary emphysema. The majority of severely affected animals die. This syndrome has been recognized in Europe and the USA and is consistently associated with RSV; nevertheless in some instances the activity of PI3 virus has also been demonstrable (Holzhauer and Van Nieuwstadt, 1976; Bryson et al., 1979a). At least one of such strains of PI3 virus produced extensive pneumonia when inoculated into calves (Bryson et al., 1979b, in press), which suggests that where dual infections had occurred, PI3 virus may have contributed to the severity of the lung damage.

Shipping fever pneumonia is characterized by depression, anorexia, high fever, rhinitis and nasal discharge, tachypnea and dyspnea. Increased lacrimation is a frequent sign, and affected animals may show evidence of thoracic pain. On auscultation there is increased harshness of lung sounds, and adventitious sounds may be detectable. Diarrhea occurs in some animals, and there

is invariably a substantial weight loss. Most cases occur within one month of arrival at the feedlot; morbidity rates recorded have varied from 4% to 44% of animals at risk. Mortality rates up to 20% have been described (Jensen et al., 1976; Yates, 1982).

While it is possible to clinically differentiate the major respiratory syndromes in which PI3 virus may participate it is not possible to diagnose the involvement of PI3 virus on clinical grounds alone.

PATHOLOGY

Although there are numerous reports of experimental infections of calves with PI3 virus, only a few have included a detailed pathological study of the respiratory tract. Where pathological examination has been carried out, the damage has varied from mild rhinitis and tracheobronchitis (Bögel, 1961) to pneumonia, which in some cases was quite extensive (Dawson et al., 1965; Omar et al., 1966; Bryson et al., 1979b).

The rhinitis and tracheitis produced by PI3 virus is usually slight and not a striking feature at necropsy. Uncomplicated PI3 virus pneumonia appears as deep red coloured zones of consolidation distributed mainly in the cranial lung lobes and in the cranioventral aspects of the caudal lobes (Fig. 124). Such zones may coalesce to produce areas of consolidation involving most of the affected lobe or lobes. In the acute stages the pneumonic areas are swollen, but in the repair stage they may be slightly depressed compared with adjacent nonconsolidated lung tissue. On section of the lung tissue catarrhal or purulent exudate may be expressed from small bronchi and bronchioli. Interlobular septa may be edematous but are seldom distended. The bronchial and mediastinal lymph nodes may be enlarged. Pleurisy nd abscess formation are usually absent.

Histopathologically, PI3 virus activity in the upper respiratory tract can result in epithelial changes such as hyperplasia, metaplasia or necrosis. Often the underlying lamina propria is infiltrated with polymorphs, macrophages and cells of the lymphocyte series. In the lower respiratory tract PI3 virus can cause severe damage to small bronchi, bronchioli and alveoli. In the early stages of the disease there is bronchitis, bronchiolitis, cellular infiltration into alveoli and thickening of interalveolar septa. Cellular exudate accumulates in bronchial and bronchiolar lumina until many of the small airways become occluded. Alveolar epithelial hyperplasia develops, leading to sometimes extensive cuboidal epithelialization. In the acute stages of PI3 virus infection eosinophilic intracytoplasmic and less commonly intranuclear inclusion bodies may be observed in bronchial, bronchiolar and alveolar epithelium. They are most commonly observed between 2 and 7 days p.i. Epithelial syncytium formation on bronchiolar and alveolar walls (Fig. 125) has been a prominent feature of infections with some strains of bovine PI3 virus (Dawson et al., 1965; Omar et al., 1966; Bryson et al., 1979b).

In the repair stage of PI3 virus pneumonia, macrophages invade bronchiolar and alveolar lumina and reepithelialization is observed. Organization of bronchiolar exudate can lead to widespread bronchiolitis obliterans, with fibrous masses causing varying degrees of obliteration of bronchiolar lumina (Bryson et al., 1983b).

In animals that die in outbreaks of PI3-virus-associated enzootic pneumonia or shipping fever, primary PI3 virus pathology may be obscured by superimposed changes produced by secondary invaders, particularly the severe exudative changes, necrosis and pleurisy produced by *Pasteurella* sp. However, in some instances, particularly with PI3 virus strains that produce large numbers
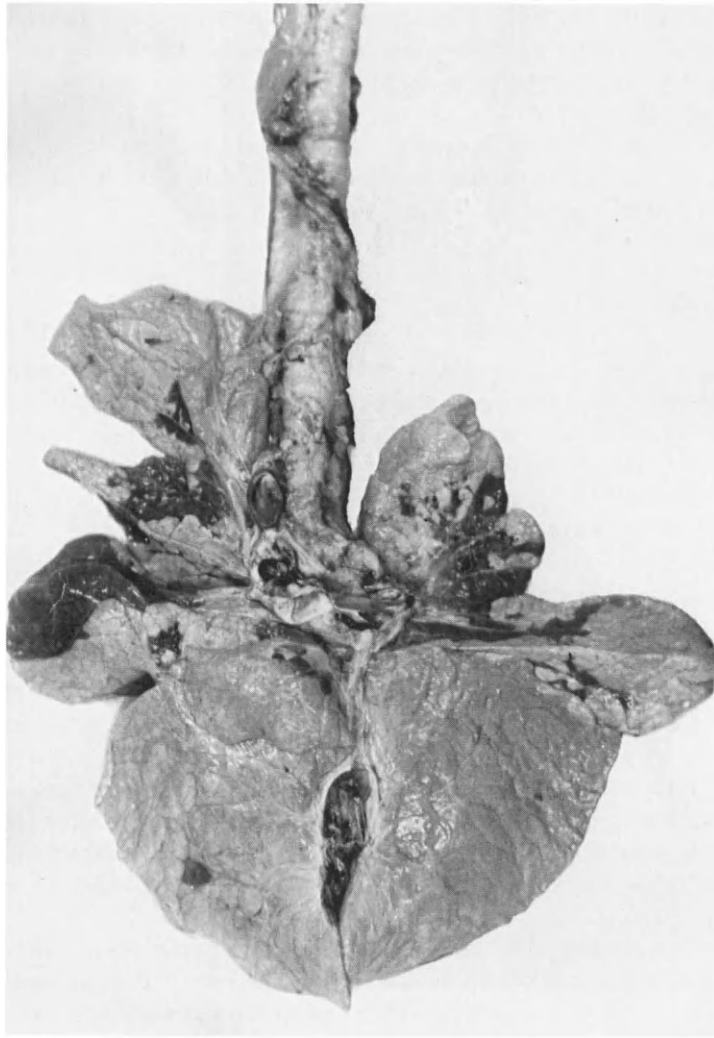
Fig. 124. Lungs of calf killed 7 days after experimental infection with PI3 virus; note pneumonic areas in the cranial lung lobes.

of syncytia, changes incriminating PI3 virus may still be detectable on careful histopathological examination (Betts et al., 1964; Bryson et al., 1978b).
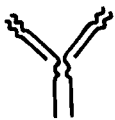
IMMUNE REACTION

Exposure of seronegative calves to PI3 virus results in the development of both systemic and local virus neutralizing antibody (Morein, 1970; Marshall and Frank, 1971; McKercher et al., 1972). IgM and IgG are the immunoglobulin classes predominantly involved in the serum antibody response, whilst anti-PI3 virus activity in nasal secretions resides mainly in the IgA class of immunoglobulin (Morein, 1970). After primary infection, high levels of serum antibody may be detectable 6 days p.i.; virus neutralizing antibody may also be detected in nasal secretions at that time (Marshall and Frank, 1971). There is some dispute as to the length of time over which serum antibodies remain at a high level (Dawson et al., 1965; Thomas, 1973); most workers have found that after a primary infection serum antibodies will persist at a high level for 3–5 months.

In a series of experiments, Marshall and Frank (1971) exposed 5–12-month-old colostrum-deprived calves to aerosols of PI3 virus and studied the develop-
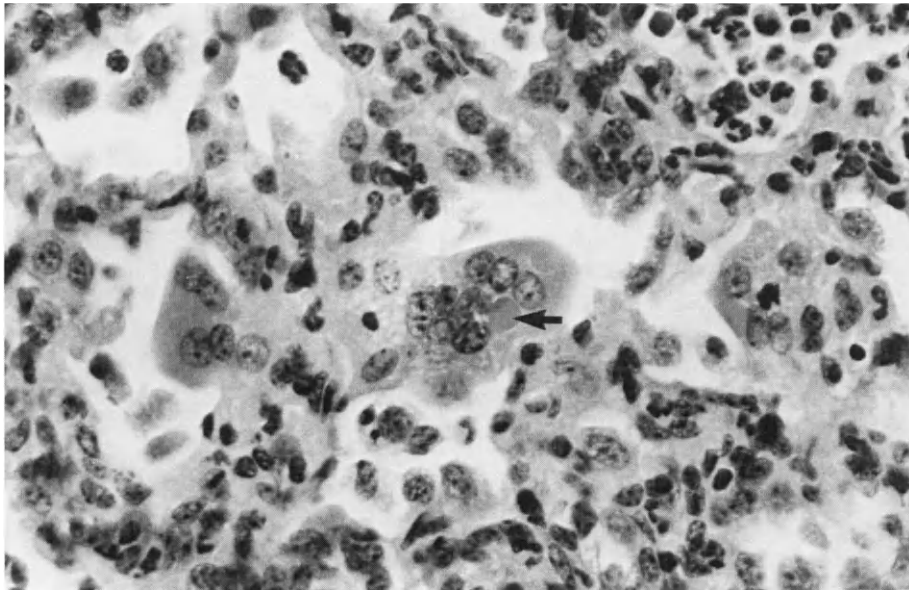
Fig. 125. Alveolar syncytium containing intracytoplasmic inclusion body (arrow) in a field case of enzootic pneumonia from which PI3 virus was isolated; H&E, × 400.

ment of virus neutralizing antibodies in serum and nasal secretions. The serum antibodies persisted for 3–5 months, but nasal antibodies declined rapidly, reaching low to subdetectable levels within 6–8 weeks. Reexposure produced a sharp increase in both serum and nasal antibody titer consistent with an amnestic response. After reexposure, high levels of nasal antibody and serum antibody persisted for 5 months, but thereafter nasal antibody levels but not serum antibody levels began to fall. In a subsequent experiment Frank and Marshall (1971) found that in reinfection experiments with bovine PI3 virus calves with high levels of serum antibody and low levels of nasal secretory antibody succumbed to clinical illness, whereas calves with low serum antibody titers and high nasal secretory antibody titers did not. These workers concluded that the degree of protection from PI3 virus-associated illness was reflected more by nasal secretory antibody than by serum antibody. However, serum antibody was associated with reducing the severity of illness once it occurred. In reinfections, PI3 virus may continue to be excreted for varying periods, and Gates et al. (1970) concluded that differences in serum or nasal antibody titers could be demonstrated to account for differences in duration of viral excretion.

As the majority of adult cattle has antibodies to PI3 virus, calves will obtain maternal antibodies to this virus. Dawson (1966) found that their persistence in calf serum depended on the initial titer of the transferred antibodies. Maternal antibodies to PI3 virus often persisted until calves were 10 weeks of age; in calves which had received a high initial level of maternal antibodies they persisted until 19–23 weeks of age. McKercher (1972) reported that antibody acquired from colostrum also appeared in the nasal secretions of young calves.

Marshall and Frank (1975) studied the clinical and immunologic responses of calves with colostral antibody against PI3 virus to infection. After aerosol exposure the colostrum-fed calves developed disease and shed virus from their nasal passages. However, the disease was less severe in the colostrum-fed calves than in colostrum-deprived calves. Both the serum and nasal antibody responses were markedly depressed compared to the response of calves which did not receive maternal antibody. A challenge exposure stimulated amnestic responses which appeared damped by the maternal antibodies, being less than

in calves which were initially seronegative. No clinical signs were observed following challenge exposure. These findings indicate that maternal antibodies may reduce the severity of PI3 virus-associated disease. In experimental systems, the most severe lesions produced have been in colostrum-deprived calves (Omar et al., 1966; Bryson et al., 1979b).

CMI also participates in the immune response. Morein and Moreno-López (1973) produced a delayed skin hypersensitivity reaction with PI3 virus antigen in seropositive cattle naturally infected with PI3 virus. Very young calves possessing exclusively maternal antibodies to PI3 virus did not show any skin hypersensitivity. Subsequently Johnson and Morein (1977) and Moreno-López (1977) demonstrated lymphocyte stimulation and leukocyte migration inhibition by PI3 virus antigen in cattle infected with PI3 virus. However, the relative importance of CMI compared with that of antibody mediated immunity in protection against PI3 virus disease is unknown.

LABORATORY DIAGNOSIS

In outbreaks of bovine respiratory disease it is not possible to diagnose PI3 virus infection on clinical grounds alone. To establish a diagnosis it is necessary to take nasal samples and paired sera from infected animals and to submit animals from the outbreak for necropsy, the latter facilitating pathological, microbiological and immunocytochemical examinations of the lower respiratory tract.

Good quality nasal samples may be nasal swabs taken into transport media or samples of nasal mucus extracted with a portable vacuum pump (Baskerville and Lloyd, 1977). The use of conventional cotton-wool-tipped swabs frequently does not yield suitable material for virus isolation; more suitable swabs have been described by Thomas and Stott (1975). Because of their content of infected cells, nasal mucus samples provide excellent material not only for virus isolation in tissue culture but also for rapid diagnosis by IF (McFerran and McNulty, 1981). To maximize the chances of PI3 virus isolation it is important to sample as early as possible in the outbreak, preferably within a few days of the animals showing clinical signs, and to sample a range of animals showing a range of clinical signs and not just the most severely affected animals. Samples should be submitted as quickly as possible to the diagnostic laboratory.

PI3 virus infection in an outbreak of respiratory disease can be detected by the demonstration of a 4-fold or greater rise in serum antibody titer to the virus between acute- and convalescent-phase serum samples. For the antibody increase (seroconversion) to be detected, paired sera taken from the same animals approximately 3–4 weeks apart must be submitted and the acute phase samples must be taken as early as possible. Submission of a batch of single samples is useless. Serological diagnosis in young calves may be complicated by persistent maternal antibodies blocking an active antibody response to virus infection (Thomas, 1973). To circumvent this, as many animals as possible should be sampled, and diagnosis should not be left to serological examination alone. In small groups at least 50%, and in larger groups 10–20% of the animals should be serologically examined. The serological tests most commonly used are HI, SN, indirect IF (Adair, 1986) and ELISA (Florent and De Marneffe, 1986). Development of IgM-specific ELISA may prove useful for serodiagnosis of PI3 virus infections in young calves with maternal antibodies, as has been shown in the case of BRSV (Westenbrink and Kimman, 1987).

In animals submitted for necropsy from outbreaks of respiratory disease it is possible to carry out histopathological examination and immunofluorescent

and immunoperoxidase staining to detect PI3 virus antigen in lung parenchyma and also to attempt virus isolation from the lower respiratory tract. Unfortunately, in many animals that die during outbreaks of enzootic pneumonia or shipping fever primary PI3 virus pathology may be obscured by secondary exudative changes in the lungs, particularly those produced by *Pasteurella* sp. Furthermore, in animals dying in the middle or latter stages of outbreaks, PI3 virus antigen may be no longer detectable in lung tissue. Special efforts therefore should be made to submit animals which die in the early stages of outbreaks for necropsy, and better still to submit a live animal in the early stages of the outbreak.

On histopathological examination suspicion of PI3 virus infection may be aroused by the finding of eosinophilic intracytoplasmic inclusion bodies in respiratory epithelium and by the presence of epithelial syncytia on bronchiolar and alveolar walls, often accompanied by hyperplasia of the alveolar epithelium. However, it should be remembered that a similar pathology is encountered in RSV pneumonia in young cattle (Bryson et al., 1983b). Furthermore, inclusion bodies in syncytia are not present at all stages of infection and some strains of PI3 virus produce few syncytia (Bryson et al., 1979b). Examination of sections therefore should be accompanied by specific staining for PI3 virus antigen using IF or immunoperoxidase methods.

PI3 viral antigen may be detected in lung tissue by direct or indirect IF examination of impression smears, cryostat sections (McNulty and Allan, 1984) and paraffin sections (Watt et al., 1982). PI3 viral antigen may also be detected in paraffin sections using immunoperoxidase methods, such as those described for the detection of BRSV (Bryson et al., 1988).

PROPHYLAXIS AND CONTROL

In the prophylaxis of PI3 virus-associated respiratory disease, measures should be taken to reduce managemental stress factors predisposing to severe outbreaks of pneumonia. Overcrowding should be avoided, adequate ventilation should be maintained at all times and large calf rearing houses should be partitioned into a number of subunits, each with its own air space. Buildings used for calf rearing should be periodically depopulated and disinfected whenever possible.

Both inactivated and modified live PI3 virus vaccines are commercially available.

Inactivated PI3 virus vaccines have been shown capable of stimulating systemic and local antibody production and reducing virus excretion post-challenge when administered systemically to seronegative calves or calves with low antibody levels to PI3 virus (Dinter, 1967; Morein, 1972; Probert et al., 1978). Two doses given some weeks apart are usually necessary to achieve this effect.

Modified live PI3 virus vaccines given by the intranasal or intramuscular route to seronegative calves or calves with low levels of antibody will also result in the production of systemic and local nasal secretory antibody (McKercher et al., 1972; Zygraich, 1979). Vaccinal virus may be shed from the nasal passages, the shedding index being much higher in the nasally vaccinated animals (McKercher et al., 1972). The antibody response (both local systemic) appears earlier and in higher titer in response to intranasal vaccine than to parenteral inoculation (McKercher et al., 1972; Woods et al., 1975). Viral shedding following experimental challenge exposure has often been found reduced in vaccinated animals.

Opinions are divided as to the comparative efficacy of intranasal and intra-

muscular vaccination with live PI3 virus vaccines. Gutekunst et al. (1969) and Gates et al. (1970) reported that intranasal vaccination resulted in better protection against experimental challenge. However, in a comparative study of the response of 3–4-month-old colostrum-deprived and conventionally reared calves to live PI3 virus vaccines designed for intramuscular and for intranasal use, McKercher et al. (1972) were unable to demonstrate any advantage of one vaccine or route of administration over the other. They did note a possible exception in the case of very young calves, which may have persisting maternal antibody levels capable of interfering with parenterally administered vaccine. In such a situation nasal administration is more likely to produce an effective response.

In the past, the absence of a reproducible challenge model capable of producing disease and pathological lesions has made evaluation of PI3 virus vaccines under experimental conditions very difficult. Stimulation of antibody production (often systemic antibodies alone) and reduction of viral shedding post-challenge have been mostly used as criteria of vaccinal protection. Such parameters may not be reliable indicators of vaccinal efficacy under field conditions.

PI3 virus vaccines have been marketed mainly for use in prophylactic regimes against shipping fever and for the control of enzootic calf pneumonia. Inactivated PI3 virus vaccines have frequently been combined with inactivated IBR vaccine and *Pasteurella* bacterins for the control of shipping fever and with inactivated preparations of other viruses thought to be involved in enzootic calf pneumonia outbreaks. Two injections of inactivated products are usually recommended. Live PI3 virus vaccines are available as single-component vaccines or as a component of multivalent preparations containing, for example, IBRV BVDV or adenovirus. The range of preparations currently available includes vaccines containing a temperature-sensitive mutant of the Umeå strain of bovine PI3 virus (Zygraich et al., 1979). Where calves more than 3–4 months of age are vaccinated with live vaccine one injection may be sufficient. However, where calves only a few days or weeks old are vaccinated (e.g. in regimes to control enzootic calf pneumonia), maternal antibodies may interfere. In such situations intranasal vaccination is indicated and revaccination may be necessary to ensure the persistence of antibody in nasal secretion (Marshall and Frank, 1975).

Reports of the use of PI3 virus vaccination in prophylactic regimes against shipping fever are numerous and do not provide unequivocal evidence of efficacy. In a comprehensive review on the published data on the efficacy of various respiratory disease vaccination regimes in feedlot calves, Martin (1983) criticizes the lack of detail and planning relating to experimental design and lack of formal analysis of results in many reports. The author found little evidence of a beneficial effect of the use of PI3 vaccines in feedlot calves in terms of reduced incidence of respiratory disease and improved weight gains and concluded that there was little data to support the use of vaccines.

Vaccination may be considered in the prophylaxis of enzootic pneumonia outbreaks, particularly when investigations of previous outbreaks on a calf rearing unit have yielded a diagnosis of PI3 virus activity. In view of the variety of organisms that can be involved in enzootic calf pneumonia, vaccination against PI3 virus alone, whilst possibly reducing the severity of the disease, is unlikely to effect control on every farm. Vaccination against a range of viruses —including PI3 virus—may be necessary before satisfactory prophylaxis is achieved.

REFERENCES

Adair, B.M., 1986. Immunofluorescence in the serological diagnosis of parainfluenza type 3 and respiratory syncytial virus infections in calves. Res. Vet. Sci., 41: 414–416.

Allan, E.M., Pirie, H.M., Selman, I.E. and Snodgrass, D.R., 1978. Some characteristics of a natural infection by parainfluenza-3 virus in a group of calves. Res. Vet. Sci., 24: 339–346.

Baskerville, A. and Lloyd, G., 1977. A method for the collection of nasal epithelial cells and secretion from domestic animals. Vet. Rec., 101: 168–170.

Ben-Ishai, Z., Naftali, V., Avram, A. and Yatziv, S., 1980. Human infection by a bovine strain of parainfluenza virus type-3. J. Med. Virol., 6: 165–168.

Bergman, R., Moreno-López, J., Mollerberg, L. and Morein, B., 1978. Parainfluenza-3 virus: difference in capacity of neuraminidase weak and strong strains to infect young calves and to elicit cellular immune response. Res. Vet. Sci., 25: 193–199.

Betts, A.O., Jennings, A.R., Omar, A.R., Page, Z.E., Spence, J.B. and Walker, R.G., 1964. Pneumonia in calves by parainfluenza virus type-3. Vet. Rec., 76: 382–384.

Bögel, K., 1961. Virological findings in calves with respiratory syndrome with special reference to parainfluenza type-3 virus. Monatsh. Tierheilk., 13: 129–135.

Briggs, R.E., Kehrli, M. and Frank, G.H., 1988. Effects of infection with parainfluenza-3-virus and infectious bovine rhinotracheitis virus on neutrophil functions in calves. Am. J. Vet. Res., 49: 682–686.

Brown, T.T. and Ananaba, G., 1988. Effect of respiratory infections caused by bovine herpesvirus-1 or parainfluenza-3-virus on bovine alveolar macrophage functions. Am. J. Vet. Res., 49: 1447–1451.

Bryson, D.G., McFerran, J.B., Ball, H.J. and Neill, S.D., 1978a. Observations on outbreaks of respiratory disease in housed calves. I. Epidemiological, clinical and microbiological findings. Vet. Rec., 103: 485–489.

Bryson, D.G., McFerran, J.B., Ball, H.J. and Neill, S.D., 1978b. Observations on outbreaks of respiratory disease in housed calves. II. Pathological and microbiological findings. Vet. Rec., 103: 503–509.

Bryson, D.G., McFerran, J.B., Ball, H.J. and Neill, S.D., 1979a. Observations on outbreaks of respiratory disease in calves associated with parainfluenza type-3 virus and respiratory syncytial virus infection. Vet. Rec., 104: 45–49.

Bryson, D.G., McNulty, M.S., Ball, H.J., Neill, S.D., Connor, T.J. and Cush, P.F., 1979b. The experimental production of pneumonia in calves by intranasal inoculation of parainfluenza type-3 virus. Vet. Rec., 105: 566–573.

Bryson, D.G., McNulty, M.S., McCracken, R.M. and Cush, P.F., 1983a. Ultrastructural features of experimental parainfluenza type-3 virus pneumonia in calves. J. Comp. Pathol., 93: 397–414.

Bryson, D.G., McNulty, M.S., Logan, E.F. and Cush, P.F., 1983b. Respiratory syncytial virus pneumonia in calves: clinical and pathological findings. Am. J. Vet. Res. 44: 1648–1655.

Bryson, D.G., Cush, P.F., McNulty, M.S., Platten, M. and Allan, G.M., 1988. An immunoperoxidase method of detecton of respiratory syncytial virus antigens in paraffin sections of pneumonic bovine lung. Am. J. Vet. Res., 49: 1121–1126.

Bryson, D.G., McNulty, M.S., Evans, R.T. and Allan, G.M., 1989. Studies on the effect of recombinant human alpha-1 interferon on experimental parainfluenza type-3 virus infections of the bovine respiratory tract. Vet. Rec., 125: 615–617.

Burroughs, A.L. and Sulerman, P.P., 1971. Forty-one isolates of parainfluenza-3 virus from dairy and beef cattle compared. Cornell Vet., 61: 96–103.

Dawson, P.S., 1964. The isolation and properties of a bovine strain (T1) of parainfluenza 3 virus. Res. Vet. Sci., 5: 81–88.

Dawson, P.S., 1966. Persistence of maternal antibodies to parainfluenza 3 virus. J. Comp. Pathol., 76: 373–379.

Dawson, P.S., Derbyshire, J.H. and Lamont, P.H., 1965. The inoculation of calves with parainfluenza 3 virus. Res. Vet. Sci., 6: 108–113.

Dinter, Z., 1967. In: First International Conference on Vaccines against Viral and Rickettsial Disease of Man. PAHO/WHO, Washington, DC, pp. 42–49.

Dinter, Z., Hermodsson, S. and Bakos, K., 1960. Studies on variants of a bovine strain of parainfluenza-3 virus (1) isolation and growth characteristics. Acta Pathol. Microbiol. Scand., 49: 485–492.

Drzeniek, R., Bogel, K. and Roll, R., 1967. On the classification of bovine parainfluenza-3 viruses. Virology, 31: 725–727.

Dunne, H.W., Ajinkya, S.M., Bubash, G.R. and Griel, L.C., 1973. Parainfluenza-3 and bovine enteroviruses as possible important causative factors in bovine abortion. Am. J. Vet. Res., 34: 1121–1126.

Elhazary, M.A.S.Y. and Darbyshire, J.B., 1979. Aerosol stability of bovine parainfluenza type-3 virus. Can. J. Comp. Med., 43: 295–305.

Florent, G. and De Marneffe, C., 1986. Enzyme linked immunosorbent assay used to monitor serum antibodies to bovine respiratory disease viruses. Vet. Microbiol., 11: 309–317.

Frank, G.H. and Marshall, R.G., 1971. Relationship of serum and nasal secretion neutralising antibodies in protection of calves against parainfluenza-3 virus. Am. J. Vet. Res., 32: 1707–1713.

Frank, G.H. and Marshall, R.G., 1973. Parainfluenza-3 virus infection of cattle. J. Am. Vet. Med. Assoc., 163: 858–860.

Gates, G.A., Cesario, T.C., Ebert, J.W., Kriel, R.L., Nulff, H.T., Poland, J.D., Gutekunst, D.E. and Chin, T.D.Y., 1970. Neutralizing antibody in experimentally induced respiratory infection in calves. Am. J. Vet. Res., 31: 217–224.

Gutekunst, D., Paton, I. and Volenec, F., 1969. Parainfluenza-3 vaccine in cattle: comparative efficacy of intranasal and intramuscular routes. J. Am. Vet. Med. Assoc., 155: 1879–1885.

Hamdy, A.H., 1966. Association of myxovirus PI3 with pneumo-enteritis of calves—virus isolation. Am. J. Vet. Res., 27: 981–986.

Hermodsson, S., Dinter, Z. and Bakos, K., 1961. Studies on variants of a bovine strain of parainfluenza-3 virus (2) haemagglutinating activity. Acta Pathol. Microbiol. Scand., 51: 75–84.

Holzhauer, C. and Van Nieuwstadt, A.P.K.M.I., 1976. Role of bovine respiratory syncytial virus in the aetiology of virus pneumonia in calves and yearlings ("Pinkengriep"). Tijdschr. Diergeneesk., 101: 1023–1031.

Ide, P.R., 1970. The etiology of enzootic pneumonia of calves. Can. Vet. J., 11: 194–202.

Jensen, R., Pierson, R.E., Braddy, P.M., Saari, D.A., Lauerman, L.H., England, J.J., Keyvanfar, H., Collier, J.R., Horton, D.P., McChesney, A.E., Benitez, A. and Christie, R.M., 1976. Shipping fever pneumonia in yearling feedlot cattle. J. Am. Vet. Med. Assoc., 169: 500–506.

Jericho, K.W.F., Darcel, C. le Q. and Langford, E.V., 1982. Respiratory disease in calves produced with aerosols of parainfluenza-3 virus and *Pasteurella haemolytica*. Can. J. Comp. Med., 46: 293–301.

Johnson, K. and Morein, B., 1977. In vitro stimulation of bovine circulating lymphocytes by parainfluenza type-3 virus. Res. Vet. Sci., 22: 83–85.

Kahn, D.E., Kita, J. and Gillespie, J.H., 1969. An electron microscopic comparison of 3 strains of bovine parainfluenza 3 virus. Cornell Vet., 59: 431–438.

Liggitt, H.D., Huston, L., Silflow, R., Evermann, J. and Trigo, E., 1985. Impaired function of bovine alveolar macrophages infected with parainfluenza-3-virus. Am. J. Vet. Res. 46: 1740–1744.

McFerran, J.B. and McNulty, M.S., 1981. Aids to diagnosis of virological diseases. Br. Vet. J., 137: 455–463.

McKercher, D.G., 1963. A comparative study of a strain of myxovirus from cattle in California affected with Shipping fever with American and European strains of parainfluenza-3 virus. Cornell Vet., 53: 262–269.

McKercher, D.G., 1972. Nasal versus parenteral vaccination for the protection of cattle against viral infection of the respiratory tract. Arch. Vet. Ital., 23: 63–74.

McKercher, D.G., Saito, J.K., Franti, C.E., Wade, E.M. and Crenshaw, G.L., 1972. Response of calves to parainfluenza-3 vaccines administered nasally or parenterally. Am. J. Vet. REs., 33: 721–730.

McNulty, M.S. and Allan, G.M., 1984. Applications of immunofluorescence in veterinary viral diagnosis. In: Advances in Virus Diagnosis. Martinus Nijhoff, Boston, pp. 15–27.

McNulty, M.S., Bryson, D.G. and Allan, G.M., 1983. Experimental RSV pneumonia in young calves: microbiologic and immunofluorescence findings. Am. J. Vet. Res., 44: 1656–1659.

Marshall, R.G. and Frank, G.H., 1971. Neutralising antibody in serum and nasal secretions of calves exposed to parainfluenza-3 virus. Am. J. Vet. Res., 32: 1699–1706.

Marshall, R.G. and Frank, G.H., 1975. Clinical and immunologic responses of calves with colostrally acquired maternal antibody against parainfluenza-3 virus to homologous viral infection. Am. J. Vet. Res., 36: 1085–1089.

Martin, S.W., 1983. Vaccination: Is it effective in preventing respiratory disease or influencing weight gains in feedlot calves. Can. Vet. J., 24: 10–19.

Mihajlovic, B., Bratanovic, U., Puhac, I., Sofrenovic, D., Gligorijevic, J., Kuzmanovic, M., Jermolenko, G. and Cvetkovic, A., 1972. Effect of some microclimatic factors on artificial infections with bovine respiratory viruses. Acta Vet. Beograd., 22: 299–308.

Morein, B., 1970. Immunity against parainfluenza-3 virus in cattle; IgA in nasal secretions. Int. Arch. Allergy, 39: 403–414.

Morein, B., 1972. Immunity against parainfluenza-3 virus in cattle; immunoglobulins in serum and nasal secretions after subcutaneous and nasal vaccination. Z. Immunol. Forsch., 144: 63–74.

Morein, B. and Moreno-López, J.W., 1973. Skin hypersensitivity to parainfluenza-3 virus in cattle. Zentralbl. Veterinärmed., 20: 540–546.

Morein, B. and Dinter, Z., 1975. Parainfluenza-3 virus in cattle: mechanisms of infection and defence in the respiratory tract. Vet. Sci., 12: 40–41.

Moreno-López, H.W., 1977. Cell-mediated immunity to parainfluenza-3 virus (PIV-3) in cattle. Evaluation of in vivo and in vitro tests. Zentralbl. Veterinärmed. B, 24: 231–240.

Omar, A.R., Jennings, A.R. and Betts, A.O., 1966. The experimental disease produced in calves by the J121 strain of parainfluenza virus type 3. Res. Vet. Sci., 7: 379–388.

Probert, M., Stott, E.J. and Thomas, L.H., 1976. Interactions between calf alveolar macrophages and parainfluenza-3 virus. Infect. Immun., 15: 576–585.

Probert, M., Stott, E.J., Thomas, L.H., Collins, A.P. and Jebbetts, J., 1978. An inactivated parainfluenza virus type 3 vaccine; the influence of vaccination regime on the response of calves and their subsequent resistance to challenge. Res. Vet. Sci., 24: 222–227.

Ray, R. and Compans, R.W., 1986. Monoclonal antibodies reveal extensive antigenic differences between the haemagglutinin–neuraminidase glycoproteins of human and bovine parainfluenza-3 viruses. Virology, 148: 232–236.

Reisinger, R.C., Heddleston, K.L. and Manthei, C.A., 1959. A myxovirus (SF-4) associated with Shipping fever of cattle. J. Am. Vet. Med. Assoc., 135: 147–152.

Rosenquist, B.D. and Dobson, A.W., 1974. Multiple viral infection in calves with acute bovine respiratory tract disease. Am. J. Vet. Res., 34: 363–365.

Rydbeck, R., Love, A., Orvell, C. and Norrby, E., 1987. Antigenic analysis of human and bovine parainfluenza virus type 3 strains with monoclonal antibodies. J. Gen. Virol., 68: 2153–2160.

Sakai, Y., Suzu, S., Shioda, T. and Shibuta, H., 1987. Nucleotide sequence of the bovine parainfluenza-3 virus genome: Its 3′ end and the genes of NP, P, C and M proteins. Nucleic Acids Res., 15: 2927–2944.

Sattar, S.A., Bohl, E.H. and Senturk, M., 1965. Viral causes of bovine abortion in Ohio. J. Am. Vet. Med. Assoc., 147: 1207–1210.

Shibuta, H., Kanda, T., Adechi, A. and Yogo, Y., 1979. Characteristics of bovine parainfluenza virus type 3. Microbiol. Immunol., 23: 617–628.

Shibuta, H., Suzu, S. and Shioda, T., 1986. Differences in bovine parainfluenza 3 virus variants studied by monoclonal antibodies against viral glycoproteins. Virology, 155: 688–696.

Shioda, T., Wakao, S., Suzu, S. and Shibuto, H., 1988. Difference in bovine parainfluenza-3 virus variants studied by sequencing of the genes of viral envelope proteins. Virology, 162: 388–396.

Slauson, D.O., Ley, J.C., Castleman, W.L. and Neilson, N.R., 1987. Alveolar macrophage phagocytic kinetics following pulmonary parainfluenza-3 virus infection. J. Leukocyte Biol., 41: 412–420.

Stevenson, R.G. and Hore, D.E., 1970. Comparative pathology of lambs and calves infected with parainfluenza virus type 3. J. Comp. Pathol., 80: 613–618.

Stott, E.J., Thomas, L.H., Collins, A.P., Crouch, S., Jebbett, J., Smith, G.S., Luther, P.D. and Coswell, R., 1980. A survey of virus infections of the respiratory tract of cattle and their association with disease. J. Hyg. (Camb.), 85: 257–270.

Swift, B.L. and Kennedy, P.C., 1972. Experimentally induced infection of in utero bovine fetuses with bovine parainfluenza 3 virus. Am. J. Vet. Res., 33: 57–63.

Thomas, L.H., 1973. Observations on the role of viruses in pneumonia of calves. Vet. Rec., 93: 384–388.

Thomas, L.H. and Stott, E.J., 1975. Comparison of three methods for sampling the bovine upper respiratory tract for viruses. Res. Vet. Sci., 18: 227–229.

Thomas, L.H., Wood, P.D.P. and Langland, J.M., 1978. The influence of disease on the performance of beef cattle. Br. Vet. J., 134: 152–161.

Tsai, K.S., 1977. Replication of parainfluenza type 3 virus in alveolar macrophages; evidence of in vivo infection and of in vitro temperature sensitivity in virus maturation. Infect. Immun., 18: 780–791.

Tsai, K.S. and Thomson, R.G., 1975. Bovine parainfluenza type 3 virus infection: Ultrastructural aspects of viral pathogenesis in the bovine respiratory tract. Infect. Immun., 11: 783–803.

Van Der Maaten, M.D., 1969. Immunofluorescent studies of bovine parainfluenza-3 virus (11) experimentally infected calves. Can. J. Comp. Med., 33: 141–147.

Van Nieuwstadt, A.P.K.M.I., Verhoeff, J., Ingh, T.S.G.A.M. and Hartman, E.G., 1982. Epizootiology of PI3 virus, bovine RS virus and IBR virus infections in dairy herds with traditional calf rearing. In: Proc. XII World Congress on Diseases of Cattle, The Netherlands, pp. 124–131.

Watt, N.J., Pirie, H.M., Allan, E.M., Selman, I.E., Petrie, L., Gibbs, H.A. and Wiseman, A., 1982. Parainfluenza type 3 virus and respiratory syncytial virus antigens in paraffin embedded sections of pneumonic lung. In: Proc. XII World Congress on Diseases of Cattle, The Netherlands, pp. 1211–1214.

Westenbrink, F. and Kimman, T.G., 1987. Immunoglobulin M specific enzyme-linked immunosorbent assay for serodiagnosis of bovine respiratory syncytial virus infections. Am. J. Vet. Res., 48: 1132–1137.

Woods, G.T., 1968. The natural history of bovine myxovirus parainfluenza-3. J. Am. Vet. Med. Assoc., 152: 771–777.

Woods, G.T., Sibinovic, K. and Marquis, G., 1965. Experimental exposure of calves, lambs and colostrum-deprived pigs to bovine myxovirus parainfluenza-3. Am. J. Vet. Res., 26: 52–56.

Woods, G.T., Grandall, R.A. and Mansfield, M.E., 1975. A comparison of immunologic response to intranasal and intramuscular parainfluenza-3 live virus vaccines in beef calves challenged experimentally in the feedlot. Res. Commun. Chem. Pathol. Pharmacol., 11: 117–128.

Yates, W.D.G., 1982. A review of infectious bovine rhinotracheitis, Shipping fever pneumonia and viral–bacterial synergism in respiratory disease of cattle. Can. J. Comp. Med., 46: 225–263.

Zygraich, N., Labmann, M., Peetermens, J. and Huygelen, C., 1979. In vivo and in vitro characteristics of a ts-mutant of bovine parainfluenza-3 virus in "Imuresp P" vaccine. Tierärztl. Umschau., 34: 555–562.

Parainfluenza-3 Virus in Sheep

J.M. SHARP
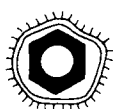
VIRUS PROPERTIES

Only one serotype of ovine PI3 virus has been recognized; it is related antigenically to both bovine and human strains of PI3 but may be distinguished from them by several serological techniques.

EPIZOOTIOLOGY

Infections of sheep by PI3 virus are common. Antibodies have been detected in the sera of sheep in many countries throughout the world and in most surveys the prevalence is over 70%. The majority of lambs acquire antibodies to PI3 via colostrum, and whilst these are present infections are rarely detected. However, such antibodies rapidly wane so that most sheep become infected within the first 12 months; certain management practices appear to delay contact with the virus until the sheep are older so that the prevalence of antibodies continues to rise with age.

Transmission of PI3 between animals has not been studied adequately, but the failure to demonstrate virus in any tissue other than the respiratory tract indicates that respiratory excretions alone are responsible. Similarly, how the virus is maintained in a flock is unclear, although outbreaks of infection not associated with the introduction of infected sheep suggest viral persistence.

It is clear from the high prevalence of antibodies that the majority of infections by this ubiquitous virus are inapparent or of a mild nature. However, a small number of observations records the association of PI3 virus with severe outbreaks of respiratory illness, but the role of PI3 virus in these is confounded by the dominating influence of *Pasteurella haemolytica*. Nevertheless, experimental findings support the observation that the virus may initiate events which predispose the sheep to infection by this bacterium. Infection of lambs with PI3 virus 4–7 days before exposure to an aerosol of *P. haemolytica* exacerbated the disease induced by the bacterium (Davies et al., 1977; Sharp et al., 1978). The clinical illness and lesions produced by the combined infection are identical with those observed in natural acute pasteurellosis (syn. enzootic pneumonia).

PATHOGENESIS

Although detailed studies of the pathology induced by PI3 virus in sheep have been made, the pathogenesis requires further elucidation.

Studies of infected lambs, killed at intervals, indicate that within the lower respiratory tract viral antigens are detected first by IF in the bronchioles and surrounding alveoli, the same sites in which the initial histological changes occur (Stevenson, 1969; Cutlip and Lehmkuhl, 1982). The principal specific lesions are inclusion body formation and bronchiolar epithelial hyperplasia followed by necrosis and desquamation. The progressive development of these lesions is associated with the appearance of illness in affected animals, and the clinical signs reflect the systemic involvement (pyrexia, anorexia, depression) or damage to the respiratory tract (tachypnea, hyperpnea, dyspnea). Both systemic and respiratory signs appear simultaneously and increase in severity with the pulmonary lesions. However, the systemic signs regress between 6 and 7 days p.i., when the extent of the pulmonary lesions is maximal; regression coincides with the appearance of specific antibodies. The respiratory signs abate more slowly as the pulmonary lesions resolve. These observations suggest that the pathogenesis of PI3 infection extends beyond the simple pathology involving the bronchiolar and alveolar epithelia (Sharp, 1977). Further support for this suggestion is provided by observations that infection with PI3 virus may reduce both growth rate and appetite (Hore, 1968; Jones et al., 1982).

It has been suggested that the epithelial destruction and accumulation of debris in the lower respiratory tract provides a focus in which secondary bacteria may localize and proliferate. It is well established that PI3 infection can predispose lambs to a severe pneumonia caused by several serotypes of *P. haemolytica* biotype A, but the mechanism is not clear. The initial lesions associated with bacterial proliferation occur in areas favoured by the virus, which supports the tissue-damage hypothesis and points to a close interaction at the cellular level (Rushton et al., 1979). However, mechanisms other than apparent tissue damage must be involved, because the pathogenicity of serotypes of *P. haemolytica* biotype T and of untypeable strains is not enhanced (N.J.L. Gilmour, personal communication, 1983). Probably PI3 virus acts within the sheep lung in the same way as other viruses act within the murine lung to impair the intrapulmonary bactericidal defences.

DISEASE SIGNS

Most PI3 virus infections are subclinical, but occasional outbreaks of acute illness occur. Such outbreaks have a sudden onset and are characterized by a high morbidity. During the early stages, affected animals have copious serous nasal and conjunctival discharges and later develop pyrexia and frequent coughing. However, the presence of other microorganisms has obscured the aspects of the illness attributable to PI3 virus. The illness following experimental inoculation largely depends on the route. A single intranasal instillation or aerosol exposure of lambs with PI3 virus results in viral replication in the upper respiratory tract without clinical signs, whereas combined intranasal and intratracheal inoculation of specific pathogen-free (SPF) lambs results in a consistent clinical picture (Wells et al., 1977a). No signs are apparent on the first 2 days after inoculation, but by day 3 a few animals appear slightly depressed, anorexic, febrile and show hyperpnea with an exaggerated jerky expiration. During days 4–6 after inoculation, the clinical signs become more pronounced and evident in an increasing proportion of lambs until, on the sixth day, over 90% of the animals appear ill. However, within 24 h the illness abates; most animals are alert, no longer febrile, and the hyperpnea and dyspnea disappear by about 9 days after inoculation (Sharp et al., 1978). Conjunctival discharges and coughing have not been observed in these experiments in SPF lambs although they have been reported in experiments in conventional animals, where other microorganisms cannot be excluded.

PATHOLOGY

Lesions associated with infection by PI3 virus are confined to the lower respiratory tract (Hore and Stevenson, 1969). Postmortem inspections of lambs inoculated intranasally and intratracheally reveal multifocal linear or patchy areas of dull red consolidation in all pulmonary lobes, which are most extensive 6–8 days after inoculation. The essential histological features are severe, hyperplastic and necrotic bronchiolitis and alveolitis, associated with infiltration of interalveolar septa by mononuclear cells. Syncytia involving epithelial cells may be seen but are not a prominent feature. These destructive changes are followed by regenerative responses but, except during the early phase of infection, the two processes are coexistent. Eosinophilic intracytoplasmic inclusions are common in bronchiolar epithelial cells and a few bronchial and alveolar cells between 3 and 6 days after inoculation. Thereafter, inclusions are difficult to demonstrate. The gross and microscopic lesions resolve fairly quickly, although a residual interstitial pneumonia and focal alveolar epithelialization may persist for a further 21 days or longer.
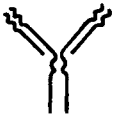
IMMUNE REACTION

Infection of sheep with PI3 virus stimulates both humoral and cellular immunity, the dynamics of which have been determined by experimental studies (Wells et al., 1977b). Following infection, antibodies are usually first detected in nasal secretions. Virus neutralizing antibodies may be demonstrated in nasal secretions after 5 days and last for 4–5 weeks; in the serum HI antibodies are not detected before 7–9 days, and virus neutralizing antibodies 2 days later. The neutralizing antibodies in nasal secretions are largely IgA, but most of the HI activity is considered to be a high-molecular-weight nonimmunoglobulin substance. In serum, IgM is clearly associated with the primary antibody response and IgG with the secondary response. In the lower respiratory tract, HI and neutralizing antibodies are considered to be mainly IgA.

The influence on immune responses of preexisting antibodies, whether from colostrum or previous infections, is not clear. Observations during natural outbreaks and experimental studies have confirmed that PI3 infections can occur without a following rise in antibody titers, and that this is particularly so when there are pre-existing antibodies.

The role of CMI in PI3 infections of sheep remains to be resolved; it has been shown that intravenous inoculation of sheep with PI3 virus will stimulate specific cytotoxic activity of peripheral blood leukocytes within 7 days (Williamson et al., 1981).

LABORATORY DIAGNOSIS

Confirmation of infection by PI3 virus can be achieved by histopathology, virus isolation, detection of viral antigens or serology. Both histopathology and virus isolation have been used but have major drawbacks, relating mainly to the transient nature of the infection.

The only histological feature that may be regarded as indicative of the PI3 virus infection is the presence of intracytoplasmic inclusions in bronchiolar epithelial cells. However, these are detected readily only between 3 and 6 days after infection and could be confused with the inclusions induced by respiratory syncytial virus (Bryson et al., 1988).

Virus isolation offers the advantage that a positive identification of the agent can be made. In respect to PI3 virus infections, the techniques are

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insensitive, as virus may be isolated readily only during the first 6 days after infection. Therefore samples for virus isolation, usually swabs or aspirates of the upper respiratory tract, should be taken from animals which have been ill for less than 24 h and from several sheep without clinical signs.

The value of histopathology and virus isolation can be improved by combining them with IF, immunoperoxidase and ELISA.

The most common laboratory aid to the diagnosis of PI3 infections is serology, which relies on detecting a 4-fold rise in antibody titers between acute and convalescent sera. This approach generally has been regarded as satisfactory, although sheep may be infected by PI3 virus without an apparent rise in serum antibody titers, particularly where there are preexisting antibodies. Also, the time at which sera are taken in relation to the infection appears to be more critical than has been appreciated. The rapid rise and fall of antibody titers in some sheep may go undetected if sera are obtained more than 2 weeks apart.

PROPHYLAXIS AND CONTROL

The available evidence suggests that PI3 virus may be one factor in the initiation of outbreaks of ovine respiratory disease, most of which also are associated with *P. haemolytica*. Therefore it would appear worthwhile to consider some means of prophylaxis and assess its value in the control of naturally occurring ovine pneumonias.

Immunity to PI3 virus can be stimulated by local or parenteral administration of PI3 antigens, which will prevent virus replication, clinical illness and pneumonic lesions (Wells et al., 1977a). Live virus inoculated either intranasally or intramuscularly stimulates both nasal secretion and serum antibodies, but the local administration induces higher mucosal antibodies and better protection. Inactivated virus given by either route fails to stimulate antibodies or confer protection unless adjuvant is used. When administered with adjuvant, inactivated whole virus and subunits given as micellar aggregates containing only the HN and F glycoproteins stimulate high antibody titers and protection (Morein et al., 1983).

Experiments to evaluate the efficacy in sheep of vaccination against PI3 in providing protection against superinfection with *P. haemolytica* have shown that it can reduce the clinical illness and pneumonic lesions induced by the bacterium (Wells et al., 1978; Davies et al., 1980).

REFERENCES

Bryson, D.G., Evermann, J.F., Liggitt, D., Foreyt, W.J. and Breeze, R.G., 1988. Studies on the pathogenesis and interspecies transmission of respiratory syncytial virus isolated from sheep. Am. J. Vet. Res., 49: 1424–1430.

Cutlip, R.C. and Lehmkuhl, H.D., 1982. Experimentally induced parainfluenza type 3 virus infection in young lambs: pathological response. Am. J. Vet. Res., 43: 2101–2107.

Davies, D.H., Dungworth, D.L., Humphreys, S. and Johnson, A.J., 1977. Concurrent infection of lambs with parainfluenza virus type 3 and *Pasteurella haemolytica*. N. Z. Vet. J., 25: 263–265.

Davies, D.H., McCarthy, A.R. and Penwarden, R.A. 1980. The effect of vaccination of lambs with live parainfluenza virus type 3 on pneumonia produced by parainfluenza virus type 3 and *Pasteurella haemolytica*. N. Z. Vet. J., 28: 201–202.

Hore, D.E., 1968. Some aspects of the pathogenesis of pneumonia in sheep. Ph.D. thesis, University of Edinburgh, Edinburgh, 135 pp.

Hore, D.E. and Stevenson, R.G., 1969. Respiratory infection of lambs with an ovine strain of parainfluenza virus type 3. Res. Vet. Sci., 10: 342–350.

Jones, G.E., Field, A.C., Gilmour, J.S., Rae, A., Nettleton, P.F. and McLauchlan, M., 1982. Effects of experimental chronic pneumonia on bodyweight, feed intake and carcass composition of lambs. Vet. Rec., 110: 168–173.

Morein, B., Sharp, M., Sundquist, B. and Simons, K., 1983. Protein subunit vaccines of parainfluenza type 3 virus: immunogenic effect in lambs and mice. J. Gen. Virol., 64: 1557–1569.

Rushton, B., Sharp, J.M., Gilmour, N.J.L. and Thompson, D.A., 1979. Pathology of an experimental infection of specific pathogen-free lambs with parain fluenza virus type 3 and *Pasteurella haemolytica*. J. Comp. Pathol., 89: 321–329.

Sharp, J.M., 1977. Studies on the role of parainfluenza virus type 3 and adenovirus in respiratory disease of sheep. Ph.D. thesis, University of Edinburgh, Edinburgh, 167 pp.

Sharp, J.M., Gilmour, N.J.L., Thompson, D.A. and Rushton, B., 1978. Experimental infection of specific pathogen-free lambs with parainfluenza virus type 3 and *Pasteurella haemolytica*. J. Comp. Pathol., 88: 237–243.

Stevenson, R.G., 1969. Immunofluorescence studies of parainfluenza 3 virus in the lungs of lambs. J. Comp. Pathol., 79: 483–490.

Wells, P.W., Sharp, J.M. and Smith, W.D., 1977a. Experimental assessment of parainfluenza type 3 virus vaccines. In: W.B. Martin (Editor), Respiratory Diseases in Cattle (Current Topics in Veterinary Medicine, 3). Martinus Nijhoff, The Hague, pp. 515–520.

Wells, P.W., Smith, W.D., Burrells, C., Dawson, A.McL. and Sharp, J.M., 1977b. Mucosal immunity in the respiratory tract of sheep. In: Immunoglobulin A Colloquium. Bristol, Biochemical Society Transactions, pp. 1574–1576.

Wells, P.W., Sharp, J.M., Rushton, B., Gilmour, N.J.L. and Thompson, D.A., 1978. The effect of vaccination with parainfluenza type 3 virus on pneumonia resulting from infection with parainfluenza type 3 virus and *Pasteurella haemolytica*. J. Comp. Pathol., 88: 253–259.

Williamson, S.E. and Gibbins, B.L., 1981. A terminal labelling assay using ^{3}H-uridine to measure cell-mediated cytotoxic responses against Parainfluenza type 3 infected target cells. J. Virol. Methods, 2: 237–247.

Chapter 32

Rinderpest Virus

G.R. SCOTT

INTRODUCTION

For centuries waves of the murrain formerly known as cattle plague and now called rinderpest regularly devastated the cattle and domestic buffalo populations of Asia and Europe and occasionally wrought havoc in north Africa. The flora and fauna of Africa south of the Sahara was changed permanently by a panzootic that spread from Eritrea and the Sudan west to the Atlantic and south to the Cape of Good Hope in the last years of the last century; the tsetse fly, for example, disappeared from most of Africa south of the Zambezi because rinderpest wiped out its hosts. Rinderpest invaded Brazil in 1920 and Australia in 1923, being introduced with livestock imported from Asia. In both countries the disease was eradicated within months by the rigorous application of quarantine measures and slaughter of infected herds. Epizootics and panzootics still occur despite the availability of excellent vaccines.
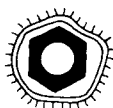
VIRUS PROPERTIES

The ultramicroscopic nature of rinderpest virus was demonstrated as early as 1902 when cattle were infected with a bacteria-free filtrate (Curasson, 1932). The virion is pleomorphic, occurring either as an enveloped plastic spheroid, 100–150 nm in diameter, or as a long filament. The surface is covered with projections, 8 nm long, spaced 8–10 nm apart. The helical nucleocapsid is tightly coiled. When unravelled the nucleocapsid emerges as a serrated thread resembling a herringbone.

Hydrated rinderpest virus is heat, light and ultrasonic sensitive. Lyophilized virus, in contrast, is relatively stable, surviving for years at − 20°C. Airborne virus survives best at low and high RH, whereas the virus rapidly loses infectivity at 50–60% RH. Infectivity is destroyed by many chemicals, including lipid solvents. Strains vary in their pH stability; most are stable between pH 7.2 and 8.0, are relatively stable between pH 4.0 and 10.2, and are rapidly inactivated at lower and higher pH values. Pragmatically, rinderpest-infected carcasses are rendered noninfectious by the pH changes that follow autolysis and inactivation is hastened by putrefaction. Contaminated premises should be treated with either 5% phenol or a lipophilic disinfectant such as 4% cresylic acid or 2% sodium orthophenylphenate.

Strains of rinderpest virus vary in their virulence, their tissue tropism and their invasiveness, and yet they are all immunologically homogenous. In addition, rinderpest virus is closely related antigenically with the viruses causing canine distemper, human measles and PPR. These viruses are classified togeth-

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er in the genus *Morbillivirus* in the family Paramyxoviridae (Kingsbury et al.,
1978). They differ from other paramyxoviruses by not having neuraminidase.
Other likely members of the genus are PMV 107 virus identified in German
cattle with encephalitis, Hh1 virus recovered from a sick hedgehog in the
United Kingdom, and phocine morbillivirus isolated from North Sea seals. The
official type species is measles virus, but recent studies using 39 monoclonal
antibodies identifying 18 epitopes on the major structural proteins pointed to
rinderpest virus being the archvirus of the genus (Norrby et al., 1985).

EPIZOOTIOLOGY

Host range

Natural infections occur only in even-toed ungulates belonging to the Order
Artiodactyla; an updated checklist has been published (Scott, 1981). Domestic
ungulates, cattle and buffaloes in particular, are most commonly attacked and
perpetuate the disease. Epizootics in goats and sheep have been observed
sporadically in Africa, Asia and Europe in the past but today are regularly
reported only from India. This peculiar restricted prevalence has been attri-
buted to the (mis)use of attenuated vaccines of strains of rinderpest vaccine
passaged serially too few times in goats. Recent explosive epizootics in goats
and sheep in Africa have proved to be caused by PPR virus.

Asiatic domestic pigs frequently suffer from and succumb to rinderpest,
whereas pigs of European origin do not, although they are susceptible and will
multiply and excrete virus. Moreover, they can be infected through eating
infected offal (Scott, 1964).

On occasion, some species of free-living wildlife are decimated; the losses are
highest in buffalo, eland, giraffe, kudu, warthog and wildebeest in Africa and
in banteng, blackbuck, gaur, nilgai and sambhar in Asia. However, overt
disease is not perpetuated for long in wildlife in the absence of large domestic
ruminants. In contrast, the results of recent serological survey lend credence
to the hypothesis that low pathogenic strains of rinderpest virus are being
maintained in wild East African ungulates (Rossiter et al., 1983).

Vectors

The role of vectors in the epizootiology of rinderpest is considered to be
minimal, although the virus has been recovered from horse flies, house flies,
stable flies, tsetse flies, mosquitoes, ticks, leeches, lice and vultures. Survival
of the virus rather than multiplication in the vector is postulated.

Geographic distribution

At the start of the 20th century rinderpest was prevalent throughout Asia,
in the fringes of Europe and in eastern and southern Africa. By mid-century
southern Africa, Europe and the USSR were no longer affected. Thereafter
enthusiastic implementation of national and international mass vaccination
campaigns hastened the global decline in the incidence of the disease, but the
goal of eradication, within sight in the mid-1970s, was not achieved. In Africa,
foci in Mauritania in the west and in southern Sudan in the east erupted. The
two epizootics merged in Nigeria with devastating economic and social conse-
quences. The eastern epizootic also invaded temporarily Egypt in the north and
Tanzania in the south. In the Middle East, Israel and Syria were invaded when
the disease spread from a focus that had smouldered in war-torn Lebanon for

13 years. Today, rinderpest is enzootic in northern equatorial Africa, the Middle East and the Indian subcontinent.

Incidence

The most complete data on the incidence of rinderpest in a country are those furnished by the government of India, which maintains in New Delhi a central office specifically monitoring the disease. A nationwide vaccination campaign in the 1950s cut the number of outbreaks from 8156 and 1956 to 960 (Khera, 1980). Between 1961 and 1970 the annual incidence rate fluctuated between 3 and 7 per 100 000 head, and since 1971 it has stabilized at 1–2 per 100 000. Similar low incidence rates appear to be the norm in other enzootic areas; in Mali, for example, the incidence in 1976 was 2 cases per 100 000 and in 1979 5 per 100 000.

There is no evidence of a seasonal incidence in either Africa or Asia.

Animals of all ages are attacked in virgin-soil epizootics, whereas the disease in enzootic areas is characterized by a specific age incidence. The adults in enzootic areas are immune either through recovery from a natural infection or from vaccination, and the sucking young are protected passively through the ingestion of antibodies in the colostrum; only the yearlings are at risk.

Economic consequences

In the past the ravages of rinderpest changed the fate of nations. The catastrophic panzootic that devastated Europe in the 1740s and 1750s by killing 200 million cattle created an awareness for the need for trained manpower to handle animal diseases. In France this resulted in support for a proposal by Claude Bourgelat to found a veterinary school at Lyons, the first in the world. Within a year after the doors of that school had opened Bourgelat and his pupils were in the field tackling rinderpest. Concurrently most continental European governments enacted legislation to control animal movements to limit the spread of the disease.

Memories were short. In the early and middle years of the 19th century a network of railways quickly spread over Europe and Russia, enabling livestock to be moved through and between countries speedily and in numbers hitherto impossible. Rinderpest panzootics were inevitable; they raged from 1857 to 1867, 1870 to 1872, in 1877 and from 1844 to 1896. The losses were enormous and governments throughout Europe were forced to establish or reestablish civil veterinary inspectorates and to promulgate fresh regulations to control animal diseases. Similarly, the origins of most of the state veterinary departments in Africa and Asia are attributable directly to the economic and political consequences of rinderpest.

The 19th-century panzootics were also responsible for the first International (now World) Veterinary Congress being convened in Hamburg in 1863 for the purpose of exchanging views, in the hope that a consensus of opinion would emerge on the nature of rinderpest and on how best it could be controlled. The foundation of the International Office of Epizootics (OIE) in Paris in the 1920s followed the havoc induced in Europe and South America by a shipload of Indian zebu cattle that were unloaded at Antwerp for shipment across the Atlantic; the zebu cattle were infected with rinderpest which spread to in-contact cattle both in Belgium and in Brazil. Similarly, the Inter-African Bureau of Epizootic Diseases was created in 1952 specifically to monitor rinderpest in Africa.

A rare modern economic appraisal of the havoc wrought by rinderpest is that by Nawathe and Lamorde (1984), who described the socioeconomic impact of the recent recurrence of rinderpest in Nigeria, a country where the cattle

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industry was valued at over 13 × 10⁹ US dollars. The damage afflicted on the industry was without parallel in this century; nearly 2 million cattle sickened and half a million died. The losses due to mortality, reduced performance, surveillance costs, replacement costs, loss of working hours and indirect losses were estimated at 2 × 10⁹ US dollars.

Estimates of the benefits of rinderpest control are also few in number. The Inter-African Bureau for Epizootic Diseases organized JP15, an international multi-million-dollar vaccination campaign aimed at eradicating the disease from Africa. Eradication was not achieved but the benefits were considerable; in northern Nigeria, for example, the additional cost of intensive vaccination as a result of JP15 was estimated at £310.912, whereas net benefits 11 years later totalled £785.127, giving a benefit–cost ratio of 2.5:1 and an internal rate of return of more than 47% annually (Felton and Ellis, 1978). Other assessments are less well documented. Nevertheless, in Sierre Leone a 4-fold increase in the cattle population was observed when the disease was eradicated. Similarly, after rinderpest disappeared from the wildlife on the Serengeti and Mara plains of East Africa in 1964 the buffalo and wildebeest populations increased several-fold within 10 years.

Speed and pattern of spread

Virgin-soil epizootics spread rapidly and frequently escalate into panzootics. However, veterinary authorities in Australia, Belgium, Brazil and, more recently, Italy have shown that a primary focus can be obliterated by prompt quarantine and slaughter procedures. Enzootic rinderpest, in contrast, spreads slowly but steadily for weeks and months through the immature and young adult stock at risk. The incidence flares up temporarily whenever the young stock are congregated around water-holes in the dry season or are assembled as recruits to flying dairy herds.

Most strains of the virus spread readily between cattle and buffaloes. Some are catholic in the range of other hosts they attack and some are remarkably selective. The problem is confounded by changes in host preference during the course of a prolonged panzootic and by variations in innate resistance in hosts. Consequently, an epizootic of rinderpest may not spread to all susceptible species at risk.

The virus is usually transmitted from sick to susceptible animals in aerosols, and normally the contact has to be close because the infectious droplets are large and short-lived. Theoretically wind-borne transmission is possible at night at high or low relative humidities, but the only hint that it might have occurred naturally was the ease with which the Pendik strain of the virus spread in East African cattle. In contrast, laboratory-attenuated strains used as vaccines are not shed in expired air or feces and consequently do not spread by contact.

Transmission through ingestion of virus-contaminated food or water is rare and any such allegation should be investigated thoroughly; the most likely source of a fresh focus is a newly arrived live animal.

Pigs can acquire infection through eating meat scraps from infected animals, and once infected they can transmit the virus in aerosols to in-contact pigs and cattle. In practice, the risk to a disease-free country from imported infected meat appears to be low, because every virgin-soil epizootic in the 19th and 20th centuries has been linked to a recent importation of animals.

Innate resistance

Innate resistance varies widely within and between species, the dominant factor in its development being selection for survival through constant associa-

tion with the disease. When this genetic selection pressure is removed, either through eradication of the disease or through vaccination, local races lose their high resistance in a few generations (Scott, 1964). Crosses between cattle with high and low resistance tend to be more resistant than the nonresistant parent but less resistant than the highly resistant parent.

The use of attenuated virus vaccines has revealed even finer shades of resistance and has shown that the races possessing the lowest resistance in Africa are Sanga-type cattle and, in Asia, Japanese Black and Yellow Korean cattle. Analysis of postvaccinal reactions has confirmed the inherited nature of the resistance; adverse reactions occurred in related cattle.

In contrast, the degree of resistance in pigs is so far inexplicable; European pigs with no ancestral association with rinderpest undergo inapparent infection, whereas Asiatic pigs in constant association with the disease have severe clinical reactions and often die. Similar inexplicable species differences are manifested in cohabitating wildlife.

Reports on an age-related resistance conflict. Curasson (1932) considered that the response of young and adult cattle were the same, but most field veterinarians believe that reactions are more severe in calves. Similarly, reports on the effect of bodily condition conflict; early workers concluded that healthy, well-nourished cattle withstood infection better than animals in poor condition. Thiery (1956), however, noted more pronounced lesions in animals in good condition than in wasted animals.

Morbidity and mortality

Morbidity and mortality are profoundly influenced by the innate resistance of the infected host, but an additional important factor is the virulence of the virus strain involved. Moreover, the degree of virulence may vary for particular hosts. The O1 Balbal strain, for example, was isolated originally from a sick eland and proved to be avirulent for cattle despite serial passage in cattle. In contrast, strains attenuated for use as vaccines to protect cattle and buffaloes have proved to be lethal when administered to captive wildlife. Other strains lethal for wildlife are also lethal for domestic stock. In general, case mortality rates in indigenous cattle reared in an enzootic area hover around 30%. When exotic cattle are exposed to the same strains the case mortality rates exceed 80%, sometimes 90%.

PATHOGENESIS

Rinderpest virus shed in an aerosol invades the tissues of a new host through the mucous membrane lining the upper respiratory tract. Thereafter, primary multiplication of the virus occurs either in the palatal tonsil or in the pharyngeal and mandibular lymph nodes (Plowright, 1968).

New virus is released from the primary multiplication site into the bloodstream, where it becomes attached to circulating mononuclear cells. This viremia is detectable 1 or 2 days before the onset of illness. Further proliferation occurs in all the lymphoid tissues, in the mucous membranes of the alimentary and respiratory tracts, and in the lungs. The virus does not multiply in the brain, kidneys or myocardium (Plowright, 1968).

The plateau phase of the virus growth curve is attained during the prodromal fever and is maintained into the erosive-mucosa phase of the disease. Virus titers fall slowly and steadily with the first appearance of circulating antibody 4 or 5 days after the onset of fever. Viremia ceases several days before virus clearance occurs from target organs (Scott, 1955; Plowright, 1968).

The duration of a rinderpest viremia is influenced by the strain of virus

involved and by the innate resistance of the host. Some viremias last 10 days and some 2 days. In general, the longer viremias are induced by the more virulent strains propagating in hosts with low innate resistances (Scott, 1955).

Affected animals shed virus in all their secretions and excretions, primarily with ocular and nasal secretions and feces. Tears are infective one day before the onset of fever and nasal secretions and feces become infective at the onset of fever (Mushi and Wafula, 1984). Virus continues to be shed throughout the fever period and for a few days after the fever regresses (Plowright, 1968).

Recovered animals are not considered to be carriers; unequivocal evidence of latency has not been found. On the other hand, at least two other morbilliviruses, canine distemper and measles virus, sometimes cause latent persistent infections that are manifested months or years later. Moreover, persistent infection of rinderpest virus has been established in Vero cells. Perhaps the controversy of the 1930s over the possible existence of carriers should be renewed, because there are several reports of the virus being recovered weeks and months after the acute clinical episode.

DISEASE SIGNS

The standard description of rinderpest is misleading, since it is based on data amassed in the virgin-soil epizootics that ravaged Europe in the 19th century. The syndrome occurs today only when exotic stock are exposed to infection. The enzootic disease in indigenous stock is less dramatic, less typical and often one or more of the cardinal features of the disease are modified or even absent.

The classic rinderpest syndrome is characterized by a short, sharp fever, erosive stomatitis, gastroenteritis, dehydration and death. A characteristic nauseating stench emanates from the typical acute case. The disease course is divisible into five phases: an incubation period, a prodromal fever, an erosive-mucosa phase, a diarrheic phase and convalescence in surviving animals (Fig. 126).

Cattle with a low innate resistance exposed naturally to rinderpest develop fever after incubation periods of 3–9 days, whereas cattle with a high innate resistance may take up to 15 days to become febrile. It cannot be stressed too
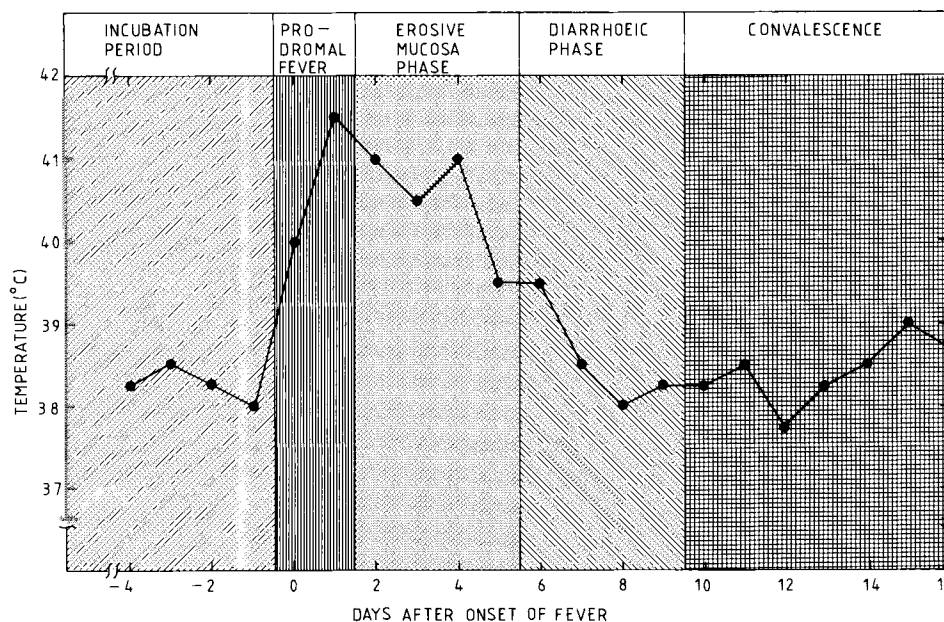
Fig. 126. The clinical phase of rinderpest.

often that cattle in the later stages of the incubation period are infectious although apparently healthy.

The onset of fever is sudden but other clinical signs are minimal. Illness becomes manifest 48 h later. The affected animal is at first restless and then stands dully apart. The muzzle is dry and the coat stares. The milk yield falls, respiration is shallow and rapid, the visible mucous membranes are congested and the outflow of serous secretions is increased. An impaired appetite is linked to poor rumination and constipation.

Mucosal erosions, the first sign suggestive of rinderpest, appear 2–5 days after the onset of fever as raised pinheads of necrotic epithelium on the mucous membranes lining the mouth, the nasal passages and urogenital tract. They are readily abraded, exposing a hemorrhagic layer of basal cells. Salivation is profuse. The erosions enlarge, coalesce and shed off thick yellow patches of necrotic cells which mix with the nasal secretions to produce a fetid mucopurulent discharge. Affected animals are still febrile and are obviously sick and uncomfortable. They drink excessively, stop eating and pass soft feces frequently.

Two to three days after the first appearance of the mucosal erosions the fever regresses and diarrhea begins. The dark brown fluid feces contain excess mucus, epithelial shreds and necrotic debris streaked with blood. The odor is offensive. Frequent straining exposes a congested and eroded rectum. Breathing is laboured and painful and often abdominal in character with a grunting exhalation.

The profuse diarrhea causes rapid dehydration. Affected animals waste away while standing with lowered heads, sunken eyes and arched backs. Most collapse and die in the diarrheic phase 6–12 days after the onset of illness, but a few linger on to die in the third week.

The convalescence is prolonged, the return to full health taking several weeks. Its onset is ill-defined; in animals that are going to recover the mucosal erosions generally resolve within a week of their first appearance and the diarrhea stops shortly afterwards. Pregnant animals abort during the convalescence, but there is no evidence that the virus is teratogenic. A common sequel that complicates the clinical picture, particularly the convalescence, is the activation of latent infections. Many pathogens have been incriminated, the most troublesome being latent protozoal infections (Curasson, 1932).

A transient mild leukocytosis often occurs during the incubation period, but the significant change is the profound leukopenia that starts before the onset of fever and persists until death, or for at least 5 weeks if the animal survives. The leukopenia is largely due to a dramatic fall in the number of lymphocytes, and to a lesser extent to the disappearance of eosinophils (Fig. 127).

Erythrocyte numbers and packed cell volumes rise significantly with the onset of diarrhea. In addition, in terminal cases blood coagulation is slow and serum separation is poor. In animals that recover both the erythrocyte numbers and the packed cell volumes regain normal levels within a week.

Total serum proteins and electrolytes decline, the chloride, potassium, sodium and bicarbonate ions being drained out in the diarrhea. Death is caused by the combined loss of body water and electrolytes.

PATHOLOGY

The gross pathology of rinderpest is suggestive but not pathognomonic, being very similar in cases of acute BVD and PPR. The carcass is emaciated and dehydrated, the eyes are sunken and the coat is rough and soiled by mucopurulent discharges and fluid feces (Maurer et al., 1956).

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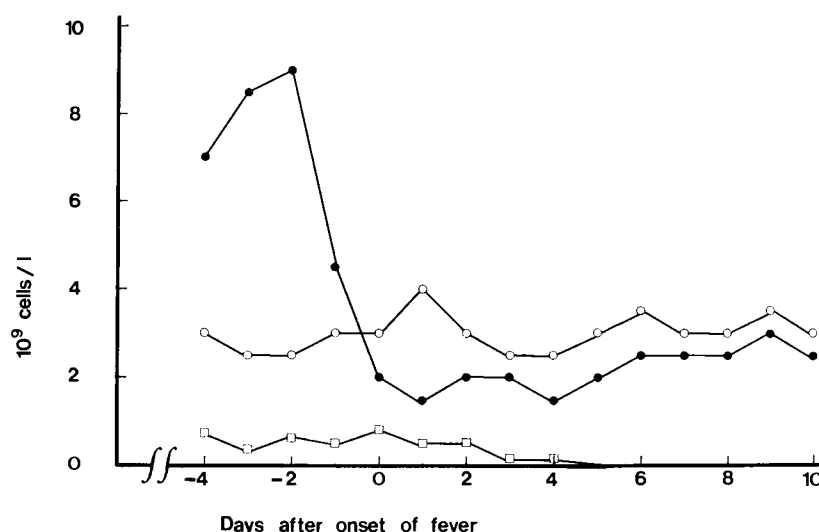
Fig. 127. Hematological changes in rinderpest; (□) eosinophils, (●) lymphocytes, (O) neutrophils.

The most spectacular gross changes occur in the alimentary and upper respiratory tracts. The mucous membranes of the mouth, throat and upper esophagus are eroded. The forestomachs are not often affected, whereas the pyloric region of the abomasum is usually edematous and the lining is eroded and sometimes ulcerated. The Peyer's patches in the small intestine are particularly conspicuous, being swollen, hemorrhagic and necrotic. Congested capillaries underlying the mucous membrane lining of the cecum, colon and rectum produce a striking pattern (called tiger stripes in Asia and zebra stripes in Africa). The mucosa itself is often ulcerated and bleeding. Gross changes in the urogenital tract are similar to those in the alimentary tract.

The nasal passages are blocked by tenacious mucopurulent debris, which also encrusts the nostrils and muzzle. The mucous membranes of the nasal passages and larynx are eroded and hemorrhagic. A prominent emphysema may be seen in animals that die late in the disease.

Early deaths are often associated with subendocardial hemorrhage in the left ventricle and subepicardial petechiae on the base of the heart and along the coronary grooves.

Dramatic histopathological changes occur in the lymphoid organs, which are denuded of lymphocytes in both the thymus-dependent and thymus-independent areas. The early significant change is the appearance of giant cells containing intracytoplasmic and intranuclear inclusions. Giant cell formation is followed by necrosis of lymphocytes, which begins in the periarteriolar region of the spleen and in the germinal centres of the lymph nodes. The necrosis proceeds to involve all the lymphocytes in the follicles. Experiments in rabbits infected with a rabbit-adapted strain of rinderpest virus have revealed that the depletion of T-lymphocytes precedes that of the B-lymphocytes. Similar events occur in the hemolymph nodes and gut-associated lymphoid tissues.

The attack on epithelial cells also results in early multi-nucleated cell formation with inclusions and proceeds to necrosis and erosion of the cells (Thiery, 1956).
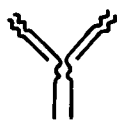
IMMUNE REACTION

The immune response to rinderpest virus is relatively uncomplicated; strains of the virus are immunologically homogenous, and there is complete

cross-protection. Surviving animals do not develop clinical signs when reexposed to the virus, although a few may support transient multiplication of the virus in the tonsillar–pharyngeal tissues. The protection is lifelong and associated with the induction of humoral antibodies which first appear as IgM immunoglobulins 5–7 days after the onset of illness. They decline within 2 weeks, being progressively replaced by IgG-class antibodies. After reaching peak values within one or more weeks antibody titers fall back to a threshold level at which they persist. Reexposure results in an anamnestic response.

Naturally infected cattle and cattle vaccinated by the intranasal route secrete specific IgA-class antibodies into the respiratory mucus (Provost, 1970). These antibodies are produced locally; they are not found when cattle are vaccinated by parenteral routes.

Antibodies in the sera of actively immune cattle are concentrated in the colostrum and are transferred to newborn calves. The half-life of the antibody is 37 days. The duration of the protection conferred passively on the calves is directly related to the amount of antibody ingested; in general, it ranges from 4 to 8 months, occasionally longer. The ingested antibodies are not secreted into the nasal mucus.

An early, if not the first, practical field exploitation of the interference phenomenon was the use of attenuated rinderpest virus vaccine to protect susceptible cattle and buffaloes during an epizootic in Burma in the middle 1930s. The protection conferred was operational several days before the appearance of humoral antibodies, but animals already incubating the disease were not protected. Since then rinderpest virus has been shown to be a potent producer of interferon in the rabbit.

Experiments in rabbits infected experimentally with adapted strains of rinderpest virus have clearly shown that thymus-dependent CMI is not essential in the recovery from infection (Yamanouchi, 1980). The role of CMI in cattle is not known but is unlikely to differ significantly from that in the rabbit.

Rinderpest virus is a potent immunosuppressive agent because it destroys T- and B-lymphocytes. The immunosuppression allows latent infections to be activated, which may complicate the clinical picture and cause diagnostic confusion. The virus suppresses both humoral and cell-mediated mechanisms, but production of memory cells is unimpaired (Yamanouchi, 1980). The lymphocyte response to mitogens is suppressed for at least 4 weeks.

The destruction of lymphoid tissues by the virus triggers auto-immunity in the rabbit; data on cattle infections are not available (Yamanouchi, 1980); an antinuclear antibody and a cold hemagglutinin antibody are produced. Histopathological evidence of auto-immune disease has not been obtained, however.

LABORATORY DIAGNOSIS

Directorates of state veterinary services in countries where rinderpest is enzootic or where the risk of its gaining entry into the country is high because of links with enzootically infected areas should issue clear and unequivocal instructions. State veterinarians, private veterinary practitioners and stockowners should be informed that any disease outbreak that resembles rinderpest must be reported and handled as if it were rinderpest until proven otherwise. Delay in instituting control measures can be economically, socially and politically disastrous. If the epizootic proves to have another cause, the cost of the then unnecessary rinderpest control measures is a small price to pay. Moreover no harm will have been done, and in any event the quarantine measures instituted to control rinderpest will limit the spread of other contagions.

Most diagnoses of rinderpest are provisional and are made by field veterina-

rians. They are based on an assessment of the history of the outbreak and on clinico–pathological observations. Confirmation should be sought when the disease episode occurs in a new area. It depends primarily on the rapid detection of virus specific antigens and less commonly on virus isolation and identification. The demonstration of early IgM-antibody and specific histopathological changes supplement the other findings. However, the key to diagnostic success is the submission of suitable samples from an adequate number of animals. Diagnostic techniques have been updated and discussed in detail by Bansal (1986) and Scott et al. (1986).

Dead animals are NOT suitable donors of samples for a diagnosis of rinderpest, because the tissues contain little or no virus or antigen. The affected herd should be examined clinically and six to ten animals in the erosive-mucosa phase should be selected. The diarrhea is a useful marker; soiled and dehydrated animals should be rejected for sampling.

The chosen animals are bled for serum and into EDTA. Simultaneously clear tears are collected on swabs inserted into the conjunctival sacs and the necrotic debris from the gum erosions is scraped off onto a spatula. Tear swabs are transported in sealed bottles containing $150\,\mu l$ of phosphate-buffered saline. Gum debris is wiped off the spatula into a convenient container. Then, if possible, at least two animals are slaughtered to collect aliquots of the lymph nodes, spleen and tonsils into sterile containers half-filled with virus-transport medium. Thin slices of these tissues together with pieces of affected mucous membranes are also collected and placed in formol–saline for histopathological examination. The fresh samples are taken on wet ice by courier to the laboratory. It is important to forward a few samples from several animals rather than several samples from too few animals.

The sampling procedures are summarized in Fig. 128.

The essential reagent for detecting rinderpest-specific antigen is hyperimmune antiserum prepared by multiple inoculations with or without adjuvants into a suitable donor. The ideal system, which avoids detection of nonrinderpest antigen-antibody reactions, is the rabbit using the Yamanouchi substrain of the Nakamura III strain of lapinized rinderpest virus; this substrain does not kill rabbits. If a lethal substrain is used then the rabbits should first be protected passively, 24 h before receiving virus. The preferred antigen detection techniques are CIEP and AGID tests. CIEP is more sensitive, and results are obtained within 40 min. IF techniques are excellent for the early detection of virus growth in cell cultures, but background fluorescence bedevils the interpretation of the impression smears and cryostat sections of most tissues from field cases. Liver sections, however, are relatively free of background fluorescence (Rossiter and Jessett, 1982).

Concurrently the leukocyte fraction of the blood sample collected in EDTA is harvested, resuspended and diluted in tissue culture maintenance medium and then inoculated into primary calf kidney cells; some cultures also receive rinderpest antiserum, and others contain flying coverslips. On the first day, the acute-phase serum samples are examined for antibody and if found positive they are reexamined after heat or 2-mercaptoethanol treatment to destroy IgM immunoglobulins.

If the CIEP and AGID test results are negative, lymph node extracts are used in CF tests. If these are also negative, spleen and tonsil samples are thawed and 10% suspensions prepared and inoculated into rinderpest-susceptible and rinderpest-immune cattle or goats.

The flying coverslips are removed 1–3 days after inoculation, fixed wet in acetone and examined by direct IF test. The other cell cultures and the inoculated animals are examined daily for three weeks. In the meantime the results of the histopathological examinations will become available.
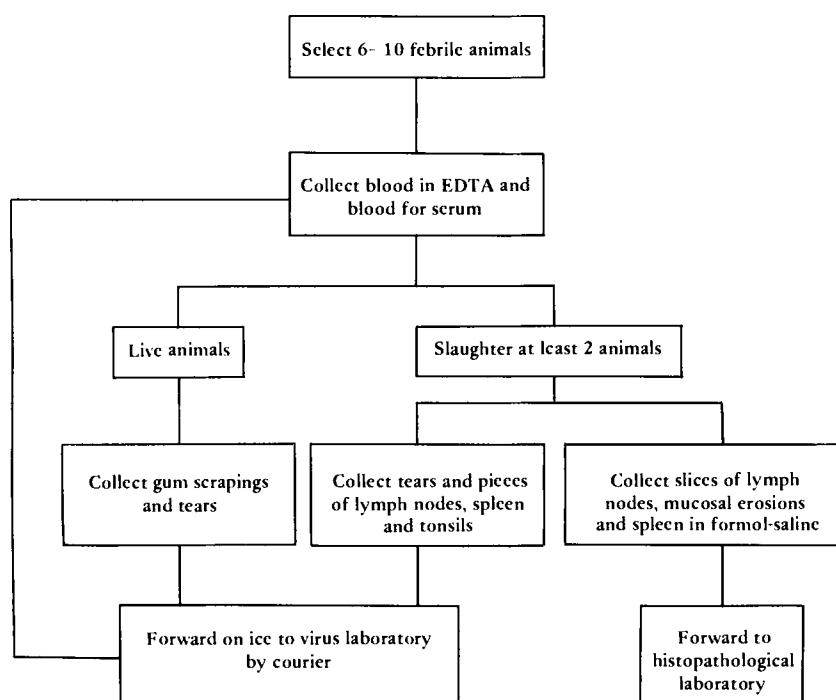
Fig. 128. Flow chart of the samples required for the confirmation of a provisional diagnosis of rinderpest.

Positive findings should be reported back immediately to the field veterinarians concerned. They should be confirmed by repeating the tests with the stored reference samples from the outbreak. The directorate of the state veterinary service should notify the directors of neighboring countries and the international clearing organisations, namely the OIE, the Inter-African Bureau for Animal Resources and the FAO.

Several diseases mimic some of the clinical manifestations of rinderpest. Two, in particular, are clinically and pathologically virtually indistinguishable, namely, BVD and PPR.

BVD has a worldwide distribution in both domestic and wild ruminants. Therefore the isolation of BVD virus from a sick or dead animal does not invalidate a provisional diagnosis of rinderpest. Fortunately CIEP and AGID tests readily differentiate rinderpest antigen from BVD antigen.

PPR has so far only been identified in the sub-Saharan area of Africa and the Middle East. It has not been recognized in the Indian subcontinent, where rinderpest in sheep and goats is not uncommon. PPR virus and rinderpest virus share antigens, which makes differentiation difficult. The simplest method is the inoculation of suspensions of lymphoid tissue from suspect cases into rinderpest-susceptible cattle or buffaloes; rinderpest virus will induce a clinical response whereas PPR virus will not. A histopathological examination of the lungs will also differentiate rinderpest and PPR; the former rarely induces viral pneumonia whereas PPR usually does so in association with giant cell formation.

The ease and frequency with which rinderpest virus activates latent and persistent infections often leads to diagnostic confusion. When animals have a high innate resistance to rinderpest virus the clinical signs of the activated infection are superimposed on the relatively low-grade signs of rinderpest. Suspicions should be aroused when animals known to be enzootically infected with protozoa die with acute manifestations of the protozoal infection.

Apart from seeking antibody activity in the IgM fraction of acute-phase

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serum, antibody tests are seldom used to confirm a diagnosis of rinderpest because the time necessary to examine paired serum samples is too long. On the other hand, antibody tests are frequently used to evaluate the efficiency of vaccination programmes and to monitor the epizootiological behavior of the natural disease, particularly in wildlife.

Antibody tests range from the very sensitive neutralization test in tissue culture to the relatively insensitive indirect AGID test; convalescent sera do not contain precipitating antibodies, hence the need in antigen-detection tests to use hyperimmune antisera. The simplest technique is HI in which the agglutination of monkey erythrocytes by measles virus is inhibited by rinderpest antibodies. It is not a sensitive technique but useful for evaluating the immune status of herds. There is also a passive hemagglutination test using erythrocytes coated with rinderpest antigen.

Very few laboratories now attempt to detect antibodies by CF. On the other hand, several groups have employed microplate ELISA for rapid screening of large numbers of sera. It is essential, however, that the tissue culture system used to produce the test virus for ELISA be free of bovine globulins.

PROPHYLAXIS AND CONTROL

Disease-free, low-risk countries maintain their freedom from rinderpest by banning imports of live domestic animals from areas where the disease is enzootic or epizootic. Unfortunately, legislation for the control of animal diseases usually fails to control the import of captive wild animals destined for zoological gardens and so-called safari parks. The last enzootic of rinderpest in Europe, for example, occurred in the zoological gardens in Rome following the importation of antelopes from East Africa (Cilli et al., 1951).

High-risk countries have geographical or commercial links with areas where the disease is enzootic. The risk varies with the degree of animal movement. In some countries, e.g. Kenya, close policing of the border areas, the establishment of controlled entry points and vaccination of all animals entering the country suffice. In other countries, e.g. Thailand, an immune barrier zone 50 km deep along the border is maintained, in which all susceptible domestic species, including pigs, are vaccinated annually. A few countries, e.g. Egypt, allow imports from neighboring countries in which the disease is enzootic but protect their own animals by annual vaccination campaigns.

Prophylaxis in areas where rinderpest is enzootic is the raison d'être of the state veterinary services in these countries. Three principal techniques are used: vaccination, control of animal movements by licensing, and disease surveillance by auxiliary staff living in the rural areas.

Although the most widely used vaccine is still the lyophilized attenuated goat-adapted virus, it is being supplanted by the cell-culture-attenuated RBKO strain developed by Plowright in Kenya. The cell-cultured virus, like the parent strain, grows only in lymphoid tissues and vaccinated animals are therefore not infectious. The vaccine is cheap to produce and easy to assay for potency and safety. It is fully attenuated for buffaloes, cattle, goats and sheep, inducing lifelong immunity. However, the field veterinarian has no way of assessing whether the injected vaccine was viable. Postvaccinal serological surveys, therefore, are essential in any vaccination campaign.

The vaccine is lyophilized and stores well at − 20°C, but its potency declines at higher temperatures and its "life" after reconstitution is less than 2 h. Provost and Borredon (1972) selected a heat-resistant clone of the cell-culture-adapted virus which retained its potency after lyophilization for 15 days at 45°C, which obviates the need for refrigeration in the field. In addition, they combined the

rinderpest vaccine with vaccine against contagious bovine pleuropneumonia. To date, this product has been used only in francophone Africa.

The use of primary calf kidney cells involves the risk of introducing bovine pathogens into the vaccine production plant and into the vaccine itself. Indian workers therefore adapted the attenuated RBKO strain to lamb kidney cells. Meantime, elsewhere in Asia several derivatives of the Nakamura III strain of lapinized rinderpest virus have been propagated in cell culture for use as attenuated virus vaccines (Sonoda, 1983).

The variable duration of the passive colostral protection of calves is a big problem in countries where the disease is enzootic. It is partially solved by vaccinating all young animals irrespective of age. Those less than 1 year old are left unmarked but the others are permanently branded or ear-notched. The unmarked young therefore have to be presented for a second vaccination a year later. In short, two calf crops are vaccinated annually.

Antiserum injected in massive doses during the incubation period or prodromal fever will prevent deaths but is useless if the animal has progressed into the erosive-mucosa phase of the disease. Treatment then consists of counteracting the depletion of body water and electrolytes. Antibiotics and sulphonamides have no effect on rinderpest virus but may inhibit activated latent infections. Similarly appropriate anti-protozoal drugs should be administered when necessary.

Although the contagious nature of rinderpest has been appreciated for more than 1000 years, the knowledge was not exploited until the chaos in the 18th and 19th centuries forced European governments to enact legislation controlling animal movements. Today epizootics in low-risk countries are obliterated by a total ban on animal movements and slaughter of all infected and in-contact suceptible species, together with thorough disinfection of contaminated premises. The first cases will probably be misdiagnosed as BVD unless there is a clear history of a live animal movement from an area where the disease is enzootic.

Outbreaks in high-risk and enzootic countries are tackled by quarantine measures and ring vaccination around the focus. Animals are vaccinated irrespective of whether or not they have vaccination brands or ear notches. Many veterinarians will also vaccinate the infected herd in the hope that attenuated virus will protect the uninfected animals by interference. Visibly sick animals are segregated.

If any disease is eradicable it is rinderpest. The goal is unlikely to be achieved in the foreseeable future because the first essential is civic stability in the countries where the disease is enzootic. In Africa, sound techniques for finding and vaccinating the calf crops of the nomadic herds have yet to be formulated. In Asia, Hinduism and Buddhism hinder the use of vaccines and brands. Finally, many of the countries where the disease is enzootic do not have the financial resources necessary to control and eradicate the disease.

REFERENCES

Bansal, R.P., 1986. Diagnosis of rinderpest with special reference to the application of modern techniques. Indian Vet. Res. Inst., Nainital, 55 pp.

Cilli, V., Mazzaracchio, V. and Roetti, C., 1951. Le foyer de peste bovine au Jardin Zoologique de Rome. Bull. Off. Int. Epizoot., 35: 444.

Curasson, G., 1932. La Peste Bovine. Vigot Frères, Paris, 344 pp.

Felton, M.R. and Ellis, P.R., 1978. Studies on the Control of Rinderpest in Nigeria. University of Reading, 40 pp.

Khera, S.S., 1980. Rinderpest eradication programme in India. In: W.A. Geering, R.T. Roe and L.A. Chapman (Editors), Proceedings of the Second International Symposium on Veterinary Epide-

miology and Economics, Canberra, 1979. Australian Bureau of Animal Health, Canberra, pp. 581–586.

Kingsbury, D.W., Bratt, M.A., Choppin, P.W., Hanson, R.P., Hosaka, Y., Ter Meulen, V., Norrby, E., Plowright, W., Rott, R. and Wunner, W.H., 1978. Paramyxoviridae. Intervirology, 10: 137–152.

Maurer, R.D., Jones, T.C., Easterday, B. and Detray, D.E., 1956. The pathology of rinderpest. In: W.A. Aitken (Editor), Proceedings Book of the 92nd Annual General Meeting of the American Veterinary Medical Association, Minneapolis, 1955. American Veterinary Medical Association, Chicago, IL, pp. 201–211.

Mushi, E.Z. and Wafula, J.S., 1984. The shedding of a virulent Kabete O strain of rinderpest virus by cattle. Vet. Res. Commun., 8: 173–179.

Nawathe, D.R. and Lamorde, A.G., 1984. Socio-economic impact of rinderpest in Nigeria. Rev. Sci. Tech. Off. Int. Epizoot., 3: 575 581.

Norrby, E., Sheshberadaran, H., McCullough, K.C., Carpenter, W.C. and Örvell, C., 1985. Is rinderpest virus the archevirus of the *Morbillivirus* genus? Intervirology, 23: 228–232.

Plowright, W., 1968. Rinderpest virus. In: S. Gard, C. Hallauer and K.F. Meyer (Editors), Virology Monographs, 3: 25–110.

Provost, A., 1970. Observations sur les muco-anticorps nasaux des bovins. Rev. Elev. Méd. Vét. Pays Trop., 23: 273–293.

Provost, A. and Borredon, C., 1972. Un vaccin mixte antibovipestique-antipéripneumonique lyophilisé utilisable sur le terrain sous refrigeration. Rev. Elev. Méd. Vét. Pays Trop., 25: 507–520.

Rossiter, P.B. and Jessett, D.M., 1982. Detection of rinderpest virus antigens in vitro and in vivo by direct immunofluorescence. Res. Vet. Sci., 33: 198–204.

Rossiter, P.B., Karstad, L., Jessett, D.M., Yamamoto, T., Dardiri, A.H. and Mushi, E.Z., 1983. Neutralising antibodies to rinderpest virus in wild animal sera collected in Kenya between 1970 and 1981. Prev. Vet. Med., 1: 257–264.

Scott, G.R., 1955. Life expectancy of rinderpest virus. Bull. Epizoot. Dis. Afr., 3: 19–20.

Scott, G.R., 1964. Rinderpest. In: C.A. Brandly and E.L. Jungherr (Editors), Advances in Veterinary Science, 9: 113–224.

Scott, G.R., 1981. Rinderpest. In: J.W. Davis, L.H. Karstad and D.O. Trainer (Editors), Infectious Diseases of Wild Mammals. Iowa State University Press, Ames, IA, 2nd edn., pp. 18–30.

Scott, G.R., Taylor, W.P. and Rossiter, P.B., 1986. Manual on the Diagnosis of Rinderpest. Food and Agriculture Organization, Rome, pp. 1–187.

Sonoda, A., 1983. Production of rinderpest tissue culture live vaccine. Jap. Agric. Res. Q., 17: 191–198.

Thiery, G., 1956. Influence du type de virus et de l'espèce affectée sur les lésions de la peste bovine. Rev. Elev.Méd. Vét. Pays Trop., 9: 109–115.

Yamanouchi, K., 1980. Comparative aspects of pathogenicity of measles, canine distemper, and rinderpest viruses. Jap. J. Med. Sci. Biol., 33: 41–66.

Chapter 33

Peste-des-Petits-Ruminants (Goat Plague) Virus

G.R. SCOTT

INTRODUCTION

In the early 1940s Gargadennec and Lalanne (1942), working in the Ivory Coast, studied a fatal disease of goats, hitherto undescribed, which they labeled "peste-des-petits-ruminants" (PPR) because of the high case mortality rate. Fourteen years later Mornet and his colleagues (1956) rediscovered the disease and found a serological relationship with rinderpest virus in addition to clinical signs and pathological changes reminiscent of rinderpest. They concluded that PPR was a strain of rinderpest virus, adapted to and pathogenic for goats and sometimes sheep, that had lost its ability to infect cattle. PPR virus has since been shown to be a distinct entity possessing antigenic links with the viruses of rinderpest, canine distemper and human measles (Gibbs et al., 1979).

PPR has many synonyms, most of them being descriptive: erosive stomatitis and enteritis of goats, goat catarrhal fever, "kata" and stomatitis–pneumoenteritis complex; in view of the disastrous nature of the infection, the term "goat plague" is justified as a more concise disease term.
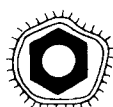
VIRUS PROPERTIES

The half-life of hydrated virus held at 37°C was about 2 h; at 50°C infectivity was destroyed within 30 min (Lefevre, 1982). However, a molar solution of magnesium sulphate significantly slows the rate of heat inactivation. PPR virus is also sensitive to lipid solvents and to low pH. Virus in lymph nodes is protected from pH changes after death, and it has been recovered from lymph nodes of carcasses held for 8 days at 4°C.

PPR virus does not hemagglutinate, but PPR antibodies will inhibit the hemagglutination of monkey erythrocytes by measles virus.

Strains of PPR virus isolated in Senegal and in Nigeria were found to be homogenous (Taylor and Abegunde, 1979). The relationships with other members of the *Morbillivirus* group is close. Cross-protection has been observed; PPR has, on occasion, protected cattle against rinderpest, and the cell-culture-adapted strain of rinderpest virus used as an attenuated rinderpest vaccine usually (but not always) protects goats against PPR.

EPIZOOTIOLOGY

Natural disease is observed in goats and less often in sheep. More older goats recover than younger goats. West African Dwarf goats react severely when

affected and may die, whereas reactions in European goats are less dramatic. Reactions in indigenous and exotic sheep are often subclinical and most diseased animals recover.

Cattle in contact with sick goats were not affected (Mornet et al., 1956). After experimental infection some exhibited low grade fever and developed antibodies. Viremias in cattle, if they occurred, were short-lived. Deer and domestic pigs have been shown experimentally to support virus multiplication. Reactions in the deer were unpredictable and ranged from inapparent infections to fulminating fatal disease. Pigs developed antibodies after inoculation without exhibiting clinical reactions, and the virus did not spread to in-contact susceptible pigs or goats. The role of pigs, therefore, in the epizootiology of PPR was considered negligible.

Many species of wild ruminants and wild pigs in areas where rinderpest is enzootic have antibodies reactive with rinderpest virus in in vitro tests. It seems that some of the antibodies in the sera of wildlife may have been induced by PPR virus.

PPR virus circulates in a belt lying across Africa immediately south of the Sahara and in the Arabian peninsula (Taylor, 1984). Most disease outbreaks occur in West Africa, but at least two epizootics have ravaged sheep and goats in the Sudan. Moreover, the description of the peracute septicemia in Sudanese goats known as "abu nini" (Otte, 1960) is reminiscent of PPR. Similar clinical episodes have been observed in sheep and goats in Ethiopia.

Antibodies to PPR virus have been detected in sera from small ruminants in Oman, Dhofar and the Yemen Arab Republic, areas of the Arabian peninsula in close commercial contact with Islamic Africa. In addition, a rinderpest-like disease caused by a morbillivirus was seen to affect sheep and goats in Saudi Arabia in 1981 and was diagnosed as PPR. Taylor (1984) expressed the opinion that further spread of PPR was inevitable, and he saw no reason to expect Kenya, Uganda or Somalia to remain free of infection. Similarly, he forecasted that PPR would spread northwards from Saudi Arabia into Jordan, Syria and Iraq.

Epizootics are most frequently observed in village flocks and herds in the humid zone of West Africa at the start of the rainy season; epizootics in the sub-Sahara zone occur in the dry season, but they are less commonly reported. Serological studies have revealed similar incidences of infection in goats and sheep; the percentages with antibodies rose progressively with age to 74% in animals over 3 years old (Taylor, 1979). Morbidity is significantly higher in goats. Similarly mortality in clinically affected goats is also much higher, ranging from 70% to 80%.

West Africans prefer goat and sheep meat to beef. Goats and sheep are the major source of animal protein, and consequently the annual toll from PPR is of utmost significance; in Nigeria alone the estimated loss is 1.5×10^6 US dollars per year (Hamdy et al., 1976). Control of PPR in many West African countries therefore has a high priority.

The onset of the rains in the humid tropics causes managemental changes; owners sell their surplus kids and tether the remaining animals indoors to protect the growing crops. The goats dislike rain and huddle close together under whatever shelter is available. This behaviour favors rapid spread of the virus by the aerosol route. Epizootics therefore tend to be explosive in character and involve all adolescent animals; sometimes an epizootic leads to the loss of the entire village herd. The source of infection is either the introduction of an animal newly purchased or the return of unsold animals from a market.

In arid and simi-arid areas surplus transhumant animals are marketed in the dry season because of a scarcity of feed. Infection is readily acquired in the markets and spreads to settled flocks and herds.

PATHOGENESIS

PPR virus in an aerosol invades the body through the tissues lining the upper respiratory tract, is disseminated before the onset of clinical signs and is shed in nasal secretions, tears, saliva and urine. When diarrhea supervenes fecal excretion occurs. As the disease progresses the quantity of excreted virus increases. Animals that recover do not become carriers.

DISEASE SIGNS

Sheep and, less commonly, goats develop subacute reactions after an incubation period of about 6 days, the illness being manifested by a low-grade fever, nasal catarrh, recurring mucosal erosions and intermittent diarrhea. After a course of 10–14 days the animals usually recover.

Acute reactions begin after an incubation period of 3–4 days. Fever and serous rhinorrhea suddenly appear but otherwise the affected animal looks normal. A few hours later depression is evident. Erosions develop in the mucous membranes lining the upper alimentary, upper respiratory and urogenital tracts 1 or 2 days after the onset of fever. Salivation is profuse and the tongue is continually protruded and retracted. The rhinorrhea becomes so mucopurulent that it often blocks the nostrils. Simultaneously mucopurulent tears mat the eyelids together. The mucosal erosions coalesce, producing extensive lesions in a further 2–3 days. Ulceration often follows. Diarrhea and pneumonia then supervene. Affected goats are a picture of misery and exude a nauseating odor (Fig. 129). Most of them die within 10 days.

Peracute reactions follow incubation periods that are often as short as 2 days. A profuse nasal catarrh precedes a sudden high fever with signs of depression, dyspnea, anorexia and constipation. The visible mucous membranes are congested and sometimes eroded. A debilitating diarrhea sets in within 3–4 days. The fever persists to within a few hours of death, which usually occurs 4–6 days after the onset of illness.

Within 24 h of the onset of fever a severe leukopenia is evident and persists

Fig. 129. Acute peste-des-petits-ruminants (photograph by M.A. Bonniwell).

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until death or for 10 days in animals that recover. Differential counts have not been published; massive destruction of lymphocytes has been observed in lymphoid tissues, and by analogy with rinderpest it is likely that the leukopenia is primarily caused by a lymphocytopenia.

Pasteurella pneumonia almost invariably supervenes in acute cases and complicates the clinical and postmortem findings. In addition, a wide range of other secondary bacterial invaders has been isolated. Experimental dual infection of goats with _Mycoplasma capri_ and PPR virus was found to be much more severe than infection by either pathogen alone (Onoviran et al., 1984). Enteric and hemoprotozoal infections are also exacerbated. Similarly activation of latent viral infections is suspected. Adenovirus and PPR virus, for example, have both been isolated from a fatal case of PPR. Many surviving goats develop labial crusts and oral plaques, some of which are attributable to exacerbated orf and others to a recrudescence of _Dermatophilus congolensis_, which in the goat has a predilection for the lips.

PATHOLOGY

Goats dying from PPR are dehydrated and soiled with feces. The periorbital and perinasal areas are encrusted with mucopurulent discharges. The spectacular gross lesions are the eroded and ulcerated mouth and throat and the secondary bronchopneumonia which often masks an underlying primary viral pneumonia manifested by areas of level red consolidation (Rowland et al., 1969). Other gross lesions are surprisingly minor in appearance; there is usually congestion of the iliocecal valve, some inconspicuous "zebra striping" of the colon, and lymphadenopathy.

Microscopic lesions are associated with the mucosal erosions, depressed lymphoid tissues and the consolidated lung lobules. The primary change is the development of multi-nucleated giant cells containing intranuclear and intracytoplasmic inclusions, the syncytia in the lung parenchyma being par-
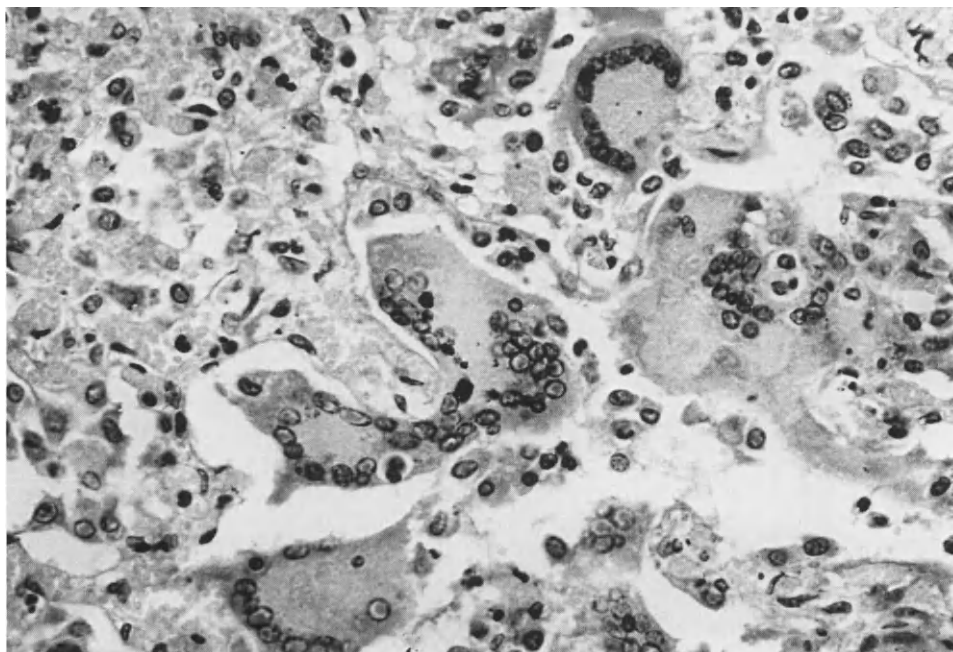
Fig. 130. Multinucleate giant cell with intranuclear inclusions within alveoles; H & E, × 450 (photograph by A.C. Rowland).

ticularly conspicuous (Fig. 130). Focal necrosis destroys epithelial cells of the alimentary tract and lymphocytes in lymphoid tissues.
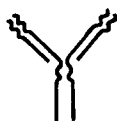
IMMUNE REACTION

Surviving goats and sheep develop immunity and resist reinfection. The duration of immunity is likely to be lifelong. It is associated with humoral antibodies which are detectable by CF, AGID, measles HI and neutralization tests. Cell-culture-adapted rinderpest virus used as an attenuated rinderpest vaccine protected most goats against challenge with PPR virus; the vaccinated animals developed rinderpest antibodies but not PPR antibodies. When the goats were challenged with PPR virus, homologous antibodies appeared anamnestically.

Colostral immunity protects kids and lambs until they are weaned. Artificial passive immunity has been used successfully to limit the spread of disease by injecting the animals at risk with hyperimmune serum.

LABORATORY DIAGNOSIS

Most diagnoses of PPR are not confirmed, being based on knowledge of the history of the outbreak, particular attention being paid to the season of the year, recent purchases from markets, the nature of the clinical signs and the lesions found post mortem.

Laboratory confirmation depends primarily on detection of antigen and isolation and identification of the virus, supplemented by histopathology. Inoculation of tissue suspensions from suspect cases into rinderpest-susceptible cattle may be necessary to differentiate PPR virus from rinderpest virus if tissue culture facilities are not available.

The preferred samples for antigen detection are lymph nodes and tonsils taken from animals killed early in the course of the disease; it is also worthwhile collecting lymph nodes from dead animals. The samples are used in agar precipitation tests against rabbit hyperimmune-rinderpest serum (if no PPR-hyper-immune serum is available) employing the CIEP or the conventional AGID tests; CF tests are also useful.

The preferred samples for virus isolation are likewise collected from animals killed while febrile and within 5 days of the onset of fever. These include blood in heparin or EDTA, lymph nodes, tonsils, lung and affected intestinal mucous membranes. Dilutions of the tissue suspensions are inoculated into primary lamb kidney cells or into known susceptible and immune goats; animal inoculations yield the quicker results. The isolated virus is identified by cross-neutralization tests using homologous (PPR) and heterologous (rinderpest) antisera.

A histopathological examination of early mucosal lesions, lymphoid tissues and lungs that reveals multinucleated giant cells with inclusions backs up the other procedures.

Unlike the sera of rinderpest-convalescent animals the sera of PPR-convalescent sheep and goats contain antibodies readily detected by CIEP and AGID tests. In addition, antibodies are detectable by HI using measles virus and by neutralization tests. Cross-neutralization tests differentiate PPR antibodies from rinderpest antibodies (Taylor, 1979).

For many years in many West African countries PPR was misdiagnosed, usually as a purulent pneumonia. By the same token today, every acute death in goats tends to be labelled PPR. It is difficult to distinguish PPR from

rinderpest without laboratory tests or cattle inoculations. Fortunately, rinderpest appears to be absent or very rare in goats and sheep in Africa, whereas PPR has not been detected in India, where rinderpest in sheep and goats is not uncommon. Diagnostic confusion has occurred with fulminating fatal cases of heartwater and contagious caprine pleuropneumonia. The ulcerated oral lesions of bluetongue have been mistaken for PPR. The labial crusts in PPR-convalescent animals have been attributed to primary orf or bluetongue reactions but are latent infections activated by PPR.

PROPHYLAXIS AND CONTROL

In the humid tropics of West Africa villages are isolated. The simplest prophylactic measure therefore is to ban the importation of live animals purchased at or brought back unsold from markets. Unfortunately most purchases are made for festive or social reasons, necessitating the sacrifice of a live animal; an animal slaughtered in the market is no substitute.

In the dry tropics it is virtually impossible to segregate transhumant animals from settled village animals. Consequently, prophylaxis is based on vaccination. Virulent cell-culture-passaged PPR virus produced durable immunity in goats when administered simultaneously with hyperimmune anti-PPR serum prepared in cattle (Adu and Joannis, 1984). An attenuated PPR virus strain has been developed by prolonged serial passage in cell culture, but it is not yet available commercially as a vaccine. The common prophylactic, therefore, is still attenuated cell-culture-adapted rinderpest virus vaccine, first used with good results by Bourdin et al. (1970). The goats developed rinderpest antibodies and withstood challenge with PPR virus. Cell-culture-adapted rinderpest virus vaccine, moreover, is nonpathogenic in newborn and pregnant goats. It is now widely used to protect sheep and goats against PPR but, on occasion, there have been failures (Bonniwell, 1980).

Tissues from PPR-infected goats have been used to produce inactivated vaccines. Results with formalin-inactivated vaccine were inconsistent, whereas chloroform-inactivated vaccine maintained its efficacy for 1 year at 4°C, and the sera of vaccinated goats fixed complement 8 months after vaccination (Nduaka and Ihemelandu, 1975).

Hyperimmune serum has been used successfully to limit the spread of infection in an outbreak but had no effect in diseased animals.

Symptomatic treatment and good nursing alleviate suffering and may save lives. It should be supplemented with antibiotics and sulphonamides to counteract bacterial infections. In addition, anti-protozoal drugs and anthelmintics should be administered where necessary.

Sick animals should be segregated immediately and the remaining animals should be vaccinated with attenuated rinderpest virus vaccine. The affected village should be quarantined until the outbreak is over. Thereafter biannual vaccination of the adolescent kids should be carried out.

REFERENCES

Adu, F.D. and Joannis, T.E., 1984. Serum–virus simultaneous method of immunization against peste-des-petits-ruminants. Trop. Anim. Health Prod., 16: 119–122.

Bonniwell, M.A., 1980. The use of tissue culture rinderpest vaccine (TCRV) to protect sheep and goats against "peste-des-petits-ruminants" in the Ashanti Region of Ghana. Bull. Off. Int. Epizoot., 92: 1233–1238.

Bourdin, P., Rioche, M. and Laurent, A., 1970. Emploi d'un vaccin antibovipestique produit sur cul-

tures cellulaires dans la prophylaxie de la peste-des-petits-ruminants au Dahomey. Note Prélimi-
naire. Rev. Elev. Méd. Vét. Pays Trop., 23: 295–300.

Gargadennec, L. and Lalanne, A., 1942. La peste-des-petits-ruminants. Bull. Serv. Zootech. Epizoot.
Afr. Occident. Fr., 5: 16–21.

Gibbs, E.P.J., Taylor, W.P., Lawman, M.J.P. and Bryant, J., 1979. Classification of peste-des-petits-
ruminants virus as the fourth member of the genus *Morbillivirus*. Intervirology, 11: 268–274.

Hamdy, F.M., Dardiri, A.H., Nduaka, O., Breese, S.S. and Ihemelandu, E.C., 1976. Etiology of the
stomatitis pneumonenteritis complex in Nigerian Dwarf goats. Can. J. Comp. Med., 40: 276–284.

Lefevre, P.C., 1982. Peste-des-petits-ruminants et Infection Bovipestique des Ovins et Caprins.
Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, Maisons-Alfort, 95 pp.

Mornet, P., Gilbert, Y., Orue, J., Thiery, G. and Mamadou, S., 1956. La "peste-des-petits-ruminants"
en Afrique Occidentale francais. Ses rapports avec la peste bovine. Rev. Elev. Méd. Vét. Pays
Trop., 9: 313–335.

Nduaka, O. and Ihemelandu, E.C., 1975. The control of pneumonia–enteritis complex in dwarf goats
of Eastern Nigeria by the use of chloroform-inactivated tissue vaccine. Bull. Anim. Health
Prod. Afr., 23: 341–348.

Onoviran, O., Majiyagbe, K.A., Molokwu, J.U., Chima, J.C. and Adegboye, D.S., 1984. Experiment-
al infection of goats with *Mycoplasma capri* and "peste-des-petits-ruminants" virus. Rev. Elev.
Méd. Vét. Pays Trop., 37: 16–18.

Otte, E., 1960. Clinical studies on "abu nini" in the Sudan: a contagious disease of goats and sheep,
possibly caused by pleuropneumonia-like organisms. Vet. Rec., 72: 140–145.

Rowland, A.C., Scott, G.R. and Hill, D.H., 1969. The pathology of and erosive stomatitis and
enteritis in West African Dwarf goats. J. Pathol., 98: 83–87.

Taylor, W.P., 1979. Serological studies with the virus of peste-des-petits-ruminants in Nigeria. Res.
Vet. Sci., 26: 236–242.

Taylor, W.P., 1984. The distribution and epidemiology of peste-des-petits-ruminants. Prev. Vet. Med.,
2: 157–166.

Taylor, W.P. and Abegunde, A., 1979. Isolation of peste-des-petits-ruminants virus from Nigerian
sheep and goats. Res. Vet. Sci., 26: 94–96.

Chapter 34

Bovine Respiratory Syncytial Virus

G. WELLEMANS
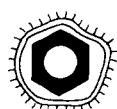
VIRUS PROPERTIES

Physical and chemical characteristics

The bovine respiratory syncytial virus (BRSV) is morphologically similar to the human RSV and pneumonia virus of mice, the only known members of the pneumovirus group (Cash et al., 1977).

The virus is of great pleomorphism. Many particles appear roughly spherical with an overall diameter of 80–450 nm (Ito et al., 1973; Mohanty, 1978). The RSV genome codes for ten virus proteins. Four proteins are associated with the virus envelope: the large glycoprotein (G, 84 k), the fusion-protein (F, 68 k), the matrix (M, 26 k) and the 22 k protein. The F protein consists of two disulphide-linked polypeptide fragments (F1, 48 k, and F2, 20 k) and causes fusion of infected cells (Walsh and Hruska, 1983). The G protein is the attachment protein (Levine et al., 1987). Three proteins constitute the virus nucleocapsid: the major nucleocapsid protein (N, 42 k), the phosphoprotein (P, 34 k) and the large protein (L, 200 k). A small protein (9.5 k) is found in association with purified virions and is candidate to be a nonstructural protein (Huang and Wertz, 1983). The nucleocapsids have a density of 1.26–1.27 g/ml, and structures of 1.32–1.36 g/ml appear during the disintegration of nucleoids. Virion RNA has a sedimentation coefficient of 52 S (Zhdanov et al., 1974).

Cell-culture-adapted virus survives quick freezing to −70°C, but Mohanty (1978) reports that isolations are more difficult from frozen than from fresh material. BRSV is sensitive to low pH, ether and chloroform and is destroyed by heating at 56°C for 30 min (Inaba et al., 1970; Paccaud and Jacquier, 1970; Rosenquist, 1974). Application of the least squares method to survival values obtained at 0.5 and 15 min indicated estimated half-lives of 1.8 and 2.8 min (Rosenquist, 1974).

The infectivity titer of a BRSV suspension decreases by about 1 log per 24 h at 37°C. The virus remains infectious for at least 10 years when stored in liquid nitrogen.

Antigenic properties

Evidence of a close antigenic relationship between the bovine agent and human RSV is confirmed by CF and IF tests (Paccaud and Jacquier, 1970; Wellemans et al., 1970).

Cross-neutralization experiments indicate that antiserum against human RSV neutralizes the homologous virus at significantly higher titers than the bovine virus (Inaba et al., 1970; Paccaud and Jacquier, 1970; Smith et al., 1975).

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Some differences, e.g. the spectrum of susceptible cells and an antigenic dissimilarity, suggest that the two viruses have different natural hosts (Inaba et al., 1970). Moreover, virus could not be recovered from the respiratory tract of mice inoculated with bovine strains (Taylor et al., 1984). However, Jacobs and Edington (1975) succeeded in an experimental infection of a calf with a human strain. This experiment was successfully repeated by Thomas et al. (1984).

The protein composition of human and bovine RSV strains is very similar, with only minor differences in mol. wt. (Cash et al., 1977). Two subgroups of human RSV can be distinguished; the major antigenic differences are located on the G protein (Mufson et al., 1985). BRSV can be classified as a separate group, sharing epitopes with most proteins of the two human subgroups, but being distinct with respect to the epitopes on the G protein (Orvell et al., 1987).

Monoclonal antibodies to the F protein showed neutralizing activity and prevented spread of the virus in vitro (Walsh et al., 1985). Passive transfer of monoclonal antibodies to the F and G proteins offered protection against challenge with RSV, while antibodies to internal proteins did not (Walsh et al., 1984).

Cultivation

In tissue culture, BRSV shows a wide range of susceptible host cells. It replicates in all cell types (kidney, testicle, thyroid, thymus, duodenum, rectum) of bovine origin as well as in cells from swine (embryonic kidney), hamster (lung, kidney), monkey (Vero) and human (embryonic lung and kidney, Hela, HEp-2). Better growth is observed in bovine than in human cell cultures (Matumoto et al., 1974). This is in contrast to the observations of Paccaud and Jacquier (1970), who did not succeed in cultivating their BRSV strains in a BHK-21 cell line and in various human cells.

Larger amounts of virus are obtained in calf kidney and testicle cell culture, and higher titers are scored in secondary than in primary cultures of the same cells (Mohanty, 1978; Wellemans, 1977). The direct IF test in which the immunoglobulins of BRSV antiserum are conjugated with fluorescein isothiocyanate detects the antigens as soon as 16–18 h after inoculation. Fluorescence is always confined to the cytoplasm. Within 24 h, fine fibrils appear, usually parallel to the long axis of the cell, and cytoplasmic granules are formed around the nucleus (Rossi and Kiesel, 1977b). After 24 h, coincident with rounding of the cells, fluorescence slowly moves to the periphery of the cytoplasm. By IF and as determined by the release of BRSV into the supernatant fluid, the minimal time for a single cycle of infection was between 24 and 26 h (Rossi and Kiesel, 1977b).

BRSV grows in organ cultures of bovine fetal trachea explants at 37°C and pH 7.2. It reaches maximum titers of 10^5 PFU/ml between 11 and 21 days after inoculation. Virus growth does not affect ciliary activity of the cultured cells (Thomas et al., 1976; Rossi and Kiesel, 1977a).

EPIZOOTIOLOGY

BRSV has been isolated in most European countries, North America, Australia, Japan and more recently in North Africa (Mahin and Wellemans, 1982).

The virus generally appears in a BRSV-free country after the introduction of an infected animal. Although the presence of virus carriers has not yet been proven (probably because of the difficult isolation of the virus), one should be aware of this possibility. Humans, especially veterinarians and animal handlers, could play a role in the transmission of the virus. Considering the success-

ful infection of a calf with a human strain (Jacobs and Edington, 1975) and the similarities between human and bovine strains, humans cannot be excluded as a virus reservoir (Berthiaume et al., 1973). However, Inaba et al. (1970) and Paccaud and Jacquier (1970) do not endorse this view.

Once a herd in a region is affected, the disease rapidly spreads from farm to farm. In an already infected region, the disease becomes endemical and affects the same herds almost every year. The animals, especially beef cattle, are most susceptible from 3 up to 9 months of age, though older animals are not always resistant (Paccaud and Jacquier, 1970; Wellemans et al., 1970; Van Bekkum et al., 1977). On the other hand, younger calves of only a few weeks of age can also be affected, and their protection by vaccination is still questionable (Wellemans, 1982). Differences in housing and other aspects of management probably have some effect; in some herds the virus is present without causing any disease, as shown by occasional seroconversions, in others one or even two outbreaks occur every year (Van Bekkum et al., 1977). However, severe disorders can be observed also in animals at pasture.

Most of the severe cases appear from October to January (Van Bekkum et al., 1977; Wellemans, 1977), but recently BRSV outbreaks have also occurred in spring and summer. Therefore, disease caused by BRSV can be expected during the whole year, as in the case of IBR. The weather, particularly a fall in atmospheric pressure, plays an important role in the outbreak of the disease (Wellemans, 1982).

PATHOGENESIS

The disease symptoms observed after experimental inoculation are not as severe as in cases of natural infection (Inaba et al., 1972; Jacobs and Edington, 1975; Smith et al., 1975; Mohanty, 1978; Elazhary et al., 1979; Thomas et al., 1984) and the infection frequently remains inapparent. Stott (1985) suggests that virus unpassaged in tissue culture may have a greater virulence for the natural host. Treatment with dexamethasone enhances lung lesions produced by bovine strains, extends the period of virus shedding and increases peak titers (Thomas et al., 1984). The concomitant presence of BVDV as well as a sudden fall in atmospheric pressure or a drop of the minimum temperature seem to aggravate the disease (Verhoeff and Van Nieuwstadt, 1984).

The first symptoms are observed 2–8 days after inoculation. The virus can be reisolated from nasal secretions for 4–10 days after inoculation, and from the nasal, tracheal and bronchial mucosae for 7–13 days after infection (Jacobs and Edington, 1975; Thomas et al., 1984).

It appears that BRSV infection can occur in the presence of circulating antibodies (Mohanty, 1978; McNulty et al., 1983). There is no evidence, however, that preexisting serum antibody causes exacerbation of the disease in young calves, as was reported in infants (Kim et al., 1969); other conclusions have been drawn by Smith et al. (1975). However, the protective effect of nasal neutralizing antibodies against infection with BRSV has been shown by Mohanty (1978). This author found that young calves with traces of neutralizing antibodies in the nasal secretions remained solidly immune to challenge. The quick appearance and the pathogenic action of BRSV on the nasal mucosa would indicate that the nasal cavity might be the point of departure to the target cells; Baskerville (1981) disagrees with this hypothesis. BRSV is unable to suppress ciliary activity, even though the virus replicates within ciliated epithelial cells of tracheal rings. It was therefore suggested that the tracheal epithelium may not be important in the pathogenesis of BRSV infection (Thomas et al., 1976; Rossi and Kiesel, 1977a).

The accumulation of cellular debris and exudate favors bacterial proliferation, which may lead to extensive purulent pneumonia chiefly in young calves. However, the sudden onset of pulmonary emphysema is not explained by BRSV infection. Immunological hypersensitivity reactions probably are involved as complicating factors, as has been shown in humans (Gardner et al., 1970).

McIntosh and Fishaut (1980) have reviewed several theories about the pathogenesis of RSV disease in infants: serum antibodies reacting with the virus, prior sensitizing infection, CMI, IgE-mediated reactions—no firm conclusions are possible about immunopathologic mechanisms in bronchiolitis.

DISEASE SIGNS

In typical outbreaks, the disease develops in two distinct episodes. Suddenly 80–90% of a certain age group may show symptoms. Affected calves are 3–9, sometimes up to 15 months old. The animals cough and there is nasal discharge and a conjunctivitis with lachrymation. The body temperature is about 40°C.

About 2 or 3 days later, when everything seems to be back to normal, the second episode begins and signs of lung emphysema appear. Some animals have difficulty in breathing, accompanied by bouts of dry coughing. Body temperature at the time these symptoms are first manifest is close to normal. The calves lose weight and have a rough hair coat. Respiration rates can go up to > 100. The breathing of the sick animals becomes increasingly rapid and shallow, and the condition is aggravated by bouts of coughing. There is little or no discharge from the nostrils. Frequently there is froth at the commissure of the lips. Ill calves may stand with a stretched neck and extended forelimbs (Fig. 131). Symptoms of abdominal breathing can develop. The animals can neither lie down nor eat and make desperate efforts to breath through the open mouth. Constipation is common and there is complete loss of appetite. The mucosae become cyanotic. On auscultation, some harshness in the breathing can be detected.

There can be up to 20% mortality in the herd. Farms specializing in baby-beef tend to have the highest losses. Mortality often occurs within a few hours.

Surviving calves recover after a few days: breathing becomes easier and
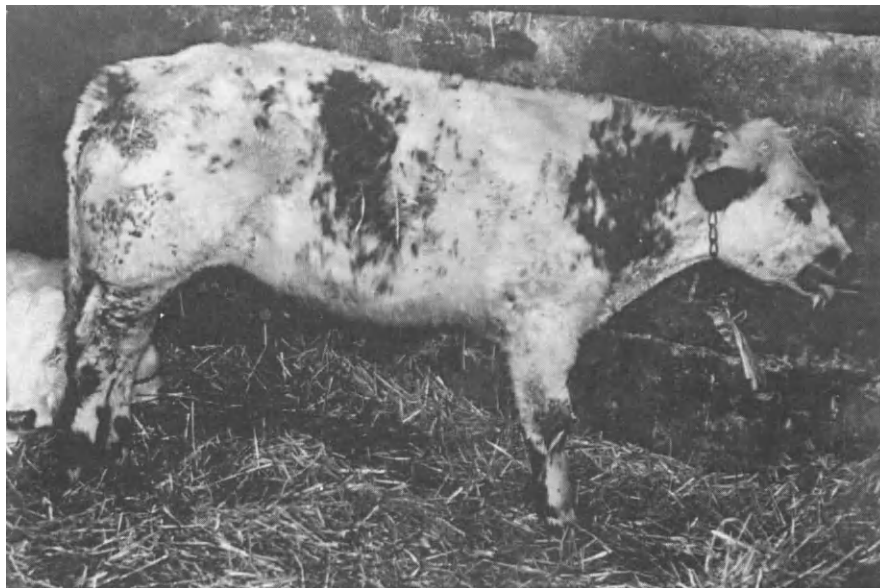
Fig. 131. Calf with BRSV infection; note the dyspnea.

Fig. 132. Pulmonary emphysema (apical lobe) with hemorrhagic lesions.

appetite returns (Holzhauer and Van Nieuwstadt, 1976; Van Bekkum et al., 1977; Wellemans, 1977; Verhoeff and Van Nieuwstadt, 1984).

A slightly different symptomatology can be observed in 6-week-old calves, especially in fattening farms. Few or no emphysema lesions are encountered, but bacterial superinfections are frequently found. Cough, high fever and serous to mucopurulent nasal discharge are the most common symptoms.

Paccaud and Jacquier (1970) noted that all animals aged less than 7 years showed signs of acute respiratory disease. Pregnant animals or good milk producers tend to be affected (Inaba et al., 1972).

In Belgium, respiratory disorders are seldom observed in cattle more than 2 years old.
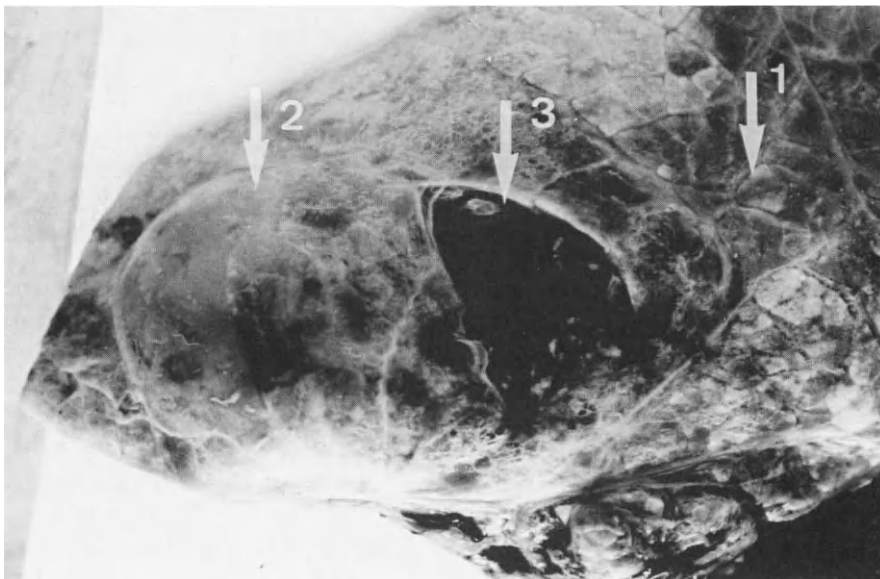
Fig. 133. Pulmonary emphysema (mediastinal lobe); (1) interlobular septa are distended; (2) large bullae; (3) pleural wall is broken.

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PATHOLOGY

In animals that have died from the disease, pathological changes are restricted to the respiratory tract; postmortem lesions are not really pathognomonic: they can also be found in cases of BVD and PI3 infection.

The lungs are voluminous and emphysematous. The apical and cranial lobes show lobular bronchopneumonia. Numerous large ecchymotic hemorrhages are present in emphysematous bullae and under the pleura (Figs. 132, 133); often foamy exudate covers the injected mucosae of the bronchi. The diaphragmatic lobes are edematous and interlobular septa are distended. Subpleural emphysema is present in all lobes but is more severe in the diaphragmatic lobe, where numerous large dissecting bullae are present. In some cases the pleural wall is broken, resulting in pneumothorax. In most cases there is also marked mediastinal emphysema and in some calves emphysema extends into the subcutaneous tissues of the shoulder, back and neck. The animals then show crepitating, asymetric swellings. Petechia are frequently seen, particularly on the larynx. In the heart, petechia are often found on the myocard (Holzhauer and Van Nieuwstadt, 1976; Wellemans, 1977; Bryson et al., 1983).

Calves of a few weeks of age present hepatization in the anterior lobes; emphysema is rare.

Histological lesions, e.g. mononuclear infiltration in the hyperplastic alveolar walls, bronchiolitis or lobular interstitial pneumonia with edema, are not pathognomonic of BRSV infection. However, in some cases, mainly in young calves, syncytia with eosinophilic inclusions are observed in the alveoles (Mohanty, 1978; Bryson et al., 1983). Two types of lesions can be found in ultrathin sections of apical and cranial lobes, with the aid of a fluorescein isothiocyanate (FITC) conjugated anti-BRSV serum. The lesions are referred to as types A and B. In type A lesions the antigen is distributed homogenously in the cytoplasm of the affected cells. These cells look undamaged, and syncytium formation is frequently seen. This type of lesions is found in calves of a few weeks of age and sometimes in older animals of the Charolais or Blonde d'Aquitaine breed (Wellemans, 1982). In type B lesions the antigen is clustered in packs. The cells are disrupted and antigen masses are spread i the alveolar or bronchial lumen. Rarely giant cells are found in these lesions (Wellemans, 1977). The type B lesions are encountered in emphysematous lungs of older calves of the Belgian Blue White breed.
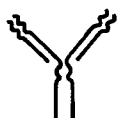
IMMUNE REACTION

Epidemiological studies have shown that there is no solid protection in humans against nasal reinfection. However, cotton rats infected with RSV develop complete resistance to pulmonary infection lasting for at least 18 months. Nasal resistance was of shorter duration and decreased from 8 months on. Immunity to RSV infection is therefore more long lasting in the lungs than in the upper tract and the level of immunity to RSV in the upper respiratory tract does not necessarily reflect resistance in the lungs (Prince et al., 1983).

Preexisting maternal antibodies to BRSV did not protect calves from infection (Mohanty, 1978; McNulty et al., 1983). On the other hand, maternal immunity was effective but transient in the lungs of young cotton rats (Prince et al., 1983). Immune factors other than neutralizing antibodies may play a role in the maternal passive immunity (Prince et al., 1983).

In experimentally infected calves, high interferon titers are detected during the early stage of infection. This is followed by a period of at least 1 week during which interferon is not detectable. After this, moderate to low inter-

feron titers reappear in most animals and persist for a number of weeks (Elazhary et al., 1981). In contrast, in children no interferon or only low levels are found (Hall et al., 1978; McIntosh, 1978), and mean levels do not fluctuate significantly in relation to disease and recovery. These discordant results are probably due to the fact that the first blood sample in children is taken in the course of the disease, whereas in calves the first sample is taken at an early beginning stage.

Antibodies have been demonstrated by VN, CF, indirect IF, ELISA and precipitation tests in sera from cattle infected with BRSV. Neutralizing antibodies appear in serum of experimentally infected calves after 7 days at extremely low titers (4–8) (Mohanty et al., 1975). In natural infections, however, the SN titers score much higher (64: Rosenquist, 1974; 256: Inaba et al., 1972) and the maximum level is reached after 3 weeks. Using indirect IF, antibodies may be evidenced as early as 3 days p.i., with maximum titers at about 10 days p.i. (Elazhary et al., 1981). In natural infections, it is not uncommon to find highly seropositive calves in the acute stage of the disease. Experimentally infected animals can react in different ways: in some calves, antibodies develop very early, as described by Elazhary et al. (1981); in others their appearance is delayed (7–9 days). In nasal mucus antibodies evidenced by indirect IF are excreted from the first week on, and they are present for at least 3 months (Wellemans, 1977).

Results of leukocyte migration–inhibition tests under agarose indicate that a CMI response is elicited after infection of calves with BRSV. The calves also develop a delayed hypersensitivity skin response (Field and Smith, 1984).

Recovery of 6–7-month-old calves from severe BRSV-associated disease was accompanied by an antibody response that was mainly directed to the F and N proteins. Calves 2–3 weeks of age with moderate levels of maternal antibodies to BRSV particularly directed to the F and N proteins became seriously ill after infection. The antibody response in these calves was severely suppressed. In sera of 4–9-month-old calves that had died in the course of infections, high anti-F and anti-N antibody levels were found. Apparently, the presence or development of antibodies to the F and N proteins is not sufficient for protection against or recovery from infections with BRSV (Westenbrink et al., 1989).

Fig. 134. CPE caused by a BRSV infection on BFK cell culture; note the presence of syncytia (S) and the "moth-eaten" aspect of the culture.

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LABORATORY DIAGNOSIS

A clinical diagnosis based on symptomatology is impossible. Only the laboratory can help the practitioner who suspects a BRSV outbreak in a farm. The laboratory diagnosis will be based on either BRSV isolation, the detection of viral antigen in suspected organs, or by evidencing a seroconversion in diseased animals.

The nasal mucus is taken with sterile cotton swabs during the initial stage of the disease (serous discharge, fever, conjunctivitis). The swabs are then put into a tube with a protein-rich medium (maintenance medium for cell cultures) and forwarded very rapidly in a cool box to the laboratory. Postmortem samples of lung tissue from very young calves should also reach the laboratory rapidly. In contrast to other viruses, e.g. IBRV, viral presence in the nasal mucous is of short duration and limited to the first stage of the disease, which often passes unnoticed.

The isolation of the virus in cell culture is difficult because of the late appearance of CPE; the time of incubation may be extremely long: 20 days (Inaba et al., 1970), 30 days (Paccaud and Jacquier, 1970), 45 days (Wellemans et al., 1970) or even 50 days (Smith et al., 1975). Secondary fetal calf kidney or testicle cells are most susceptible. Virus growth depends on the age of the cell culture, and the medium has to be replaced frequently.

The first changes noted are small areas where four or five cells become ballooned with shrinkage of cytoplasm. In the following days, syncytia develop in these cultures. They become opaque while contracting, and holes appear in the cell monolayer. The aspect becomes more and more granular, resembling a mycoplasma-infected cell culture (Fig. 134).

In preparations fixed with Bouin solution and stained with H & E, homogenous eosinophilic inclusion bodies are observed in the cytoplasm of syncytial cells. These inclusions are less polymorphic than in cells infected with PI3 virus.

The cells grown on glass slide of Leighton tubes are fixed in aceton at − 20°C and stained with an antiserum to BRSV conjugated with fluorescein isothiocyanate. Plaques of fluorescent cells confirm the presence of the virus (Fig. 135).

Isolation of BRSV is difficult and of long duration; it is not recommended as a routine procedure (Edwards et al., 1984).

Viral antigen can be detected in nasal mucus and in lung tissue. Nasal
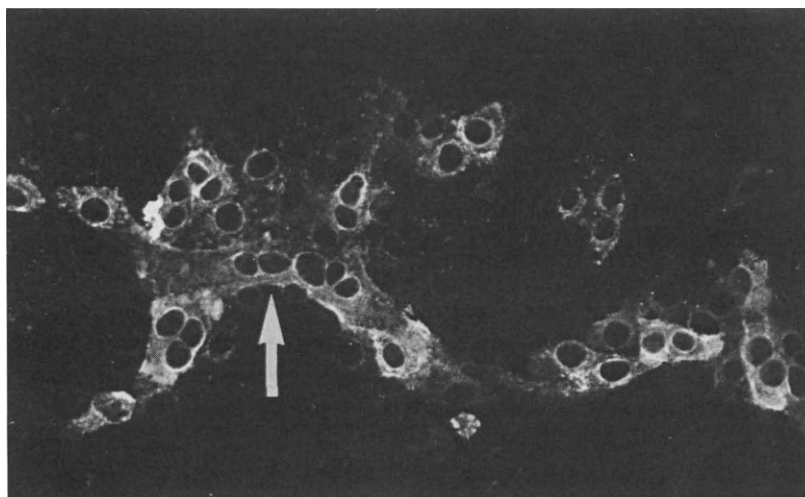
Fig. 135. Direct IF staining of a BRSV infected BFK culture; note the presence of a syncytium.
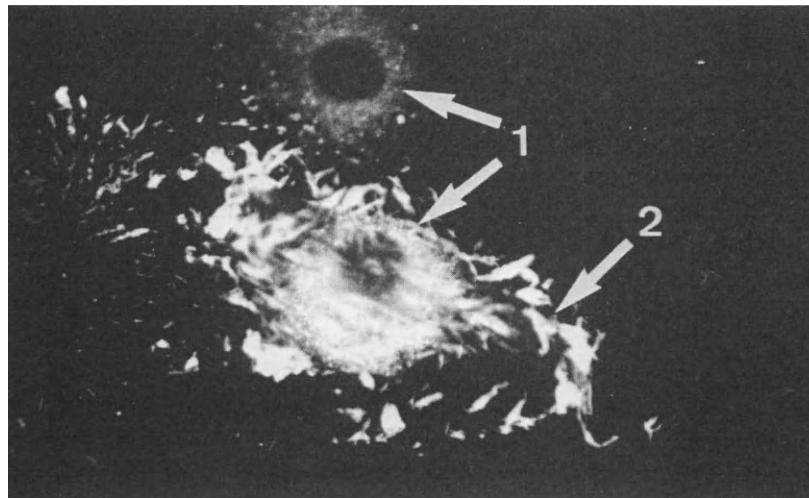
Fig. 136. Direct IF staining of an infected cell; two types of fluorescence present: (1) granular, around the nucleus; (2) filamentous, in the rest of the cytoplasm.

mucus can be taken with a swab. Though the most characteristic emphysema lesions are situated in the mediastinal lobes of the lungs, antigen has to be searched for mainly in the apical and cardiac lobes. The samples have to be forwarded quickly and under refrigeration to the laboratory. For the direct IF test, monospecific hyperimmune serum is required that is marked with fluorescein isothiocyanate using the classical technique.

Typical small, round cells in which fluorescence is confined to the cytoplasm are detected in the nasopharyngeal specimens (McNulty et al., 1983). Fluorescence is not seen in ciliated columnar epithelial cells, although many are present in most specimens (Thomas and Stott, 1981). Problems of nonspecific fluorescence are encountered with the method when applied to nasopharyngeal material but not in the examination of lung material (Thomas and Stott, 1981). Evans blue (1:10.000) can be used to reduce nonspecific fluorescence.

Ultrathin sections of lung fragments, preferably from the apical and cardiac lobes, are stained with conjugated serum. Fluorescent antigen is detected in this material for up to 48 h post mortem, even after freezing. However, it is noteworthy that the viral antigen disappears after longer freezing periods, even at low temperatures ($- 100°C$). In most of the lung specimens, antigen is found located in the alveoli and sometimes in the bronchioles. Two types of
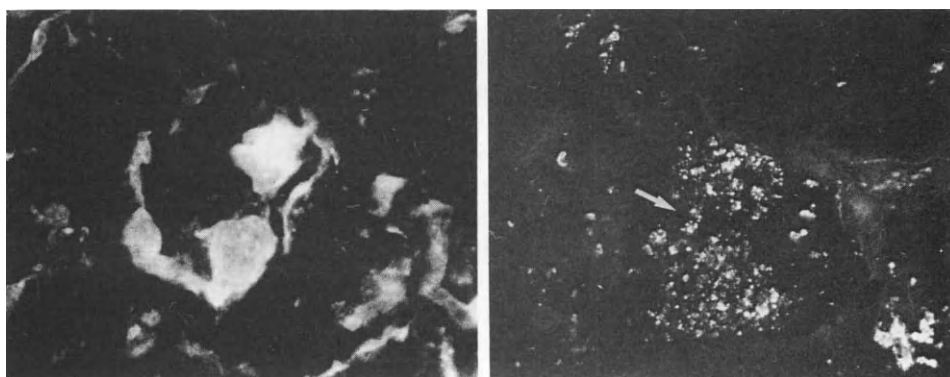
Fig. 137 (left). Direct IF staining on sections of the lung of a young calf; the affected cells of the alveolus appear intact (type A).

Fig. 138 (right). Direct IF staining on ultrathin sections of the lung of an older calf; lesions of alveolitis with disrupted cells (type B).

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fluorescence are distinguished (Fig. 136): type A, in which the antigen is spread homogenously throughout the cytoplasm. The cells look intact and syncytia are frequently observed (Fig. 137); and type B, in which the antigen is aggregated in granular packs. The cells are disrupted and antigenic masses are spread in the alveolar and bronchial lumen. Rarely giant cells are found in these lesions (Fig. 138). Type A lesions are encountered in 2–3-week-old calves and also in older cattle of the Charolais and Blonde d'Aquitaine breed; type B lesions are found in emphysematic lungs of older animals of the Belgian Blue White breed.

ELISA is applied more and more for the detection of viral antigens. Hornsleth et al. (1981) described its use for RSV diagnosis and confirmed its reliability.

An increase of anti-BRSV antibodies can be evidenced in paired sera taken from the same animals in the acute stage and 15 days to 3 weeks later. Neutralizing antibodies appear as a consequence of a BRSV infection. SN titers in convalescent animals rarely exceed 4–16 according to Bartha (1976), whereas Rosenquist (1974) and Inaba et al. (1970) report titers of 256. Despite the improved micromethods the SN test remains a time-consuming technique with difficult reading of the results; it is used in research work rather than for routine diagnosis.

Numerous authors have used the CF test (e.g. Takahashi et al., 1975; Holzhauer, 1978). Untreated infected cell culture fluid, fluorocarbon-treated and ether-treated material are equally suitable as antigen. Human RSV antigen is commercially available. Best specific reactions are obtained with 5% fresh normal calf serum added to the diluent of complement (Wellemans et al., 1970; Takahashi et al., 1975). Neutralizing and complement fixing antibody titers are closely related. The CF test is very reliable for the diagnosis of BRSV infection (Takahashi et al., 1975).

Seroconversion can be detected by AGID. After infection, more than 50% of the animals have immunodiffusion antibodies (Zygraich and Wellemans, 1981). However, this test is only seldom used because of the difficulty in obtaining precipitating antigen and the 3-day delay before the test can be read.

Espinasse et al. (1978) have adapted passive hemagglutination (PHA) to the detection of BRSV antibodies. The preliminary results are encouraging and appear equivalent to the SN scores.

Anti-BRSV antibodies in serum, nasal mucus and organ extracts can be evidenced rapidly and specifically using the indirect IF test (Wellemans, 1977; Potgieter and Aldridge, 1977). The production of a batch of slides covered with antigen-containing cells, which can be stored in a freezer for a long time, makes this test highly reproducible.

After experimental inoculation, the antibodies appear generally from the 7th day on and the titers rise quickly to levels of $\geqslant 1280$ at the end of the second week. Titers exceeding 5000 are frequently found. In other cases, however, a very slow titer evolution is noted, with a maximum score of 135 — although a BRSV infection could be confirmed on postmortem examination of the lungs.

No valid diagnosis can be made without paired sera. However, very high indirect IF or CF antibody levels in single samples are indicative of a BRSV infection in severe respiratory distress. The increase of anti-BRSV antibodies reflects an infection but no conclusions can be drawn about the pathogenic role of the virus. Striking seroconversions have been observed in animals vaccinated 2–3 months before, although no respiratory problems were noticed (Wellemans, 1982). Serological examination is useful but the possibility of atypical seroconversions exists.

PROPHYLAXIS AND CONTROL

BRSV carriers have not yet been proven to exist but it seems possible they will, as was shown for other bovine pathogens, e.g. IBRV (Bitsch, 1973), coronavirus (Van Opdenbosch et al., 1979) and BVDV (Coria and McClurkin, 1978). The fact that the disease becomes enzootic after its introduction in a BRSV-free area supports this hypothesis. Therefore it is advisable to forbid the introduction of diseased animals into a BRSV-free region.

Modification of breeding and fattening conditions (habitat, environment, feeding) has to be taken into consideration, although it is our experience that the most severely affected herds are not always the most badly managed ones.

The vaccination of young children with an inactivated RSV vaccine caused severe hypersensitivity reactions (Kim et al., 1969). In cattle, Mohanty et al. (1981) used a formalin-inactivated and Freund-adjuvanted vaccine. There was no evidence after challenge that vaccinal serum antibodies caused exacerbation of disease in young calves. The vaccine did not induce a nasal antibody response. All but one of the five vaccinated calves appeared to be protected against the disease after challenge exposure. Stott et al. (1984) used a vaccine containing cells persistently infected with BRSV and fixed with glutaraldehyde to retain viral antigens on the cell surface. The glutaraldehyde-fixed cells were combined with incomplete Freund's adjuvant or Saponine Quil-A. After challenge, virus was recovered from all control calves but from only one out of twelve vaccinated calves.

A BRSV strain (Poum 2) at the 94th passage level in BFK cell cultures is presently used in Belgium (Wellemans, 1982) and in the Netherlands (Holzhauer, 1982) for immunization of young cattle. The animals (at least 3 months old) are vaccinated intramuscularly in August–September and boostered 3 weeks later. Some vaccination problems that are not attributable to the vaccine virus strain have been described (Wellemans, 1982). This strain has been shown to be safe in over a million vaccinated animals (Zygraich, 1982) and the results of protection are satisfactory (Holzhauer, 1982; Wellemans, 1982).

REFERENCES

Bartha, A., 1976. Isolation of respiratory syncytial virus from cattle with a respiratory disease. Magy. Allatrv. Lapja, 31: 99–102.

Baskerville, A., 1981. Mechanisms of infection in the respiratory tract. N.Z. Vet. J., 29: 235–238.

Berthiaume, L., Joncas, J., Boulay, G. and Pavilanis, V., 1973. Serological evidence of respiratory syncytial virus infection in sheep. Vet. Rec., 93: 337–338.

Bitsch, V., 1973. Infectious bovine rhinotracheitis virus infection in bulls, with special reference to preputial infection. Appl. Microbiol., 26: 337–343.

Bryson, D.G., McNulty, M.S., Logan, E.F. and Cush, P.F., 1983. Respiratory syncytial virus pneumonia in young calves: clinical and pathologic findings. Am. J. Vet. Res., 44: 1648–1655.

Cash, P., Wunner, W.H. and Pringle, C.R., 1977. A comparison of the polypeptides of human and bovine respiratory syncytial viruses and murine pneumonia virus. Virology, 82: 369–379.

Coria, M.F. and McClurkin, A.W., 1978. Specific immune tolerance in an apparently healthy bull persistently infected with bovine viral diarrhea virus. J. Am. Vet. Med. Assoc., 172: 449–451.

Edwards, S., Newman, R.H. and Stanley, M., 1984. Respiratory syncytial virus diagnosis. Vet. Rec., 114: 101.

Elazhary, M., Galina, M., Roy, R.S., Fontaine, M. and Lamothe, P., 1979. Experimental infection of calves with bovine respiratory syncytial virus (Quebec strain). Can. J. Comp. Med., 44: 390–395.

Elazhary, M., Silim, A. and Roy, R.S., 1981. Interferon, fluorescent antibody and neutralizing antibody responses in sera of calves inoculated with bovine respiratory syncytial virus. Am. J. Vet. Res., 42: 1378–1382.

Espinasse, J., Le Layec, C. and Faye, P., 1978. Hemagglutination passive: application de la méthode au diagnostic sérologique des affections respiratoires virales des jeunes bovins. Rec. Méd. Vét., 154: 227–232.

Field, E.W. and Smith, M.H., 1984. Cell-mediated immune response in cattle to bovine respiratory syncytial virus. Am. J. Vet. Res., 45: 1641–1643.

Gardner, P.S., McQuillin, J. and Court, S.D.M., 1970. Speculation on pathogenesis in death from respiratory syncytial virus infection. Br. Med. J., 1: 327–330.

Hall, C.B., Douglas, R.G., Simons, R.L. and Geiman, J.M., 1978. Interferon production in children with respiratory syncytial, influenza and parainfluenza virus infections. J. Pediat., 93: 28–32.

Holzhauer, C., 1978. Bronchopneumonia of Yearling Cattle. PhD thesis, Utrecht University, Utrecht, The Netherlands, 192 pp.

Holzhauer, C., 1982. Prevention of pinkengriep by vaccination, a field trial. Proceedings XII Congress on Diseases of Cattle, Amsterdam, September 1982, Vol. 1, pp. 166–170.

Holzhauer, C. and Van Nieuwstadt, A., 1976. The etiological role of bovine respiratory syncytial virus in "pinkengriep". Tijdschr. Diergeneesk., 101: 1023–1031 (in Dutch).

Hornsleth, A., Brenoe, E., Friis, B., Knudsen, F.U. and Uldall, P., 1981. Detection of respiratory syncytial virus in nasopharyngeal secretions by inhibition of enzyme-linked immunosorbent assay. J. Clin. Microbiol., 14: 510–515.

Huang, Y. and Wertz, G., 1983. Respiratory syncytial virus mRNA coding assignments. J. Virol., 46: 667–672.

Inaba, Y., Tanaka, Y., Sato, K., Ito, H., Omori, T. and Matumoto, M., 1970. Nomi virus, a virus isolated from an apparently new epizootic respiratory disease of cattle. Jap. J. Microbiol., 14: 246–248.

Inaba, Y., Tanaka, Y., Sato, K., Omori, T. and Matumoto, M., 1972. Bovine respiratory syncytial virus. Studies on an outbreak in Japan, 1968–1969. Jap. J. Microbiol., 16: 373–383.

Ito, Y., Tanaka, Y. and Omari, T., 1973. Structure of bovine respiratory syncytial virus. Arch. Ges. Virusforsch., 40: 198–204.

Jacobs, J.W. and Edington, N., 1975. Experimental infection of calves with respiratory syncytial virus. Res. Vet. Sci., 18: 299–306.

Kim, H.W., Canchola, J.G., Brandt, C.D., Pyles, G., Chanock, R.M., Jensen, K. and Parrot, R.H., 1969. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am. J. Epidemiol., 89: 422–434.

Levine, S., Klaiber-Franco, R. and Paradiso, P., 1987. Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. J. Gen. Virol., 68: 2521–2524.

McIntosh, K., 1978. Interferon in nasal secretions from infants with viral respiratory tract infections. J. Pediat., 93: 33–36.

McIntosh, K. and Fishaut, J.M., 1980. Immunopathologic mechanisms in lower respiratory tract disease of infants due to respiratory syncytial virus. Prog. Med. Virol., 16: 94–118.

McNulty, M.S., Bryson, D.G. and Allan, G.M., 1983. Experimental respiratory syncytial virus pneumonia in young calves: microbiologic and immunofluorescent findings. Am. J. Vet. Res., 44: 1656–1659.

Mahin, L. and Wellemans, G., 1982. Serological evidence for the intervention of bovine respiratory syncytial virus in a respiratory disease outbreak in moroccan cattle. Zentralbl. Veterinärmed. B, 29: 76–79.

Matumoto, M., Inaba, Y., Kurogi, H., Sato, K., Omori, T., Goto, Y. and Hirose, O., 1974. Bovine respiratory syncytial virus: host range in laboratory animals and cell cultures. Arch. Ges. Virusforsch., 44: 280–290.

Mohanty, S.B., 1978. Bovine respiratory viruses. Adv. Vet. Sci. Comp. Med., 22: 83–109.

Mohanty, S.B., Ingling, A.L. and Lillie, M.G., 1975. Experimentally induced respiratory syncytial viral infection in calves. Am. J. Vet. Res., 36: 417–419.

Mohanty, S.B., Rockemann, D.D., Davidson, J.P., Sharabrin, O.I. and Forst, S.M., 1981. Effect of vaccinal serum antibodies on bovine respiratory syncytial viral infection in calves. Am. J. Vet. Res., 42: 881–883.

Mufson, K., Orvell, C., Rafnar, B. and Norrby, E., 1985. Two distinct subtypes of human respiratory syncytial virus. J. Gen. Virol., 66: 2111–2124.

Orvell, C., Norrby, E. and Mufson, M., 1987. Preparation and characterization of monoclonal antibodies directed against five structural components of human respiratory syncytial virus subgroup B. J. Gen. Virol., 68: 3125–3135.

Paccaud, M.F. and Jacquier, C., 1970. A respiratory syncytial virus of bovine origin. Arch. Ges. Virusforsch., 30: 327–342.

Potgieter, L.N.D. and Aldridge, R.L., 1977. Use of indirect fluorescent antibody test in the detection of bovine respiratory syncytial virus antibodies in bovine serum. Am. J. Vet. Res., 38: 1341–1343.

Prince, G.A., Horswood, R.L., Camargo, E., Koenig, D. and Chanock, R.M., 1983. Mechanisms of immunity to respiratory syncytial virus in cotton rats. Infect. Immun., 42: 81–87.

Rosenquist, B.D., 1974. Isolation of respiratory syncytial virus from calves with acute respiratory disease. J. Infect. Dis., 130: 177–182.

Rossi, C.R. and Kiesel, G.K., 1977a. Susceptibility of bovine macrophage and tracheal-ring cultures to bovine viruses. Am. J. Vet. Res., 38: 1705–1708.

Rossi, C.R. and Kiesel, G.K., 1977b. Bovine respiratory syncytial virus infection of bovine embryonic lung cultures: a kinetic study by the fluorescent antibody technique. Am. J. Vet. Res., 38: 1901-1904.

Smith, M.H., Frey, M.L. and Dierks, R.E., 1975. Isolation, characterization and pathogenicity studies of a bovine respiratory syncytial virus. Arch. Virol., 47: 237-247.

Stott, E.J. and Taylor, G., 1985. Respiratory syncytial virus from cattle with a respiratory disease. Arch. Virol., 84: 1-52.

Stott, E.J., Thomas, L.H., Taylor, G., Collins, A.P., Jebbett, J. and Crouch, S., 1984. A comparison of three vaccines against respiratory syncytial virus in calves. J. Hyg. (Camb.), 93: 251-261.

Takahashi, E., Inaba, Y., Kurogi, H., Sato, K., Goto, Y. and Omori, T., 1975. Diagnosis of bovine respiratory syncytial virus infection by complement fixation test. Nat. Inst. Anim. Health Q., 15: 179-185.

Taylor G., Stott, E.J., Hughes, M. and Collins, A.P., 1984. Respiratory syncytial virus infection in mice. Infect. Immun., 43: 649-655.

Thomas, L.H. and Stott, E.J., 1981. Diagnosis of respiratory syncytial virus infection in the bovine respiratory tract by immunofluorescence. Vet. Rec., 108: 432-435.

Thomas, L.H., Stott, E.J., Jebbett, J. and Hamilton, S., 1976. The growth of respiratory syncytial virus in organ cultures of bovine foetal trachea. Arch. Virol., 52: 251-258.

Thomas, A.P., Stott, E.J., Collins, A.P., Crouch, S. and Jebbett, J., 1984. Infection of gnotobiotic calves with a bovine and human isolate of respiratory syncytial virus. Modification of the response by dexamethasone. Arch. Virol., 79: 67-77.

Van Bekkum, J.C., Holzhauer, C., Straver, P.J. and Van Nieuwstadt, A., 1977. Viral infections of the respiratory tract in cattle in the Netherlands. Bull. Off. Int. Epizoot., 88: 69-79.

Van Opdenbosch, E., Wellemans, G., Dekegel, D. and Strobbe, R., 1979. Neonatal calf diarrheoa: complex viral etiology. Vlaams Diergeneesk. Tijdschr., 48: 512-526.

Verhoeff, J. and Van Nieuwstadt, A., 1984. BRS-virus, PI3 virus and BHV 1 infections of young stock on self-contained dairy farms: epidemiological and clinical findings. Vet. Rec., 114: 288-293.

Walsh, E. and Hruska, J., 1983. Monoclonal antibodies to respiratory syncytial virus proteins: identification of the fusion protein. J. Virol., 47: 171-177.

Walsh, E., Schlesinger, J. and Brandiss, M., 1984. Protection from respiratory syncytial virus infection in cotton rats by passive transfer of monoclonal antibodies. Infect. Immun., 43: 756-758.

Walsh, E., Brandiss, M. and Schlesinger, J., 1985. Purification and characterization of the respiratory syncytial virus fusion protein. J. Gen. Virol., 66: 409-415.

Wellemans, G., 1977. Laboratory diagnosis methods for bovine respiratory syncytial virus. Vet. Sci. Commun., 1: 179-189.

Wellemans, G., 1982. Evaluation du programme de vaccination anti-virus respiratoire syncytial bovin en Belgique. Proceedings XII World Congress on Diseases of Cattle, Amsterdam, September 1982, Vol. 1, pp. 146-152.

Wellemans, G., Leunen, J. and Luchsinger, E., 1970. Isolement d'un virus (220/69) sérologiquement semblable au virus respiratoire syncytial (RS) humain. Ann. Méd. Vét., 114: 89-93.

Westenbrink, F., Kiman, T. and Brinkhof, J., 1989. Analysis of the antibody response to bovine respiratory syncytial virus protein in calves. J. Gen. Virol., 70: 591-601.

Zhdanov, V.M., Dreizin, R.S., Yankevici, O.D. and Astakhova, A.K., 1974. Biological characteristics of respiratory syncytial virus. Rev. Roum. Virol., 25: 277-280.

Zygraich, N., 1982. Vaccination against RS virus: a five year experience with Rispoval. Proceedings of XII World Congress on Diseases of Cattle, Amsterdam, September 1982, Vol. 1, pp. 189-195.

Zygraich, N. and Wellemans, G., 1981. Immunological markers of an attenuated bovine respiratory syncytial virus vaccine. Zentralbl. Veterinärmed. B., 28: 355-362.

Chapter 35

Ovine and Caprine Respiratory Syncytial Virus

G. WELLEMANS

A syncytial virus was isolated from a pygmy goat with respiratory tract disease (Smith, 1970). This goat isolate did not produce hemadsorption of guinea pig erythrocytes, contained ribonucleic acid and was sensitive to lipid solvents and heat (56°C) and labile at pH 3.0. SN tests revealed that the virus is antigenically related to bovine and human strains of RSV. The morphogenesis and structure of caprine RSV are compatible with characteristics of the *Pneumovirus* genus of the Paramyxoviridiae family (Lehmkuhl et al., 1980). However, cross-neutralization tests indicate that the caprine isolate is distinct from BRSV (Smith et al., 1979).

In a serological survey (Berthiaume et al., 1973), 81% of sheep from different farms in Canada had complement-fixing antibodies to RSV. This prevalence was confirmed by other authors (Fulton et al., 1982; Elazhary, 1984; Morgan et al.,1985). Signs of illness, lesions and seroconversions were observed in lambs experimentally inoculated with RSV of bovine origin. Signs of illness were mild and consisted of fever and hyperpnea. Multifocal interstitial pneumonia and bronchiolitis were seen in lambs necropsied during the period of clinical response (Cutlip and Lehmkuhl, 1979; Lehmkuhl and Cutlip, 1979). The role of RSV in small ruminants is far from clear (Morgan et al., 1985) and there is no evidence that natural infection results in clinical disease (Berthiaume et al., 1973). Mild lesions caused by RSV infection could predispose the lung to invasion by opportunistic bacteria (Al-Darraji et al., 1982). Seemingly, RSV infection facilitates *Pasteurella haemolytica* pulmonary lesions in lambs and results in a decrease of Fc receptors on alveolar macrophages (Trigo et al., 1984).

REFERENCES

Al-Darraji, A.M., Cutlip, R.C. and Lehmkuhl, H.D., 1982. Experimental infection of lambs with bovine respiratory syncytial virus and *Pasteurella haemolytica*: pathologic studies. Am. J. Vet. Res., 42: 224–229.

Berthiaume, L., Joncas, J., Boulay, C. and Pavilanis, V., 1973. Serological evidence of respiratory syncytial virus infection in sheep. Vet. Rec., 93: 337–338.

Cutlip, R.C. and Lehmkuhl, H.D., 1979. Lesions in lambs experimentally infected with bovine respiratory syncytial virus. Am. J. Vet. Res., 40: 1479–1482.

Elazhary, M.A.S.Y., Silim, A. and Dea, S., 1984. Prevalence of antibodies to bovine respiratory syncytial virus, bovine diarrhea virus, bovine herpes 1 and bovine parainfluenza 3 virus in sheep and goats in Quebec. Am. J. Vet. Res., 45: 1660–1662.

Fulton, R.W., Downing, M.M. and Hagstad, H.V., 1982. Prevalence of bovine Herpes 1, Bovine viral diarrhea, parainfluenza 3, bovine Adenoviruses 3 and 7, and goat respiratory syncytial virus antibodies in goats. Am. J. Vet. Res., 43: 1454–1457.

Lehmkuhl, H.D. and Cutlip, R.C., 1979. Experimental respiratory syncytial virus infection in feeder-age lambs. Am. J. Vet. Res., 40: 1729–1730.

Lehmkuhl, H.D., Smith, M.H. and Cutlip, R.C., 1980. Morphogenesis and structure of caprine respiratory syncytial virus. Arch. Virol., 65: 269–276.

Morgan, K.L., Kyle, R.A.M., Martin, H.T. and Duncan, A.L., 1985. Serum antibodies to respiratory syncytial virus in goats in U.K. Vet. Rec., 116: 239–240.

Smith, M.H., Lehmkuhl, H.D. and Philips, S.M., 1979. Isolation and characterization of a respiratory syncytial virus from goat. Am. Ass. Vet. Lab. Diagn. 22nd Annual Meet., San Diego, CA, pp. 259–268.

Trigo, F.J., Breeze, R.G., Liggit, H.D., Evermann, J.F. and Trigo, E., 1984. Interaction of bovine respiratory syncytial virus and *Pasteurella haemolytica* in the ovine lung. Am. J. Vet. Res., 45: 1671–1678.

Rhabdoviridae

Animal rhabdoviruses (from Greek rhabdos = rod) are oblong particles with one rounded and one planar end, which results in a bullet-shaped virion morphology. Particles are of varying length (130–380 nm) and diameter (between 50 and 95 nm). The membrane carries surface projections 5–10 nm in length and about 3 nm in diameter. In thin sections an axial channel is seen. The nucleocapsid measures about 50 nm in diameter and consists of a helical structure (20 × 700 nm), tightly coiled into a cylinder. This confers a characteristic cross-striation (spacing 4–5 nm) to the particle in electron microscopic preparations. Rhabdoviruses of animals have been assembled in two genera, named *Vesiculovirus* and *Lyssavirus*. When including the probable members, some 30 viruses may belong to this family.

The genome of rhabdoviruses consists of one molecule of noninfectious linear single-stranded RNA of negative polarity; the mol.wt. is between 3.5 and 4.6×10^6. Virion proteins are associated with the membrane (Matrix protein and surface projection Glycoprotein) and the nucleocapsid (Nucleocapsid protein, Non-Structural and Large protein). The L and NS proteins constitute the virion transcriptase.

Virion density is 1.19–1.20 g/cm^3 in CsCl and 1.17–1.19 g/cm^3 in sucrose. Sedimentation values between 550 and 1000 S have been determined.

Viral replication is entirely cytoplasmic. The virion transcriptase copies the viral RNA into several positive stranded mRNA species which are encountered in polysome complexes. Virion RNA replication involves an RNA nucleoprotein intermediate. Morphogenesis is by budding from intracytoplasmic or peripheral membranes.

Vesiculovirus

Some viruses of this genus cause vesicular stomatitis. This disease was formerly known to occur primarily in horses but is now more common in cattle; it also occurs in pigs. It is a disease of the New World and a zoonosis. The vesicular stomatitis virus group is represented by serotypes Indiana and New Jersey. The classical Indiana virus is the type species and the subtype 1. It is antigenically related to subtype 2 (Cocal and Argentina) and subtype 3 (Alagoas from Brazil).

Lyssavirus

In areas of endemic rabies, domestic ruminants may be victims of rabies virus, which has an extraordinarily broad range of susceptible animal host species. The famous Negri bodies are intracytoplasmic acidophilic inclusion bodies seen in the brain cells of animals with rabies. They represent accumulations of viral ribonucleoprotein and are pathognomonic. Direct IF is more reliable and a faster diagnostic test.

Bovine ephemeral fever (BEF) virus is loosely grouped among the "probable members" of the family and occurs in vertebrates and invertebrates.

Chapter 36

Vesicular Stomatitis Virus

R.P. HANSON* and B. McMILLAN
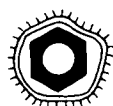
VIRUS PROPERTIES

Rhabdoviruses are enveloped and rapidly destroyed by lipid solvents. They are inactivated in minutes at 50–60°C (Galasso, 1967) and rapidly by low or high pH (Fong and Madin, 1954). The virus can be preserved for years by low temperature storage (– 60°C) and by lyophilization. All common disinfectants, formalin, phenols and quaternary ammonium compounds rapidly inactivate vesicular stomatitis virions.

The virus contains the envelope glycoprotein that is type-specific and the nucleoprotein and matrix protein that are shared to a degree by viruses of the *Vesiculovirus* group (Meyers and Hanson, 1962; Brown et al., 1966; Kelly et al., 1972).

Indiana virus is antigenically diverse (Federer et al., 1967). Cocal virus isolated in Trinidad from mites and rodents (Jonkers et al., 1964; Thormar, 1967) shares both neutralizing and complement fixing antigens with Indiana virus of Cotton (1927). Federer et al. (1967) examined a collection of Indiana virus strains from North and South America and classified them as Indiana type 1 (the original isolate from the USA), Indiana type 2 (Cocal from Trinidad), and Indiana type 3 (Alagoas from Brazil). Cocal and Alagoas viruses isolated from arthropods and wild mammals were initially believed not to attack livestock under natural conditions. However, both are pathogenic for horses and to a lesser extent for cattle. Alagoas has on occasion been recovered from cattle with severe vesicular stomatitis in northeastern Brazil. Thus the antigenic subtype of an isolate is not an indicator of pathogenicity for livestock.

New Jersey virus isolates are antigenically similar irrespective of the host species or the geographic location (Hanson, 1952; Tesh, 1979).

The oligonucleotide fingerprint obtained from Cocal virus is readily distinguishable from that of the Indiana strain (Clewley et al., 1977). RNA fingerprints from the Indiana serotypes are unlike those of the New Jersey serotype. Hybridization studies conducted on several antigenically similar New Jersey isolates RNA has shown that New Jersey isolates can be differentiated into two groups (Reichman et al., 1978) having less than 25% homology. More genetic difference exists between the two antigenically similar New Jersey groups than among the three antigenic subtypes of Indiana virus.

Isolates of both Indiana and New Jersey viruses differ in their physical, biological and genetic properties. Gonzales (1977) studied seven isolates of Indiana type 1 virus and 13 isolates of New Jersey virus that were recovered

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from livestock at different places in Colombia over a 10-year period. Comparison of the growth characteristics in vivo and in vitro showed that the isolates varied in their incubation times, thermostability and ability to generalize. Lauerman (1967), in studies carried out for the purpose of obtaining a suitable vaccine virus for cattle, found that large plaque variants of New Jersey and small plaque variants of Indiana virus were more virulent for laboratory hosts than their respective opposites. Whether this would be true for all isolates of the two viruses remains to be determined. Other investigators have used treatments to selectively eliminate wild-type virus and obtain heat-sensitive mutants (Pringle, 1970; Pringle et al., 1971).

Vesicular stomatitis viruses can be propagated in guinea pigs (Cotton, 1926; Henderson, 1960), mice (Karstad and Hanson, 1958), chicken embryos (Skinner, 1954; Holbrook and Patterson, 1957) and in continuous cell cultures such as Vero and BHK-21. In cell culture the viruses are cytocidal (Sellers, 1955; McClain and Hacket, 1958) and produce clear and turbid plaques (Lauerman, 1967).

Whether a laboratory strain represents the wild-type virus should be questioned. Most investigators who have isolated vesicular stomatitis virus from animals having the natural disease report difficulty in propagating the virus initially in chicken embryos at a temperature of 37°C (Sigurdsson, 1943; Karstad and Hanson, 1958). Incubation at 34–35°C gave more virus isolations and higher titers. Yet after two or three passages in embryos the virus has always been adapted for growth at 37–38°C. Biological properties, particularly the ability to generalize in the host, is lost following adaptation to growth in chicken embryos (G.J. Castañeda, personal communication, 1983).

EPIZOOTIOLOGY

Epizootiologic investigations rely on the ability of the investigator to identify the disease entity by its clinical signs, by isolation of the agent, or the detection of specific antibody. The epizootiology of vesicular stomatitis is made complex by the fact that there are two antigenically distinct viruses, Indiana and New Jersey, which induce vesicular lesions in the mouths and on the feet of cattle, horses, and swine. Furthermore, Indiana virus has three distinguishable serotypes.

One of the Indiana serotypes occurs from the USA through Central America into Brazil. The second serotype (Cocal virus) is limited to South Caribbean countries and the third serotype (Alagoas) is found only in Brazil. The New Jersey virus, which has one serotype, ranges from Canada south to Argentina.

The host range in nature is also dependent on the strain and serotype of the virus. Clinical disease in livestock is regularly induced by New Jersey virus and by Indiana, serotype 1. Indiana serotype 2 sometimes produces disease in horses and serotype 3 does so in cattle. There are differences, probably determined by strain, of host specificity between epizootics. Most commonly horses and cattle are affected without disease appearing in swine, but sometimes only swine are affected. Differences also occur in the nature of the disease produced. In some epizootics there may be primarily foot lesions, in others mouth lesions, teat lesions, or no predilection for an anatomical site at all. Gonzales (1977) has shown that isolates of New Jersey and of Indiana viruses recovered from different epizootics can differ in their ability to induce localized or generalized disease in laboratory mice and lesions in laboratory rabbits and guinea pigs. Virus isolations and demonstration of virus-specific antibody indicates that many wild mammals and man can become infected with vesicular stomatitis viruses.

Antibodies are found in the general population of rural areas where the disease is enzootic. However, the infection is most frequently recognized

among livestock handlers during epizootics or in laboratory personnel. Disease in man has been documented in individuals working in virus laboratories (Hanson et al., 1950; Fellowes et al., 1955; Johnson et al., 1966; Laserna, 1968) or employed as clinicians (Patterson et al., 1958). Serologic studies in Georgia, New Mexico and Panama have revealed that 25–90% of farmers or livestock handlers have antibodies (McCroan, 1956; Hanson and Brandly, 1957; Brody et al., 1967; Fields and Hawkins, 1967). Since the disease in man resembles influenza and is unlikely to be diagnosed, the rural cases may be as clinically apparent as the laboratory infections. The primary source of infection among laboratory workers appears to be aerosol, while contamination of hands, eyes and nose is probably the source for clinicians, farmers and livestock handlers.

With the exception of white-tailed deer, there is no evidence of clinical disease occurring in wild mammals that have been found to have specific antibodies. In the USA the raccoon, skunk and bobcat have become infected during epizootics. In the tropics (Kuns, 1962) Indiana antibodies, in contrast to New Jersey antibodies, are found frequently in arboreal and semiarboreal mammals such as the kinkajou, two- and three-toed sloths, night monkeys and marmosets. Among terrestrial mammals in the tropics, the agouti and the rabbit have antibodies only to New Jersey virus. From the wide range of wild animal species affected one might conclude that disease in livestock is incidental to the perpetuation of the disease, and that transmission is dependent upon some mechanism other than animal-to-animal contact.

The manner in which the virus is transmitted from one animal to another has remained controversial for 70 years. It is clear from experimental studies that virus can be transmitted from an infected to a suceptible cow by contaminating an abrasion in a susceptible site (mucosa of the gum or tongue, skin of the teat orifice or skin bordering the hoof) with saliva or any exudate from a lesion. Efforts to infect susceptible animals through the intact mucosa or skin have been unsuccessful (Sorensen, 1953; Karstad, 1957).

Theiler (1901) explained the transmission of an agent that does not infect through the intact mucosa by suggesting that awns of pasture grasses could produce tiny wounds during mastication that would allow virus entry. There are few alternative ways of preparing an entry site. Internal parasites which penetrate the mucosa of the gastrointestinal tract rarely attempt entry in the susceptible tissues of the buccal cavity. However, increased success in infecting swine fed both ascarids and virus has been reported (Hanson and Karstad, 1956). The biting arthropods that are vectors in the transmission of togaviruses and bunyaviruses probably do not transmit vesicular stomatitis virus, since they do not feed on mucosal surfaces. Insects may introduce vesicular stomatitis viruses either as mechanical or biological vectors into susceptible sites of the skin at the teat orifice or within the narrow band of skin adjacent to the hoof of cattle (Ferris et al., 1955; Mussgay and Suarez, 1962; Bergold et al., 1968). Mouth lesions would be produced only if the virus was able to generalize as a result of viremia. Indiana virus was isolated from an *Aedes* mosquito during an outbreak of vesicular stomatitis in cattle in New Mexico (Sudia et al., 1967). Other investigators have established that Indiana virus can be regularly isolated from *Phlebotomus* flies, that they support viral replication, and that transovarial transmission occurs in these flies (Shelekov and Peralta, 1967; Johnson et al., 1969; Tesh et al., 1972). New Jersey virus was recovered from several flying arthropods in Colorado during the 1982 epizootic (T.C. Walton, unpublished data) but the virus has not been shown to replicate in mosquitoes or phlebotomines.

Vesicular stomatitis of cattle, horses and swine is a disease that recurs annually or at 2- or 3-year intervals in tropical and subtropical countries and at longer intervals in the temperate regions of North and South America.

While Lauerman (1967) observed what appeared to be a centrifugal move-
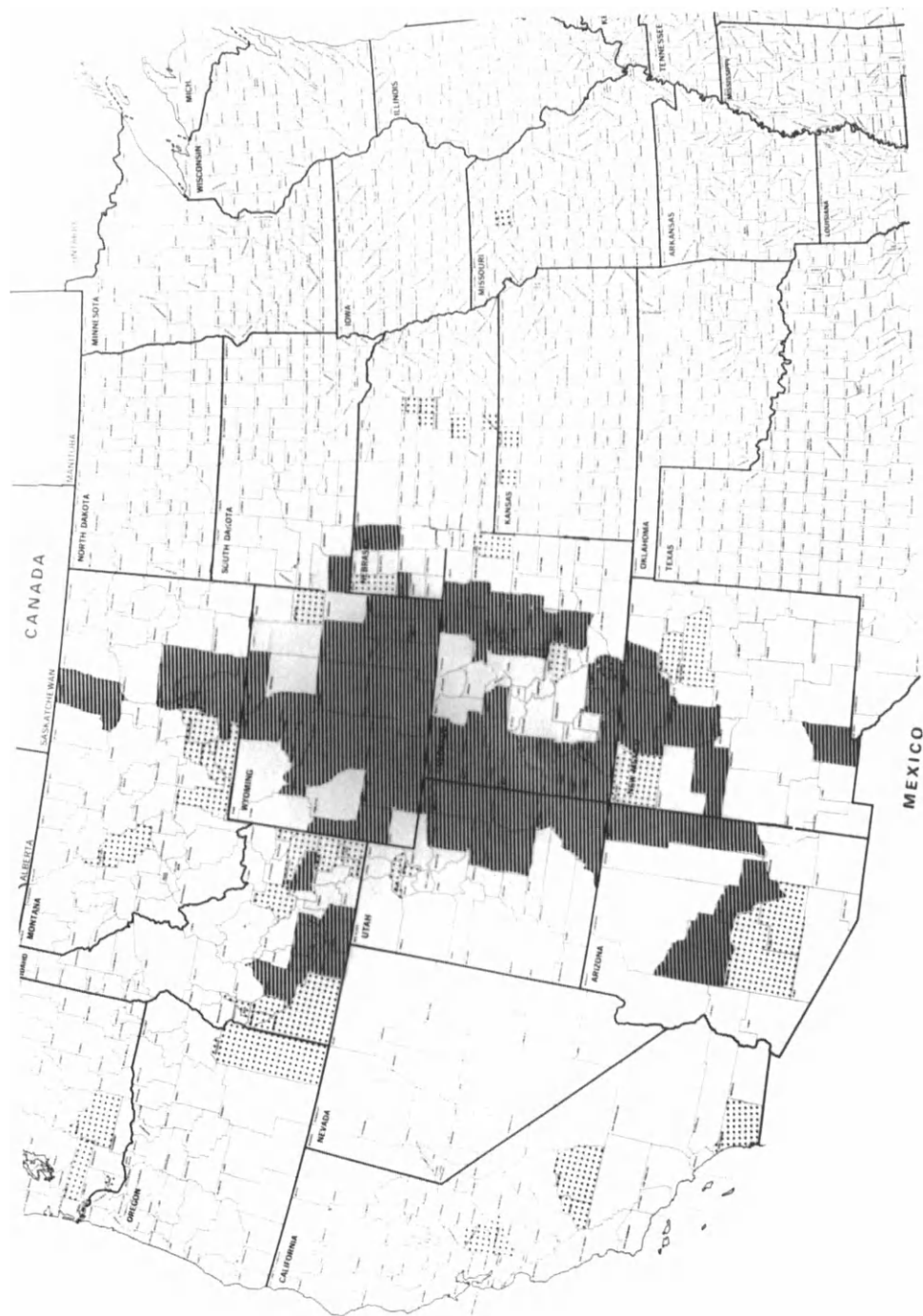
Fig. 139. Epizootic of New Jersey type vesicular stomatitis in the USA, 1982; hatched and dotted areas identify counties in which cases were diagnosed before and after October, respectively. Over 500 premises were found to be infected during the epizootic that lasted from July to February; the epizootic did not extend into Canada but reached south into southern Mexico.

ment from the disease foci in Georgia, most observers have found that the disease does not spread along roads and fails to reach all susceptible herds in a neighborhood as is characteristic of communicable disease. This suggests that the virus is not transmitted directly between livestock.

The recurrence of vesicular stomatitis in one area and the continual absence from another has been observed in both North and South America. In the USA the disease has recurred several times in the southern Appalachians, the upper Mississippi River Valley, the eastern Rocky Mountain states and along the Gulf of Mexico. In Wisconsin seven of ten northwestern counties had the disease in 1937 and again in 1949. Sixty southern and eastern Wisconsin counties, including those with the largest cattle populations, did not become infected during either outbreak (Brandly et al., 1951).

Vesicular stomatitis virus has the seasonal prevalence of an arthropod-borne virus. It appears late in the warm season in the temperate zones and usually disappears with the onset of hard frost. In the tropics it appears at the end of the rainy season and disappears when the countryside becomes arid (Kuns, 1962; Lauerman, 1967). However, the period of virus activity is probably longer than the reported cases indicate (Karstad et al., 1956). During the 1982–83 outbreak in the USA infections continued for 4 months after the onset of freezing weather (Fig. 139). This outbreak tended to affect animals at elevations less than 2200 m and seemed to follow rivers and river valleys. The population of black gnats and deer flies was high during the late summer and early fall of 1982. No explanation was found for the continuation of this outbreak into the winter months beyond the period of arthropod activity.

While vesicular stomatitis is endemic in the tropical and subtropical areas of the Americas (Jonkers et al., 1964; Kuns, 1964), disease in livestock is seasonal. If active transmission continues year round, it must involve hosts other than livestock, or the virus must persist in a latent condition in some host. Antibodies to Indiana virus are common among forest mammals in Panama (Tesh et al., 1969). In the areas of Georgia where vesicular stomatitis virus is endemic, antibodies can be found in several forest mammals and particularly feral swine as well as in domestic horses, cattle, and swine (Karstad et al., 1956; Hanson and Karstad, 1958). Whether the infection of wildlife occurs between the periods of active transmission in livestock is not known. In coastal Georgia clinical disease is seldom seen except in swine or imported cattle. There is little evidence of vesicular stomatitis virus persistence in the USA outside the coastal plain (Hanson, 1952). The virus appears to be transported into these temperate regions from the warm endemic refugia, which in some cases may be more than 1000 miles away.

Within its extensive range vesicular stomatitis is restricted to favorable habitats that occupy only a fraction of the entire land mass (Hanson, 1952). In the upper Mississippi river valley it appears in aspen parklands, a narrow ecotone separating hardwood–coniferous forests to the northeast and open prairies to the southwest. In the mountainous regions of the USA the disease moves up and down valleys but rarely infects animals kept on higher pastures. In tropical areas it spreads at irregular intervals into high valleys and upper slopes but recurs with considerable regularity on the lowland savannas (Kuns, 1962; Hanson et al., 1968).

In both tropical and temperate regions, shade-producing trees, high humidity and natural surface water are common to pastures in which cattle become infected (Kuns, 1962; Jonkers, 1967). The disease does not invade treeless prairies (Hanson, 1952). Others have reported that cattle in pastures of dry, short grass are not affected, while animals in nearby pastures which have more moisture and taller grasses become infected. On every occasion in which the disease appeared in livestock in the USA, it has failed to reach major cattle

populations that were fully susceptible, while invading herds in areas in which it had been observed years before. Outside of these regions, disease has sometimes occurred in horses and cattle that were being shipped (Mohler, 1918; Hanson, 1952; APHIS Newsletter, 1982). These are circumstances in which man appears to ensure transmission through abrasion-inducing fomites.

In connection with the still unknown reservoirs for the virus, it should be mentioned that vesicular stomatitis virus was found to multiply efficiently in the leafhopper (*Peregrinus maidis*), the vector of maize mosaic virus, a plant rhabdovirus (Lastra and Esparza, 1976).

PATHOGENESIS

Although the Indiana and New Jersey serotypes of vesicular stomatitis virus are distinct, the clinical signs of the infections are indistinguishable. Vesicles appear on the epithelial tissues of the mouths or teats of infected animals. The natural portals of entry are abrasions of the mucosal epithelium in the mouth, on the teats or the coronary band above the hoof. The incubation stage of the disease is usually less than 24 h but in some cases may be as long as 48 h. Vesicles appear, presumably at the site of infection, and enlarge, sometimes coalescing to form large vesicles. The severity of the lesion is probably dependent upon the strain of virus and environmental factors that influence the resistance of the infected animal. In general the vesicular stomatitis viruses cause high morbidity but low mortality. The morbidity is evident mainly as decreased productivity in dairy herds but can also take the form of slowed weight gain in beef and swine operations. Asymptomatic infections occur in the field (Brandly et al., 1951) and have been reproduced experimentally (Seibold and Sharp, 1960).

Persistently high antibody titers in experimentally infected mice have been reported. These antibodies are apparently associated with virus replication (Sorensen, 1953) and are perhaps attributable to an immune response directed against host antigens incorporated in the viral envelope (Hecht and Paul, 1982). However, these antibodies do protect the animals from reinfection with vesicular stomatitis virus.

DISEASE SIGNS

Prior to eruption of the vesicles, the animals may appear lethargic or weak and consume less feed and water, but more often infected animals do not present any signs of illness.

The primary clinical signs, cessation of eating and salivation, appear with the rupture of the oral vesicles. Any attempt to examine the lips or tongue markedly increases the flow of saliva. The animal gives the appearance of wanting to eat, making motions as if starting to eat, but stopping short of taking food in the mouth. If there are lesions around the hoof, the animals are disinclined to move and limp.

Increase in the body temperatures may occur in cattle 8–10 h before the vesicles erupt but dissipates rapidly thereafter. Calves may exhibit a biphasic temperature rise. The timing of the initial phase coincides with the temperature rise in adult animals. The second phase is observed in calves which develop vesicles in the mouth and on the tongue. It is detectable at the end of the erosive period and may be more related to the low level of fluid intake than to an effect of the virus.

While the vesicular or the erosive stages are not diagnostic they are easily

recognizable in the field. With proper laboratory support the veterinarian can rapidly diagnose vesicular stomatitis virus infections.

PATHOLOGY

Ribelin (1958) and Chow et al. (1951) describe the classical pathology of vesicular stomatitis virus infections. In the cases studied, infection occurred through an abrasion or virus was injected intradermally. Virus spread through the intracellular spaces to adjacent cells. The macular stage was rarely evident and the papules were often not well defined. Vesicles appeared in 24–48 h and were readily ruptured, particularly when they coalesced with adjoining vesicles. The fluid of the vesicle contained few polymorphonuclear leukocytes. Most of the leukocytes were caught as part of the infiltrates within the tissue at the base of the vesicles. The erosive stage followed the vesicular stage, lasted a week or longer, and this is the stage that is more often seen by clinicians than the transitory earlier stages.

The first sign of disease at the microscopic level is a loosening of the prickle cells of the Malphighian layer; this progresses until the intracellular bridges are stretched and rupture by fluid accumulation. The vacuoles coalesce to form the vesicle; the epithelial cells disarranged by the intracellular edema undergo degenerative changes. Edema and hemorrhage in the dermal papulae, engorgement of the lymph vessels and blood vessels, and perivascular leukocytic infiltration are usually observed. Sweat glands and sebaceous glands are not affected, although there is sometimes hemorrhaging around the hair shaft. With the exception of the liver, where congestion is sometimes apparent, significant lesions are not seen in other tissues. The eroded epithelium is open to secondary infections. Though this may occur, repair is usually rapid and well underway within a week of the time vesicles are observed.

Gross vesiculation is not always the consequence of experimental infection in cattle. Seibold and Sharp (1960) found that most of the vesicles become dehydrated in situ by seepage of the intracellular edema through the stratum corneum, and the epithelium eroded in the manner of a dry necrotic, mucosal lesion. They concluded that depending on the virus strain and the host reaction, either intracellular edema or necrosis could be favored, and only when the proper combination of these two processes occurred was gross vesiculation produced. While clinical mastitis is reported in the field it is not easy to reproduce experimentally (Easterday et al., 1954).

The course of disease in swine (Chow and McNutt, 1953; Patterson et al., 1956), horse (Lauerman, 1967) and deer (Karstad and Hanson, 1957) is similar to that described for cattle.
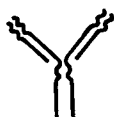
IMMUNE REACTION

Neutralizing and CF antibodies are detectable in bovine serum samples 10–14 days p.i. The titers increase until the fourth or fifth week and then persist at high levels for several months before gradually declining (Sorensen, 1953). The CF antibodies disappear before the neutralizing antibodies, which may persist for years. Recovered cattle are refractory to reexposure within a month and for at least a year. The degree of this immunity can be determined by inoculation of serial dilutions of the virus into separate sites in the mucosa of the tongue (Castañeda et al., 1982). On the basis of information from enzootic areas in Georgia and Panama it appears that natural reinfections resulting in disease are rare. Neutralizing antibodies have been detected in cattle that were

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not reexposed to the virus for up to 7 years after infection (Sorensen, 1953). Their persistence and the duration of immunity requires further study.

LABORATORY DIAGNOSIS

Clinical specimens for virus isolation should contain either vesicular fluid or tissue scrapings from the diseased area (Karstad and Hanson, 1958). Paired sera, one of which was drawn during the clinical stage of the disease and the other 10–14 days later, can be used to establish the presence and the identity of the disease.

Demonstration of antigen in fluids or scrapings can be done using the tissue CF test. This technique is rapid, sensitive and can be completed within a day of receipt of the specimen. Virus isolation can be accomplished in mice (Karstad and Hanson, 1958), the chick embryo (Skinner, 1954) or BHK-21 cell cultures. The virus kills mice inoculated intracerebrally in 4–5 days, kills 10-day-old embryos inoculated intra-allantoically in 2 days and produces plaques or CPE in cell cultures (BHK-21 or Vero) in 2–4 days.

Fluorescent antibody, serum CF and SN tests may be used to confirm vesicular stomatitis virus isolation. However, these tests may be negative during the 10–14 days between appearance of lesions and development of detectable antibody. Newer methods such as the ELISA with monoclonal antibody (LeFrancois and Lyles, 1982) can also be used to detect vesicular stomatitis virus antigens or antibodies.

PROPHYLAXIS AND CONTROL

Treatment of vesicular stomatitis virus infections of cattle consists of good nursing care and rest. When the disease is present on neighboring farms, it is good practice to remove animals from woodlot-pastures and confine them to barns or dry feedlots.

Vaccines for vesicular stomatitis virus are used primarily in dairy cattle. Live embryo-attenuated vaccine was tested in Georgia (Lauerman, 1967), Panama (Lauerman and Hanson, 1963) and Peru (Strozzi and Ramos-Saco, 1953) and protected cattle from the New Jersey type for periods of at least one year. Venezuela has been using an attenuated vaccine on a large scale for years and has observed a significant reduction in field cases of the disease (Castañeda et al., 1982). This vaccine was prepared by multiple passages of the virus through embryonated chicken eggs or cell cultures. The vaccine induced protective antibody when introduced intramuscularly in cattle and did not cause clinical signs or result in virus shedding. When the same vaccine was used in swine, vesicular lesions were occasionally evident and the virus was shed. Less information is available on inactivated vaccines (Holbrook and Geleta, 1957).

There are no specific treatments for vesicular stomatitis. An abundance of water and soft feed should be kept before the animals to avoid excessive weight loss during the febrile period and to reduce injury to the mucosal tissues. Although secondary infections may occur in the abraded epithelial tissues, they are infrequent and are easily treated with broad-spectrum antibiotics. However, mastitis has been reported to be less responsive to treatment and can be a serious problem. W.R. McCallon (personal communication) found that losses in dairy herds for this reason in southeastern USA frequently exceeded $10 000.

REFERENCES

Bergold, G.H., Suarez, O.M. and Munz, K., 1968. Multiplication in and transmission by *Aedes aegypti* of vesicular stomatitis virus. J. Invert. Pathol., 11: 406–428.

Brandly, C.A., Hanson, R.P. and Chow, T.L., 1951. Vesicular stomatitis with particular reference to 1949 Wisconsin epizootic. Proc. 88th Annu. Meet. Am. Vet. Med. Assoc., pp. 61–69.

Brody, J.A., Fischer, G.F. and Peralta, P.H., 1967. Vesicular stomatitis virus in Panama. Human serologic patterns in a cattle-raising area. Am. J. Epidemiol., 86: 158–161.

Brown, N.F., Cartwright, B. and Almeida, J.D., 1966. The antigens of vesicular stomatitis virus. I. Separation and immunogenicity of three complement-fixing components. J. Immunol., 96: 537–545.

Castañeda, G.J., Bernal, L.C., deRodriguez, J.R., Espinoza, M., deChaurell, A. and deBlanco, G.C., 1982. Development and use of vaccines against vesicular stomatitis. In: Primera Conferencia International Sobre el Impacto de las Enfermidades Virales en el Disarrollo de Los Paises Latinoamericanos y la Region del Caribe. Rio de Janeiro, Brazil, March, 1982.

Chow, T.L. and McNutt, S.H., 1953. The pathology of vesicular stomatitis of swine. Am. J. Vet. Res., 14: 420–424.

Chow, T.L., Hanson, R.P. and McNutt, S.H., 1951. The pathology of vesicular stomatitis in cattle. Proc. Am. Vet. Med. Assoc., pp. 119–124.

Clewley, J.P., Bishop, D.H.L., Kang, C., Coffin, J., Schnitzlein, W.M., Reichman, M.E. and Shope, R.E., 1977. Oligonucleotide fingerprints of RNA species obtained from rhabdoviruses belonging to the vesicular stomatitis virus subgroup. J. Virol., 23: 152–156.

Cotton, W.E., 1926. The causal agent of vesicular stomatitis proved to be a filter-passing virus. J. Am. Vet. Med. Assoc., 70: 168–181.

Cotton, W.E., 1927. Vesicular stomatitis. Vet. Med., 22: 169–175.

Easterday, B.C., Hanson, R.P. and Simon, J., 1954. Experimental viral bovine mastitis. Am. J. Vet. Rev., 20: 819–824.

Federer, K.E., Burrows, R. and Brooksby, J.B., 1967. Vesicular stomatitis virus—the relationship between some strains of the Indiana serotype. Res. Vet. Sci., 8: 103–117.

Fellowes, O.N., Dimopoullos, G.T. and Gallis, J.J., 1955. Isolation of vesicular stomatitis virus from an infected laboratory worker. Am. J. Vet. Res., 16: 523–626.

Ferris, D., Hanson, R.P., Dicke, R.J. and Roberts, R.H., 1955. Experimental transmissions of vesicular stomatitis by Diptera. J. Infect. Dis., 96: 184–192.

Fields, B.N. and Hawkins, K., 1967. Human infection with the virus of vesicular stomatitis during an epizootic. N. Eng. J. Med., 277: 989–994.

Fong, J. and Madin, S.H., 1954. Stability of vesicular stomatitis virus at varying H ion concentration. Proc. Soc. Exp. Biol. Med., 86: 676–678.

Galasso, G.J., 1967. Quantitative studies on the quality, effects of aggregation at thermal inactivation of vesicular stomatitis virus. Proc. Soc. Exp. Biol. Med., 124: 43.

Gonzalez, G., 1977. Studies of certain biological characteristics of Colombian strains of vesicular stomatitis. PhD Thesis, University of Wisconsin-Madison, 178 pp.

Hanson, R.P., 1952. The natural history of vesicular stomatitis virus. Bacteriol. Rev., 16: 179–204.

Hanson, R.P. and Brandly, C.A., 1957. Epizootiology of vesicular stomatitis. Am. J. Public Health, 47: 205–209.

Hanson, R.P. and Karstad, L., 1956. Enzootic vesicular stomatitis. Proc. U.S. Livest. Sanit. Assoc., 60: 288–292.

Hanson, R.P. and Karstad, L., 1958. Further studies on enzootic vesicular stomatitis. Proc. U.S. Livest. Sanit. Assoc., 61: 300–307.

Hanson, R.P., Rasmussen, A.R., Brandly, C.A. and Brown, J.W., 1950. Human infection with the virus of vesicular stomatitis. J. Lab. Clin. Med., 36: 754–758.

Hanson, R.P., Estupinan, J. and Castañeda, G.J., 1968. Vesicular stomatitis in the Americas. In: Proc. Pan American Health Organization Conference on Vesicular Diseases and Zoononses, Washington, DC, March 1968.

Hecht, T.T. and Paul, W.E., 1982. Limitation of VSV infection by the hosts' response to VSV-associated cellular antigens. J. Immunol., 129: 1736–1741.

Henderson, W., 1960. Foot and mouth disease and related vesicular diseases. Veterinarian, 26: 726–730.

Holbrook, A.A. and Geleta, J.N., 1957. Vesicular stomatitis infection with inactivated vaccines of chicken embryo origin. Proc. U.S. Livest. Sanit. Assoc., 66: 359–364.

Holbrook, A.A. and Patterson, W.E., 1957. The use of chicken in the differential diagnosis of vesicular exanthema and vesicular stomatitis. J. Am. Vet. Med. Assoc., 131: 196.

Johnson, K.M., Vogel, J.E. and Peralta, P.H., 1966. Clinical and serological response to laboratory-acquired human infection by Indiana type vesicular stomatitis virus (VSV). Am. J. Trop. Med. Hyg., 15: 244.

Johnson, K.M., Tesh, R.B. and Peralta, P.H., 1969. Epidemiology of vesicular stomatitis virus: some

new data and a hypothesis for transmission of the Indiana serotype. J. Am. Vet. Med. Assoc., 155: 2133–2140.

Jonkers, A.H., 1967. The epizootiology of the vesicular stomatitis viruses: a reappraisal. Am. J. Epidemiol., 86: 286.

Jonkers, A.H., Shope, R.E., Aitken, T.H.G. and Spence, L., 1964. Cocal virus, a new agent in Trinidad related to vesicular stomatitiis virus, type Indiana. Am. J. Vet. Res., 25: 236–242.

Karstad, L.H., 1957. Enzootic vesicular stomatitis. MSc Thesis, University of Wisconsin-Madison, 84 pp.

Karstad, L. and Hanson, R.P., 1957. Vesicular stomatitis in deer. Am. J. Vet. Res., 18: 162–166.

Karstad, L.H. and Hanson, R.P., 1958. Primary isolation and comparative titrations of five field strains of vesicular stomatitis virus in chicken embryos, hogs and mice. Am. J. Vet. Res., 19: 233–236.

Karstad, L.H., Adams, E.V., Hanson, R.P. and Ferris, D.H., 1956. Evidence for the role of wildlife in epizootics of vesicular stomatitis. J. Am. Vet. Med. Assoc., 129: 95–96.

Kelley, J.M., Emerson, S.U. and Wagner, R.R., 1972. The glycoprotein of vesicular stomatitis virus is the antigen that gives rise to and reacts with neutralizing antibodies. J. Virol., 10: 1231–1235.

Kuns, M.L., 1962. The epizootiology of vesicular stomatitis in middle America. PhD Thesis, University of Wisconsin-Madison, 193 pp.

Kuns, M.L., 1964. Estudios ecologicos de vesiculo-estomatitis en la Republica de Panama. Congreso Medical Veterinarias Centro American Panama, 1', Panama 1964, Cuadernos, 2: 2.

Laserna, Z.B., 1968. Infeccion con virus estomatitis vesicular en Personal de laboratorio. Vet. Colomb. (Bogota), 2: 209–213.

Lastra, J.R. and Esparza, J., 1967. Multiplication of vesicular stomatitis virus in the leafhopper *Peregrinus maidis* (Ashm.), a vector of a plant rhabdovirus. J. Gen. Virol., 32: 139–142.

Lauerman, L.H., 1967. Vesicular stomatitis in temperate and tropical America. PhD Thesis, University of Wisconsin-Madison, 193 pp.

Lauerman, L.H. and Hanson, R.P., 1963. Field trial vaccination against vesicular stomatitis in Panama. Proc. U.S. Livest. Sanit. Assoc., 67: 483–490

LeFrancois, L. and Lyles, D.S., 1982. The interaction of antibody with the major surface glycoproteins of vesicular stomatitis virus. I. Analysis of neutralizing epitopes with monoclonal antibodies. Virology, 121: 157–167.

McClain, M.E. and Hackett, A.J., 1958. A comparative study of the growth of vesicular stomatitis virus in the tissue culture systems. J. Immunol., 80: 356–361.

McCroan, J.E., 1956. Vesicular stomatitis infection in man. U.S. Dept. Health, Education and Welfare. Morbidity and Mortality, 5:1.

Meyers, W. and Hanson, R.P., 1962. Studies on the antigenic relationships within and between serotypes of vesicular stomatitis by immunodiffusion. Am. J. Vet. Res., 23: 896–899.

Mohler, J.R., 1918. Vesicular stomatitis of horse and cattle. U.S. Dept. Agric., Bulletin 662.

Mussgay, M. and Suarez, O., 1962. Multiplication of vesicular stomatitis virus in *Aedes aegypti* (L.) mosquitoes. Virology, 17: 202–203.

Patterson, W.C., 1956. Experimental infections with vesicular stomatitis in swine. Proc. 59th Annu. Meet. U.S. Livestock Sanit. Assoc., p. 368.

Patterson, W.C., Mott, L.O. and Jenney, E.W., 1958. A study of vesicular stomatitis in man. J. Am. Vet. Med. Assoc., 133: 57–66.

Pringle, C.R., 1970. Genetic characteristics of conditional lethal mutants of vesicular stomatitis virus induced by 5-fluorouracil, 5-azacytidine and ethyl methyl suphonate. J. Virol., 5: 559–567.

Pringle, C.R., Duncan, I.B. and Stevenson, M., 1971. Isolation and characterization of temperature sensitive mutants of vesicular stomatitis virus, New Jersy serotype. J. Virol., 8: 834–841.

Reichman, M.E., Schnitzlein, W.M., Bishop, D.H.L., Lazzerini, R.A., Beatrice, S.T. and Wagner, R.R., 1978. Classification of the New Jersey serotype of vesicular stomatitis into two subtypes. J. Virol., 25: 446–449.

Ribelin, W.E., 1958. The cytopathogenesis of vesicular stomatitis infection in cattle. Am. J. Vet. Res., 19: 66–73.

Seibold, H.R. and Sharp, J.B., 1960. A revised concept of the pathologic changes of the tongue in cattle with vesicular stomatitis. Am. J. Vet. Res., 21: 35–51.

Sellers, R.F., 1955. Growth and titration of the viruses of foot-and-mouth disease and vesicular stomatitis in kidney monolayer tissue culture. Nature, 176: 547–549.

Shelokov, A.I. and Peralta, P.H., 1967. Vesicular stomatitis virus, Indiana type: an arbovirus infection of tropical sandflies and humans? Am. J. Epidemiol., 56(1): 149–157.

Sigurdsson, B., 1943. The influence of host and temperature incubation of infection of the chick embryo with vesicular stomatitis virus. J. Exp. Med., 78: 17–24.

Skinner, H.H., 1954. Infection of chickens and chick embryos with the viruses of foot and mouth disease and of vesicular stomatitis. Nature (London), 174: 1052.

Sorensen, D.K., 1953. Virus infectivity, transmission and immunity studies of vesicular stomatitis in cattle and sheep. PhD Thesis, University of Wisconsin-Madison, 78 pp.

Strozzi, P. and Ramos-Saco, T., 1953. Teat vesicles as primary and almost exclusive lesions in an extensive outbreak of vesicular stomatitis (N.J. strain) in milking cows. J. Am. Vet. Med. Assoc., 123: 415–418.

Sudia, W.D., Fields, B.N. and Calisher, C.H., 1967. The isolation of vesicular stomatitis virus (Indiana strains) and other viruses from mosquitoes in New Mixico, 1965. Am. J. Epidemiol., 86: 598–602.

Tesh, R.B., 1979. Antigenic variations among strains of the New Jersy serotype of vesicular stomatitis virus. Am. J. Vet. Res., 40: 1156–1158.

Tesh, R.B., Peralta, P.H. and Johnson, K.M., 1969. Ecological studies of vesicular stomatitis virus. I. Prevalence of infection among animals and humans living in an area of endemic vesicular stomatitis virus activity. Am. J. Epidemiol., 90: 255–261.

Tesh, R.B., Chaniotis, B.N. and Johnson, K.M., 1972. Vesicular stomatitis virus (Indiana serotype) transovarial transmission by phlebotomine sandflies. Science, 175: 1477–1479.

Theiler, S., 1901. Eine contagiöse Stomatitis des Pferdes in Süd-Afrika. Dtsch. Tierärztl. Wochenschr., 9: 131–132.

Thormar, H., 1967. A comparison of cocal and vesicular stomatitis virus, serotypes New Jersey and Indiana. Virology, 31: 323–327.

Chapter 37

Rabies Virus

G.M. BAER
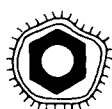
VIRUS PROPERTIES

Rabies is caused by a rhabdovirus, a bullet-shaped RNA virus (Murphy, 1975), with a diameter of about 80 nm and a length of 180–200 nm. Its surface is covered with 6–7-nm spikes which aid viral binding to cells. The gonome consists of linear, single-stranded negative-sense RNA of about 12 000 nucleotides. The noninfectious RNA and attached proteins form a helical nucleocapsid. The enveloped virions bud from cytoplasmic membranes, both at the cell surface and into the endoplasmic reticulum (Fig. 140). The virus genome codes for five structural proteins: L, N and NS (= M_1) are associated with the nucleocapsid, M (= M_2) and G with the envelope. The L protein most likely functions as transcriptase (Kawai, 1977). The G protein forms surface spikes and mediates adsorption and penetration of the virus into cells. It is the only protein that induces neutralizing antibodies (Cox et al., 1977) and is also the rabies virus hemagglutinin. Probably due to these functions it also bears a pathogenicity marker detectable by monoclonal antibody (Dietzschold et al., 1983).

Like most other enveloped viruses, rabies virus is quite fragile. It is readily inactivated by organic solvents and detergents, and by media having pH values below 4 or above 10. It is also sensitive to UV light and heat. Temperatures above 55°C destroy the virus within minutes. The half-life at physiological temperatures ranges from a few hours to several days. The detrimental influence of temperature and other physical agents is greatly modified by the stabilizing effect of polypeptides and other compounds (Michalski et al., 1976).

Rabies virus strains isolated from naturally infected hosts are usually called "street virus". The term "fixed virus" is used for certain strains maintained over many passages by intracerebral inoculation of laboratory rodents and rabbits. "Fixed viruses" are highly virulent even after peripheral inoculation. The attenuated viruses LEP (low egg passage), HEP (high egg passage), and Street Alabama Dufferin (SAD) immunize after peripheral inoculation of most species, but LEP and SAD kill when given intracerebrally. Polyclonal antisera from immunized animals do not readily differentiate between rabies virus strains in ordinary cross-neutralization tests. Only monoclonal antibodies clearly distinguish between strains with different passage histories (Wiktor and Koprowski, 1978: Flamand et al., 1980), isolates of different geographic origin (Sureau et al., 1983), and between rabies viruses circulating in different host populations (Smith et al., 1984).

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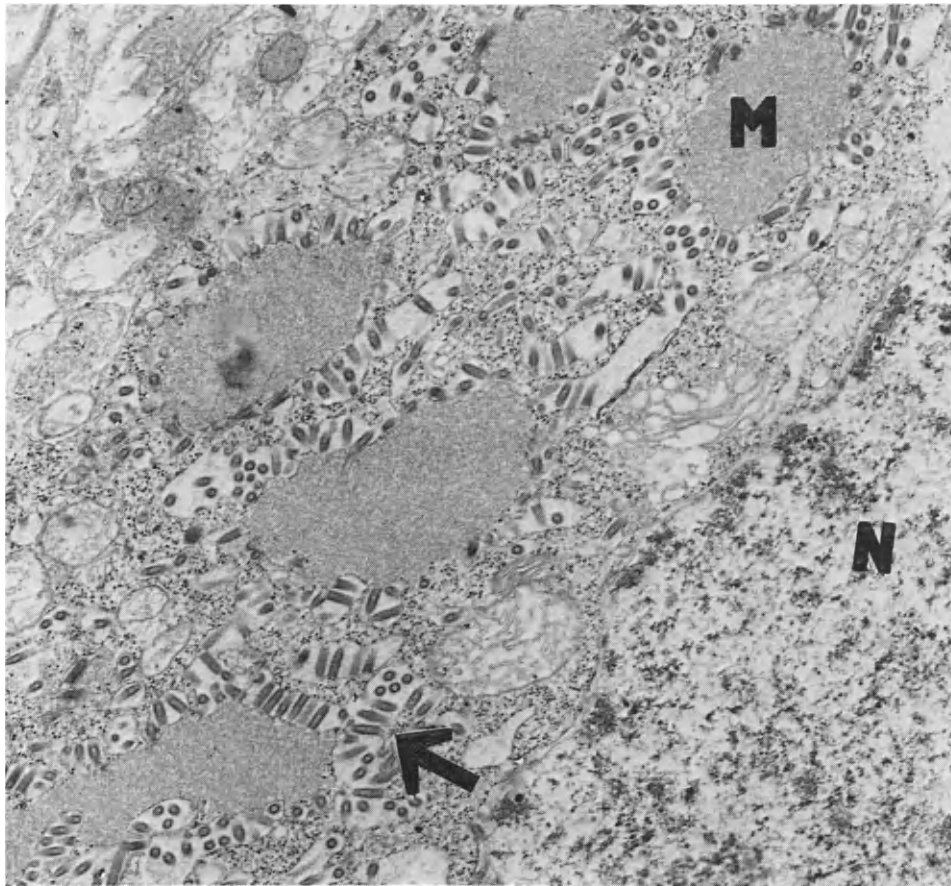
Fig. 140. Neurons of a mouse inoculated with an Ethiopian dog rabies isolate; cell packed with randomly spaced viral matrices (M) and virus particles (arrow) budding from the endoplasmic reticulum membranes; N = nucleus, × 21 000 (courtesy of Dr. Makonnen Fekadu, Centers for Disease Control, Atlanta, GA).

EPIZOOTIOLOGY

Rabies occurs on all continents except Australia and the Antarctic. Only a few countries are free from the disease (for instance, Great Britain, The Netherlands, Japan, Jamaica). One can readily distinguish between areas where dogs are the main hosts and areas where rabies is maintained by cycles in terrestrial wild animals or, in the case of Latin America, vampire bats also. In areas where wildlife rabies is common, such as Europe, Canada and the USA, cattle are the domestic species most commonly found rabid. In countries with endemic dog rabies, cattle are also commonly infected, although reporting is often deficient. Most cattle that die of rabies worldwide have been infected by vampire bats. Bovine vampire rabies is limited to Latin America, since vampires are found there only. The disease caused by such bats is dramatic, with an estimated half a million to a million cattle dying of rabies annually (Acha, 1967) at a cost of hundreds of millions of dollars (Table 23).

Vampire paralytic rabies was reported as early as in the 16th century, when Spanish conquistadores told of many Spanish soldiers dying after bat bites, and of cattle epizootics attributed to bat bites (Licenciado Palacio del Rey D. Felipe, 1576; Molina Solis, 1943; Baer, 1975). The epidemiology of vampire paralytic rabies has been amply studied (Hurst and Pawan, 1931, 1932; De

TABLE 23

Bovine paralytic rabies in the Americas[a]

Country	Number of cases per year	Estimated annual mortality	Number of cattle vaccinated per year	Annual loss (US $ equivalent)
Argentina	18 000 (1964)	50 000	100 000 (1965)	10 000 000 (1964)
Brazil	32 200 (1965)	200 000	1 300 000 (1965)	22 000 000 (1965–66)
Bolivia	20 000 (1965)	50 000	5 000 (1965)	1 500 000 (1965)
British Honduras	815 (1962)	2 000	200 (1962)	100 000 (1961)
Costa Rica	132 (1964)	10 000	18 000 (1963)	365 000 (1962)
Colombia	5 300 (1964)	50 000	150 000 (1963)	1 260 000 (1964)
Ecuador	930 (1962)	5 000	4 500 (1962)	850 000 (1963)
El Salvador	1 080 (1961)	3 000	7 000 (1964)	108 000 (1961)
French Guiana	600 (1958)	1 000	—	60 000 (1958)
Guatemala	1 120 (1964)	12 000	8 000 (1964)	168 000 (1964)
Guyana	2 000 (1957)	3 000	30 000 (1963)	43 000 (1959)
Honduras	348 (1960)	6 000	5 000 (1963)	87 000 (1960)
Mexico	1 502 (1963)	90 000	1 000 000 (1963)	10 400 000 (1964)
Nicaragua	831 (1962)	10 000	8 000 (1964)	200 000 (1962)
Panama	218 (1962)	8 000	5 000 (1963)	115 000 (1962)
Paraguay	320 (1963)	5 000	2 000 (1964)	94 000 (1963)
Surinam	733 (1963)	2 000	5 013 (1963)	55 000 (1963)
Trinidad	2 (1965)	500	24 047 (1963)	5 000 (1961)
Uruguay	83 (1965)	2 000	—	63 000 (1965)
Venezuela	215 (1965)	5 000	53 032 (1963)	119 000 (1960)
TOTAL	86 439	514 500	2 724 792	$47 592 000

[a]From Acha (1967).
—No information available.

Verteuil and Urich, 1936; Prieto and Baer, 1972). The attack rates per herd in the few outbreaks studied varied between 1% and 70% (Table 24). Good reporting is hampered by the lack of supporting laboratory diagnoses. In Argentina, for instance, the ratio of estimated bovine deaths to each laboratory-confirmed case is 25, in spite of the extensive diagnostic laboratory network (Lopez Adaros et al., 1969). In the State of Oaxaca, Mexico, Mancisidor (1965) found

TABLE 24

Individual and mean herd mortality in epizootics of bovine paralytic rabies[a]

Mean herd mortality (%)[b]	Herd mortality range (%)[c]	Area and country
1.0	Ns	Valencia Village, Trinidad
3.0	Ns	Chaco, Paraguay
6.6	1.5–50	Tuxtepec, Oaxaca, Mexico
9.0	Ns	Santa Cruz Valley and San Juan, Trinidad
15.6	2–53.3	Magdalena and La Guajíra, Colombia
20.0	Ns	Michoacan, Mexico
9.6	5–17.6	Oxapampa, Peru
33.0	Ns	Vicente, Oaxaca, Mexico
70.0	Ns	25 Leguas, Paraguay

[a]Adapted from Baer (1975).
[b]Approximate cattle mortality (total, not by herds).
[c]Ns, not stated.

TABLE 25

Rabies in dogs and cattle in the USA, 1981–1985

	1981	1982	1983	1984	1985
Dog	216	153	132	97	94
Cattle	465	296	204	154	213

that less than 1% of the 9000 cattle dying of paralytic rabies were confirmed by laboratory examination. In another outbreak in the same area only one cow was officially reported rabid of a total of 571 dying over a 15-month period (Prieto and Baer, 1972). Yet even that percentage is high compared to that in some outbreaks; the most severe and extensive outbreak of bovine paralytic rabies ever reported began in Southern Bolivia in 1953 and was only published as a mimeograph report (Serrudo, 1968). Over 250 000 cattle died of the disease in the Cercado de Tarija Valley and near the Yungas between 1954 and 1958. The disease then spread to the northern Argentine provinces of Jujuy, Salta, Misiones, and Santiago del Estero between 1959 and 1968 (Lopez Adaros et al., 1969), leaving entire zones without any cattle.

In comparison to these studies on outbreaks of vampire paralytic rabies, almost no epidemiological studies have been carried out on cattle rabies in other parts of the world, since cases there are sporadic and caused by dogs (or terrestrial wildlife such as foxes or skunks). This is true even though over 40% of all domestic animal rabies in the USA between 1980 and 1982 was in cattle (Centers for Disease Control, 1985) and the number of rabid cattle reported in the last 5 years exceeded the number of rabid dogs (Table 25).

Similarly, European data from 1983 show that cattle accounted for one-third of domestic animal rabies vs. 12% in dogs and 29% in cats (Schneider, 1983).

In both these areas wild animals such as skunks and foxes are the main transmitters of the disease to cattle (Figs. 141–144). Usually few animals per herd are affected (Table 26). In areas with endemic canine rabies, almost nothing is known of the epidemiology of bovine rabies; it is thought, however, that the problem is a sizable one. Surprisingly, bovine rabies is rarely reported in countries with endemic canine rabies, i.e. most of Asia and Africa; since the reporting of canine cases is emphasized, cases in other species are largely overlooked. In Nigeria, for instance, Okoh (1981) states that "it is highly probable that rabies in cattle and other farm livestock is widespread but

TABLE 26

Incidence of bovine rabies cases per reported attack(s)[a] by rabid animals in New York State (exclusive of New York City), January 1943–June 1955

	Number of cases per herd										Totals
	1	2	3	4	5	6	7	8	9	10	
No. herds with cases reported	1190	283	82	46	18	17	9	5	3	6	1659
% Herds in which attacks reported	71.7	17.1	4.9	2.8	1.1	1.0	0.5	0.3	0.2	0.4	100

[a] All onsets in a herd within a 2-month period after reported attack.
Source: WHO Working Document (Dean, 1956).

TABLE 27

Mortality of cattle experimentally injected with virus of vampire bat origin

Amount of virus (MICLD$_{50}$)	No. deaths/No. injected
20 000 000	3/3
4 000 000	3/3
800 000	1/3

Adapted from Sureau et al. (1971).

underreported and only when deaths are unusual and take the form of an epidemic or occur in government herds are veterinary authorities notified".

Cattle appear to be quite resistant to experimental inoculation with rabies strains from vampire bats (Table 27) but are relatively susceptible to the injection of other strains (Abelseth, 1966; Artois et al., 1984). The apparent variability in susceptibility may be explained rather by the limited studies than by any real difference in strain invasiveness. One unexplained difference, however, is the susceptibility of cattle to infection by vampire bites but their resistance to the injection of virus from vampire bats. The presence of antibodies in cows during and after an outbreak of vampire rabies (Lord et al., 1975) suggests that cattle have an intermediate resistance and are very unlike the highly susceptible fox, where almost no animals survive with antibodies (Sikes, 1962).

PATHOGENESIS

The incubation period of experimentally infected cattle varies from 20 to 150 days (this excludes one period of 611 days in an animal injected with virus from a vampire bat (Abelseth, 1975)). The average period observed in cattle bitten by vampire bats was 60–75 days (Carneiro, 1936), while that after skunk or fox bites was 35–45 days. This may be due to the amount of virus introduced and the site of the bite, since pastured cattle are often bitten on the nose after they have approached paralyzed rabid skunks, while vampire bat bites are mainly on the neck, ears, fetlocks and vulva (Baer, 1975).

Cattle excrete virus in their saliva when ill. In naturally infected animals the majority (44 out of 70) had virus in the saliva or salivary glands (Rueff et al., 1978). In bovine rabies experiments virus excretion has also been readily demonstrated (Martell et al., 1974; Pepin et al., 1984). In one of these studies (Pepin et al., 1984) 13 out of 15 of the animals had virus in the submaxillary salivary glands, but only six of these excreted virus in the saliva.

The distribution of virus in the rabid bovine has not been extensively studied. In the salivary glands the virus titer appears to vary with the strain of infecting virus. In cases where the virus was from vampire bats, the highest titers were in the parotid glands (Martell et al., 1974), whereas in animals infected with rabies virus from foxes (Rueff et al., 1978), the submaxillary glands were mostly involved. Virus distribution in other organs appears to be limited. In the brain the levels in the cerebellum are high (at least in cows dying with bovine paralytic rabies) and virus titers are higher in the midbrain and forebrain (Martell et al., 1969). Rabies virus has never been isolated from meat, although one would expect low levels there, since virus spreads centrifugally along nerves late in infection; such virus would be inactivated by cooking before it is consumed. Virus in the milk of rabid cows would be expected to be at low levels or absent, since there are few direct glandular nerve connections to the milk source; moreover, virus in milk would be highly diluted.
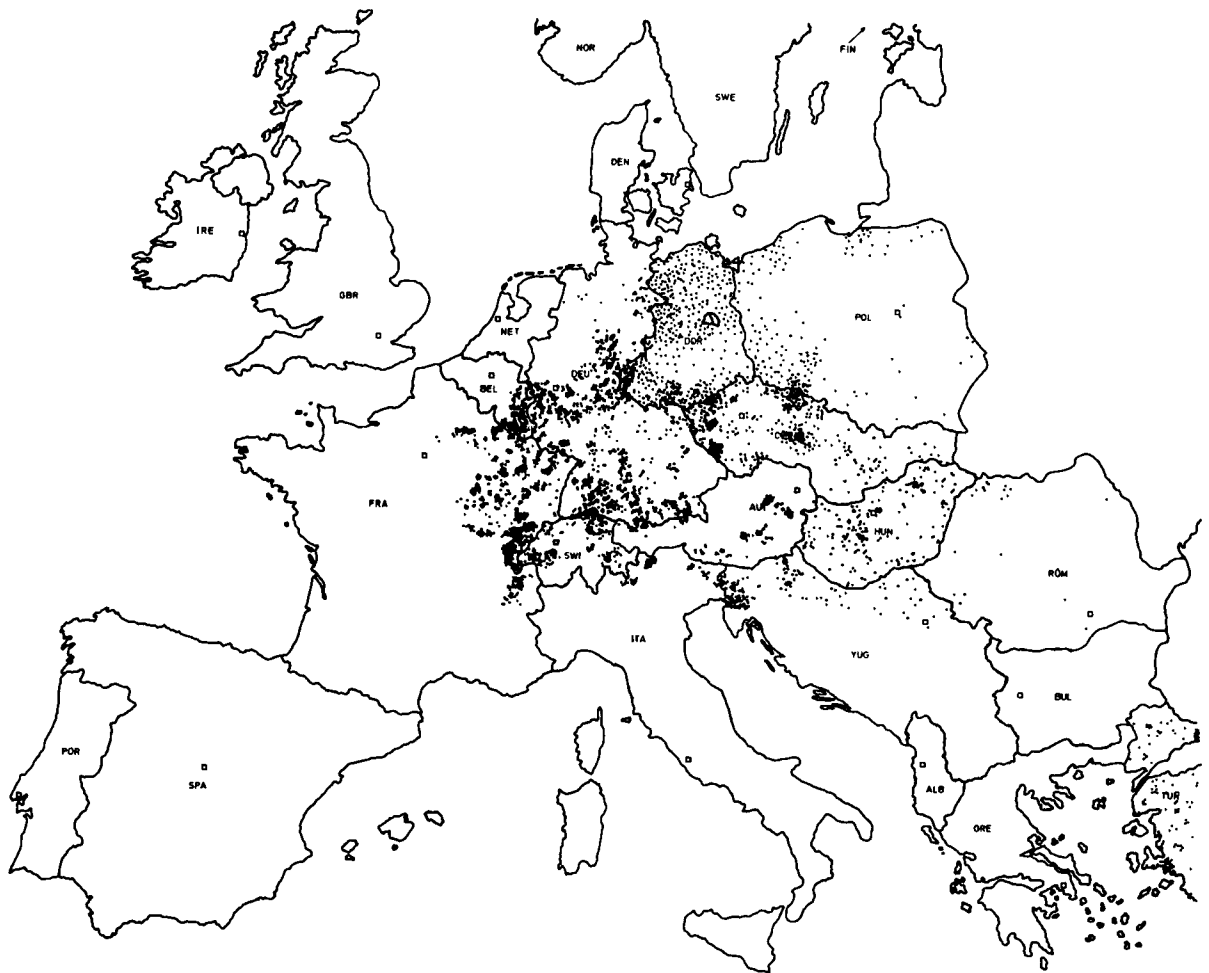
Fig. 141. Fox rabies cases in Europe, third quarter 1982; total of 4838 cases reported.

Fig. 142. Counties in the USA reporting fox rabies, 1982; 25 positive states, 84 positive counties, total of 222 cases reported; ●, 1–5 cases; X, 6–10 cases; ■, > 10 cases (symbols are plotted at the population center of each county).
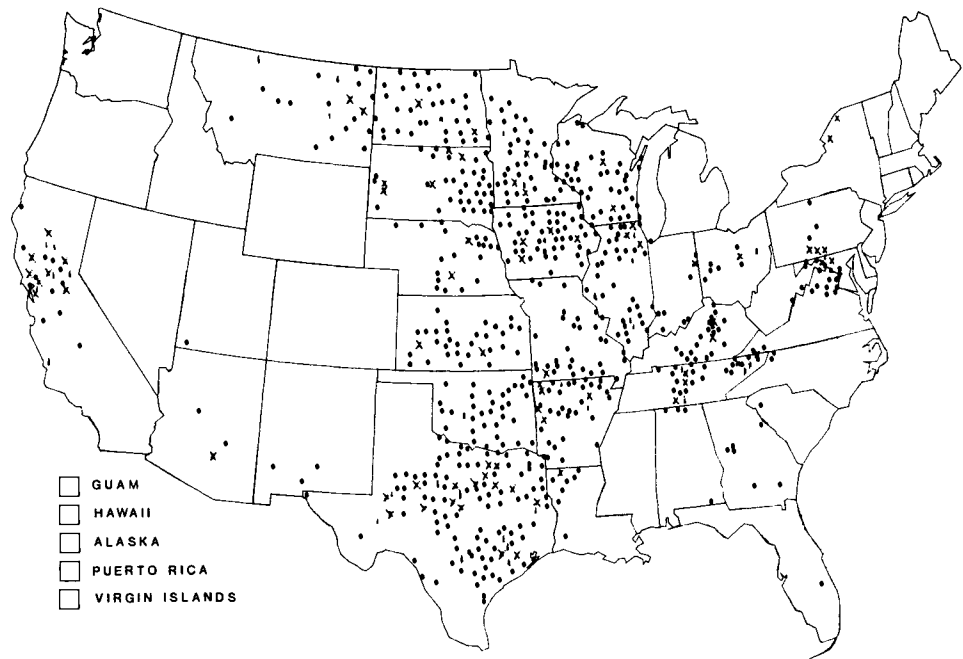
Fig. 143. Counties in the USA reporting skunk rabies, 1983; 32 positive states, 687 positive counties, total of 2388 cases reported; ●, 1–5 cases; X, 6–10 cases; I, > 10 cases (symbols are plotted at the population center of each county).
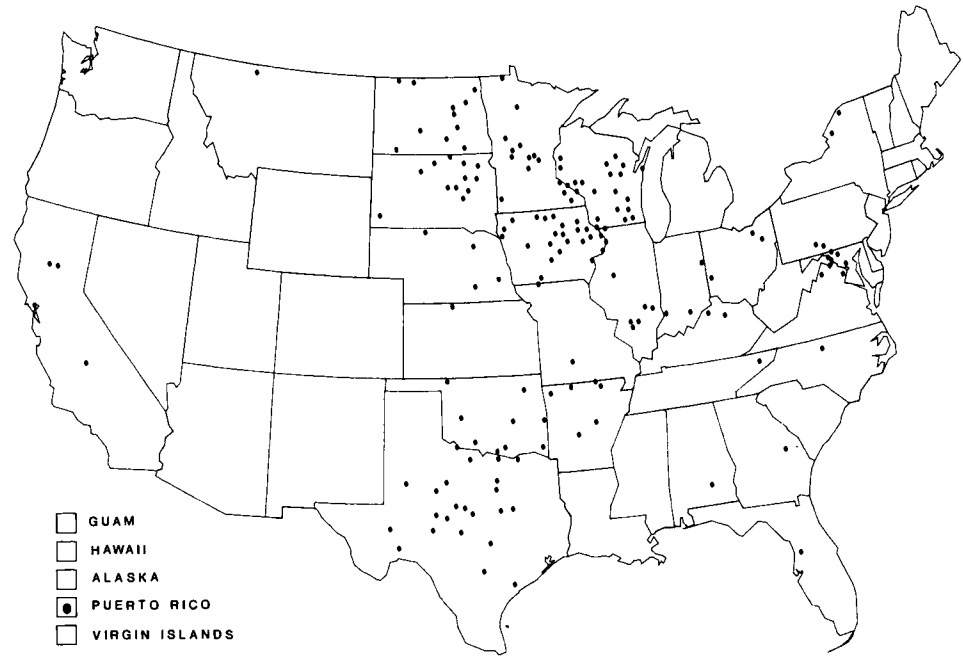
Fig. 144. Counties in the USA reporting cattle rabies, 1983: 28 positive states, 165 positive counties, total of 205 cases reported: ●, 1–5 cases (symbols are plotted at the population center of each county).

DISEASE SIGNS

The signs of rabies in cattle infected by foxes, dogs or skunks include a short premonitory period, usually hours to a few days, in which diagnosis is very difficult, with nonspecific signs predominating, such as mild temperature rises, malaise, anorexia, and an abrupt cessation of lactation (Starr, 1956). In one review (Barnard, 1979) the most obvious signs noted were salivation (92%), bellowing (69%), aggressiveness (47%), paresis or paralysis (30%) and straining (12%). The affected animals may attack and butt others in the herd, or remain by themselves. Attacks at moving objects are more common than other aggressive behavior. Most human exposures occur during the early period, when the veterinarian (or owner) suspects choke or another digestive disorder, and searches for some object in the throat of the affected animal.

The excited (furious) phase follows the prodromal period, when a definite change of behavior occurs that includes irritability and increased nervousness, often with tenesmus and bellowing. One review describes the signs as follows:

"... Cattle assume a "stupid" expression with dropping ears and a wild stare, intently following all movement in their vicinity. Frequently they show persistent tenesmus, a sign easily misinterpreted as being indicative of gastrointestinal disturbance. There may be rapid transition to an aggressive state in which they lunge at other cattle and moving objects. Often they emit a continuous and characteristic hoarse bellow. They frequently exhibit progressive ataxia associated particularly with weakness of the hindquarters and are ultimately overcome by paralysis, coma and death. Cattle may exhibit marked sexual excitability... " (Swanepoel and Foggin, 1978).
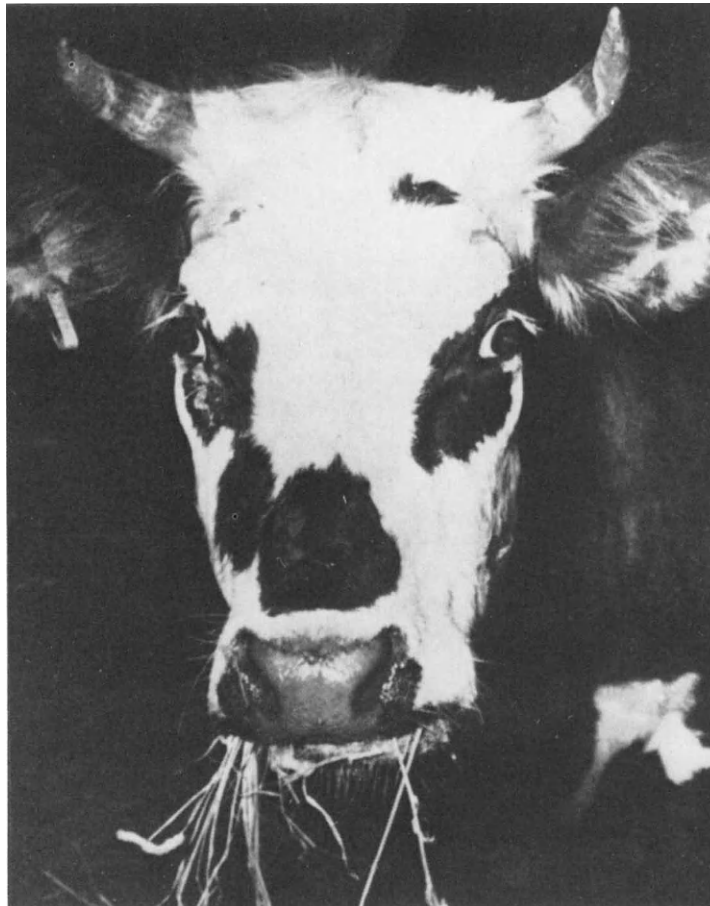
Fig. 145. A rabid cow.

Starr (1956) wrote that "a typical sign is observed in pastured animals: the patient is usually alone and may be lying in a natural position. If disturbed quietly, it will get up quickly and trot off, lifting its feet with a mincing gait. After going a short distance it may stop, turn its head, stand like a statue and stare at its disturbers. It will get away if possible, but is dangerous if cornered or . . . . confined". The cow attempts to eat and drink but cannot swallow, and hay or grass may be held in the mouth for long periods of time. The animal becomes unusually alert with the head up and the eyes wide open (Fig. 145). As mentioned, bellowing may increase during this period and tenesmus appears, both continuing up to death. The bellow is very characteristic; high, long, and somewhat shrill. Saliva may drool from the mouth. Self-mutilation is rare but dramatic when observed. Rabid cattle may show signs of pain, including sudden movements and attacks in which they drop suddenly and roll over on their side or sternum, with the head back and over the shoulder in a U-shaped curve, as is seen in milk fever. Sexual excitement is very common, with the affected animals attempting to repeatedly mount other animals in the herd. This excitement stage is usually short, and is followed by paralysis, dehydration, prostration, and death, thought to be caused by respiratory paralysis.

The signs of disease in cattle infected by vampire bats are quite different, with paralysis predominating (Haupt and Rehaag, 1921). The premonitory signs are virtually pathognomonic, with the animal becoming slightly uncoordinated in the rear quarters, a sign that gave rise to the names of the disease "derriengue" (limping illness) or "mal de cederas" (hip ill). It is at this early point that ranchers rush their cattle to market. Paralysis ensues, and the animal falls repeatedly and then tries to rise. Once paralysis becomes extensive the animal often pulls the head over and back towards the shoulder, as described above, and finally ends in opisthotonos with an extensive paddling motion of the front legs.

PATHOLOGY

Gross postmortem pathology in domestic animals other than dogs gives no indication for rabies. Histopathology of the brain shows lesions of encephalitis with neuronal degeneration in the midbrain and medulla. Cellular infiltration is mostly slight. Negri bodies in the neurons of the hippocampus and cerebellum are pathognomonic. The presence of viral antigen can be confirmed by IF.

LABORATORY DIAGNOSIS

The preferred diagnostic method is the fluorescent antibody technique (Goldwasser and Kissling, 1958; Dean and Abelseth,1973) developed in the late 1950s; before then rabies was diagnosed by direct staining of brain impressions (especially with Sellers' stain) to detect Negri bodies, but these inclusions were only seen in 70–80% of infected animals (Kissling, 1975). Subsequent mouse inoculation permitted the separation of the remaining 20–30% of positive cases from the negative ones, but this procedure took 7–20 days. This changed when the fluorescent antibody technique was introduced (McQueen, 1959), a method virtually 100% effective in diagnosing the disease and performed in hours.

PROPHYLAXIS AND CONTROL

Many inactivated and attenuated rabies vaccines have been developed for cattle. Those most commonly used worldwide are the ERA strain (an att-

TABLE 28

Inactivated rabies vaccine produced on tissue culture, for use in cattle

Product name	Produced by	Marketed by	Dosage	Age at primary vaccination	Booster recommended
RABGUARD-TC	Norden	Norden	1 ml	3 months	Annually
IMRAB	Merieux	Pitman-Moore	2 ml	3 months	Annually

Source: CDC, 1986.

enuated vaccine) (Abelseth, 1964), HEP Flury vaccine (Koprowski and Cox, 1948; Carneiro et al., 1955), and inactivated suckling mouse brain vaccine (Fuenzalida et al., 1969). Attenuated products must be properly handled in the field to assure immunity, whereas inactivated products are more stable under adverse conditions. This may explain many of the vaccine failures that have occurred with the attenuated vaccines (Palacios and Dumith Arteaga, 1964). Only inactivated tissue culture vaccines are currently licensed for use in cattle in the USA and some European countries (Table 28; Petermann et al., 1967; Centers for Disease Control, 1986), although other vaccines have been used in the past.

Few studies have been made on the post exposure efficacy of rabies vaccines, but those published have indicated that vaccine is of *no value* if used *after* the bite (Moegle et al., 1976).

There is no surveillance of human rabies vaccination per se, and only a general idea can be drawn of the number of people vaccinated for exposure to rabid cows. In one study, 8% of all persons vaccinated for rabies in part of the USA were treated for exposure to rabid cows (Helmick, 1983). Most cattle in Europe and North America develop rabies after exposure to wild animals, and the bovine cases occur where the cases in wild animals are concentrated. Veterinarians and others who treat sick cows should always consider rabies in the differential diagnosis of CNS disorders in cattle and seek medical assistance if they are exposed. Preexposure rabies vaccination is available for veterinarians (Centers for Disease Control, 1984).

Human deaths after exposure to rabid cattle are very rare (since cattle do not bite in the manner dogs do); 13 cases have been recorded (Baer, 1980). The most recent death involved a Swiss veterinarian who examined a rabid cow in 1977 (Grani et al., 1978).

REFERENCES

Abelseth, M.K., 1964. An attenuated rabies vaccine for domestic animals produced in tissue culture. Can. Vet. J., 5: 279–286.

Abelseth, M.K., 1966. The growth of rabies virus in tissue culture and its use as a living attenuated vaccine for domestic animals. Thesis, University of Minnesota, Minneapolis, MN, 120 pp.

Abelseth, M.K., 1975. Bovine vaccines—past and present. In: G.M. Baer (Editor), The Natural History of Rabies. Academic Press, New York, Vol. II, pp. 203–219.

Acha, P.N., 1967. Epidemiology of paralytic bovine rabies and bat rabies. Bull. Off. Int. Epizoot., 67: 343–382.

Artois, M., Aubert, M.F.A., Blancou, J. and Pericard, M., 1984. Rage experimentale du chat sensibilité—symptomes—excrétion du virus. Rev. Méd. Vét., 135: 281–287.

Baer, G.M., 1975. Bovine paralytic rabies and rabies in the vampire bat. In: G.M. Baer (Editor), The Natural History of Rabies. Academic Press, New York, Vol. II, pp. 155–175.

Baer, G.M., 1980. Human Rabies after Exposure to Rabid Cattle. Rabies Information Exchange, Number 3, December 1980. Centers for Disease Control, Lawrenceville Fac., Atlanta, GA, pp. 33–34.

Barnard, B.J.H., 1979. Symptoms of rabies in pets and domestic animals in South Africa and Southwest Africa. J. S. Afr. Vet. Assoc., 50: 109–111.

Carneiro, V., 1936. Rabies epizootics in the Americas: the role of hematophagous bats Arch. Inst. Biol., 7: 273–322 (in Portugese).

Carneiro, V., Black, J. and Koprowski, H., 1955. Rabies in cattle. V. Immunization of cattle in Brazil against exposure to street virus of vampire bat origin. J. Am. Vet. Assoc., 127: 366–369.

Centers for Disease Control, 1984. Rabies Prevention—United States. Recommendation of the Immunization Practices Advisory Committee (ACIP). Morb. Mortal. Wkly. Rep., 33: 397–416.

Centers for Disease Control, 1985. Rabies Surveillance, Annual Summary 1984. Centers for Disease Control/Public Health Service/US Dept of Health and Human Services, Atlanta, GA.

Centers for Disease Control, 1986. Compendium of Animal Rabies Vaccines, 1986 (Prepared by The National Association of State Public Health Veterinarians, Inc.). Morb. Mortal. Wkly. Rep., 34: 770–774; 779–781.

Cox, J.H., Dietzschold, B. and Schneider, L.G., 1977. Rabies virus glycoprotein. II. Biological and serological characterization. Infect. Immun., 16: 743–759.

Dietzschold, B., Wunner, W.H., Wiktor, T.J., Lopez, A.D., Lafon, M., Smith, C. and Koprowski, H., 1983. Characterization of an antigenic determinant of the glycoprotein which correlates with pathogenicity of rabies virus. Proc. Natl. Acad. Sci. U.S.A., 80: 70–74.

Dean, D.J., 1956. The epidemiology of bovine rabies in New York State. Expert Committee on Rabies, WHO Working Document (WHO/Rabies/56), World Health Organization, Geneva.

Dean, D.J. and Abelseth, M.K., 1973. The fluorescent antibody test. In: M.M. Kaplan and H. Koprowski (Editors), Laboratory Techniques in Rabies. World Health Organization, Geneva, pp. 73–84.

De Verteuil, E. and Urich, F.W., 1936. The study and control of paralytic rabies transmitted by bats in Trinidad. Trans. R. Soc. Trop. Med. Hyg., 29: 317–354.
in Trindad. Trans. R. Soc. Trop. Med. Hyg., 29: 317–354.

Flamand, A., Wiktor, T.J. and Koprowski, H., 1980. Use of hybridoma monoclonal antibodies in the detection of antigenic differences between rabies and rabies-related virus proteins. I. The nucleocapsid protein. II. The glycoprotein. J. Gen. Virol., 48: 97–109.

Fuenzalida, E., Acha, P.N., Atanasiu, P., Larghi, O. and Szyfres, B., 1969. Rabies vaccination of cattle. Proc. 73rd Annu. Meet. U.S. Anim. Health Assoc., pp. 307–322.

Goldwasser, R.A. and Kissling, R.E., 1958. Fluorescent antibody staining of street and fixed rabies virus antigen. Proc. Soc. Exp. Biol. Med., 98: 219–223.

Grani, R., Wandeler, A., Steck, F. and Rosli, R., 1978. Tollwut bei einem Tierarzt. Schweiz. Med. Wochenschr., 108: 593–597.

Haupt, H. and Rehaag, H., 1921. Durch Fledermäuse verbreitete seuchenhafte Tollwut unter Viehbeständen in Santa Catharina (Süd-Brasilien). Z. Infektionskr. Hyg. Haustiere, 22: 104–127.

Helmick, C.G., 1983. The epidemiology of human rabies postexposure pyophylaxis. J. Am. Med. Assoc., 250: 1990–1996.

Hurst, E.W. and Pawan, J.L., 1931. An outbreak of rabies in Trinidad without history of bites and with the symptoms of acute ascending myelites. Lancet, II: 622–628.

Hurst, E.W. and Pawan, J.L., 1932. A further account of the Trinidad outbreak of acute rabies myelitis: History of the experimental disease. J. Pathol. Bacteriol., 35: 301–321.

Kawai, A., 1977. Transcriptase activity associated with rabies virion. J. Virol., 24: 826–835.

Kissling, R.E., 1975. The fluorescent antibody test in rabies. In: G. M. Baer (Editor), The Natural History of Rabies. Academic Press, New York, Vol. I, pp. 401–416.

Koprowski, H. and Cox, H.R., 1948. Studies on chick embryo adapted rabies virus. I. Cultural characteristics and pathogenicity. J. Immunol., 60: 533–554.

Licenciado Palacio del Rey D. Felipe, II., 1576. Cited in: Constantine, D.G., 1970. Bats and their relation to health. In: W.A. Wimsatt (Editor), Biology of Bats. Academic Press, New York, Vol. 2.

Lopez Adaros, H., Silva, M. and La Mata, M., 1969. Seminario Sore Rabia para Los Paises de la Zona IV. Pan American Health Organization, Buenos Aires, Argentina, pp. 161–181.

Lord, R., Delpietro, H., Fuenzalida, E., Oviedo de Diaz, A. M. and Lazaro, L., 1975. Presence of rabies neutralizing antibodies in wild carnivores following an outbreak of bovine rabies. J. Wildl. Dis., 11: 210–213.

Mancisidor Ahuja, A., 1965. El uso de una vacuna autogena en el control de un brote de derriengue en Mexico. Tec. Pec. (Mexico), 5: 27–29.

Martell, M.A., Batalla, D. and Baer, G.M., 1969. Estudio de immunofluorescencia de diferentes segmentos de encefalos de bovinos muertos de rabia paralitica o derriengue en forma natural e inoculados experimentalmente. Tec. Pec. (Mex.), 12–13: 24–27.

Martell, M.A., Batalla, D., Baer, G.M. and Benignos, J., 1974. Experimental bovine paralytic rabies "Derriengue". Vet. Rec., 527–530.

McQueen, J.L., 1959. Rabies diagnosis special application of fluorescent antibody techniques. Proc. 63rd Annu. Meet. U.S. Livest. Sanit. Assoc., pp. 356–363.

Michalski, F., Parks, N.F., Sokal, F. and Clark, H.F., 1976. Thermal inactivation of rabies and rhabdoviruses: stabilization by the chelating agent ethylenediaminetetra-acetic acid at physiological temperatures. Infect. Immun., 14: 135–143.

Moegle, H., Schneider, L.G., Ritter, H. and Muller, B., 1976. Zur postinfektionellen Immunisierung Tollwut-exponierter Rinder. Ein Impfversuch an vier Kuhen. Berl. Munchn. Tierärztl. Wochenschr., 89: 360–362.

Molina Solis, J.F., 1943. Historia del descubrimiento y conquista del Yucatan, 3: 38.

Murphy, F.A., 1975. Morphology and morphogenesis. In: G.M. Baer (Editor), The Natural History of Rabies. Academic Press, New York, Vol. 1, pp. 33–61.

Okoh, A.E.J., 1981. Rabies in farm livestock in Nigeria. Int. J. Zoonoses, 8: 51–56.

Palacios, G.C. and Dumith Arteaga, G., 1964. Proc. Annu. Meet. on Rabies, Venezuela, December 1964, pp. 1–12.

Pepin, M., Blancou, J. and Aubert, M.F.A., 1984. Rage expérimentale des bovins. Sensibilité-symptomes-reactions immunitaires-lésions-excrétion du virus. Ann. Rech. Vét., 42: 211–217.

Petermann, H.G., Lang, R., Branche, R., Soulebot, J. P. and Mackowiak, C., 1967. Realization d'un nouveau vaccin rabique a partir de culture cellulaire. C. R. Acad. Sci. (Paris), 265: 2143–2144.

Prieto, J.F. and Baer, G.M., 1972. An outbreak of bovine paralytic rabies in Tuxtepec, Oaxaca, Mexico. Am. J. Trop. Med. Hyg., 21: 219–225.

Rueff, C., Blancou, J. and Andral, L., 1978. La salive des bovins enragés est-elle virulente? Rev. Méd. Vét., 129: 895–909.

Schneider, L.G., 1983. Rabies Bulletin Europe/Information Surveillance Research, WHO Collaborating Centre for Rabies Surveillance and Research, Federal Research Institute for Animal Virus Diseases, Tubingen, Germany.

Serrudo, L., 1968. CEPANZO Bulletin, Fall 1968. Pan American Health Organization, Buenos Aires, Argentina, p.12.

Sikes, R.K., 1962. Pathogenesis of rabies in wildlife. I. Comparative effect of varying doses of rabies virus inoculated into foxes and skunks. Am. J. Vet. Res., 23: 1041–1047.

Smith, J.S., Sumner, J.W., Roumillat, L.F., Baer, G.M. and Winkler, W.G., 1984. Antigenic characteristics of isolates associated with a new epizootic of raccoon rabies in the United States. J. Infect. Dis., 149: 769–774.

Starr, L.E., 1956. Rabies In: M.G. Fincher, W.J. Gibbons, K. Mayer and S.E. Park (Editors), Diseases of Cattle. American Veterinary Publishers, Evanston, IL, pp. 646–653.

Sureau, P., Arellano, C. and Batalla, D., 1971. Evaluacion de la eficacia de la vacuna antirrabica cepa "ERA" en bovinos. II.Duraction de Inmunidad. Tec. Pec. (Mexico), 18: 16–21.

Sureau, P., Rollin, P. and Wiktor, T.J., 1983. Epidemiological analysis of antigenic variations of street rabies virus: detection by monoclonal antibodies. Am. J. Epidemiol., 117: 605–609.

Swanepoel, R. and Foggin, C.M., 1978. The occurrence, diagnosis, treatment and control of rabies in Rhodesia. Cent. Afr. J. Med., 24: 107–115.

Wiktor, T.J. and Koprowski, H., 1978. Monoclonal antibodies against rabies virus produced by somatic cell hybridization: Detection of antigenic variants. Proc. Natl. Acad. Sci. U.S.A., 75: 3938–3942.

Chapter 38

Bovine Ephemeral Fever Virus

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INTRODUCTION

Bovine ephemeral fever (BEF) has been recognized under this name and various others for approximately 100 years. Other names applied to it are three day(s) sickness, stiffsiekte and bovine epizootic fever. From the very early years of this century, ephemeral fever has been assumed to be a vector-borne disease, but the final steps to prove this have yet to be taken. The "ephemeral" nature of the disease, which is a reflection of its behavior in an individual animal, and its largely unpredictable epizootic behaviour both present difficulties for the investigator. It is a disease that affects tens of millions of cattle and buffaloes and exists in many countries. The fact that very few animals die as a result of infection with BEFV has diverted attention from its tremendously disruptive effects on production and animal husbandry. Its clinical signs are by no means "ephemeral", as the description later in this chapter shows.

The taxonomic position of BEFV has been resolved recently by Calisher et al. (in press). BEFV is one of several rhabdoviruses, namely Kimberly, Berrimah, Malakal and Puchong viruses, which form an antigenically related cluster within the lyssaviruses. Thus the physical characteristics of the BEFV virion, the natural history of the virus and the pathogenesis of the consequent disease will form a model for the "newer" viruses.
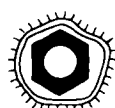
VIRUS PROPERTIES

The structure of BEFV was described by various workers as that of a rhabdovirus, on the basis of electron photomicrographs. However, there was doubt for some time, because Japanese workers initially described the virus as having double-stranded RNA. The apparent inconsistency has now been resolved and BEFV is known to have the single-stranded RNA of the rhabdoviruses (Della-Porta and Snowdon, 1980).

In preparations of BEFV, three different shapes can be found. Their approximate diameter is 73 nm, which is close to the usual diameter of 70 nm given for animal rhabdoviruses. The longest virions are bullet-shaped with a length of approximately 183 nm, having parallel sides and a precisely coiled helical nucleocapsid with a 4.8 nm interval. Particles are also found which have a truncated bullet shape, while others appear as blunt cones. The length of the shorter particles varies from 70 to 140 nm. These shorter virions are considered to be defective and to cause interference with the growth of BEFV in tissue cultures. This results in low yields when tissue cultures are inoculated with

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high multiplicities. Conversely, yields are increased when diluted inocula are used to infect cultures.

BEFV is very sensitive to lipid solvents. It is inactivated at pH levels below 5 or above 10. Since a pH level below 5 is rapidly attained in a beef carcass when lactic acid levels in muscle rise after slaughter, meat is of no consequence as a means of virus survival, aside from the impossibility of transmission from infected meat. Any disinfectant producing either a high or a low pH will inactivate the virus, but, apart from laboratory use, disinfectants have no role to play in the control.

The general characteristics of Kimberley virus are consistent with those of BEFV, but the other BEF serogroup viruses remain to be fully described. The pathogenicity of Kimberley virus for cattle has been partially investigated and strains tested so far appear to be nonpathogenic. Each BEF group virus was isolated from blood collected from a sentinel cow with a subclinical infection. It must be assumed, for the present, that these viruses are not of direct significance in the production of ephemeral fever disease, though they may produce heterotypic antibodies in natural infections. This means that these viruses may cause false positive results in serological surveys.

EPIZOOTIOLOGY

There are a number of species of ruminants in which neutralizing antibodies to BEFV have been found, but only cattle and water buffaloes (*Bubalus bubalis*) suffer clinical disease, and BEFV has been isolated only from cattle. In Australia, antibodies have been found in feral water buffaloes, feral and domesticated red deer (*Cervus elaphas*), feral Chital deer (*Axis axis*) and Rusa deer (*Cervus timorensis*). In India, Malaysia and Indonesia, domesticated buffalo have been found to have antibodies. The African ruminant species known to be infected as the result of antibody studies also fit this general pattern. They are the African buffalo (*Syncercus kaffer*), waterbuck (*Kobus ellipsiprymnus*), hartebeest (*Alcelaphus buselaphus*) and wildebeest (*Connochaetes taurinus*). Thus the number of species experiencing subclinical infection is greater than that known to suffer disease. The numbers and variety of species of ruminants in Africa which could be involved in the epizootiology of BEFV is considerable. The numbers of buffaloes are such that they constitute a significant reservoir of infection in parts of tropical Australia, but the small populations of deer are of negligible importance. The deer in Asia and Europe have not yet been examined for antibodies. No natural antibodies have been detected in any species of marsupial, horses, sheep, pigs, cats, dogs or rodents.

The production of symptoms or viremia by experimental infection of sheep, dogs, cats and mice has been reported. The experimental infection of rats and rabbits results in the production of antibodies, but not a detectable viremia. None of these species play any part in the epizootiology. Ephemeral fever is not a zoonosis. The owners of herds through which epizootic ephemeral fever has passed have not developed neutralizing antibodies to BEFV, although there would have been ample exposure to infection. Furthermore, BEFV has been handled by numerous laboratory workers without any evidence of infection. Since control and infected cattle may be closely housed together, BEF must be considered as a noncontagious infection of cattle.

BEFV does not occur in the Americas, the Pacific Islands, New Zealand or Europe. It can be assumed that it has occurred in all countries of Africa, the Arabian peninsula and all countries of Asia south of a line formed by Lebanon, Iraq, Iran, Pakistan, India, Bangladesh, Burma, southern and eastern China and Japan; not all parts of these countries have infected cattle. Although

Papua–New Guinea experienced an epizootic in 1956, ephemeral fever has not been observed since then, and extensive serological surveys support the theory that it died out spontaneously (St. George, 1981; St. George and Standfast, 1988).

Infection with BEFV can be subclinical or clinical. In the tropical areas in which BEFV infection is enzootic, subclinical infections occur commonly with occasional clinical expression. In more temperate regions of the northern and southern hemispheres where epizootics are seen, subclinical infection may still occur. Subclinical infections have been proven by the isolation of BEFV from the blood of sentinel cattle not showing clinical signs, in both enzootic and epizootic areas.

Epizootics can be widely spaced, as in Japan and in Australia between 1937 and 1967. Such infrequent epizootics tend to involve cattle of all ages. In contrast, since 1967, in Australia, epizootics have occurred at 2–3-year intervals, so that most clinical cases in recent years have been in younger animals. In a naive population all ages could be affected.

There is strong epizootiological evidence that BEFV is vector-borne. Major epidemics in Australia had the appearance of travelling with a wave front in a north–south direction with only local lateral spread.

Outbreaks of ephemeral fever occur in summer. A strong association with recent rainfall is reported from Africa and Australia. The onset of winter in temperate regions terminates an epizootic. This pattern is compatible with that of a vector-borne disease.

The other important evidence of the involvement of a vector is the observation that in the northern hemisphere disease occurs as far north as Syria, Iraq, Iran in western Asia and south of the 38°N parallel of latitude in Japan. In the southern hemisphere an epizootic rarely goes further south than 25°S in western and central Australia and 36°S on the eastern side of the continent, which has a higher rainfall. In other words, there is a discontinuity of occurrence on a continuous landmass, with contiguous cattle populations. The barrier to spread north or south of these limits is neither quarantine, topography, nor lack of suitable hosts. This indirectly indicates insect vectors with climatic constraints on their distribution.

The speed of spread of an epizootic ranges from slow and apparently erratic, to extremely swift. The first recorded outbreak in southern Africa took about one year to move from Transvaal to Cape Province (covering a distance of approximately 1000 km). The 1909 outbreak in Egypt moved 1000 km down the Nile Valley from Aswan to the Delta in the summer months. In Australia, outbreaks have varied in their speed of apparent movement, taking from 6 weeks in 1968 to 2 years in other epizootics to cover north–south distance of approximately 3000 km. The general direction of spread has always been away from the equator in countries that have experienced the epizootic pattern (China, Japan, Australia, Egypt and South Africa).

To complicate the issue, the general pattern in Australia has altered three times. Initially, there were swift moving, widely spaced epizootics 20 years apart, followed by epizootics at 2–3-year intervals for 10 years. Currently epizootics are localized and slow moving with either a high or low incidence of the disease in most years in the north-east quadrant of the continent. This pattern is tending towards that seen in the tropics where the disease occurs frequently, but not as explosive epizootics.

The factors that influence the pattern of spread of disease include the immunity level of the population, the virulence of the virus, the presence of vector species and their relative transmission efficiency, vector habitat, geographical barriers, and climate. Within a herd, competition from related and unrelated arboviruses spreading in the same time frame in the same vector

species may be an additional factor. In one herd, viremia with a Simbu group orbivirus or another rhabdovirus appeared to confer temporary protection on cattle against BEFV infection. This may contribute to the apparent erratic nature of some outbreaks.

The immunity level of the cattle population can be measured by serological tests using a statistically based sample or a system of sentinel animals. However, the significance of low titers to the various "standard" strains used in Asia, Africa and Australia will have to be reconsidered in the light of the discovery that BEFV is part of a group of antigenically related viruses that can engender heterotypic antibodies. The immunity of a national herd is also changed by vaccination, as has occurred in the southern part of Japan, where mass vaccination has reduced the pool of nonimmune cattle.

Maternal antibody is passed in the colostrum to calves, whether generated by natural infection or vaccination. Even a low level of maternal antibody appears protective. Calves with maternal antibody during one epizootic are fully susceptible at the succeeding epizootic. The presence of maternal antibodies can be a confusing factor in assessing the number of animals in a herd at risk in an enzootic area.

There is a wide variation in the virulence of BEFV strains. Strains of avirulent BEFV have been isolated from cattle, others produce disease in virtually all inoculated animals. So far, no strain has been isolated with a high fatality rate under experimental conditions. Within a particular outbreak, the variation in severity of clinical signs is wide, but whether this is due to variation in virulence of BEFV or host reaction is unknown.

The insect species from which BEFV has been isolated have not been proven to function as vectors anywhere in the world. Isolations of BEFV have been made from a pool of *Culicoides* spp. in Kenya, from *Culicoides brevitarsis* in Australia and from two widely different genera of mosquitoes in Australia, *Anopheles bancroftii* and a pool of Culicine species. As the disease occurs in an immense area of the world, several species of mosquitoes and *Culicoides* must be involved as vectors. There will be a range of different efficiencies between these vectors, so that the mere presence of an insect species from which BEFV has been isolated will not allow definitive conclusions concerning its role in epizootic spread.

Ephemeral fever can spread in a wide range of habitats, from the tropics to very temperate latitudes; from lush rice-growing regions and rainforests to arid central Australia (which has an average annual rainfall of 100–250 mm) and, in Africa, from sealevel to the highlands of Kenya. The factors in these environments affecting vector distribution remain to be identified.

The oceans appear to have prevented the entry of BEFV into the Americas and the oceanic islands. This barrier could be breached should viremic animals be brought into an area when potential vectors are active, if migratory birds carry the virus, or if live infected insects are introduced. Water barriers of up to 50 km are not effective in preventing spread of BEF, and there is speculation that water gaps of several hundred km are not effective over a span of years. Thus BEFV may have crossed from the Indonesian archipelago to northern Australia, or from the Asian mainland across the Yellow Sea to Japan. An important factor must be the general direction of the wind and the weather pattern, as this affects the movements of flying mammals, birds and insects.

Climate does have an influence on the occurrence of ephemeral fever in that it imposes a northern and southern limit, and also a summer/winter division. In some tropical areas where there is a distinct wet and dry season but not a summer/winter temperature change, the disease occurs in the wet months or the monsoon season. In the Nile Valley, the disease has spread south to north in the summer, a time when rainfall is almost unknown. It is possible in such an area that the vector populations may be linked to river level changes.

There is no objective information on the relative susceptibility of European or Zebu cattle, or of breeds within these species, all of which seem susceptible. Cows, bulls and steers are equally susceptible to infection. Clinical signs tend to be more severe in very fat animals. Calves have milder clinical signs and are less commonly affected in nature, though they are easily infected experimentally.

PATHOGENESIS

Numerous experiments have been conducted under insect-proof conditions and have shown that direct cow-to-cow transmission does not occur, even when cattle are in very close contact. In contrast, the disease is easily transmitted by the intravenous injection of blood taken from a viremic bovine. The disease has not been transmitted when nasal or ocular discharge or saliva from infected animals was smeared on mucous surfaces or injected intravenously.

The presence of BEFV in the bloodstream makes the virus available to biting midges. *Culicoides* feed by lacerating the surface of the skin and taking up the blood that pools in the wound from cuts in the capillary bed. Mosquitoes feed by probing with their mouthparts to locate a capillary before penetrating into these small vessels and sucking blood. BEFV multiplies for an unknown period in the body of the arthropod and eventually reaches the salivary glands. When the vector takes a second or a later blood meal the virus is transferred to the wound with the saliva. Whether BEFV is inoculated directly into the bloodstream or reaches it via the lymphatics is unknown.

The natural incubation period is also unknown, but there is some epizootiological evidence that 4–8 days are required, which is consistent with the 3–10 days after experimental inoculation. Viremia in natural cases often persists no more than 1 or 2 days and in experimental cases 3 days, generally coincidental with fever. BEFV appears to occur mostly in the white cell fraction of the blood and has been demonstrated by IF in neutrophils in the blood stream or joint transudates, in alveolar macrophages and in some visceral organs. However, the peak of viremia is at least 24 h before the neutrophil or temperature rise.

There is no evidence that BEFV persists in blood or tissues after the acute phase of illness; neither chronic infections nor latency can be suspected. The disease is seasonal, and during the cooler months or dry season the virus is suspected to be carried in the invertebrate vector rather than in cattle, buffaloes or deer.

Many signs of the disease are due to the host response rather than to direct pathological damage by the virus. Factors influencing the host response are age, bodily condition, stage of lactation, and whether the animal is forced to exercise during illness. Heavy or lactating animals are affected more severely than lean, nonlactating or young animals.

In the first stage of the disease, a sudden drop in milk production, fever, neutrophilia, lymphopenia and increase of plasma fibrinogen are linked, but the mechanism by which BEFV induces these changes is not yet determined. In the second phase muscle stiffness, depression, and falling plasma calcium and zinc levels are noted. Whether calcium-deficit-related signs are displayed by a particular animal is probably dependent on a decline of ionized calcium in the bloodstream. The ruminal stasis that occurs in more severely affected cattle may be a factor in reduced calcium uptake, but no explanation is available as to why homeostatic mechanisms do not compensate. Ruminal movement ceases as plasma calcium level falls and returns when it rises.

The rapid clinical recovery that is characteristic of ephemeral fever, and to which the disease owes this name, occurs in both experimental and natural

disease. In experimental cases the disappearance of clinical signs precedes by 3 or 4 days the detection of antibody. An explanation other than neutralization by antibody must be sought for the end of viremia and recovery. High interferon levels in the plasma are attained during illness.

DISEASE SIGNS

The clinical severity of the disease varies greatly from outbreak to outbreak, and even within a herd, so that not all the signs described occur in any one animal, or even one herd.

Typically, one or several cattle will show signs about one week ahead of the main wave of the disease. The passage of ephemeral fever through a herd takes 2–4 weeks and longer in large herds. This means that animals at various stages of illness may be seen at one time.

The clinical course is highly variable but can be described in four main phases: (a) onset of fever; (b) period of disability; (c) recovery or onset of complications; (d) sequelae.

Onset of fever: In natural infections, there is a rapid development of fever. Pyrexia reaches a peak of 40–41.5°C within a few hours of a detectable temperature rise. The pyrexia is biphasic but the first peak may not be noticed. A small proportion of cattle shows triphasic or polyphasic fever. Concurrently, slight changes in behaviour or stance may be noted by the herdsman, and milk production may drop suddenly. This phase may last half a day.

Period of disability: This phase usually lasts 1 or 2 days. The first peak of fever has passed, but the rectal temperature is still elevated. The most characteristic signs are severe depression, anorexia, a tucked-up appearance, general muscle stiffness and lameness, with or without joint swelling. There may be ocular and nasal discharge and salivation. The animal is usually standing, but may be in sternal recumbency, although able to rise if forced to do so. Milk production may have virtually ceased, and the quality of the remaining milk is altered. The heart and respiratory rates are increased. Dry rales may be detected in the lungs, progressing to moist rales the following day. These signs are all associated with a generalized inflammation.

Superimposed on these signs may be others which are associated with hypocalcemia, such as muscular fibrillation, an incoordinated gait, inability to rise, ruminal stasis, constipation, loss of swallowing reflex, salivation, and refusal to drink. While the animal is in a sternal recumbency the head may be turned to one flank. The affected animal may show varying degrees of these effects of hypocalcemia.

Recovery or onset of complications: In the majority of instances recovery occurs after 1–2 days of mild to severe disability. Recovery can be gradual or quite dramatic, and animals that were thought close to coma can be moving about normally a few hours later and be completely recovered a day later. Death ensues in less than 2% of the uncomplicated cases, but can occur without an obvious cause being detected postmortem. It can occur during acute disease or at the stage when most animals are recovering uneventfully.

Sequelae: Except for cows late in lactation, milk yield increases steadily after recovery (except when bacterial mastitis supervenes) but not above 80–90% of pre-illness levels (Fig. 146). Subcutaneous emphysema, particularly along the backline, occurs unpredictably. The emphysema may be a consequence of overbreathing, atalectasis and breakdown in lung structure, but this remains to be proven. This complication occurs in clusters of animals in particular herds. Bacterial pneumonia is another complication, particularly in stormy weather, as is dehydration in very hot weather.
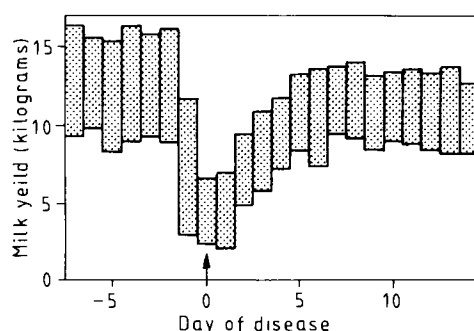
Fig. 146. Daily milk yield of a dairy herd that suffered a BEF outbreak measured before, during and after the occurrence of clinical signs; the bar chart illustrates the range of milk yield change; it should be emphasized that the fall in milk yield began before characteristic clinical signs became obvious.

Ataxia of the hindlimbs is not rare, particularly in bulls. The prognosis for animals that cannot rise within 2 days of the onset of symptoms is poor, although there are instances where full recovery has followed weeks of careful nursing.

Abortion sometimes occurs in the late stages of pregnancy. Such cows may not lactate until the following pregnancy. BEFV does not appear to cross the placenta. Fertility in the female is unimpaired. Bulls may have reduced semen quality for a period of up to 6 months. Early treatment avoids this complication. If an animal with well-developed ephemeral fever signs is forced to exercise, or is travelled on foot or in a vehicle, the risk of a fatal outcome or of complications is increased.

PATHOLOGY

Most of the material avialable for postmortem examination is derived from experimental cases.

BEF displays inflammations of all serous surfaces. The generalized changes noticed are swelling of the lymph nodes and effusion of fluid flecked with fibrin clots into the pericardial, pleural, peritoneal and joint cavities. The involvement of the joints is highly variable, but may include the articular surfaces of the limbs and of the spinal column.

If death occurs in the acute stages, there is evidence of inflammation in the nasal mucosa, turbinates, sometimes the larynx and also a patchy inflammation of the mesentery. The lungs may show a patchy engorgement especially in the diaphragmatic lobes. The spleen and kidneys may be engorged. The bladder and urine appear normal. There are no gross or microscopic brain lesions to account for the nervous signs.

The histopathology was summarized by Basson et al. (1970). The organs show some congestion of small vessels but nothing that is pathognomonic of the disease. The gross changes in the lungs relate to areas of alveoli and bronchioles containing fluid and leukocytes alternating with small areas of atalectasis. The brain shows only some degree of congestion of blood vessels but no microscopic neural lesions. Thus physical pathological changes do not account for all the clinical signs.

In the few cases where a thorough examination has been made of animals that have been permanently partially paralyzed, Wallerian degeneration has been found in the cervical section of the spinal cord (Hill and Schultz, 1977). In paralyzed animals the muscles show only degeneration consistent with disuse.

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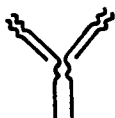
IMMUNE REACTION

One attack of clinical ephemeral fever confers permanent immunity in almost all cattle, whether it is a natural event or experimentally induced. There are known exceptions where cows have had two, and rarely three episodes of disease in the same outbreak with a 2–4-week spacing, or in successive outbreaks 2 or more years apart. In each instance, there was no virological or serological confirmation of the double or triple attacks, but the observers were familiar with the syndrome. There are two conclusions that can be drawn from these observations: infection with pathogenic virus should be preventable by vaccination and possibly more than one virus can produce the syndrome.

The serological response to natural infection is variable. The titers of neutralizing antibody in recovered cattle, as measured by laboratory strains of BEFV, bear no relationship to the severity of clinical signs. The titers in recovered animals vary from 10 to 400. Very rarely titers in excess of 40 000 are found in natural infections. In two instances BEFV was isolated from the blood of cows, and there was virtually no subsequent serological response, as measured in neutralization tests with standard and homologous viruses.

During recovery from clinical disease, many naturally infected cattle produce what appears to be a secondary immune response. Antibody is detectable before clinical signs have disappeared and rises to a plateau level in less than 5 days. The explanation for this phenomenon seems to be prior infection with an antigenically related virus. There is some evidence that Kimberley virus acts in this way. Since there are several viruses in the BEF complex, further work is necessary before the effect on immunity of these other viruses is known.

In experimental cases where a single strain of virus is used, neutralizing antibody is first detected 3 or more days after clinical recovery (9–10 days postinoculation) and the titer rises in a primary response pattern.

LABORATORY DIAGNOSIS

Almost all diagnoses of BEF are made on clinical grounds. A diagnosis can be confirmed by the isolation of the causative virus, but this procedure is slow and not very efficient at present. There are various other tests, which are not definitive but much more rapid. They do not provide a positive confirmation but are useful in discounting the diagnosis if negative. Once clinical signs are sufficiently developed for a veterinarian to be required to visit an animal, fever and an elevated leukocyte count may already be declining. Therefore, samples should be taken from other animals in the herd that have fever but have not yet developed characteristic signs. The owner or herdsman should be able to point out individual cattle which have altered gait or behaviour pattern.

A sample of blood taken from the sick animal is required in four parts, two air-dried smears for a differential leukocyte count and an IF test, 5–10 ml in an anticoagulant, and 20 ml that is allowed to clot. The anticoagulant must not be EDTA if a calcium estimation is to be done, otherwise lithium or sodium heparin, or sodium citrate may be used.

The blood should be collected aseptically and kept at 4–10°C until it is received in the laboratory. In countries where the disease is enzootic, central veterinary laboratories should be able to attempt transmission to susceptible cattle or the isolation of BEFV in suckling mice or tissue culture. As the procedure is expensive it may only be warranted at the beginning of an outbreak. If ephemeral fever is suspected in a country where it has never occurred previously the appropriate action is to collect blood samples and notify the
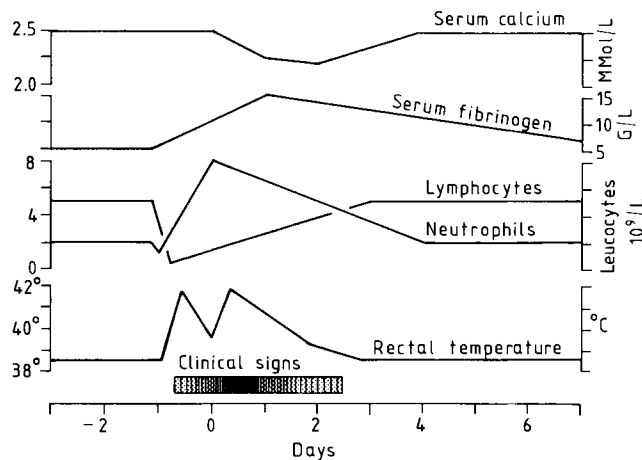
Fig. 147. Changes in serum calcium and fibrinogen values, blood neutrophil and lymphocyte counts and rectal temperatures all related to the period of naturally occurring clinical ephemeral fever; the representation is a schematic based on approximately 30 cases.

regulatory authorities. The subsequent action will then depend on that country's policy.

A total leukocyte count is carried out on the blood sample taken in anticoagulant. In combination with the blood smear this will allow calculation of a differential leukocyte count. During the first 2 or 3 days of illness, the neutrophil count is usually elevated into the range of 8–18 $\times$ 10^9 per l, with a high proportion of immature or banded forms. A depression in the absolute leukocyte count to 1–2 $\times$ 10^9 per l occurs at the time of neutrophil increase. A rise in monocytes to 0.7–0.8 $\times$ 10^9 per l is a late manifestation and continues in the recovery phase. While not pathognomonic of ephemeral fever, if the leukocyte changes do not conform to the pattern as illustrated in Fig. 147 the disease in the cow is unlikely to be ephemeral fever. Hemoglobin levels and packed cell volume should be near normal. The clotted blood from an animal with ephemeral fever contracts poorly and has a whitish appearance, so that the serum yield is reduced. This is the reason for collecting a substantial volume of blood for serology.

Serum biochemistry can be helpful. Fibrinogen levels are raised approximately to 2–4 times normal within the range of 10–20 g/l. The decline to normal takes 1–2 weeks (see Fig. 146). Serum calcium may be at the low end of the normal range, or subnormal, depending on the stage of illness and whether the cow is showing specific signs of hypocalcemia.

There is no rule as to when serum samples should be taken for confirmation by VN tests. The first serum samples should be taken as early in acute illness as possible, the second 2–3 weeks later. While occasional animals fail to develop neutralizing antibodies to laboratory strains of BEFV, most will do so. There may be a low titer (≤ 4) of antibody in the sample taken during acute illness. A 4-fold rise in a paired sample is considered diagnostic.
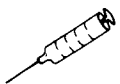
PROPHYLAXIS AND CONTROL

The advent of vaccines for the control of ephemeral fever has come late in the history of the disease. The difficult task of first isolating the causative virus was achieved at Onderstepoort, South Africa, in 1966 by Van der Westhuizen (Van der Westhuizen, 1967). A complication in vaccine manufacture soon became apparent when some strains of BEFV lost antigenicity, as well as

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virulence upon passage in mice or tissue cultures. However, certain strains have been adapted to tissue cultures and retain their antigenicity. Live virus attenuated vaccines with adjuvant have been made successfully in South Africa, Japan and Australia. These vaccines protect against experimental challenge with virulent virus. In Japan, a killed vaccine has been also used to boost initial immunity produced by a live virus vaccine. Vaccination has been carried out on a large scale in Japan, but until another epizootic occurs there the economic value may not be assessed. Data on the protection achieved against natural disease in South Africa, Australia or other countries with live virus adjuvant vaccines has not yet been published.

An experimental killed vaccine produced in Australia failed to protect cattle against intravenous challenge with virulent virus. Its value as a protection against natural disease has not been tested. The serum neutralizing antibody titers in vaccinates were in excess of 40 000. A high antibody titer is not necessarily protective against BEFV infection.

In many instances a vaccination program, once initiated must be continued for many years before natural challenge occurs. The costs, availability and administration of vaccines has to be assessed in relation to potential loss not only from country to country, but also within a country; no universal recommendation can be made.

Ephemeral fever is a benign disease in that most animals will recover spontaneously. Unlike for most virus diseases treatment for the clinical effects of ephemeral fever is available and the severity of the disease can be ameliorated. The first principle is to rest the sick animal, preferably for one week. The second is to give nothing by mouth, unless the swallowing reflex has been observed to be functional.

Treatment revolves around the generalized inflammation and the depression of serum calcium. The inflammation can be treated with phenylbutazone given intramuscularly for eight hourly periods of up to 3 days, depending on response. Treatment with calcium borogluconate is warranted when signs of hypocalcemia are present. While the initial dose is given intravenously, to effect, further maintenance doses can be given subcutaneously. Late treatment has little beneficial effect. The prognosis depends on the duration of illness. Anti-inflammatory treatment is essential. Supplementary antibiotic treatment to avoid secondary pneumonia or mastitis are warranted in valuable animals. The choice of antibiotic must be with regard to the bacteria known to cause pneumonia or mastitis in the particular geographical area. If paralysis has been established for more than a day or so, treatment is unlikely to be effective. The animal then appears to have impairment of nerve function and the chances of recovery are poor.

The basic lesion of ephemeral fever is inflammatory and resolution of the damage takes time: therefore specific treatment supplements rest but does not replace it. If the treated animal is stressed, relapse is likely. If treatment is delayed until the animal has reached the stage of either atalectasis or coma the prognosis is poor. If the animal is severely affected it is better to provide shelter and care where it is, rather than to force it to move any distance to shelter.

REFERENCES

Basson, P.A., Pienaar, J.G. and Van der Westhuizen, B., 1970. The pathology of ephemeral fever: a study of the experimental disease in cattle. J. South. Afr. Vet. Med. Assoc., 40: 385–397.
Calisher, C.H., Karabatsos, N., Zeller, H., Digoutte, J-P., Tesh, R.B., Shope, R.E., Travassos da Rosa, A.P.A. and St. George, T.D. (in press). Intervirology: antigenic relationships among rhabdoviruses from vertebrates and hematophagous arthropods.

Della-Porta, A.J. and Snowdon, W.A., 1980. Bovine ephemeral fever virus. In: D.H.L. Bishop (Editor), Rhabdoviruses. CRC Press, Boca Raton, FL, Vol. 3, pp. 167–191.

Hill, M.W.M. and Schultz, K., 1977. Ataxia and paralysis associated with bovine ephemeral fever infection. Aust. Vet. J., 53: 217–221.

St. George, T.D., 1981. Ephemeral fever. In: E.P.J. Gibbs (Editor), Virus Diseases of Food Animals. Academic Press, London, Vol. 2, pp. 541–564.

St. George, T.D. and Standfast, H.A., 1988. Bovine ephemeral fever. In: T.P. Monath (Editor), The Arboviruses: Epidemiology and Ecology. CRC Press, Boca Roton, FL, Vol. 2, pp. 71–86.

Van der Westhuizen, B., 1967. Studies on bovine ephemeral fever. 1. Isolation and preliminary characterization of a virus from naturally and experimentally produced cases of bovine ephemeral fever. Onderstepoort. J. Vet. Res., 34: 29–40.

Retroviridae

This family owes its name to the unique enzyme reverse transcriptase (from Latin retro = backwards) which transcribes a DNA copy from the viral RNA genome. The subfamilies Oncovirinae, Spumavirinae and Lentivirinae are further subdivisions of this family which contains more than 20 viruses.

Retrovirions are spherical enveloped particles measuring 80–100 nm in diameter and possessing glycoprotein surface projections of approximately 8 nm. The internal structure is complex, consisting of an icosahedral core which in turn contains a helical ribonucleoprotein strand. In thin sections the outer envelope, an inner shell and a nucleoid are distinguishable. The nucleoid is located acentrically in the genus Type B Oncovirinae and concentrically in the genus Type C Oncovirinae. Type species of the former is the mouse mammary tumor virus; the latter comprises the mammalian and avian subgenera of leukosis and leukemia viruses.

The genome of retroviruses is an inverted dimer of linear single-stranded RNA with a positive polarity; the monomers are held together at their respective 5' ends by hydrogen bonds, probably by base-pairing. A cellular tRNA serves as a primer for reverse transcription and is bound to a specific primer attachment site about 100 residues from the 5' end of the genome. It is not known whether both copies of the genome are functional. The genetic information is contained in the genes gag (coding for the nonglycosylated internal proteins with *group* specific *antigenic* determinants), pol (coding for the reverse transcriptase or *poly*merase) and env (coding for the *env*elope glycoproteins). The gene sequence is 5'- gag–pol–env -3'. Some retroviruses incorporate genetic information for nonstructural proteins from the host cell which are important in pathogenesis, especially in oncogenicity. They are either inserted into a complete retroviral genome or substitute deleted viral sequences. In this latter case the virus is rendered defective by the deletions and it depends on a nontransforming helper virus for the production of infectious progeny.

Virions contain four internal nonglycosylated structural proteins (including the gag-determinants which define the subgenera), the reverse transcriptase enzyme and two A envelope proteins (env gene products).

Retroviruses band between 1.16 and 1.18 g/cm^3 in sucrose gradients; the sedimentation coefficient of the virion is about 600 S.

Upon entry into the cell and uncoating, replication starts with the reverse transcription of virion RNA into DNA, which is then made double stranded. This structure is integrated into the chromosomal DNA of the host cell at nonspecified sites. Virus can only replicate when the DNA is integrated. Cellular RNA polymerase II transcribes the integrated provirus into virion RNA mRNAs. Both RNA splicing and posttranslational cleavage of proteins have been shown to occur in retrovirus-infected cells. Virus is released from the cells by budding.

The Retroviridae are the only family of animal RNA viruses with an oncogenic potential; some may be immunosuppressive (e.g. the lentiviruses causing AIDS in man and animals) or nonpathogenic (e.g. the spumaviruses); for a summary see Table 29.

TABLE 29

Exogenous Retroviridae

Oncoviruses EM: nucleoid of C type; cell-transforming
   acute, via v-oncogene[a]
      avian, feline and murine sarcoma viruses
   chronic, via c-oncogene[b]
      avian, feline and murine leukemia viruses
   chronic, via *tat* gene[c]
      BLV, HTLV-1, HTLV-2, STLV-1
Lentiviruses EM: nucleoid similar to that of D type; cytolytic[d]
   Visna/maedi
   Ovine progressive pneumonia virus (OPPV) ⎤———— p30 (reciprocal)
   CAEV ⎦
   EIAV ⎤
      └————————————————— p24, p17 (nonrecripocal)
      HIV-1, HIV-2, STLV-III (SIV)
                       ———— p24:p26 (reciprocal)
   BIV ⎤
   FTLV ⎦

[a] Viral (v) oncogene integrated into the viral genome.
[b] Viral genome inserted into cellular chromosome proximally to a cellular (c) oncogene.
[c] Situated between *env* gene and 3'LTR. The viral genome has no v-oncogene and is not inserted proximally to a c-oncogene.
[d] Replication strictly regulated by four or five accessory genes, often overlapping (*sor, tat, art/trs, 3'orf,* R).
Lines: antigenic relationships; p24 and p17 are core proteins of HIV, p26 is a core protein of BIV.

Bovine leukemia virus (BLV) is a C-type oncovirus that preferentially infects lymphocytes of the B lineage in cattle (but T lymphocytes in sheep). In most cells in vivo BLV persists in the proviral state but its replication can be induced (in vitro), e.g. by mitogens. BLV is related to human T-lymphotropic and leukemia viruses (HTLV-1 and HTLV-2). These viruses together with a similar simian virus (STLV-I) form a group of chronic leukemia viruses with as yet unknown mechanisms of leukemogenesis.

Lentiviruses are agents of slow diseases — visna and maedi (caused by the same virus) in sheep as well as caprine arthritis–encephalitis. These viruses are interrelated by sharing at least one antigen — the internal protein, p30. The growth of a visna virus in cell culture is abundant when compared to growth in vivo, which is markedly restricted. It is unclear if integration of DNA is required for replication, since most of the DNA is extrachromosomal.

Visna virus and caprine arthritis–encephalitis virus (CAEV) are related to HTLV-3/LAV, the agent primarily involved in etiology of AIDS (acquired immune deficiency syndrome). These viruses together with a similar simian virus (STLV-3), the equine infectious anemia virus and the above-mentioned leukemia viruses were placed into a group of *trans*-acting retroviruses, different from other retroviruses (for review, see Wong-Staal and Gallo, 1985). In connection with BLV and related leukemia viruses, the *trans*-acting transcriptional activity is believed to be the major mechanism of cell transformation.

The virus causing ovine pulmonary adenomatosis (*jaagsiekte*) was recently identified as a retrovirus. It does not seem to be related to either the known lentiviruses or oncoviruses.

Spumaviruses are nonpathogenic, causing persistent but silent infections. Cells of infected cultures form syncytia and have vacuolated cytoplasm, thus showing a "foamy" appearance. One member of this subfamily is bovine syncytial virus, important only in connection with differential diagnosis in the laboratory.

REFERENCE

Wong-Staal, F. and Gallo, R.C., 1985. Human T-lymphotropic retroviruses. Nature, 317: 395–403.

Chapter 39

Bovine Leukosis Virus

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INTRODUCTION

The discussions contained in this chapter relate primarily to the adult or enzootic form of bovine leukosis (EBL). This is the only form of the disease in which bovine leukemia virus (BLV) has been shown to play an etiological role. The other forms of leukosis, commonly referred to as sporadic, include calf lymphoma, thymic lymphoma (sometimes considered together as juvenile lymphomas) and skin lymphoma. There is no evidence that these less frequently occurring types of lymphoid neoplasms are related to BLV or to other, as yet unisolated and unidentified, infectious agents. They will be discussed only to the extent that information regarding differential diagnosis is required.
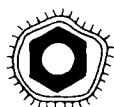
VIRUS PROPERTIES

Bovine leukemia virus is classified as a type-C oncovirus in the retroviridae family and thus shares characteristics with the leukemia viruses of other species.

The BLV virion is apparently quite fragile and, in the laboratory, infectivity is greatly decreased by routine manipulations such as ultracentrifugation or subjecting the virus to a single freeze–thaw cycle. The virus is readily destroyed by heating to 56°C for 30 min. It has also been shown that BLV is inactivated in milk heated to temperatures of 60°C for more than 1 min and by routine pasteurization temperatures. Attempts to preserve virus by lyophilization have failed. The viral envelope contains lipid, thus rendering the virus susceptible to destruction by organic solvents. Extensive tests of the effects of various disinfectants on BLV have not been conducted, but binary ethyleneimine and N-acetylethyleneimine have been found to be effective inactivating agents for virus preparations (Miller and Van der Maaten, 1980). The reader is also reminded that much of the transmission of virus among cattle does not result from exposure to cell-free virus but rather from the transfer of lymphocytes containing BLV genomes. Thus, the possibility that BLV infectivity in this unexpressed form may be more or less susceptible to inactivation must be kept in mind. From a strictly practical viewpoint, the success of eradication programs, where positive animals were removed from infected herds but no specific disinfection or sanitation was practiced, indicates that BLV does not persist in the environment for any appreciable length of time (Van der Maaten and Miller, 1979).

Studies to date have failed to reveal important antigenic differences among BLV isolates from various parts of the world, and so it is presently assumed

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that antigenic subtypes, if they exist, remain to be discovered. There have been some studies that identiy viral subtypes at the genomic level (Burny et al., 1980), but these apparently do not result in differences in antigenic properties of the virus particle.

No significant antigenic relationships between BLV and leukemia virus of other domestic animal species have been detected to date, and so BLV, at present, remains antigenically unique in this respect. There is evidence, however, that BLV shares structural and functional properties, and perhaps even minor antigenic determinants, with the human T-cell leukemia viruses, which has led to the suggestion that these agents may comprise a new group of retroviruses that have arisen from a common ancestor (Burny et al., 1984).

EPIZOOTIOLOGY

Cattle, including the European breeds (*Bos taurus*) and zebu breeds (*Bos indicus*) are considered to be the primary and important host of BLV. Sheep (Kettmann et al., 1984) have been found infected with BLV and death losses from lymphosarcoma have occurred, but these instances have been so rare that they must be regarded as resulting from the accidental introduction of BLV rather than as evidence that sheep are a significant reservoir of the virus. More recently, studies in South America have reported serological evidence of infection (but not disease) in water buffalo and capybara, a large rodent. The reported prevalence of infection among the buffalo and capybara was, however, well below that of the cattle tested in the same study. Other studies in which common domestic animals, laboratory animals, wild animals and wild and domestic birds were examined have failed to provide evidence of BLV antibodies in these species. Information to date thus supports the hypothesis that BLV is propagated and maintained primarily in the cattle population and that infections in other species are accidental introductions. The possibility of transmission to other species must, of course, be considered in the formulation of control programs. The frequency with which transmissions occur may be related to the presence of vectors capable of transmitting the infection, not only within the cattle population but among other species as well. Ticks and biting flies have been incriminated but, in consideration of the persistent presence of BLV-infected cells in the blood, other biting insects could also spread the infection. The available evidence indicates that arthropods are merely transport vectors and not true biological vectors of BLV; transmission has been obtained with *Ixodes ricinus* adults that have been fed on BLV-infected cattle as nymphs.

The potential human health hazards associated with BLV have been a constant concern of the meat and dairy industries. Some epidemiological studies have indicated that certain malignancies and lymphoid neoplasms are more prevalent among persons engaged in dairying or other farm-related occupations. None of these studies, however, has shown any direct correlation between the observed malignancies and BLV infections in the cattle present on the farms (Burridge, 1981). Furthermore, all attempts to obtain direct evidence of human infection, including the application of serological, virological, or biochemical methods, have yielded negative results. There have been indications of a distant antigenic relationship between BLV and some of the human T-cell leukemia viruses. This has resulted in the identification of antibodies reacting with BLV in a few human subjects known to be infected with human T-cell leukemia virus; current evidence indicates that this reactivity is due to the apparent common ancestry of BLV and the human T-cell leukemia virus and not a consequence of BLV transmission to man (Burny et al., 1984; Thiry

et al., 1985). Thus the information available to date indicates that BLV is not a human health hazard.

BLV infections among cattle are worldwide in distribution. However, there are certain countries, such as Switzerland, where infection has not been identified or where it is no longer considered to be present. The Netherlands, Austria and the British Isles (Mussgay et al., 1980) apparently have only a low prevalence of infection, and other countries or regions may be recognized in the future as being BLV-free.

BLV-related economic losses arise from deaths and restrictions on the movement of BLV-infected animals in international commerce. The incidence of tumors among BLV-infected cattle is quite low (vide infra) so these losses are not of great significance on a national basis. Because they often occur in multiple-incidence herds, they may represent significant losses to certain herd owners. The economic significance related to restrictions regarding movement of infected cattle and their products are, of course, directly related to the export of breeding stock, semen, embryos, or similar materials.

Early epidemiological observations had indicated that EBL is a horizontally transmitted infectious disease. Studies conducted after BLV was isolated and serological tests were available confirmed these observations by providing additional evidence of the slowly progressing contact transmission in herds (Mussgay et al., 1980). The virus does not gain entrance to the germ line and hence true genetic transmission does not occur, but there is ample evidence of in utero transmission. Various studies have indicated that 3–20% of the calves from infected dams are BLV-infected at birth, possibly depending upon genetic susceptibility of the cattle (Ferrer and Piper, 1981). Thus, although in utero infections may perpetuate the virus from one generation to the next, they are not regarded as the most preponderant mode of transmission. Calves which are not infected in utero but are born to infected cows are probably only occasionally infected as a result of ingesting colostrum or milk that contains infected lymphocytes (Ferrer and Piper, 1981). They are protected from contact infection until the levels of colostral antibody wane, usually 4–6 months after birth (Van der Maaten et al., 1981). Contact virus transmission may occur among young animals during the rearing period but, because there are usually only small numbers of infected animals present, the prevalence of infection remains low. An increased rate of transmission is noted when the calves reach breeding age and are reintroduced into a highly infected adult population (Van der Maaten and Miller, 1979).

Factors that influence the epizootiological pattern have not been identified in the USA, but the prevalence of BLV infection is higher among dairy cattle than among beef cattle. Husbandry practices (greater opportunity for animal-to-animal contact) and genetic factors may be responsible for this. High prevalence of BLV infection is also often reported from tropical or semitropical areas. It can only be speculated at present that the high prevalences are due to the large numbers of insect vectors in these areas.

The term morbidity is perhaps inappropriate to describe animals infected with BLV because in the majority there is no evidence of disease, only of seroconversion and persistent virus infection. The prevalence of infection varies from country to country as described above, and even from farmstead to farmstead within countries or regions. It is not rare to encounter herds where 80–90% are infected and, within the same geographic region, to find other herds that are virtually BLV-free (Miller and Van der Maaten, 1980).

Tumor development among BLV-infected animals is influenced by virologic, genetic and immunologic factors, but none of these is understood at the present time. Evidence presented indicates that the BLV genome is quite susceptible to genetic mutation, and that the clusters of tumor cases observed during a short

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period may have resulted from the transmission of a particularly oncogenic form of BLV that subsequently underwent changes and lost this strong tumorigenic activity. There are also some indications that cellular and humoral immune mechanisms, particularly those specific for BLV-related antigens, may be suppressed in the infected animal (Yamamoto et al., 1984). It is widely recognized, however, that tumor development is age-related, and only rarely are tumors found in animals less than 2 years old. It is thus apparent that herd management decisions may profoundly influence the tumor rate, because older animals may be routinely culled, whereby the size of the population with the highest risk of developing lymphosarcoma is decreased. One estimate of tumor rate from a study in a region of France indicates that 0.4% of BLV-infected cattle may be expected to develop tumor in any given year (Crespau et al., 1978). As indicated above, the immunological interactions involved in tumor suppression have not been elucidated, but it is obvious that BLV-specific antibody has no protective effect. Antibodies are present in all infected cattle, regardless of whether or not they have tumors.

The possibility that genetic factors influence the development of tumors in BLV-infected animals has long been recognized, because numerous tumor cases were recorded within certain cow families or sire groups (Ferrer et al., 1978). Recent studies have confirmed this genetic susceptibility for tumor formation; they also provided evidence that persistent lymphocytosis as a response to BLV infection may be under a similar, but separate, genetic control mechanism. Finally, susceptibility to infection with BLV may also be related to heritable factors (Ferrer et al., 1978).

PATHOGENESIS

The susceptible host probably encounters BLV in the form of infected lymphocytes, not as cell-free virions. Experimental inoculations with lymphocytes have proven that BLV infections can be established by oral (only in newborn calves) and intranasal exposure, or as a result of intrauterine instillation. All of these studies involved the inoculation of large quantities of infectious material, and they never resulted in the infection of all animals exposed (Miller and Van der Maaten, 1980). Thus it must be concluded that these are relatively inefficient routes of entry for BLV. Conversely, the parenteral route, particularly intracutaneous inoculation of relatively small doses of infectious material, has proven very successful in establishing BLV infections. From these experiments it has been concluded that insects or other blood-sucking ectoparasites might play a role in BLV transmission. Obviously, other factors such as minor trauma, surgery with contaminated instruments, the insertion of ear tags or other identification procedures that pierce the skin (tattooing) could transfer infected lymphocytes from one individual to another. Vaccination or venipuncture must also be considered as potential avenues to BLV transmission, particularly if the needle is contaminated with blood, a situation more likely to happen during collection of blood specimens than during vaccinations (Straub, 1981). Premunition procedures and blood transfusions, in which BLV-infected donors are used, would be other means of BLV dissemination.

Once the infected lymphocyte enters the host organism, a cycle of virus replication is initiated in the infecting lymphocyte in probably the same manner as when these cells are cultured *in vitro*. Virus produced by the infecting lymphocyte contacts susceptible cells, probably of lymphoid or stem-cell origin, and additional replicative cycles are initiated. There is little evidence that specific lymphoid sites are involved in this early stage of replication; experi-

mental studies have failed to implicate the regional lymph node, bone marrow, thymus, or spleen as fulfilling critical roles. The plasma of recently infected cattle contains cell-free virus on occasion, but not routinely; so it seems that a prolonged true viremia is not a requisite step in the initiation of infection. The BLV infection apparently becomes established in the lymphoid tissues during the first few weeks and virus can usually be reisolated from blood lymphocytes between the third and fifth week after experimental inoculation. The level of infectivity present in the blood can vary considerably from animal to animal. At the time that virus appears in the blood of recently infected cattle, or within a few to several days thereafter, BLV-specific antibody can usually be detected. The infection and antibody response, which may vary somewhat due to physiological changes in the host, such as pregnancy and parturition, persist for life.

As stated above, little or no cell-free virus is produced in the tissues of infected cattle, and thus most infectivity must escape from the persistently infected host in the form of infected lymphocytes (Ressang et al., 1982). Therefore, blood is probably the most potent source of infectivity. Milk and colostrum have also been found to contain BLV, undoubtedly due to their lymphocyte content. Saliva, urine, semen, feces and expired air have also been examined and occasionally been found to contain infectivity or BLV antigen (Ferrer, 1980; Lucas and Roberts, 1982), but, on a quantitative basis, they must be considered of far less importance than blood (Straub, 1981; Ressang et al., 1982). It should be recognized, however, that inflammation or tissue damage that results in hemorrhage into any of these materials may increase their cellular content, thereby altering their infectivity.

The molecular mechanisms involved in cell transformation and tumor development remain to be elucidated. BLV does not carry cellular oncogenes within its genome like the rapidly transforming sarcoma viruses. It also does not integrate at specific sites into the host genome, and it is unlikely that it transforms cells by activating specific transforming cellular sequences near the integration site, as has been described for e.g. avian retroviruses. BLV is integrated at various (if not at random) sites in the cell genome; one of the BLV gene products is capable of activating cellular genes at some distance from the integration sites and thus brings about the malignant transformation (Burny et al., 1984). The longstanding observation that BLV is much more oncogenic in sheep than in cattle has not yet been explained at the molecular level. The observation that BLV-infected ovine lymphocytes, unlike bovine lymphocytes, contain considerable quantities of unintegrated DNA transcripts of BLV RNA is a significant difference and may provide a clue for determining the mechanisms responsible for the differences in oncogenicity in these ruminant species (Kettmann et al., 1984).

DISEASE SIGNS

The initiation of BLV infection is often associated with a transitory lymphocytosis, which is clinically inapparent. There have also been reports of abnormalities in immunoglobulin production, particularly of IgM (Meiron et al., 1985), and of decreased cytotoxic responses to BLV-infected target cells (Yamamoto et al., 1984). Most tests of immunologic responses of BLV-infected animals to a variety of antigens have, however, failed to identify abnormalities. There has been one paper that presents evidence that BLV-infected cows were culled from a dairy herd at a higher rate than their nonBLV-infected herdmates (Thurmond et al., 1985), but other reports indicate that BLV-infected cattle do not differ from their uninfected herdmates in milk production, reproductive

performance, incidence of mastitis or productive life span. Herds with chronic health problems have been tested for BLV antibodies and a high prevalence of infection was found. It was then assumed that an etiologic association exists between the BLV infection and health problems present in the herd. However, no data are available to support such a conclusion.

Persistent lymphocytosis is associated with BLV infections, and lymphocyte counts have in the past been used with some success to identify herds infected with BLV (Bendixen, 1965). Various studies have indicated that 29–85% of BLV-infected cattle develop lymphocytosis. Hematological examinations thus have limitations in sensitivity and furthermore disregard other possible causes of persistent lymphocytosis. They have therefore generally been supplanted with the more sensitive and specific serological tests (Miller and Van der Maaten, 1977). Cattle with persistent lymphocytosis remain clinically normal and, although these animals seem to have a greater risk of developing tumors, persistent lymphocytosis is regarded as neither an initial nor a requisite step in the development of the malignant lymphoid neoplasm. It is now generally accepted that the predisposition to develop persistent lymphocytosis and tumor formation are determined by separate genetic factors, but that these factors tend to occur together (Ferrer et al., 1978; Ferrer, 1980).

Development of lymphosarcoma is a rare result of BLV infection. Tumor formation is sometimes accompanied by increased numbers of circulating lymphocytes, but this leukemic manifestation is distinct from the persistent lymphocytosis mentioned above. Hematological studies will be of value in arriving at a diagnosis of malignant lymphoma. Although preferred sites for tumor development appear to exist, there is no absolute tissue predilection, and so clinical signs of disease may be extremely varied. A presumptive diagnosis of lymphosarcoma can sometimes be made on the basis of tumors in subcutaneous or retrobulbar lymph nodes, or enlargements may be detected by rectal or vaginal palpation. There also may be signs related to organ involvement, such as cardiac distress, digestive disturbances, or neurological manifestations. In other instances, there may be only vague indications of disease, such as decreased production, inappetence, weight loss, weakness, and generalized debility. Surgical exploratory procedures and examination of biopsy specimens may be of value in some cases, but diagnosis will ultimately depend upon the skill and experience of the attending clinician and will require laboratory confirmation.

PATHOLOGY

The definition of leukosis given by pathologists is "a proliferation of leukocyte forming tissue." In veterinary literature, the term is commonly used to indicate conditions of uncontrolled proliferation, i.e. neoplasia. In some species, such as the chicken, this malignant state can involve any class of leukocytes, but in cattle only lymphocytes are affected. Therefore, the pathologic diagnosis frequently made is lymphosarcoma or malignant lymphoma. The term leukemia is also used; however, this designation is not accurate because primary bone marrow involvement is extremely rare in the cow.

When diagnosing leukosis in cattle, it is important to recognize which of the clinical forms of the disease is involved. Three forms of bovine lymphosarcoma (calf, thymic, and skin) are included in a category designated sporadic bovine leukosis (SBL), because there is no epidemiologic evidence to indicate that they are infectious diseases (Hugoson, 1967). Only the enzootic form of lymphosarcoma in adult cattle is caused by BLV.

A differential diagnosis of the four clinicopathologic types of lymphosar-

coma can usually be made on the basis of a clinical examination and an evaluation of the case history. The calf form usually occurs in animals that are less than 6 months old, and the characteristic feature of this form is its generalized nature. Virtually all the lymph nodes are enlarged and, at necropsy, lymphoid tumor cell infiltration is frequently visible in the liver, spleen, and bone marrow. As a result of the marrow involvement, peripheral blood often shows an elevated leukocyte count due to the presence of numerous malignant lymphocytes. This clinical-pathological manifestation is (rarely) also encountered in animals up to 2–3 years of age. The cases, which are BLV antibody negative, are regarded as atypical manifestations of the calf form. The second form of SBL, thymic lymphosarcoma, typically occurs in animals between 6 and 18 months of age. Primary tumor involvement is restricted to the thymus, although thoracic lymph nodes may also be enlarged. Skin leukosis, the third type of SBL, is the only form of bovine lymphosarcoma in which a clinical recovery can be expected. The cutaneous tumors are usually accompanied by lymphadenopathy and are seen in young adults, 1–3 years of age. The lesions persist for only a few weeks or months and then complete remission occurs. Bendixen (1965) reported that in all cases of the cutaneous form the malignancy eventually recurs with involvement of vital organs and a fatal termination. In our experience, however, some cattle recovered from skin leukosis and remained healthy for as long as 12 years after regression of the lesions. Because nothing is known concerning the etiology or pathogenesis of any form of SBL, we cannot be sure that the cutaneous cases we have seen represent the syndrome described by Bendixen.

The adult form of lymphosarcoma, as the name implies, is seen in cattle over 2 years old, with a peak incidence at 5–18 years. The disease is most frequently recognized due to a visible enlargement of one or more of the peripheral lymph nodes. Involvement of retrobulbar lymphoid tissue may result in protrusion of the eyeball, which can lead to corneal ulcerations. Enlarged nodes or tumor masses may also be detected in the peritoneal cavity or retroperitoneal areas during rectal examination. When lymphadenopathy is not apparent upon clinical examination, there is usually no chance to make a definitive diagnosis until a necropsy is performed. At this time, internal tumors, frequently in the mesenteric or retroperitoneal lymph nodes, may be obvious. Tumor cell proliferation in the white pulp can cause massive enlargement of the spleen, which sometimes leads to rupture of the capsule and death due to internal hemorrhage. When the lymphoid tissues are not grossly affected, a careful examination of other tissues is required. One of the organs frequently affected is the heart, especially the right atrium. The abomasum is another common location of tumor; in many cases the visible manifestation of neoplasia is ulceration at the mucosal surface. The kidneys and uterus should also be examined. If paralysis or neurologic signs were noted, examination of the brain and spinal cord are indicated. It is advisable to substantiate necropsy findings with histopathologic study. The microscopic examination should confirm that the lesions are composed of proliferating lymphoid cells. Lesions which are thought at gross examination to be tumors can be found microscopically to be some type of proliferative inflammation. It is also important to differentiate other types of tumors which may be primary in one of the organs mentioned above and may have metastasized into lymphoid tissues.

Microscopically, the diagnosis of lymphosarcoma depends on a subjective evaluation of the involved tissue with primary consideration given to established criteria for the characterization of neoplasia, e.g. anaplasia, invasiveness, unrestrained growth, metastasis, etc. The tumors of lymphoid leukosis may be classified pathologically according to the predominant stage of cell differentiation, e.g. reticular, lymphoblastic, lymphocytic, etc.; however, this

type of classification has not proven helpful in clinical or epidemiologic investigations.
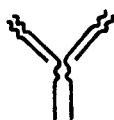
IMMUNE REACTION

The humoral immune response to BLV infection has been well characterized. After experimental infection, antibody first appears within 3–9 weeks, but the average time is 4–6 weeks as determined by AGID (Miller and Van der Maaten, 1977) and perhaps several days sooner as determined by highly sensitive techniques such as RIA. An infected animal can make antibody to several viral proteins, but the reaction to two antigens is of particular importance: to the major structural protein of the virus core (p24), and to one of the glycosylated envelope proteins (gp51). Antibody to gp51 is believed to be responsible for the virus-neutralizing activity that is found in sera of BLV-infected cattle. The production of neutralizing antibody does not lead to elimination of BLV from an animal because of the mechanism of BLV persistence (integration of DNA copies of viral nucleic acid into host chromosomes). The gp51 antibody is also capable of lysing BLV-infected cells in vitro; however, the reaction requires the presence of rabbit complement, and it is not known if antigen-producing cells are similarly affected in vivo.

The constant presence of the viral genome apparently is accompanied by a persistent production of antigen. The result of this viral activity are antibody titers that usually remain high throughout the infected animal's life span. It has been shown that in most BLV-infected cattle the titers to gp51 antigen are about 10-fold higher than the titers to p24. Antibody titers to both antigens are higher in cattle with tumors than in animals that are asymptomatic, and there is frequently a relative increase in titers of antibody to p24 in cattle with tumors. These differences are neither great nor consistent enough, however, to be used for diagnostic purposes.

Serum antibody to BLV has been found to be IgG_1 or IgA. Infection appears to alter serum levels of IgM but there are no reports of a viral specific antibody that has the physical–chemical characteristics of this globulin. The IgM alterations do not present a consistent pattern (Meiron et al., 1985).

The BLV antibody level in a cow's serum drops precipitiously at the time of calving, returning to normal within a few weeks (Miller and Van der Maaten, 1980). The decreased antibody content of serum is apparently the result of a selective concentration of the immunoglobulin in colostrum. This maternal antibody can confer immunity to newborn calves that should protect them against a contact infection for the first 4–6 months of life.

To date there has been no evidence of a significant CMI response to BLV infection. It has been shown that when BLV-infected lymphocytes are cultured in vitro and produce virus particles, other (noninfected) lymphocytes in the culture undergo blastogenesis. This response is a physiologic and morphologic alteration of lymphoid cells that represents a specific reaction to the presence of viral antigens. It is not known if a similar response occurs in vivo; furthermore, there is no evidence to suggest that this or any other type of cellular immune reaction plays a role in the pathogenesis of BLV.

LABORATORY DIAGNOSIS

The diagnosis of bovine leukosis is aimed at two objectives. One goal is the detection of BLV infection in cattle, another goal is the diagnosis of lymphosarcoma.

For the diagnosis of BLV infection, serological tests are preferred. A complete herd test of all cattle over 6 months old is most desirable. However, if the herd size is large, it may be possible to test only a representative sample of the animals. In this case, attention should be given to selecting animals in age groups that are characteristic of the herd, because BLV prevalence tends to increase with age. There is little reason to test calves that are less than 6 months old, because a serological test does not distinguish between passive immunity due to colostral antibody and calves that have been infected (Van der Maaten et al., 1981).

A herd serosurvey can be used to estimate the prevalence of BLV infection. This information may be useful when determining the desirability and feasibility of a control program. With regard to the testing individuals, the main purpose will be to satisfy a health certificate requirement. On occasion, the test may be used to aid a clinician in making a differential diagnosis of lymphosarcoma (adult form). It is important in this situation to recognize the limitations of a serological test, i.e. it can only be used to diagnose infection with BLV. Because most infected animals do not develop lymphosarcoma, a positive serological test cannot be used to confirm a presumptive diagnosis of lymphosarcoma. A negative test, however, is helpful because it indicates that the animal in question probably has no clinical EBL.

Serological tests developed for the diagnosis of BLV infection include AGID, CF, RIA, ELISA and VN. The RIA, ELISA, and VN tests have the greatest sensitivity, but the AGID test is the most widely used because of its greater practicality. The AGID test is used especially in regional laboratories where the small number of samples tested may not justify a more sophisticated technique.

As we indicated previously, serological tests are of no value for diagnosing BLV infection in young calves from infected cows, because such calves will have passive antibody from the dam's colostrum. If it is deemed essential to determine the BLV status of such animals, techniques to identify virus, rather than antibody, must be used. These include in vitro culture of lymphocytes from the animal in question and the subsequent visualization of C-type virus particles by EM, or attempts to identify viral antigens by AGID, competition RIA, antigen-capture ELISA, or other serological methods. Alternatively, BLV might be identified by the cocultivation of the lymphocytes with specific indicator cells in which the virus will stimulate syncytium formation. These techniques require specific equipment and reagents and are only available in certain central laboratories. An additional method to identify BLV infectivity involves the inoculation of test material into BLV-negative sheep or cattle which are then kept in isolation and examined serologically. This is not only the most sensitive virological test method available but it can be used to test a large volume of inoculum, even if contaminating microorganisms are present. However, this is an expensive and time-consuming procedure that would only be applied in special situations. From the foregoing discussion it must be concluded that the virological test methods will find only limited application in practical field situations.

The diagnosis of clinical EBL (lymphosarcoma) requires a pathological and serological investigation. The tissues submitted for histopathological study may be obtained by biopsy or at necropsy. The specimens should be collected in 10% formalin or other suitable tissue fixative and submitted to a veterinary pathologist. A diagnosis of lymphosarcoma may be made on such material, but the designation of EBL can only be applied if a serological test confirms the existence of a BLV infection. The serological test is necessary because SBL can occur (rarely) in adult cattle and the clinicopathological features of such cases may be indistinguishable from those of EBL. It is important to remember the

effect of BLV infection on hematologic values in cattle, i.e. that many animals will develop a persistent lymphocytosis. In these animals the examination of blood smears may also occasionally reveal the presence of abnormal lymphocytes. An uninformed diagnostician may try to use a high lymphocyte count and cellular morphologic evaluation to substantiate a diagnosis of lymphoblastic or lymphocytic leukemia. However, as we have described in the section on pathogenesis, these conditions are natural sequelae of BLV infection in many cattle and they cannot be directly correlated with the presence of lymphoid neoplasia.

PROPHYLAXIS AND CONTROL

There have been only a few attempts to develop a vaccine for the prevention of BLV infection in cattle. Thus far, the only immunogen which appears to be effective is a surface glycoprotein of the virus (Miller and Van der Maaten, 1980). Unfortunately this is also the antigen that is used in the serological tests that identify infected cattle. Therefore it is unlikely that such a vaccine would be acceptable to countries that are using the serological tests in a BLV control or eradication program.

When control measures for EBL were initiated, the hematological tests was the only method available for the identification of infected animals. This test was later replaced by the more sensitive and specific serological tests which also detect animals with asymptomatic BLV infection. Control usually requires slaughter of either an entire infected herd or just the individual infected animals, sometimes with their offspring. Because such practices are costly, efforts have been made to reduce the spread of BLV by segregating infected cattle from noninfected animals. This is a relatively effective control measure but is not used extensively, probably because of the extra labor costs required for maintenance of two separate herds, especially when milking cattle are involved. For trade purposes, it may be more practical to obtain BLV-free calves and keep them isolated from the infected herd. The easiest way to obtain such calves is to select those born to noninfected dams. In some herds, however, the prevalence of BLV infection may be so high that this is not feasible. In these situations there are several possibilities for obtaining virus-free calves: (1) allow the calves to nurse their dams and then use a virological test to detect which calves are infected; (2) allow the calves to nurse their dams, wait until colostral antibody wanes (4–6 months of age) and then use serological tests to identify noninfected calves; (3) feed the calves colostrum from BLV-negative dams so serological tests can be used immediately to identify calves that were either infected in utero or during the birth process. The last two methods are probably the most practical ones (Van der Maaten and Miller, 1979).

Another suggested method for securing BLV-negative calves from infected cows is to use embryo transfer. Research in this area is limited, but reports thus far indicate that such an approach would be successful. The relative costs of using embryo transfer compared to calf rearing methods have not been assessed (Bouillant et al., 1981; Eaglesome et al., 1982; Olson et al., 1982).

Regarding the role of veterinarians in BLV control, the need for good hygienic practices when blood sampling, vaccinating, and performing surgical procedures is obvious, because any transfer of blood (or other material that may contain lymphocytes) involves a risk of spreading the virus. The veterinarian should also educate his client regarding these practices in everyday management of the herd. The possible role of insects as mechanical blood vectors should also be emphasized (pest control). It is important to recognize that these efforts are merely going to reduce the risk of exposure for noninfected animals; unless the BLV carriers are removed from the premises there will

be continual transmission of BLV in the herd and some level of infection will be maintained.

REFERENCES

Bendixen, H.J., 1965. Bovine enzootic leukosis. Adv. Vet. Sci. Comp. Med., 10: 129–204.

Bouillant, A.M.P., Ruckerbauer, G.M., Eaglesome, M.D., Samagh, B.S., Singh, E.L., Hare, W.C.D. and Randall, G.C.B., 1981. Attempts to isolate bovine leukemia and bovine syncytial viruses from blood, uterine flush fluid, unfertilized ova and embryos from infected donor cattle. Ann. Rech. Vét., 12: 385–395.

Burny, A., Bruck, C., Chantrenne, H., Cleuter, Y., Dekegel, D., Ghysdael, J., Kettmann, R., Leclercq, M., Leunen, J., Mammerickx, M. and Portetelle, D., 1980. Bovine leukemia virus: molecular biology and epidemiology. In: George Klein (Editor), Viral Oncology. Raven Press, New York, NY, pp. 231–289.

Burny, A., Bruck, C., Couez, D., Deschamps, J., Ghysdael, J., Kettmann, R., Mammerickx, M., Marbaix, G. and Portetelle, D., 1984. Enzootic bovine leukemia: its relevance as a model system for human T-cell leukemia. In: R.C. Gallo, M.E. Essex and L. Gross (Editors), Human T-cell Leukemia/Lymphoma Virus. Cold Spring Harbor Laboratory, New York, NY pp 17–24.

Burridge, M.J., 1981. The zoonotic potential of bovine leukemia virus. Vet. Res. Commun., 5: 117–126.

Crespeau, F., Sarat, J.P., Vuillaume, A., Levy, D. and Parodi, A.L., 1978. A two-years sero-epidemiological survey of bovine leukemia virus (BLV) infection in a high incidence area of the south-west of France. Ann. Rech. Vét., 9: 747–754.

Eaglesome, M.D., Mitchell, D., Betteridge, J.J., Singh, E.L., Samagh, B.S. and Hare, W.C.D., 1982. Transfer of embryos from bovine leukemia virus-infected cattle to uninfected recipients: Preliminary results. Vet. Rec., 111: 122–123.

Ferrer, J.F., 1980. Bovine lymphosarcoma. Adv. Vet. Sci. Comp. Med., 24: 1–68.

Ferrer, J.F. and Piper, C.E., 1981. Role of colostrum and milk in the natural transmission of bovine leukemia virus. Cancer Res., 41: 4906–4909.

Ferrer, J.F., Marshak, R.R., Abt, D.A. and Kenyon, S.J., 1978. Persistent lymphocytosis in cattle: its cause, nature and relation to lymphosarcoma. Ann. Rech. Vét., 9: 851–857.

Hugoson, G., 1967. Juvenile bovine leukosis. Acta Vet. Scand., Suppl. 22: 1–106.

Kettmann, R., Mammerickx, M., Portetelle, D., Gregoire, D. and Burny, A., 1984. Experimental infection of sheep and goat with bovine leukemia virus: localization of proviral information in the target cells. Leukemia Res., 8: 937–944.

Lucas, M.H. and Roberts, D.H., 1982. Transmission of bovine leukosis virus (BLV). Curr. Top. Vet. Med. Vet. Sci., 15: 264–266.

Meiron, R., Brenner, J., Gluckman, A., Avraham, R. and Trainin, 1985. Humoral and cellular responses in calves experimentally infected with bovine leukemia virus (BLV). Vet. Immunol. Immunopathol., 9: 105–114.

Miller, J.M. and Van der Maaten, M.J., 1977. Use of glycoprotein antigen in the immunodiffusion test for bovine leukosis virus antibodies. Eur. J. Cancer, 13: 1369–1375.

Miller, J.M. and Van der Maaten, M.J., 1980. The biology of bovine leukemia virus infection in cattle. Cold Spring Harbor Conf. Cell Proliferation, 7: 901–909.

Mussgay, M., Dietzschold, B., Lorenz, R., Matheka, H.-D., Matthaeus, W., Straub, O.C., Weiland, F., Wilesmith, J.W., Frenzel, B. and Kaaden, O.R., 1980. Some properties of bovine leukemia virus, its use in seroepidemiological studies, and eradication of the disease from infected herds. Cold Spring Harbor Conf. Cell Proliferation, 7: 911–925.

Olson, C., Rowe, R.F. and Kaja, R., 1982. Embryo transplantation and bovine leukosis virus: preliminary report. Curr. Top. Vet. Med. Vet. Sci., 15: 361–369.

Ressang, A.A., Mastenbroek, N. and Quak, J., 1982. Studies on bovine leukosis IX. Excretion of bovine leukosis virus. Zentralbl. Veterinärmed. B, 29: 137–144.

Straub, O.C., 1981. Enzootic bovine leukosis. In: E.P.J. Gibbs (Editor), Virus Diseases of Food Animals, Vol. 1, International Perspectives. Academic Press, New York, NY, pp. 693–718.

Thiry, L., Sprecher-Goldberger, S., Jacquemin, P., Cogniaux, J., Burny, A., Bruck, C., Portetelle, D., Cran, S. and Clumeck, N., 1985. Bovine leukemia virus-related antigens in lymphocyte cultures infected with AIDS-associated viruses. Science, 227: 1482–1484.

Thurmond, M.C., Maden, C.B. and Carter, R.L., 1985. Cull rates of diary cattle with antibodies to bovine leukemia virus. Cancer Res., 45: 1987–1989.

Van der Maaten, M.J. and Miller, J.M., 1979. Appraisal of control measures for bovine leukosis. J. Am. Vet. Med. Assoc., 175: 1287–1290.

Van der Maaten, M.J., Miller, J.M. and Schmerr, M.J.F., 1981. Effect of colostral antibody on bovine leukemia virus infection of neonatal calves. Vet. Res., 42: 1498–1500.

Yamamoto, S., Onuma, M., Kodama, H., Mikami, T. and Izawa, H., 1984. Suppression of natural cytotoxic activity of lymphocytes from cattle and sheep during the progress of bovine leukosis. Vet. Microbiol., 9: 105–111.

Chapter 40

Maedi-Visna Virus

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INTRODUCTION

Maedi and visna are two disease manifestations in sheep, both caused by a virus belonging to the lentivirus subgroup of retroviruses. Maedi is an Icelandic word meaning dyspnea and is used for a slow progressive interstitial pneumonia; the term visna (meaning wasting) is used for a slow progressive inflammatory disease of the central nervous system resulting in paresis or paralysis. These diseases, together with pulmonary adenomatosis and scrapie, were classified by Sigurdsson (1954, 1962) as slow infections when he formulated his criteria of this special group of infectious diseases. The criteria for slow infections were:

— A very long period of latency (meaning incubation period) lasting months or years.

— A rather regular progressive course after clinical signs have appeared, usually ending in serious disease and death.

— Sigurdsson's third criterion, namely limitation of the infection to a single host species and lesions in only one organ or tissue system, has since been modified in the light of more recent knowledge, as he had anticipated.

In this chapter only the essential features of maedi-visna will be presented. For further information the reader may consult several reviews dealing with the virus and the infection (Gudnadóttir, 1974; Haase, 1975; Pálsson, 1976; Thormar, 1976; De Boer and Houwers, 1979; Pétursson et al., 1979; Dawson, 1980; Georgsson et al., 1980; Narayan et al., 1983; Nathanson et al., 1983; Haase, 1986; Cheevers and McGuire, 1988; Narayan and Clements, 1989).

VIRUS PROPERTIES

The maedi-visna virus (MVV) exhibits typical retrovirus morphology (Harter, 1976). The fully formed virion has a diameter of approximately 100 nm and contains an electron-dense nucleoid (about 30–40 nm). Between the internal nucleoid and the outer membrane an internal structure can sometimes be seen in electron micrographs. The membrane is covered by knobs about 10 nm in length, most readily seen with negative staining. Electron microscopy of infected tissue culture cells shows that the assembly of virions is completed by budding from the cytoplasmic membrane (Fig. 148). MVV has a buoyant density of 1.15–1.16 g/ml in sucrose, the sedimentation coefficient is about 600 S and the isoelectric point of the virion is 3.8 (Haase, 1975).

The virus is readily inactivated by ethyl ether, chloroform, formaldehyde, ethanol and phenol and by treatment with trypsin. It is stable at − 50°C for months and relatively resistant to repeated freezing and thawing. Incubation

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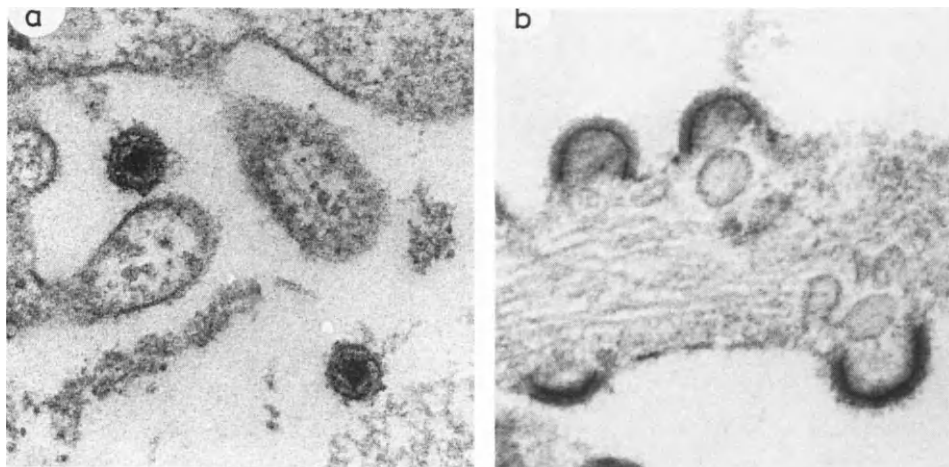
Fig. 148. MVV in cell culture, × 80000: (a) extracellular virions (arrows), an electron-dense core surrounded by two membranes, spikes on the surface; (b) virus budding from the plasma membrane.

at 50°C in 1% serum inactivated 90% of the infectivity in 10–15 minutes, as does storage at 4°C for 4 months. The infectivity is relatively stable at pH values between 5.1 and 10 (Thormar, 1965).

The structural proteins of MVV are quite similar in general to those of other lentiviruses, except for certain differences in the glycoproteins (Haase and Baringer, 1974). A major virus component (40% of the virion mass) consists of a single protein of 25–30000 daltons (p30) which is a constituent of the virus core; a glycoprotein of an apparent mass of 135000 daltons (gp135) is thought to be associated with the virus surface knobs. These two proteins form the two major precipitation lines in immunodiffusion tests with sera from infected sheep. The envelope of MVV has been reported to contain neuraminic acid. The virus does not seem to be hemagglutinating but will inhibit hemagglutination by influenza virus (August et al., 1977).

Several strains of MVV have been isolated in various countries. They cross-react strongly in various serological tests, e.g. immunodiffusion, CF and ELISA. The major internal antigen p30 of various strains seems identical or strongly cross-reactive, but different isolates of virus can vary in neutralization tests, even isolates from the same animal.

The basic biological features of MVV are quite similar to those of other animal lentiviruses and the human lentivirus HIV, although immunodeficiency is not seen in the ovine disease (Pétursson et al., 1989).

EPIZOOTIOLOGY

MVV infections are found in sheep only. Although a related virus can cause disease in goats (caprine arthritis-encephalitis) there is little evidence to show that sheep will contract the infection from goats under natural conditions. Studies in Norway and Australia indicate that the goat disease may exist for a number of years in a region without being transmitted to sheep. It is unclear to what extent visna–maedi infection of sheep will be transmitted to goats under natural conditions. MVV has been transmitted to goats by parenteral injections and the goat virus to sheep (Banks et al., 1983). It has not been possible to infect small laboratory animals. The virus will grow in cultured cells from several species, including bovine and human cells (Thormar, 1976). No evidence for laboratory infection of humans has ever been found. There are no known reservoir hosts or vectors.

There seems to be a widespread geographic distribution of maedi or closely related diseases of sheep (Pálsson, 1976). The pulmonary disease has been

known by different names in various countries, lungers or ovine progressive pneumonia in the USA, zwoegerziekte in the Netherlands, Graaf–Reinet disease in South Africa and la bouhite in France. It has also been found in Germany, Belgium, Spain, Italy, Greece, Hungary, Bulgaria, Switzerland, Israel, Kirgizia in the Soviet Union, Canada, Kenya, India and Peru. In the last two decades the disease has been introduced with imported sheep to Denmark, Norway, Sweden, Great Britain and Morocco. In many cases the imported sheep were of the Texel breed.

Together with pulmonary adenomatosis (jaagsiekte), visna and maedi were brought into Iceland with the importation of Karakul sheep from Halle in Eastern Germany in 1933 (Pálsson, 1976). The diseases were not known in the Karakul flock. In Iceland the local breed, primitive short-tailed hill-sheep, were extremely susceptible to the new diseases. In the beginning losses due to maedi were overshadowed by those due to jaagsiekte in areas where the two diseases coexisted, since jaagsiekte has a shorter incubation period and clinical course. Losses due to maedi gradually increased over several years and reached 15–30% annually in some flocks. Clinical signs of visna were seen in the southwestern part of Iceland but not in the northeastern part, where maedi spread from a separate focus. This may indicate that genetic differences in the virus and/or in the sheep population may determine which manifestation of the infection is dominant. In most other countries the neurological signs of visna have not been apparent, although brain lesions have been found in maedi-infected flocks by histological surveys (Ressang et al., 1966). The incidence of maedi varies widely between countries and individual flocks. Serological tests may show from a few percent up to 80–90% of adult sheep to be infected. Annual losses due to clinical maedi have been much lower generally than in Icelandic flocks, but information on the economic consequences of the infection is difficult to obtain. The infection has come to play an increasing role in trade with live sheep, especially when they are to be imported. Serological screening of flocks is required by many countries before import licences are granted, since experience shows that flocks may be infected even if clinical cases have not been seen. The initial spread of the infection is often insidious and the infection may have spread widely before any clinical cases are detected, especially where it is customary to cull ewes at a relatively early age. Husbandry methods such as housing of sheep may facilitate the spread of the disease.

PATHOGENESIS

Most of the studies on the pathogenesis of visna have been done by infecting sheep intracerebrally with virus. From field studies it is evident that the virus spreads laterally, probably by the respiratory route. Crowding definitely favors the spread of the infection. However, where the infection is enzootic the most important route of infection seems to be from ewe to lamb with colostrum (De Boer et al., 1979). There are conflicting reports concerning transmission to the fetus in utero but it rarely seems to occur under natural conditions (Hoff-Jørgensen, 1977; De Boer et al., 1979). The virus is found in the lungs, CNS, spleen and lymph nodes, salivary glands and in white blood cells. The mammary gland is infected and virus has been isolated from milk. No evidence for transmission with semen has been obtained, but rams have been known to spread the infection by transmission to ewes (Krogsrud and Udnes, 1978). In experimental visna there is severe restriction of virus replication in various tissues (Haase et al., 1977). Many cells contain the viral genome in DNA provirus form as demonstrated by in situ hybridization with virus-specific probes. In a smaller fraction of cells the DNA is transcribed into RNA, but cells

containing virus protein detected by usual methods such as IF are rare. It is difficult to demonstrate budding or fully formed virus in the CNS by EM (Georgsson et al., 1977). The reasons for this restriction of virus replication in vivo are unknown, but it is apparently not due to the immune responses of the host.

The production of lesions in visna seems to be immunologically mediated, probably by a CMI response to virus antigen on infected cells (Pétursson et al., 1979). The exact mechanism of pathogenesis of the pulmonary lesions in maedi is unknown, but some features of the pathological lesions speak for an immunopathological process operating in the lungs also. An important fact is that the infected animals rarely, if ever, rid themselves of virus. Each infected animal continues to harbor the virus and remains a constant threat to any uninfected contact animal.

DISEASE SIGNS

The first clinical symptom of *maedi* is failure to thrive. The affected animals tend to lag behind and show signs of respiratory distress when flocks are driven to pasture. Later an increased rate of respiration is observed even at rest. Coughing is not prominent and there is no discharge from the nostrils. This is of help in differential diagnosis, since pulmonary adenomatosis, another virus-induced lung disease of sheep, is characterized by coughing fits and copious discharges of fluid from the nostrils. Gradually the sheep become cachectic in spite of good appetite and lie down most of the time in the terminal phase of the disease. The clinical course may last for months or even more than a year but is apparently accelerated when the sheep are exposed to stress such as lambing and lactation. Clinical maedi is rarely seen in sheep younger than 3–4 years (Pálsson, 1976).

The clinical picture of *visna* is dominated by neurological symptoms. The affected sheep tend to lag behind the flock when driven due to lameness of one or both hindlegs. Weight loss is usual in spite of a healthy appetite. Gradually the paresis of the hindlegs progresses whereas the front legs are usually not affected, at least not before the final stage of the disease. Sometimes a slight tremor of the head and of the muscles around the mouth is observed and occasionally blindness. The course of the clinical phase may last for several months or even years but ends in prostration and death.

Maedi and visna may coexist in the same flock or even in the same individual sheep, but maedi is by far the most common clinical manifestation. Only in some flocks in Iceland did visna dominate. Recently arthritis has been observed in flocks in the USA afflicted with progressive pneumonia (Oliver et al., 1981), and a chronic indurative mastitis is also associated with MVV infection (Van der Molen et al., 1985).

PATHOLOGY

In *maedi* the lesions primarily affect the lungs and the mediastinal lymph nodes (Georgsson et al., 1976). The lungs are increased in size, often weighing 2–3 times more than normal (Fig. 149). They do not collapse when the thoracic cavity is opened and exhibit a dull greyish-blue or greyish-brown color rather than the normal pink. They are compact to the touch — somewhat like a rubber sponge but less elastic. Usually in advanced maedi the whole lung is diffusely affected. On the cut surface the tissue is rather dry and homogenous in color and consistency. The tracheobronchial and mediastinal lymph nodes are enlar-
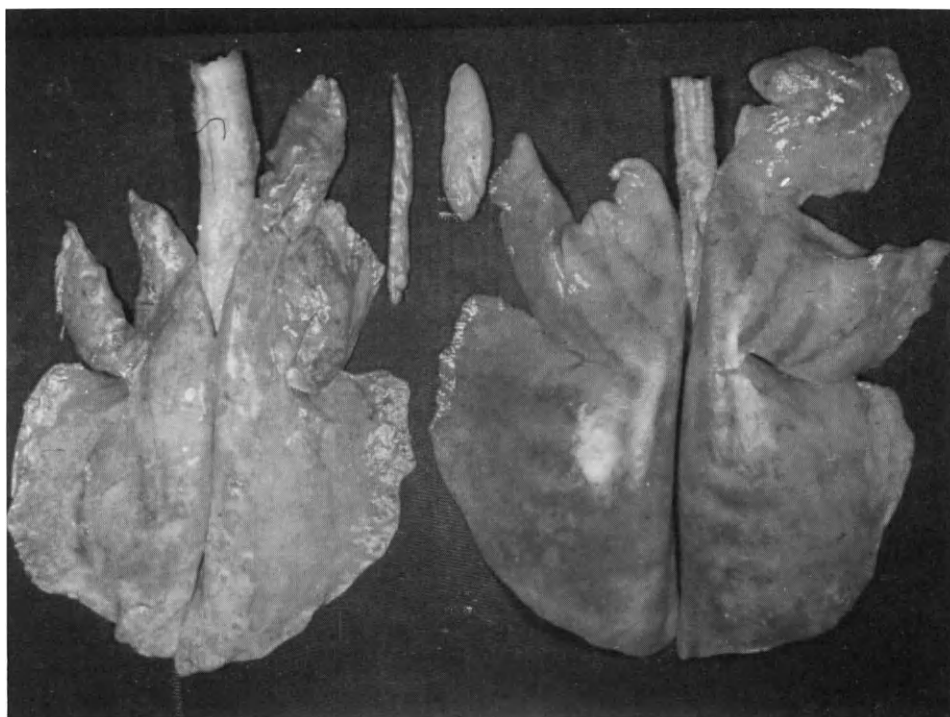
Fig. 149. Maedi lungs (1370 g) with enlarged mediastinal lymph node on the right; normal lung (450 g) with corresponding mediastinal lymph node on the left.

ged, often 3–5 times the normal size. Microscopically, the lesions consist of diffuse thickening of the interalveolar septa, which gradually encroaches upon alveoli, leading to their obliteration with almost total consolidation in some areas. The thickening of the alveolar septa is mainly caused by cellular infiltrates consisting of lymphocytes, monocytes and/or macrophages in varying proportions, with a few plasma cells. Polymorphonuclear leukocytes are not a feature of these chronic inflammatory changes. Hypertrophy and/or hyper-
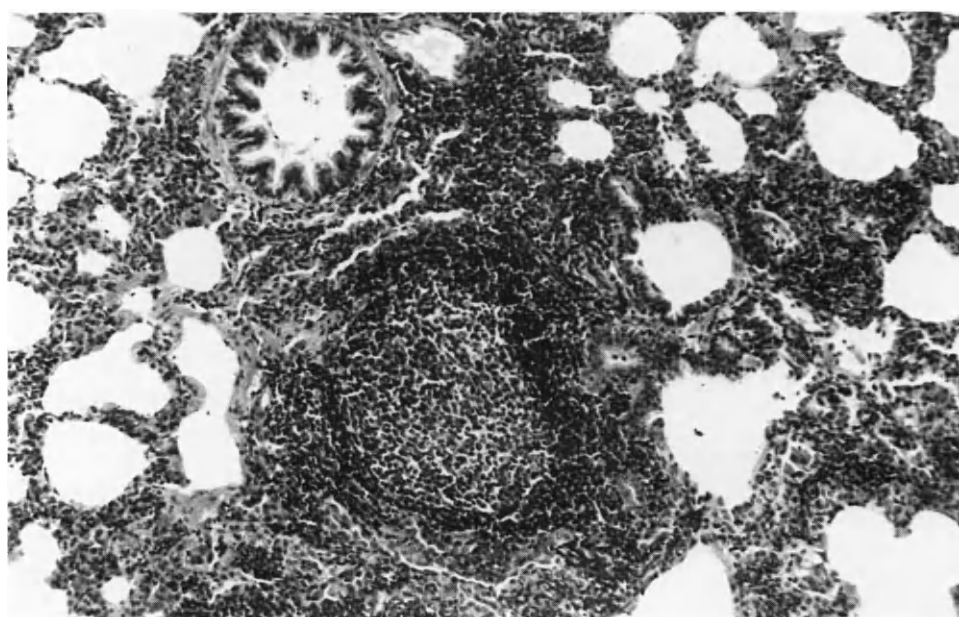
Fig. 150. Maedi: chronic interstitial inflammation with peribronchial lymphoid hyperplasia; Masson-trichrome, × 120.

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plasia of smooth muscles in interalveolar septa is a usual feature of the disease; there is an increase in reticular fibers but fibrosis is not prominent. A regular and typical finding is a sometimes very prominent accumulation of lymphoid tissue, peribronchially and often perivascularly as well. This is often in the form of regular lymph follicles with active germinal centers (Fig. 150). Transformation of the squamous epithelium of the alveoli into cuboidal cells if often noted, and sometimes irregular cyst-like cavities lined with cuboidal epithelium are seen. They may bear a superficial resemblance to lesions found in pulmonary adenomatosis. The lesions of maedi are not pathognomonic, and early lesions may be difficult to evaluate especially in lungs infested with lungworms. Other lung diseases of sheep such as pneumonia due to other virus infections and mycoplasma may pose problems in differential diagnosis. Therefore it is desirable to use virus isolation and serological tests to confirm the diagnosis of maedi.

The lesions of *visna* have mainly been studied in experimental infections, but they seem to correspond closely to those found in the natural disease (Georgsson et al., 1976). The cardinal features are chronic inflammation of the brain and the spinal cord. The lesions are mainly distributed around the ventricular system affecting both grey and white matter (Fig. 151). Subependymal and perivascular infiltrates of lymphocytes, monocytes and/or macrophages with some plasma cells are typical, and an astrocytic reaction is seen bordering the lesions. The inflammatory changes are sometimes quite severe with liquefaction necrosis and occasionally coagulative necrosis. Destruction of myelin
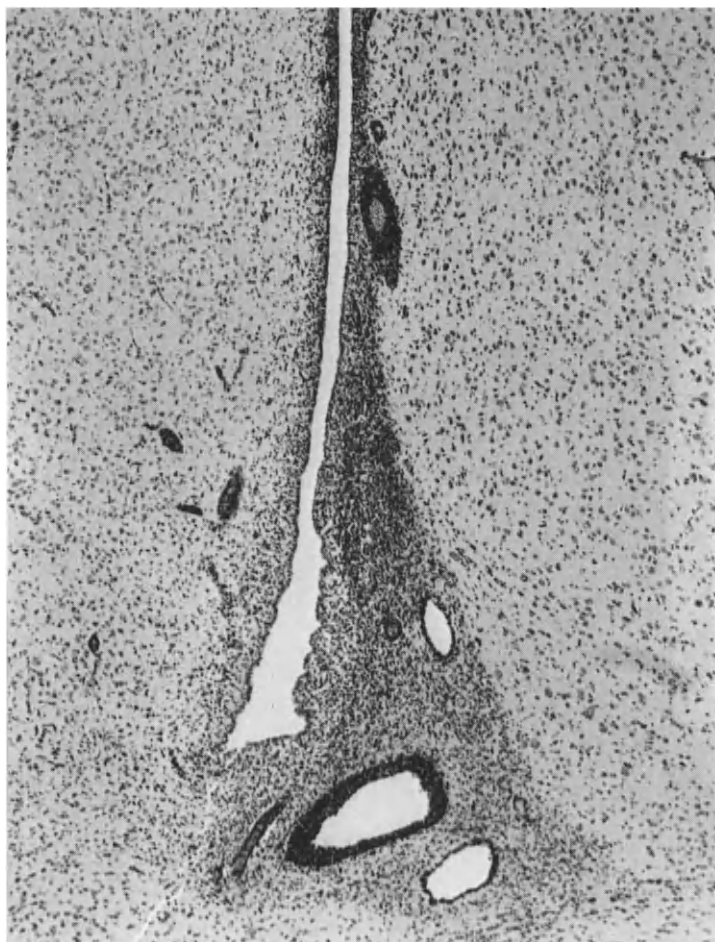
Fig. 151. Visna: periventricular inflammation around the lateral ventricle with perivascular inflammatory cuffs extending into the adjacent neuroparenchyma; H & E, × 50.

together with the axons is seen in the necrotic foci, but primary demyelination is also observed where the myelin sheath is selectively destroyed, leaving the axons intact (Georgsson et al., 1982). This feature of primary demyelination makes experimental visna an interesting model in research on demyelinating diseases of humans of unknown etiology, e.g. multiple sclerosis. The meninges of the brain and the spinal cord also show inflammatory changes and the choroid plexus is affected in varying degree, sometimes with massive proliferation of lymphoid tissue with active germinal centers. There is little destruction of neurons, and no changes have been seen in the peripheral nervous system. The lesions in visna resemble caprine arthritis–encephalitis and other virus encephalitides of animals, e.g. demyelinating canine distemper.
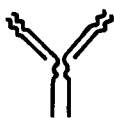
IMMUNE REACTION

Sheep respond to infection with MVV by production of antibodies, which can be detected by means of various tests. The VN test was the first to be used and can be carried out mixing sera and virus and assaying for infectivity in cultured sheep cells. Although a useful tool in experimental work, the VN test has several drawbacks in diagnostic work. It is time-consuming and expensive, and due to antigenic variation of MVV false negative results may occur. In experimental animals neutralizing antibodies are usually first detected 2–3 months after infection, increasing in titer for a few weeks and then remaining fairly constant for months or even years (Pétursson et al., 1976). CF tests have also been useful in detecting antibodies in infected animals (Gudnadóttir and Kristinsdóttir, 1967). Some laboratories have obtained results with this test, others report difficulties due to anticomplementary reactions of sera and difficulty in producing good antigens consistently. It is very important to test all samples in parallel with control antigen from uninfected cell cultures. The first antibody rise after experimental infection can be detected after 3–4 weeks using CF, and the titers tend to remain constant for years after they have reached a plateau. More positive animals are detected in an infected herd using CF rather than VN tests (Gudnadóttir et al., 1968). Both neutralizing and CF antibodies seem to belong to the IgG1 subclass of sheep immunoglobulins but represent two distinct subpopulations of IgG1 molecules, since the neutralizing antibodies can be separated from the CF fixing molecules by ion-exchange chromatography (Pétursson et al., 1979).

Antibodies against the glycoprotein antigen and the p30 core antigen can be demonstrated by AGID (Cutlip et al., 1977). More than 90% of sheep experimentally infected with visna show a precipitation line against the glycoprotein antigen; a lower proportion shows a second line corresponding to the p30 antigen (Pétursson et al., 1979). This test is simple to carry out, but different preparations of antigen may contain varying relative amounts of glycoprotein and p30 antigens. The ELISA with semipurified virus has given good results and may become the test of choice (Houwers et al., 1982). Some laboratories have experienced difficulties with high backgrounds in sera from normal animals. IF tests using virus-infected cells in culture have also been used successfully to measure virus-specific antibodies (Thormar, 1969).

A comparative study has shown indirect ELISA, indirect IF and AGID tests to be equally good in detecting maedi-specific antibodies in sera from infected sheep (Dawson et al., 1982).

Evidence of CMI in experimentally infected sheep has been obtained by use of lymphocyte blast transformation. The response seems to be irregular and to vary with time (Sihvonen, 1984); this test is therefore not suitable for diagnostic work.

There is evidence from experimentally infected sheep that the CNS lesions are immunologically mediated at least in the early stages of the disease. The lesions could be prevented by installing an immunosuppressive regime, and there is some evidence that they are produced by a CMI response to virus-induced antigens in the brain (Nathanson et al., 1976). The pathogenesis of the primary demyelination seen in visna has not been fully explained, but findings of the viral genome and antigen in glial cells, astrocytes and especially oligodendrocytes (Stowring et al., 1985) indicate that an immune attack on infected oligodendrocytes may be responsible for the primary demyelination.

LABORATORY DIAGNOSIS

In living animals the only practical laboratory diagnostic methods are tests for antibodies against the virus. ELISA and AGID tests are best considered as herd tests since individual animals may show false negative results, for example if they are recently infected. If visna is suspected a spinal tap may reveal an increase in the number of cells in the spinal fluid, consisting mainly of macrophages and lymphocytes with a few plasma cells (Georgsson et al., 1979). In maedi-affected sheep anemia and hypergammaglobulinemia have been reported, but these are neither specific nor constant features of the disease. Attempts to isolate virus from the buffy coat of the blood are occasionally successful but this technique is hardly practical as a diagnostic test (Pétursson et al., 1976).

At autopsy the histopathology of the lungs and the brain may reveal typical lesions, but these may be minimal or absent in some animals. Then virus isolation in cell culture remains the final means for detection of infection.

The best methods of isolating virus are explant cultures in plasma clot and cocultivation of cells from infected animals with susceptible cell strains such as cultivated plexus choroideus or testis cells (Pétursson et al., 1976). Most cultivated sheep cells are permissive for MVV in vitro. The CPE consists of rounded individual cells with filiform extensions and multi-nucleated giant cells that arise by fusion of several virus-infected cells. Eventually the cell sheet degenerates and is lysed. Some field strains of maedi or the progressive pneumonia form of the disease in the USA are not strongly cytolytic and do not produce higher titers in vitro (Quérat et al., 1984). The isolates can be characterized as belonging to the MVV group by their typical morphology in EM, the presence of reverse transcriptase and most simply by serological comparison with known MVV strains. The spleen, lungs, mediastinal lymph nodes and the plexus choroideus are the organs of choice in attempts to isolate virus (Pétursson et al., 1976).

PROPHYLAXIS AND CONTROL

There is no vaccine available for protection against maedi and visna. Limited attempts to produce such a vaccine have not been successful. The most important preventive measures are thorough serological tests of the flocks of origin when sheep are aquired from another herd. In Iceland the disease was eradicated by ruthless stamping out of flocks in areas where the disease had been detected and replacement of the sheep with lambs brought in from unaffected regions. Strict control of movement of live animals with division of the country into fenced-off quarantine areas led to the complete eradication of the disease in 1965. In the process, pulmonary adenomatosis was also eradicated. Today Iceland is the only country where the disease has been eradicated. These

drastic measures were necessary because of the heavy losses experienced and the lack of information about the disease at that time (Pálsson, 1976).

These methods are impractical in most other countries, but other less severe measures are available. They all require extensive, repeated serological testing and restrictions of movement of sheep between flocks. Some rely upon culling of seropositive animals with repeated tests over a period of several years (Krogsrud et al., 1978). These methods are slow and most suitable when the proportion of infected animals in a herd is low (Hoff-Jørgensen, 1978). In heavily infected flocks the main route of infection is from the mother ewe to her lamb by means of the colostrum. Attempts to clean up infected herds by removing lambs from the ewe at birth and raising them artificially, in isolation from the infected flock, have been successful in the Netherlands and in the USA (Houwers et al., 1983; Molitor et al., 1979).

REFERENCES

August, M.J., Harter, D.H. and Compans, R.W., 1977. Characterization of visna virus envelope neuraminic acid. J. Virol., 22: 832–834.

Banks, K.L., Adams, D.S., McGuire, T.C. and Carlson, J., 1983. Experimental infection of sheep by caprine arthritis–encephalitis virus and goats by progressive pneumonia virus. Am. J. Vet. Res., 44: 2307–2311.

Cheevers, W.P. and McGuire, T.C., 1988. The lentiviruses: maedi/visna, caprine arthritis–encephalitis, and equine infectious anemia. Adv. Vet. Res., 34: 189–215.

Cutlip, R.C., Jackson, T.A. and Laird, G.A., 1977. Immunodiffusion test for ovine progressive pneumonia. Am. J. Vet. Res., 38: 1081–1084.

Dawson, M., 1980. Maedi/visna: A review. Vet. Rec., 106: 212–216.

Dawson, M., Biront, P. and Houwers, D.J., 1982. Comparison of serological tests used in three state veterinary laboratories to identify maedi–visna virus infection. Vet. Rec., 111: 432–434.

De Boer, G.F. and Houwers, D.J., 1979. Epizootiology of maedi/visna in sheep. In: D.A.J. Tyrrell (Editor), New Perspectives in Clinical Microbiology. Vol. 2. Aspects of Slow and Persistent Infections. Martinus Nijhoff Publishers, The Hague, pp. 198–220.

De Boer, G.F., Terpstra, C., Houwers, D.J. and Hendriks, J., 1979. Studies in epidemiology of maedi/visna in sheep. Res. Vet. Sci., 26: 202–208.

Georgsson, G., Nathanson, N., Pálsson, P.A. and Pétursson, G., 1976. The pathology of visna and maedi in sheep. In: R.H. Kimberlin (Editor), Slow Virus Diseases of Animals and Man North-Holland, Amsterdam, pp. 61–96.

Georgsson, G., Pálsson, P.A., Panitch, H., Nathanson, N. and Pétursson, G., 1977. The ultrastructure of early visna lesions. Acta Neuropathol. (Berl.), 37: 127–135.

Georgsson, G., Martin, J.R., Pálsson, P.A., Nathanson, N., Benediktsdóttir, E. and Pétursson, G., 1979. An ultrastructural study of the cerebrospinal fluid in visna. Acta Neuropathol. (Berl.), 48: 39–43.

Georgsson, G., Nathanson, N., Pétursson, G., Martin, J.R. and Pálsson, P.A., 1980. The Immunopathogenesis of visna. In: F. Clifford Rose and P.O. Behan (Editors), Animal Models of Neurological Disease. Pitman Medical, London, pp. 378–389.

Georgsson, G., Martin, J.R., Klein, J., Pálsson, P.A., Nathanson, N. and Pétursson, G., 1982. Primary demyelination in visna. An ultrastructural study of Icelandic sheep with clinical signs following experimental infection. Acta Neuropathol. (Berl.), 57: 171–178.

Gudnadóttir, M., 1974. Visna–maedi in sheep. Progr. Med. Virol., 18: 336–349.

Gudnadóttir, M. and Kristinsdóttir, K., 1967. Complement-fixing antibodies in sera of sheep affected with visna and maedi. J. Immunol., 98: 663–667.

Gudnadóttir, M., Gislason, G. and Pálsson, P.A., 1968. Studies of natural cases of maedi in search for diagnostic laboratory methods. Res. Vet. Sci., 9: 65–67.

Haase, A.T., 1975. The slow infection caused by visna virus. Curr. Top. Microbiol. Immunol., 72: 101–156.

Haase, A.T., 1986. Pathogenesis of lentivirus infections. Nature, 322: 130–136.

Haase, A.T. and Baringer, J.R., 1974. The structural polypeptides of RNA slow viruses. Virology, 57: 238–250.

Haase, A.T., Stowring, L., Narayan, O., Griffin, D. and Price, D., 1977. Slow persistent infection caused by visna virus: Role of host restriction. Science, 195: 175–177.

Harter, D.H., 1976. The detailed structure of visna-maedi virus. In: R.H. Kimberlin (Editor), Slow

Virus Diseases of Animals and Man. North-Holland, Amsterdam, pp. 45–60.

Hoff-Jørgensen, R., 1977. Slow virus infections with particular reference to maedi–visna and enzootic bovine leukaemia. Vet. Sci. Comm., 1: 251–263.

Hoff-Jørgensen, R., 1978. Maedi–visna in Danish sheep. Bull. Off. Int. Epizoot., 89: 527–530.

Houwers, D.J., Gielkens, A.L.J., Schaake, Jr., J., 1982. An indirect enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to maedi–visna virus. Vet. Microbiol., 7: 209–219.

Houwers, D.J., De Boer, G.F., Schaake, Jr., J. and König, C.D.W., 1983. Maedi–visna control in sheep. I. Artificial rearing of colostrum-deprived lambs. Vet. Microbiol., 8: 179–185.

Krogsrud, J. and Udnes, H., 1978. Maedi (progressive interstitial pneumonia in sheep). Diagnosis, epizootiology, prevention and control programme in Norway. Bull. Off. Int. Epizoot., 89: 451–464.

Molitor, T.W., Light, M.R. and Schipper, I.A., 1979. Incidence of ovine progressive pneumonia in the North Dakota State University sheep flocks, determined by agar-gel immunodiffusion. Farm Res., 37: 24–26.

Narayan, O. and Clements, J.E., 1989. Biology and pathogenesis of lentiviruses. J. Gen. Virol., 70: 1617–1639.

Narayan, O., Strandberg, J.D., Griffin, D.E., Clements, J.E. and Adams, R.J., 1983. Aspects of the pathogenesis of visna in sheep. In: C.M. Mims (Editor), Viruses and Demyelinating Diseases. Academic Press, London, pp. 125–140.

Nathanson, N., Panitch, H., Pálsson, P.A., Pétursson, G. and Georgsson, G., 1976. Pathogenesis of visna. II. Effect of immunosuppression upon early central nervous system lesions. Lab. Invest., 35: 444–451.

Nathanson, N., Georgsson, G., Lutley, R., Pálsson, P.A. and Pétursson, G., 1983. Pathogenesis of visna in Icelandic sheep. Demyelinating lesions and antigenic drift. In: C.A. Mims (Editor), Viruses and Demyelinating Diseases. Academic Press, London, pp. 111–124.

Oliver, R.E., Gorham, J.R., Parish, S.F., Hadlow, W.J. and Narayan, O., 1981. Ovine progressive pneumonia: Pathologic and virologic studies on the naturally occurring disease. Am. J. Vet. Res., 42: 1554–1559.

Pálsson, P.A., 1976. Maedi and visna in sheep. In: R.H. Kimberlin (Editor), Slow Virus Diseases of Animals and Man. North-Holland, Amsterdam, pp. 17–43.

Pétursson, G., Nathanson, N., Georgsson, G., Panitch, H. and Pálsson, P.A., 1976. Pathogenesis of visna. I. Sequential virologic, serologic and pathologic Studies. Lab. Invest., 35: 402–412.

Pétursson, G., Martin, J.R., Georgsson, G., Nathanson, N. and Pálsson, P.A., 1979. Visna. The biology of the agent and the disease. In: D.A.J. Tyrrell (Editor), New Perspectives in Clinical Microbiology, Vol. 2. Aspects of Slow and Persistent Infections. Martinus Nijhoff Publishers, The Hague, pp. 198–220.

Pétursson, G., Pálsson, P.A. and Georgsson, G., 1989. Maedi/visna in sheep: host–virus interactions and utilization as a model. Intervirology, 30 (Suppl. 1): 36–41.

Quérat, G., Barban, V., Sauze, N., Filippi, P., Vigne, R., Russo, P. and Vitu, C., 1984. Highly lytic and persistent lentiviruses naturally present in sheep with progressive pneumonia are genetically distinct. J. Virol., 52: 672–679.

Ressang, A.A., Stam, F.C. and De Boer, G.F., 1966. A meningoleukoencephalomyelitis resembling visna in Dutch zwoeger sheep. Path. Vet., 3: 401–411.

Sigurdsson, B., 1954. Observations on three slow infections of sheep. Br. Vet. J., 110: 255–270.

Sigurdsson, B., 1962. Atypically slow infectious diseases. In: Livre Jubilaire du Dr. Ludo van Bogaert. Acta Med. Belg., pp. 738–753.

Sihvonen, L., 1984. Late immune responses in experimental maedi. Vet. Microbiol., 9: 205–213.

Stowring, L., Haase, A., Pétursson, G., Georgsson, G., Pálsson, P.A., Lutley, R., Ross, R. and Szuchet, S., 1985. Detection of visna virus antigens and RNA in glial cells in foci of demyelination. Virology, 14: 311–318.

Thormar, H., 1965. A comparison of visna and maedi viruses. I. Physical, chemical and biological properties. Res. Vet. Sci., 6: 117–129.

Thormar, H., 1969. Visna and maedi virus antigen in infected cell cultures studied by the fluorescent antibody technique. Acta Pathol. Microbiol. Scand., 75: 296–302.

Thormar, H., 1976. Visna–maedi virus infection in cell cultures and in laboratory animals. In: R.H. Kimberlin (Editor), Slow Virus Diseases of Animals and Man. North-Holland, Amsterdam, pp. 97–114.

Van der Molen, E.J., Vecht, V. and Houwers, D.J., 1985. A chronic indurative mastitis in sheep, associated with maedi–visna infection. Vet. Q., 7: 112–119.

Chapter 41

Caprine Arthritis–Encephalitis Virus

O. NARAYAN and L.C. CORK

INTRODUCTION

Caprine arthritis–encephalitis (CAE) is a disease complex that affects domestic goats of all ages and probably all breeds. In young animals the disease is characterized by paresis which progresses rapidly to paralysis, and in adults by a high prevalence of chronic persistent arthritis and mastitis. On rare occasions, adults also develop a slowly progressive fatal pneumonia–encephalitis characterized clinically by dyspnea and progressive paralysis. The entire disease complex is caused by a lentivirus that shares antigenic determinants with the etiologic agent of maedi–visna of sheep, but is distinct from all other retroviruses. Serologic surveys show that the CAE virus (CAEV) is widely disseminated in goat herds in North America, Europe and Australia and that the incidence of infection in individual herds may be close to 100% even though only a small fraction of these animals shows clinical disease. Except for goats, and possibly sheep, there is no other known host for this virus.
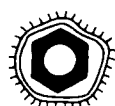
VIRUS PROPERTIES

The CAE agent is a nononcogenic retrovirus and a member of the lentiviruses (Clements et al., 1980; Crawford et al., 1980a; Sundquist, 1981). The polypeptides of biological significance in the virion are structurally very similar to those of maedi–visna virus (MVV) (Clements et al., 1980). They consist of a single glycoprotein with a molecular weight of 140 000 daltons (gp140) which is located in the virus envelope and is the antigen normally responsible for eliciting virus neutralizing antibodies. The other proteins in the virion are nonglycosylated; the p28 protein is in the core of the virion, making up 75% of its mass. This protein shares many antigenic sites with its counterpart of MVV (Narayan et al., 1980).

The virus is usually cultivated in early subcultures of outgrowths of synovial membranes of goats. The growth cycle of the virus extends from 15 to 20 h in these cells. Most of the mature virions are produced by budding from the cell membrane, although large numbers of particles are produced by budding from the endoplasmic reticulum into intracytoplasmic vesicles. Replication of the virus in cell cultures is accompanied by cell fusion (multinucleated giant cell formation), which is the major cytopathic effect of the virus (Narayan et al., 1980; Klevjer-Anderson and Cheevers, 1981). These cytopathic effects have not been seen in lesions in diseased animals.

The lentiviruses of goats and sheep are susceptible to most forms of chemical inactivation because of the fragile lipoprotein envelope of the virion. Thus,

detergent soap solutions, phenolic and quaternary ammonium compounds, in addition to formalin and hypochlorite would all be effective as disinfectants. The agent is inactivated by heat at 56°C for 10 min. Since colostrum and milk from infected animals usually contain virus-infected cells, these products should therefore be used with caution to prevent oral infection of kids. Presumably these products can be sterilized by adequate heating procedures.

EPIZOOTIOLOGY

CAE is a relatively new disease whose epizootiology is still being evaluated. Epizootiological studies have been complicated by the fact that a single virus can cause three different diseases in different age groups of the host. These include: (1) a rapidly progressive leukoencephalitis and pneumonia in newborn and young goats (Cork et al., 1974b); (2) chronic arthritis (Crawford et al., 1980) and mastitis (Cork and Narayan, 1980) in adult goats; (3) a sporadic slowly progressive pneumonia–encephalitis in adult goats (Sundquist et al., 1981). The neurological disease in kids occurs in animals less than 4 months of age and may have an incidence of up to 20%. The arthritic disease may have a prevalence of 10–20%. Most infected animals do not show clinical disease, although many may have pathologic lesions (L.C. Cork and T.B. Crawford, unpublished data, 1974). Using the immunodiffusion technique which recognizes the p28 polypeptide of this virus, Crawford and Adams (1981) examined over 1000 serum samples from different areas in the USA and showed that 81% of the animals were infected. Similar high levels of infection have been reported in goat herds in several countries in Europe.

Further complications in evaluating the epizootiology of CAEV comes from the fact that the agent is closely related serologically to ovine MVV, posing the question whether the goat disease could be caused by the sheep agent or vice versa. The recent demonstration that arthritis and mastitis also occur as a complication, albeit rarely, of maedi–visna of sheep (Oliver et al., 1981) keeps the issue of cross-species infection alive. No other animal species is known to be susceptible to infection with this virus.

One of the main methods of dissemination of CAEV may be via the feeding of infected colostrum, as previously established for the spread of MVV in sheep (De Boer et al., 1979). Crawford and Adams (1981) showed that kids which were deprived of colostrum remained free of infection for at least 2 years, whereas kids which had nursed or were fed colostrum showed evidence of infection by day 60. Transplacental infection of fetuses by viremic maternal blood does not seem to be a major method of virus spread. Exposure of newborn kids to virus in colostrum is probably responsible for the high incidence of leuko encephalitis seen in this age group of animals.

The fact that many individual goat herds show almost 100% infection can be explained by a combination of factors. Firstly, the infection in goats persists, often for life, and thus these animals become important virus shedders either via colostrum or via respiratory secretions. Secondly, infection is usually subclinical and thus virus spread is insidious. In this respect the mastitis which develops in mature goats is even more insidious than the arthritis, because the inflammatory changes occurring in the mammary glands cannot be seen except by histological examination. Thirdly, dairy goat husbandry usually includes hand rearing of kids. This fosters virus dissemination by bottle feeding of infected milk as well as by physical contact due to handling of kids. Further horizontal spread of virus probably occurs among kids, because these animals are often housed together in close quarters.

PATHOGENESIS

CAEV and MVV have three biological properties which lend themselves perfectly to persistent infection. First, these viruses can sequester themselves in host cells by integrating their proviral DNA into host cell DNA. In this integrated state the agents elude immunologic elimination. Second, these viruses replicate preferentially in macrophages (Narayan, 1980; Narayan et al., 1982). Field strains of the viruses replicate most efficiently in macrophage cultures, and the highest concentration of infectivity in pathologically affected tissues are found in the extracts of macrophages in these target tissues. Third, these viruses do not usually induce virus neutralizing antibodies (Narayan, 1980; Griffin and Narayan, 1981; Klevjer-Anderson and McGuire, 1982). Virus replication can thus continue independent of any control by the humoral immune system.

Exposure of goats and sheep to their respective retroviruses results in a persistent systemic infection during which virus infects and replicates at minimal levels in cells of the monocyte–macrophage series. Animals develop a low-level viremia in which virus is associated almost exclusively with monocytes. Monocytes are derived from bone marrow and circulate in blood for a variable period before localizing in particular tissues where they mature into resident macrophages (Blusse van oud Alblas and Van Furth, 1979). Studies on infected monocytes show that they contain the viral genome but no infectious particles. When these cells are cultivated in vitro they mature into macrophages, which then produce infectious particles (Narayan et al., 1983a). The inference from these studies is that monocytes may be important in dissemination of infection to various tissues when the cells "home" for specific tissues. Support for this comes from studies which showed that in naturally or experimentally infected animals virus could be isolated from monocytes and also from macrophages in spleen, lung, synovium and, in lactating does, from macrophages in the colostrum and milk (Kennedy-Stoskopf et al., 1985).

Examination of animals with clinical disease — goats and sheep in the encephalitic phase of disease (leukoencephalitis and visna, respectively) or sheep with dyspnea (maedi, progressive pneumonia, zwoegerziekte) or goats with arthritis — all show the typical low-grade systemic infection, i.e. nonproductive infection in monocytes. However, homogenates of tissues with lesions nearly always contain cell-free infectivity, indicating that virus replication occurs at an amplified level in the these tissues in contrast to the low level of replication in other tissues. Further, comparison of the amount of infectivity in extracts of maedi lung tissue with the infectivity in extracts of alveolar macrophages lavaged from the same lungs showed similar titers. This suggests that the macrophages in the organs with lesions may be the site for increased virus replication (Narayan et al., 1983b).

Newborn goats are particularly susceptible to infection of the CNS with CAEV. However, the isolation of virus from the CNS of persistently infected adult animals is relatively rare except from animals with encephalitic disease (Narayan et al., 1983b). This applies even to animals with chronic arthritis and/or pneumonia. Experimental infection corroborates the rarity of CNS involvement, since adult sheep and goats inoculated via parenteral routes develop typical systemic infection without infection of the CNS. Intracerebral inoculation of virus, however, results in virus replication in brain with acute leukoencephalitis, simulating the encephalitic phase of naturally occurring disease. Moreover, i.c. inoculation results also in the low-grade systemic infection seen after parenteral inoculation. This would be expected, given the "spill over" of i.c. inocula into the blood stream.

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Whereas macrophages are important cells for virus replication, the immune reponses of infected animals play other crucial roles in pathogenesis. As mentioned above, the failure of the animal to develop antiviral antibodies cripples a major arm of the host defense system and thus indirectly potentiates persistent infection. The cellular immune response plays an even greater role in the disease because of the strong implication for cell-mediated injury. The basic pathologic lesion in all affected organ systems consists of infiltration and proliferation of mononuclear cells together with necrosis of cells normally populating this tissues, e.g. oligodendroglia in brain, synovial cells in joints (Cork et al., 1974a; Adams et al., 1980b). Administration of antithymocyte serum to both sheep (Nathanson et al., 1976) and goats (Cork and Narayan, 1980b) during the early phases of infection prevent development of these lesions without affecting virus recovery. Present indications for pathogenesis therefore are that infected monocytes bring virus to target organs and cause infection with virus replication in indigenous cells in these organs. Viral antigens produced by these cells elicit the CMI response which causes the immunopathologic consequences.

DISEASE SIGNS

CAEV is associated with at least three disease syndromes in nature. The most frequently encountered is the arthritic disease of adult goats (Crawford and Adams, 1981). The arthritis is insidious in onset and usually slowly progressive over a period of months to years. In a survey of animals in Washington State these authors noted that disease may appear suddenly and remain static, whereas in others it is either rapidly progressive or chronic and slowly progressive over a period of several years. Joints, bursae and tendon sheaths are targets for disease, but the commonest and most severe localization is usually seen in the carpal ("knee") joints, giving rise to the colloquial description of "big knee" disease. The hock and stifle joints are involved to a lesser extent. In severe cases, swelling of the atlantal and supraspinous bursae also become evident. Disease is usually seen in animals between 2 and 9 years of age and, generally, the longer the disease duration the greater the tissue damage. Affected goats are usually thin to emaciated and have long, coarse, poor hair coats (Fig. 152). Lactating does show a reduction in milk production. Depending on disease severity, affected animals show signs varying from lameness and reluctance to walk, to severe restriction in joint movements, leading to recumbency. Decreased locomotion often leads to overgrown distorted hooves which compound gait abnormalities (Fig. 153), whereas recumbency sometimes leads to skin ulceration, abscessation and osteomyelitis. In some cases long-standing severe arthritis becomes compounded by mineralization, and ligaments and tendons may rupture, resulting in inability to stand.

Except in septic complications, arthritic animals are afebrile, alert and maintain healthy appetites despite severe loss in physical condition. In early cases of arthritis there is an excess of synovial fluid which causes distention of joint capsules, bursal spaces and tendon sheaths. The fluid is less viscous than normal; it is blood tinged or straw colored and highly cellular. Whereas normal synovial fluid may have a mononuclear cell count of less than 500 cells/mm^3, fluid from arthritic joints may have up to 500 000 cells/mm^3. These cells consist of lymphocytes, plasma cells and macrophages. Fibrin, fragments of synovial membrane and mineralized debris are often found in the fluid.

The histopathological changes seen in natural and experimentally induced arthritis have been described by Adams et al. (1980b), Crawford et al. (1980a, b), and Cork and Narayan (1980a). The earliest lesions are characterized by
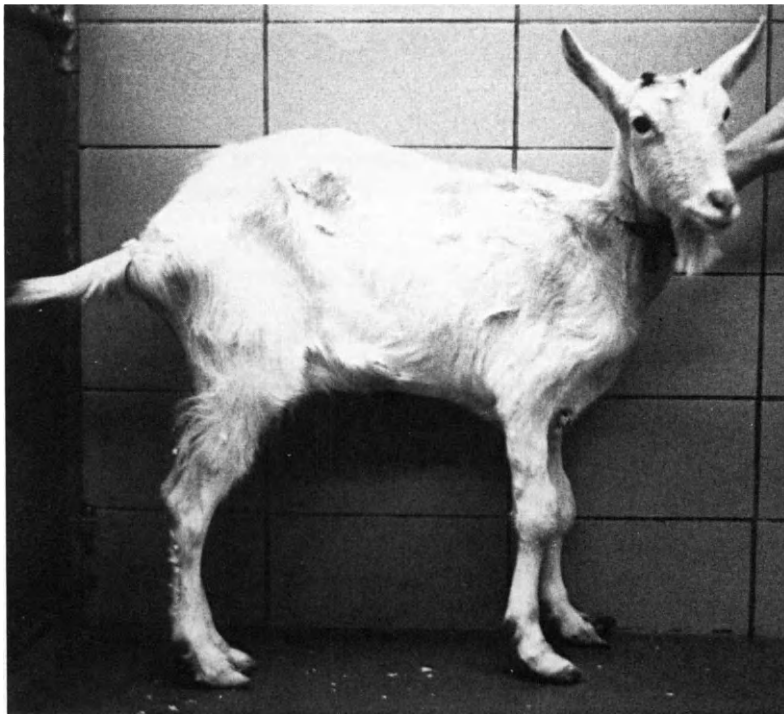
Fig. 152. Goat with CAE showing typical unthrifty appearance and arthritis in carpal joints.

proliferation of the synovial membrane with development of villous projections into the lumen of the joint. There is an accompanying inflammatory response characterized by perivascular cuffs which are made up of the same type of mononuclear cells as seen in the synovial fluid (Fig. 154).

Later lesions consist of necrosis of the collagen structures, including the

Fig. 153. Carpal joints of goat with CAE are enlarged and may show mineralization of periarticular tissue radiographically; hooves are overgrown and misshapen due to decreased activity and abnormal gait.

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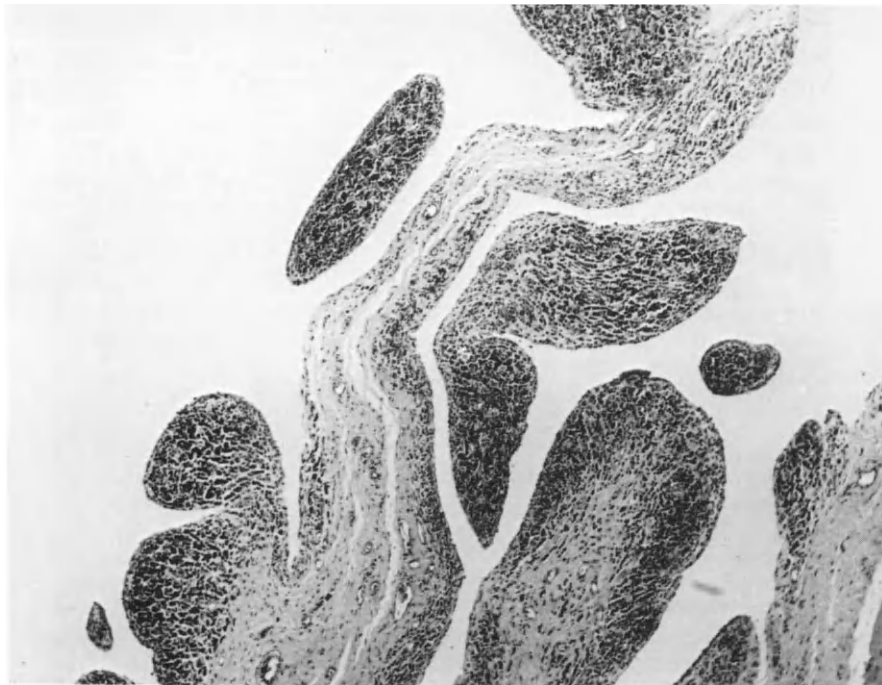
Fig. 154. Synovial lining from carpus of goat infected with CAEV; synovial villi are hypertrophied and thickened by increased fibrous connective tissue; a massive infiltration of lymphocytes, macrophages and plasma cells is within the villi and is most prominent in perivascular regions; × 40.
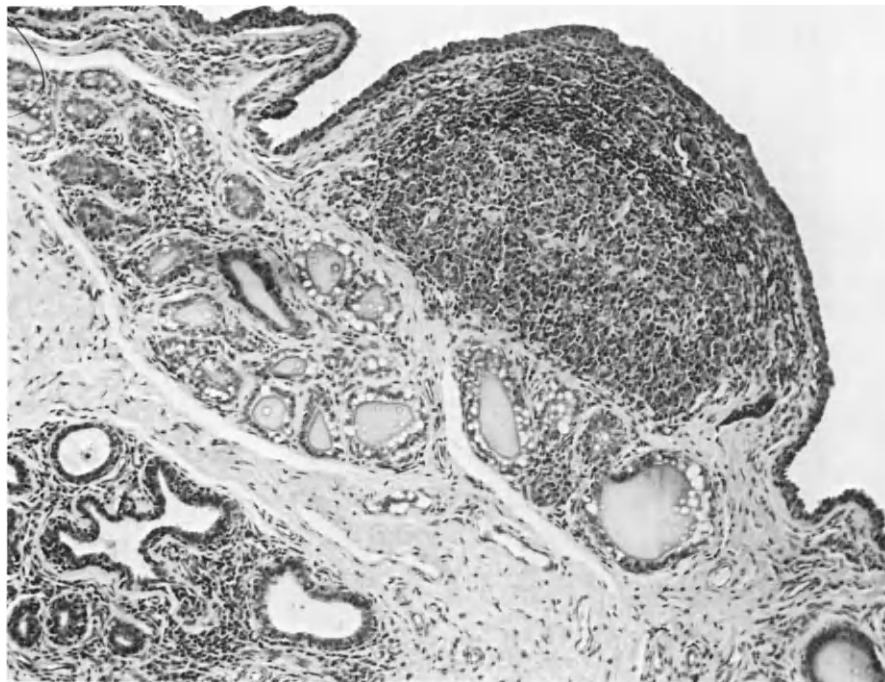
Fig. 155. Mammary gland from doe infected with CAEV; the glandular tissue has focal, interstitial and perivascular infiltrations of mononuclear cells; a germinal center has formed adjacent to the duct; × 175.

joint capsules, tendons and tendon sheath, ligaments and bursae. Inflammatory cells in these late lesions often become organized into germinal centers with more extensive perivascular cuffing in which plasma cells are predominant. Severe lesions consist of extensive necrosis compounded by mineralization of necrotic debris and subsequent fibrosis. It is at this stage of cartilagenous degeneration that tendons and ligaments rupture.

In addition to arthritis, lactating does often have chronic inflammatory lesions in the mammary glands (Cork and Narayan, 1980a). These lesions consist of marked lymphoid hyperplasia, which in the chronic cases become lymphoid nodules as seen in the joints. Many of these nodules are arranged adjacent to the lactiferous ducts (Fig. 155). The inflammatory cells are of the same type as seen in the arthritic joints. Both the arthritic and mastitic lesions can be reproduced when young adult goats are inoculated with CAEV (Cork and Narayan, 1980a).

Rapidly progressive neurological disease of young goats is the other major disease syndrome associated with this virus infection (Cork et al., 1974b). Kids are usually 2–4 months old when the earliest signs of disease are recognized, and they progress rapidly to paralysis within the following 2–4 weeks. The earliest clinical sign is often posterior paresis or ataxia or "weakness in hind quarters", which could be either uni- or bilateral. A few may show circling signs or appear blind. Within weeks, posterior paresis may progress to include the front legs. Kids with tetraparesis become recumbent and are usually killed. If given good nursing care, however, these animals recover from the acute disease and survive, although with residual paralysis. Torticollis and deviations of the head have been observed in these survivors. Goats with encephalomyelitis usually have rough hair coats and may have muscle atrophy. However, they are afebrile, alert and maintain a good appetite (Fig. 156). Survivors of the neurologic disease develop arthritis similar to that seen in adult goats (L.C. Cork, unpublished data, 1974).

Fig. 156. Goat kid with CAE and posterior paresis; affected kids are alert and afebrile and will continue to nurse or graze; note atrophy of muscles in hind leg.

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PATHOLOGY

Pathologic changes consist of demyelinating encephalomyelitis (Cork et al., 1974a). During the acute stage of disease animals develop a pleocytosis, in which up to 100000 mononuclear cells/mm³ can be found in the cerebrospinal fluid. Starting at the pial or ependymal surfaces of the brain, lesions consisting of perivenous accumulations of mononuclear cells expand into the parenchyma (Fig. 157). This inflammation is accompanied by destruction of myelin and proliferation by glial cells. Lesions in the spinal cord follow the same pattern, radiating from the perivascular regions and giving rise to demyelination. In the late stages inflammatory cells may become organized into germinal centers or focal accumulations of glial cells. Paralyzed animals may have residual demyelinated areas with no further evidence of ongoing inflammation. These large foci of demyelination also occur in the CNS of goats with late onset disease in adults (see below) and also in sheep with visna (Georgsson et al., 1982).

Kids with neurological disease also often develop a transient subclinical interstitial pneumonia and the lungs fail to collapse completely at autopsy. Histologically, these lesions consist of lymphoid hyperplasia with frequent nodular arrangements, thickening of alveolar septa and pronounced infiltration by macrophages. These lesions are typical of chronic interstitial pneumonia which, in sheep, is called progressive pneumonia in the USA, maedi in Iceland and zwoegerziekte in the Netherlands (Dawson, 1980).

The third disease manifestation in infected goats is a sporadic slowly progressive paralytic disease in adult animals ("goat visna") (Sundquist et al., 1981). Goats 1–5 years old are affected and disease starts as a slight abberration in gait which progresses over a course of months to paresis and paralysis. In some cases the disease stabilizes for a few weeks and then relapses with a progressive course. Similar to young animals with rapid onset leukoencephalomyelitis and adults with arthritis, these adult goats with paralytic di-
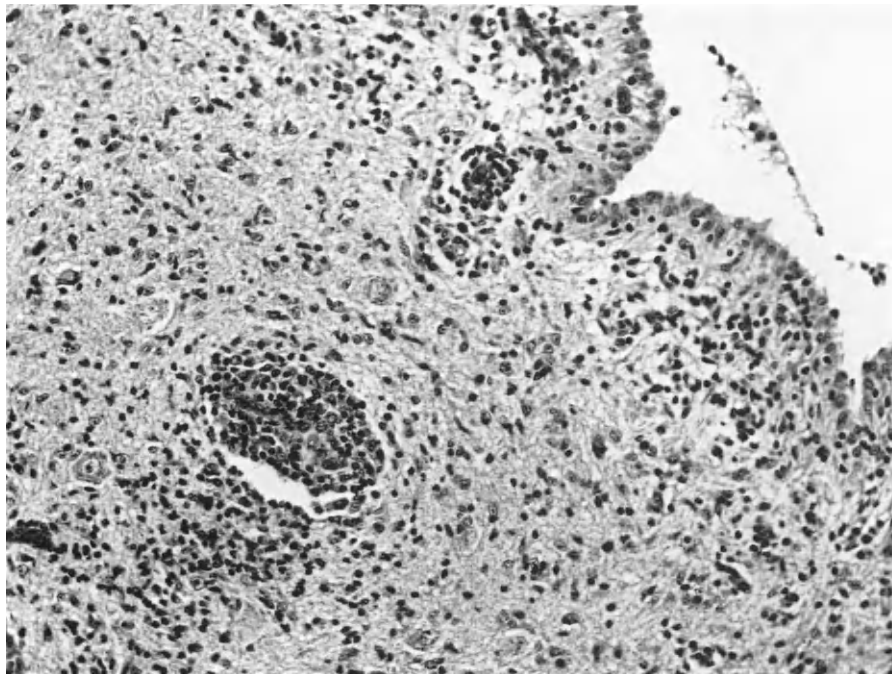
Fig. 157. Brainstem from goat kid with CAE; ependymal lining is hyperplastic or disrupted, adjacent neuroparenchyma is diffusely infiltrated with mononuclear cells; broad perivascular cuffs of macrophages, lymphocytes and plasma cells are prominent; × 200.

sease are afebrile, alert and maintain a good appetite. They have normal withdrawal and menace reflexes. Arthritis has not been reported with this disease.

The basic pathologic lesions in these adult goats are similar to those seen in young animals. Spread of inflammation from the ventricles, glial proliferation and myelin destruction all accompany necrotizing malacic lesion. The localization of lesions to periventricular areas of the mid-brain, cerebellum, pons, medulla and spinal cord are typical of the disease in goats and remarkably similar to lesions of visna in sheep (Sigurdsson et al., 1962). Furthermore, these animals often have interstitial pneumonia of the same type seen in kids with rapid onset leukoencephalomyelitis and in sheep with maedi.

Presumably this disease complex in adult goats is similar to that described by Stavrou et al. (1969) in Germany. However, in the latter case the "granulomatous" pathologic changes noted in the brain were also seen in the eyes in a form of iridocyclitis. It is possible that these optic lesions could represent disease initiated by a particular strain of CAEV in yet another target organ. However, the viral specificity of these lesions has not been confirmed. It should be noted, moreover, that none of the currently known strains of CAEV or MVV cause lesions in the optic system or peripheral nervous system.
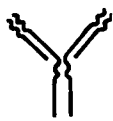
IMMUNE REACTION

Both the humoral and cellular immune responses to the virus play important roles in the infection, but neither seems to have any benefit to the host. Very little is known about IgM antibody responses of goats and sheep to these lentiviruses. However, large amounts of virus-specific antibody are present in the IgG class and these are found specifically in the IgG_1 fraction (Griffin and Narayan, 1981; Johnson et al., 1983). The affinity of these antibodies for gp140, and their lack of virus neutralization, has not been evaluated but the antibodies bind strongly to the p28 polypeptide. This antibody binding can be seen in a variety of tests including immunoprecipitation, AGID, ELISA, IF and CF tests. As mentioned above, these immunoglobulins do not neutralize infectivity of the virus. The reasons for this are not understood. In contrast, the glycoprotein of laboratory-adapted Icelandic visna virus elicits excellent neutralizing antibodies; however, these antibodies are specific for Icelandic visna virus and do not neutralize field isolates of MVV or the CAE agents. Furthermore, the neutralizing antibodies to the Icelandic visna virus serve for the selection of antigenic mutants of this virus, providing an explanation of antigenic drift, or variation, among these agents (Narayan et al., 1981).

Experimental infection of goats with CAEV results in appearance of the virus-specific, nonneutralizing antibodies in the serum a few weeks later. Levels of the antibody fluctuate with time but are present for life (Adams et al., 1980a). Virus-specific IgG1 antibodies have been found also in the cerebrospinal fluid of sheep infected with visna virus (Griffin et al., 1978b) and in synovial fluid of CAEV-associated arthritic goats (Johnson et al., 1983). Current data suggests that these antibodies are produced locally by plasma cells in the lesions. Whether these local antibodies are in the form of immune complexes and play a role in the disease process is not known. Presumably the colostrum and milk of infected does also contain antibodies. These antibodies do not enhance infectivity of virus in cell culture systems (O. Narayan, unpublished data, 1980). Whether they enhance infection in the animal is not known.

The cellular immune response in sheep infected with visna virus (Griffin et al., 1978a), maedi virus (Larsen et al., 1982), and goats infected with CAEV (Adams et al., 1980a) is virus specific. Using the phenomenon of virus-induced

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lymphocyte proliferation in vitro as an index of CMI responses, these investigators have shown that the CMI response correlates with the onset of encephalitis, pneumonia and arthritis, and persists as long as inflammation persists. Whether sensitized lymphocytes are important in the causation of the inflammatory disease cannot be answered directly because histocompatibility differences among the animals preclude adoptive immunization experiments. However, the beneficial effects of administration of antithymocyte serum to infected animals supports the concept that lesions are immunologically mediated. These studies prove at least that the virus does not cause primary cytopathic effects in tissues as it does in cell cultures.

Although CAEV and MVV have similar pathogenic mechanisms and share major antigenic determinants, the CAEV agent seems distinct. In competitive hybridization assays of radiolabeled RNA from the goat agent, Icelandic visna virus and progressive pneumonia virus, the CAEV RNA showed only 20% homology with the RNA of the sheep viruses (Roberson et al., 1982). Similar differences between the polypeptides of CAEV and the sheep viruses have been observed. However, more strains of CAEV need to be analyzed in these tests and the viruses should be compared with current field strains of MVV (rather than laboratory adapted strains) before the species specificity of the goat agent can be appreciated with certainty.

LABORATORY DIAGNOSIS

Since animals become persistently infected after exposure to the virus, demonstration of antiviral antibody indicates infection. The immunodiffusion technique is the test of choice for demonstration of antibody (Crawford and Adams, 1981) and is widely used in diagnostic laboratories in the USA and Europe. The ELISA technique is probably more sensitive but its efficacy is only now being evaluated (Adams et al., 1980a; Houwers et al., 1982). Single samples of freshly procured serum are all that is required for establishing serological diagnosis.

Virus isolation techniques can also be used for diagnostic purposes: 10–20 ml of blood are collected in anticoagulant solutions (heparin, Alsever's etc.) and sent to the laboratory in plastic syringes. Here the buffy coat cells are separated and cocultivated with normal goat synovial membrane cultures. The coculture is maintained for at least 2–3 weeks at 37°C and examined frequently for development of viral CPE. Alternatively, viral target tissues may be cultivated directly and the cellular outgrowths examined for CPE. Positive serological or virus isolation results are self explanatory for infection in the animal. However, since not all infected animals show clinical disease, identification of infection in clinically normal animals does not prognosticate eventual disease. These animals shed virus and would have to be culled from herds in any attempt to control the disease.

PROPHYLAXIS AND CONTROL

Not only do humoral antibodies fail to neutralize CAEV in cell culture, but hyperimmunization of goats and sheep with live or inactivated virus preparations does not protect these animals against subsequent infection with live virus (Narayan et al., 1984). Control measures therefore rest entirely on identification of infected animals by serological means and elimination of such animals from the herd. Present data suggests that formites and infected premises play minimal roles in maintaining the agent. Given the physical charac-

teristics of these agents and the virus life cycle, direct contact and/or feeding of infected milk would be the most important factor in virus dissemination. Although sexual transmission has not been proven, it is conceivable that infected macrophages on the surfaces of mucosal membranes could transfer during mating. Maintenance of closed herds with serological monitoring at regular intervals would therefore be the only sure method of keeping out infection by CAEV.

REFERENCES

Adams, D.S., Crawford, T.B., Banks, K.L., McGuire, T.G. and Perryman, L.E., 1980a. Immune responses of goats persistently infected with caprine arthritis–encephalitis virus. Infect. Immun., 28: 421–427.

Adams, D.S., Crawford, T.B. and Klevjen-Anderson, P., 1980b. A pathogenic study of early connective tissue lesions of viral caprine arthritis–encephalitis. Am. J. Pathol., 99: 257–271.

Blusse van oud Alblas, A. and Van Furth, R., 1979. Origin kinetics and characteristics of pulmonary macrophages in the normal steady state. J. Exp. Med., 149: 1504–1518.

Clements, J.E., Narayan, O. and Cork, L.C., 1980. Biochemical characterization of the virus causing leukoencephalitis and arthritis in goats. J. Gen. Virol., 50: 423–427.

Cork, L.C. and Narayan, O., 1980. The pathogenesis of viral leukoencephalomyelitis–arthritis of goats. Lab. Invest., 42: 596–602.

Cork, L.C., Hadlow, W.G., Gorham, J.R., Piper, R.C. and Crawford, T.B., 1974a. Pathology of viral leukoencephalomyelitis of goats. Acta Neuropathol., 29: 281–292.

Cork, L.C., Hadlow, W.J., Crawford, T.B., Gorham, J.R. and Piper, R.C., 1974b. Infectious leukoencephalomyelitis of young goats. J. Infect. Dis., 129: 134–141.

Crawford, T.B. and Adams, D.S., 1981. Caprine arthritis–encephalitis: Clinical features and presence of antibody in selected goat populations. J. Am. Vet. Med. Assoc., 178: 713–719.

Crawford, T.B., Adams, D.S., Cheevers, W.P. and Cork, L.C., 1980a. Chronic arthritis in goats caused by a retrovirus. Science, 207: 997–999.

Crawford, T.B., Adams, D.S., Sande, R.D., Gorham, J.R. and Hanson, J.B., 1980b. The connective tissue component of the caprine arthritis–encephalitis syndrome. Am. J. Pathol., 100: 443–450.

Dawson, M., 1980. Maedi–visna. A review. Vet. Rec., 106: 212–216.

De Boer, G.F., Terpstra, C., Houwers, D.J. and Hendriks, J., 1979: Studies in epidemiology of maedi–visna in sheep. Res. Vet. Sci., 26: 202–208.

Georgsson, G., Martin, J.R., Klein, J., Palsson, P.A. and Nathanson, N., 1982. Primary demyelination in visna. Acta Neuropathol. (Berl.), 57: 171–178.

Griffin, D.E. and Narayan, O., 1981. Immune responses of sheep to CNS infection with visna virus. In: J.E. Butler (Editor), The ruminant Immune System. Plenum Press, New York, p. 883.

Griffin, D.E., Narayan, O. and Adams, R.J., 1978a. Early immune responses in visna, a slow viral disease of sheep. J. Infect. Dis., 139: 340–350.

Griffin, D.E., Narayan, O., Bukowski, J., Adams, R.J. and Cohen, S.R., 1978b. The cerebrospinal fluid in visna, a slow viral disease of sheep. Ann. Neurol., 4: 212–218.

Houwers, D.J., Gielkens, A.L.J. and Schaake, J., 1982. An indirect enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to maedi–visna virus. Vet. Microbiol., 7: 209–219.

Johnson, G.C., Adams, D.S. and McGuire, T.C., 1983. Pronounced production of polyclonal IgG1 in the synovial fluid of goats with caprine arthritis–encephalitis virus infection. Infect. Immun., 41: 805–815.

Kennedy-Stoskopf, S., Narayan, O. and Strandberg, J.D., 1985. The mammary gland as a target organ for infection with caprine encephalitis arthritis virus. J. Comp. Pathol., 95: 609–617.

Klevjer-Anderson, P. and Cheevers, W.P., 1981. Characterization of the infection of caprine synovial membrane cells by the retrovirus caprine arthritis–encephalitis virus. Virology, 110: 113–119.

Klevjer-Anderson, P. and McGuire, T.C., 1982. Neutralizing antibody response to rabbits and goats of caprine arthritis–encephalitis virus. Infect. Immun., 38: 455–461.

Larsen, H.J., Hyllseth, B. and Krogsrud, J., 1982. Experimental maedi virus infection in sheep: Early cellular and humoral immune response following parenteral inoculation. Am. J. Vet. Res., 43: 379–383.

Narayan, O., Clements, J.E., Strandberg, J.D., Cork, L.C. and Griffin, D.E., 1980. Biological characterization of the virus causing leukoencephalitis and arthritis in goats. J. Gen. Virol., 50: 69–79.

Narayan, O., Clements, J.E., Griffin, D.E. and Wolinsky, J.S., 1981. Neutralizing antibody spectrum determines the antigenic profiles of emerging mutants of visna virus. Infect. Immun., 32: 1045–1050.

Narayan, O., Wolinsky, J.S., Clements, J.E., Strandberg, J.D., Griffin, D.E. and Cork, L.C., 1982. Slow virus replication: Role of macrophages in the persistence and expression of visna viruses of sheep and goats. J. Gen. Virol., 59: 345–356.

Narayan, O., Kennedy-Stoskopf, Sheffer, D., Griffin, D.E. and Clements, J.E., 1983a. Activation of caprine arthritis encephalitis virus expression during maturation of monocytes to macrophages. Infect. Immun., 41: 67–73.

Narayan, O., Strandberg, J.D., Griffin, D.E., Clements, J.E. and Adams, R.J., 1983b. Aspects of pathogenesis of visna in sheep. In: C.A. Mims (Editor), Symposium on Viruses and Demyelinating Diseases. Academic Press, New York, pp. 125–140.

Narayan, O., Scheffer, D., Griffin, D.E., Clements, J.E. and Hess, J., 1984. Lack of neutralizing antibodies to caprine arthritis encephalitis lentivirus in persistently infected goats can be overcome by immunization with inactivated *Mycobacterium* tuberculosis. J. Virol., 49: 349–355.

Nathason, N., Panitch, H., Pálsson, P.A., Pétursson, G. and Georgsson, G., 1976. Pathogenesis of visna. II. Effect of immunosuppression upon early central nervous system lesions. Lab. Invest., 35: 444–451.

Oliver, R.E., Gorham, J.R., Parish, S.F., Hadlow, W.T. and Narayan, O., 1981. Ovine progressive pneumonia: Pathologic and virologic studies on the naturally occurring disease. Am. J. Vet. Res., 42: 1554–1559.

Roberson, S.M., McGuire, T.C., Klevjer-Anderson, P., Gorham, J.R. and Cheevers, W.P., 1982. Caprine arthritis–encephalitis virus is distinct from visna and progressive pneumonia viruses as measured by genome sequence homology. J. Virol., 44: 755–758.

Sigurdsson, B., Palsson, P.A. and Van Bogaert, L., 1962. Pathology of visna. Acta Neuropathol., 1: 343–362.

Stavrou, D., Deutchlander, N. and Dahme, E., 1969. Granulomatous encephalomyelitis in goats. J. Comp. Pathol., 79: 393–396.

Sundquist, B., 1981. Goat visna virus: Isolation of a retrovirus related to visna virus of sheep. Arch. Virol., 68: 115–127.

Sundquist, B., Jonsson, L., Jacobson, S.O. and Hammerberg, K.E., 1981. Visna virus meningoencephalomyelitis in goats. Acta Vet. Scand., 22: 315–330.

Chapter 42

Jaagsiekte (Ovine Pulmonary Adenomatosis) Virus

D.W. VERWOERD

INTRODUCTION

Jaagsiekte was first recognized as an infectious disease in South Africa more than a century ago (Tustin, 1969; Verwoerd et al., 1985). Nevertheless, its viral etiology was elucidated only recently. For many years all attempts in various parts of the world to isolate the causative organism failed. The involvement of a latent virus was therefore generally suspected. When several groups isolated a herpesvirus, first from affected lungs and then by the spontaneous activation of a latent virus in cell cultures of tumor cells, it seemed to be the answer. Transmission studies, serological evidence and molecular hybridization studies eliminated the ovine herpesvirus as etiological agent, however (De Villiers and Verwoerd, 1980).

The first indication of a possible retrovirus involvement was the observation of particles possessing type C morphology in jaagsiekte lungs (Perk et al., 1971). This was followed by biochemical evidence for the presence of particles with reverse transcriptase activity in lung extracts (Perk et al., 1974) and the demonstration of morphologically typical retroviruses in cell cultures established from adenomatous lungs (Malmquist et al., 1972). However, in neither case were any transmission studies done and no attempt was made to exclude the possibility of the particles being maedi–visna-related retroviruses. Jaagsiekte and maedi–visna are now known to coexist in many countries and even to be present in the same animal, a phenomenon that caused much confusion in the early literature (Palsson, 1976; Markson et al., 1983; Snyder et al., 1983; Houwers and Terpstra, 1984).

Transmission of jaagsiekte with lung extracts containing reverse transcriptase activity was first reported by Martin et al. (1976) and confirmed by Perk (1982). Serial transmissions of the disease over a number of years in newborn lambs, using concentrated and purified virus as inoculum, and the demonstration of an inverse relationship between virus concentration and the incubation period of the disease constitutes the most convincing evidence that jaagsiekte is indeed caused by a retrovirus (Verwoerd et al., 1980).

VIRUS PROPERTIES

All attempts to cultivate the jaagsiekte retrovirus (JSRV) in a variety of cell cultures have been unsuccessful to date. Therefore, the viral properties reported to date were determined for virus isolated from lungs washes or tumor extracts. Partial purification was achieved by a combination of fluorocarbon treatment and density gradients or gel-filtration (Verwoerd et al., 1980, 1983).

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It was demonstrated, however, that the virus isolated in this way consists mainly of immune complexes with IgA, which could affect some of the properties reported (Verwoerd et al., 1983). Because of the lack of an in vitro culture system, all purification and characterization experiments with JSRV were carried out using reverse transcriptase activity as indicator of viral concentration. The characteristics of the viral transcriptase have been well documented (Herring et al., 1983).

The morphology of JSRV has been studied by EM both in thin sections of tumor tissue or viral pellets and by negative staining of purified virus (Payne et al., 1983). An average size of 104 nm for the mature virion was obtained with the various techniques.

Mature virions are almost homogeneously electron dense with a close-fitting envelope. A slightly eccentric nucleoid, usually round in shape, can sometimes be seen (Fig. 158). In a few particles penetrated by stain an intermediate layer, or inner coat, could be distinguished.

Fixed negatively stained virions often appear hexagonal in shape and the surface is covered with spikes (Fig. 158, insert). The spikes consist of a knob on a narrow spine and are 10–12 nm long.

The overall morphology of JSRV is typical for retroviruses. In finer detail and in morphogenesis, however, it can be distinguished from all the other known members of this family. Intracytoplasmic immature or A particles are not found in type C or in MVV-infected cells, where a crescent-shaped core is first seen at the cell surface at the start of the budding process. Type A particles of type B MMTV and type D retroviruses are less electron dense and have a double ring structure, which only seems to mature (condense) after budding.

The nucleoids of most retroviruses are centric, with MMTV and MVV being the exceptions in possessing eccentric core structures. JSRV is intermediate in having a slightly eccentric nucleoid. MVV cores have a very characteristic ovoid or conical shape with a clear intermediate layer and electron-lucent perinucleoidal space, both absent in the case of JSRV.
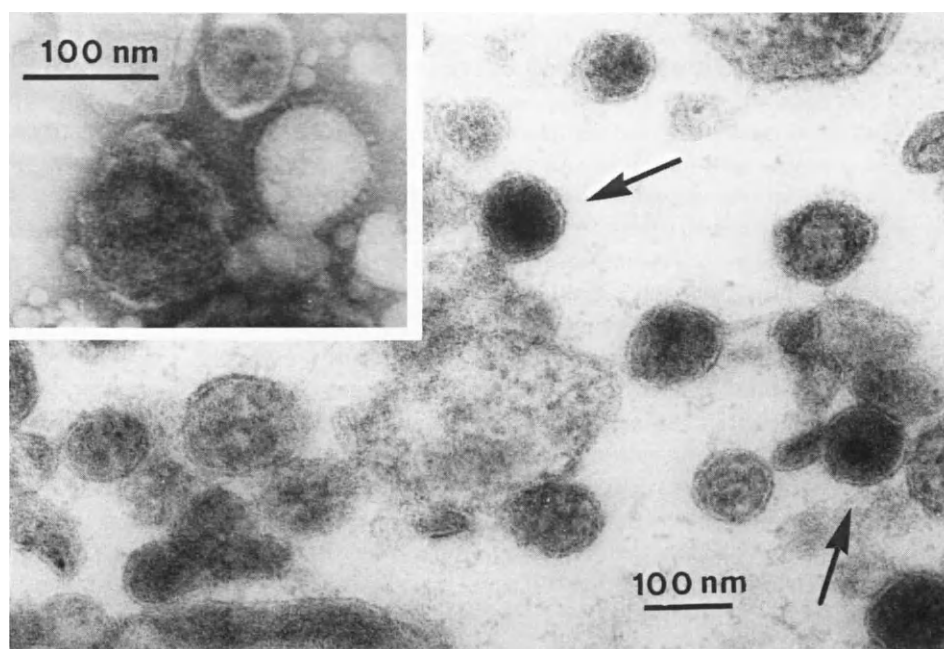
Fig. 158. Mature virions of JSRV showing an electron-dense inner structure with close fitting envelope and slightly eccentric nucleoid (arrows); positively stained section of a pellet of semi-purified virus from lung fluid. Insert: Negatively stained JSRV particle showing surface structure with spikes (courtesy A. Payne).

The surface structure of JSRV, as illustrated by negative staining, also differs significantly from the others. Only type B retroviruses possess a comparable spiked surface layer. Morphologically, therefore, JSRV seems to be most closely related to MMTV, which is perhaps not too suprising in view of the similarity of the tumors they produce.

Retrovirus capsids are complex in structure, consisting of a core particle or nucleoid, an intermediate layer and an envelope or outer coat. Each of these layers contains two or more polypeptides, bringing the total number of viral proteins to at least six. Polyacrylamide gel electrophoretic fractionation of JSRV capsids consistently yielded nine proteins ranging in size from 84 000 to 32 000 daltons (Fig. 159). Detergent treatment of the virus followed by isopyknic centrifugation in sucrose gradients resulted in the isolation of high density (1.21 g/ml) core particles (Verwoerd et al., 1983).

The total number of polypeptides found in JSRV is comparable to that of other retroviruses, especially if the two double bands represent variant forms of one protein. A significant difference, however, is the absence of polypeptides smaller than 30 000 daltons in JSRV. All the other retroviruses studied to date contain two or more major components in the 10–20 000 dalton range.

In morphology and morphogenesis JSRV most closely resembles type B MMTV. These viruses also share other characteristics, such as a relatively high density and the possession of a magnesium-dependent reverse transcriptase. A limited serological relationship was found, but molecular hybridization
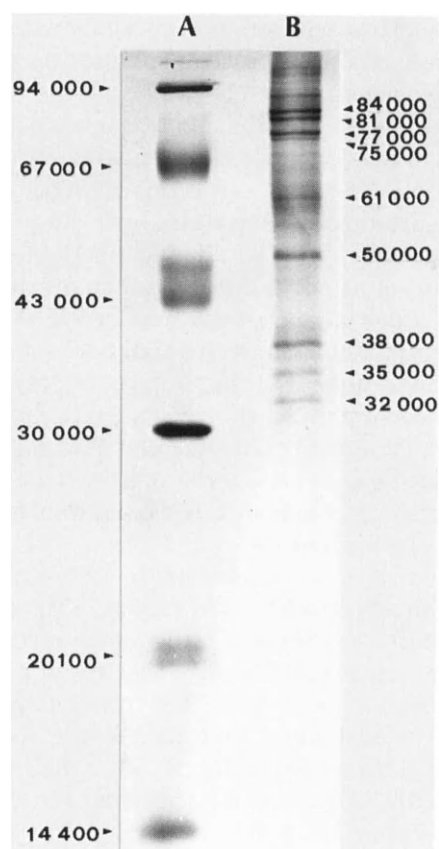
Fig. 159. Polypeptide composition of JSRV; lane A consists of molecular weight markers, lane B of dissociated complete virus.

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studies failed to reveal any significant homology of the viral genomes (D.W. Verwoerd, unpublished data, 1983).

In a serological study, JSRV was compared with representatives of type B, type C and type D retroviruses as well as with MVV, CAEV and BLV. A neutralization test based on the reverse transcriptase activity was used as a group-specific assay and an indirect ELISA for detecting low levels of cross-reaction with sera prepared against whole disrupted virus. Neither of these tests revealed any relationship between JSRV and the above-mentioned retroviruses (Verwoerd et al., 1983). However, a sensitive immunoblot technique demonstrated a cross-reaction between antisera prepared against the group-specific P27 antigen of type D MPMV and against intact MMTV, respectively, and a P25 antigen derived from jaagsiekte lungs (Sharp and Herring, 1983). No cross-reactions with MVV-related lentiviruses were found, and therefore the reaction can be used to distinguish between JSRV and lentiviruses. This has become important in view of the recent isolations of nonpathogenic lentiviruses from jaagsiekte lungs (Irving et al., 1984; Payne et al., 1986), and the experimental infection of a naturally infected jaagsiekte case with MVV (Dawson et al., 1985).

EPIZOOTIOLOGY

The domestic sheep is the animal mainly affected by jaagsiekte. A few reports of the disease in goats have been published, but none were very convincing. Experimental infection of newborn goat kids were recently obtained, but the lesions were atypical, well-circumscribed nodules (Sharp et al., 1986; Tustin et al., 1988). Attempts by various workers to infect laboratory animals have all failed, with the exception of successful transplantation of cultured tumor cells into nude mice (Verwoerd et al., 1977).

Jaagsiekte is almost ubiquitous in its distribution, Australasia being the only continent where it does not occur. The disease is endemic in most European (east and west), African, Middle-East and South American countries with a substantial sheep population. Sporadic cases have also been diagnosed in the USA and Canada and in many countries with smaller numbers of sheep.

The incidence of the disease in an affected flock depends on the susceptibility of the population, type of flock management and breed of sheep. An average incidence of about 5% is typical for South African flocks, but it can be as high as 20%. A survey in Scotland indicated that approximately 20% of all sheep older than 1 year necropsied at the Scottish Veterinary Investigation Centres had jaagsiekte lesions in their lungs (Hunter and Munro, 1983).

When infection is introduced into a clean flock, the incidence of the disease usually increases for a number of years, then stabilizes and finally decreases gradually, indicating some form of acquired resistance.

The role of acquired resistance is best illustrated by the experience in Iceland. After the importation of an affected Karakul ram in 1933, the first case of jaagsiekte in the apparently fully susceptible indigenous sheep occurred during the next year. By 1935 the farmer had already lost 54% of his flock. By 1938 one third of the country's flocks were affected with an incidence of 50–60%. This very high incidence must have been due to the total lack of previous exposure of the Icelandic sheep to jaagsiekte. After the initial epizootic the incidence decreased rapidly (Sigurdsson, 1958), and the disease was eventually eradicated by a strict slaughter policy.

Being an airborne infection, close contact can be expected to facilitate spread of the disease. Indeed, in South Africa the incidence of the disease is

highest in those parts of the country where, due to the climate, animals are housed overnight, and low under extensive farming conditions.

In Iceland it was shown beyond doubt that genetic predisposition exists. The Gottorp breed was found to be most susceptible with some farmers loosing as many as 90% of their sheep, whereas only 10% of the Adalbol breed on the same farm would succumb (Dungal et al., 1938).

In Great Britain, jaagsiekte mainly occurs in the eastern and southern parts of Scotland, with very few cases occurring in neighbouring England (J.M. Sharp, personal communication, 1983), suggesting possible breed differences. In South Africa, the merino, the karakul and various cross-breeds have been found to be equally susceptible (Tustin, 1969), but there are some indications that English breeds are less susceptible (D.W. Verwoerd, unpublished data, 1982).

In its natural form the disease has been classified as a slow infection, as symptoms are rarely seen in animals younger than a year or two. However, once symptoms are seen the disease is probably irreversible, the progessive proliferation of cancerous cells eventually leading to death.

After experimental transmission, when concentrated virus is injected intra-tracheally into newborn lambs, the disease can be much more acute. Symptoms are sometimes seen after 1–2 weeks and death can follow after 4–5 weeks (Verwoerd et al., 1980). An interesting phenomenon is the considerable variation between experimental animals. In one experiment, twins of the same sex were injected with identical inocula. One animal died at 6 weeks with acute jaagsiekte, the other did not develop any symptoms and when slaughtered after 2 years had only a few small adenomatous nodules in its lungs (D.W. Verwoerd unpublished data, 1984).

The mortality of the disease can therefore not be regarded as 100%, as some individuals seem to have the ability to contain the tumorous lesions.

PATHOGENESIS

Jaagsiekte has been transmitted experimentally by co-habitation, by droplet infection with an aerosol spray and by inoculations of extracts of adenomatous lungs or of purified virus by intrapulmonary, intrapleural, intratracheal and intranasal routes (Tustin, 1969; Wandera, 1971). Infection is therefore clearly via the respitory system and requires no vector.

Replication of the virus has only been observed in the type II alveolar epithelial cell (Payne et al., 1983), and no evidence has been found to date of virus or viral antigens in any other organ, including the blood. Infection with JSRV, even experimental infection leading to acute disease, is not associated with any fever reaction. It is therefore probable that the infection remains localized in the lung and does not lead to a viremia. This concept is supported by the observation that circulating antibodies are only rarely found.

The type II alveolar epithelial cells, and possibly the nonciliated bronchial epithelial or Clara cells are transformed to neoplastic cells which proliferate and eventually fill the alveolar spaces. Normal lung tissue is progressively replaced by solid tissue, leading to death from asphyxia.

The tumor cells, in addition to producing virus, are also surfactant-producing secretory cells. Typical jaagsiekte lungs are therefore edematous in appearance and a copious amount of clear viscous fluid is produced and accumulates in the air passages. This accumulation compounds the distress of the animal in breathing and gives rise to coughing. This, in turn, leads to droplet formation

and the dissemination of virus in an aerosol form which can be inhaled by other animals.

It is not yet known whether endogenous JSRV is present as a provirus in the genome of all sheep cells. The fact that an acute disease can be produced by experimental infection suggests that it is more probably exogenous. However, this is not necessarily so; MMTV, for example, has been shown to be transmitted horizontally as an exogenous agent although it is present endogenously. Being a retrovirus with an obligatory DNA replicative intermediate, JSRV can be expected to occur in a latent form, integrated into the cell genome, whether endogenous or exogenous in nature. No evidence for vertical transmission, i.e. through the germ cell line, or even through intra-uterine infection has been found.

DISEASE SIGNS

The incubation period in natural infections can vary from months to years, judged by the age at which symptoms are first detected (Tustin, 1969).

In experimental infections the incubation period can be reduced to weeks or even days by increasing the dose, by purifying the virus (presumably removing bound antibodies) and by inoculating newborn lambs (Verwoerd et al., 1980; Herring et al., 1983). No age limit for susceptibility has been determined, but we found that the efficiency of experimental infection progressively decreases up to the age of about 6 months, and a number of attempts to infect adult animals failed (D.W. Verwoerd, unpublished data, 1978). These observations suggest that natural infection may occur predominantly in young lambs.

Symptoms are only seen when lesions are well established and consist mainly of various manifestations of dyspnea. The first sign is usually an increased rate of respiration with typical jerky expiratory movements. These symptoms increase with exercise and chasing animals is an established diagnostic aid. The term jaagsiekte refers to the characteristic increase in respiratory rate, however, and not to the chasing of animals, as sometimes believed. Ultimately animals show acute respiratory distress, breathing with nostrils dilated and mouth opened, sometimes accompanied by signs of circulatory failure.

A second group of symptoms derives from the increase in secretory activity in the lungs. Coughing is first observed and moist rales can be heard on auscultation of the chest. In the later stages clear viscous fluid can be seen running from the nose, especially if the hind part of the animal is raised. Loss of appetite and emaciation are also terminal signs, commonly seen in chronic cases. The duration of the disease depends mainly on the age of the animal when the symptoms appear. In lambs younger than 6 months it is usually acute and animals die within a few days. In older animals the course is more chronic and symptoms can last for months or even years.

Characteristically there is a complete absence of fever, but it should be stressed that natural cases of the disease are almost invariably complicated by secondary infections, in which case a fever can be present. In South Africa pasteurellosis is the most common complication and often the cause of death.

PATHOLOGY

There is some controversy about the histological classification of jaagsiekte lesions. It may be classified as a bronchiolo-alveolar adenocarcinoma, implying some malignancy. Some workers prefer to call it a carcinoma (Perk, 1982).

Generally, however, the broad term adenomatosis, including anything from hyperplastic to neoplastic proliferation with metastatic potential, is preferred. The reason for this difference in opinion is the difference in frequency of metastases observed in various countries. In Iceland no metastases were found, in South Africa it is very rare, and in Israel a high incidence was reported. No explanation for this discrepancy can be given at present.

The pathology of jaagsiekte has been reviewed extensively (Tustin, 1969; Wandera, 1971; Perk, 1982). In advanced cases the lungs can double in size and increase to three or more times their normal weight (Fig. 160). One or both lungs may be affected, and early lesions are usually found in the ventral parts of the anterior lobes.

Early lesions are small white-colored nodules which progressively expand to form solid greyish-white areas of consolidation. On cutting, the surface is moist and there is continuous oozing of clear fluid from the tumor tissue. Bronchi and bronchioli are usually filled with froth. Mediastinal lymph nodes are often enlarged. In chronic cases fibroplasia can be present.

There is a distinct difference in appearance between natural and experimental cases. In natural cases the tumors usually grow by expansion from one or a few primary lesions. Secondary intrapulmonary spread does not seem to play an important role. In experimental cases, where a high concentration of virus is injected, the tumour is multi-centric in origin, with nodules distributed throughout the lungs.

The smallest microscopic lesion consists of an alveolus lined by cuboidal or columnar epithelial cells. These cells proliferate, filling the lumen of the alveolus and forming acinar or papilliform masses completely obliterating the normal alveolar structure of the lung (Fig. 161). Similar proliferations are sometimes seen in the bronchioli. In more chronic cases fibroplasia of the interstitial tissue can be prominent. Proliferation of alveolar macrophages is a feature of advanced lesions, also in the absence of secondary infections. An increased number of lymphocytes and granulocytes, in contrast, is usually indicative of an inflammatory response to a secondary infection.

Electron microscopy confirmed that the tumor cells consist of the type II granular pneumocytes. The secretory granules in tumor cells varied in app-
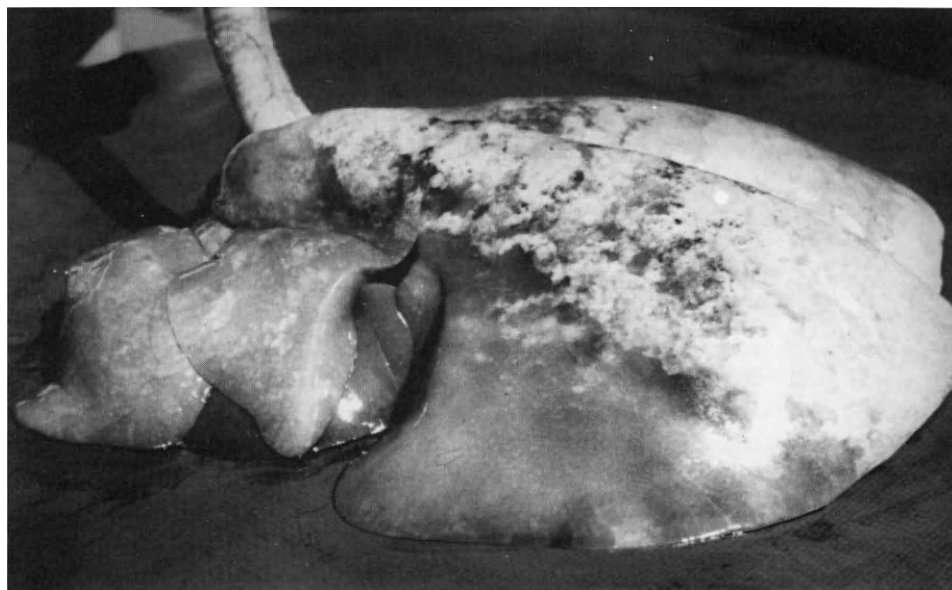
Fig. 160. Typical appearance of jaagsiekte adenomatous lungs; note the marble-like appearance of consolidated lesions interspersed between normal tissue.
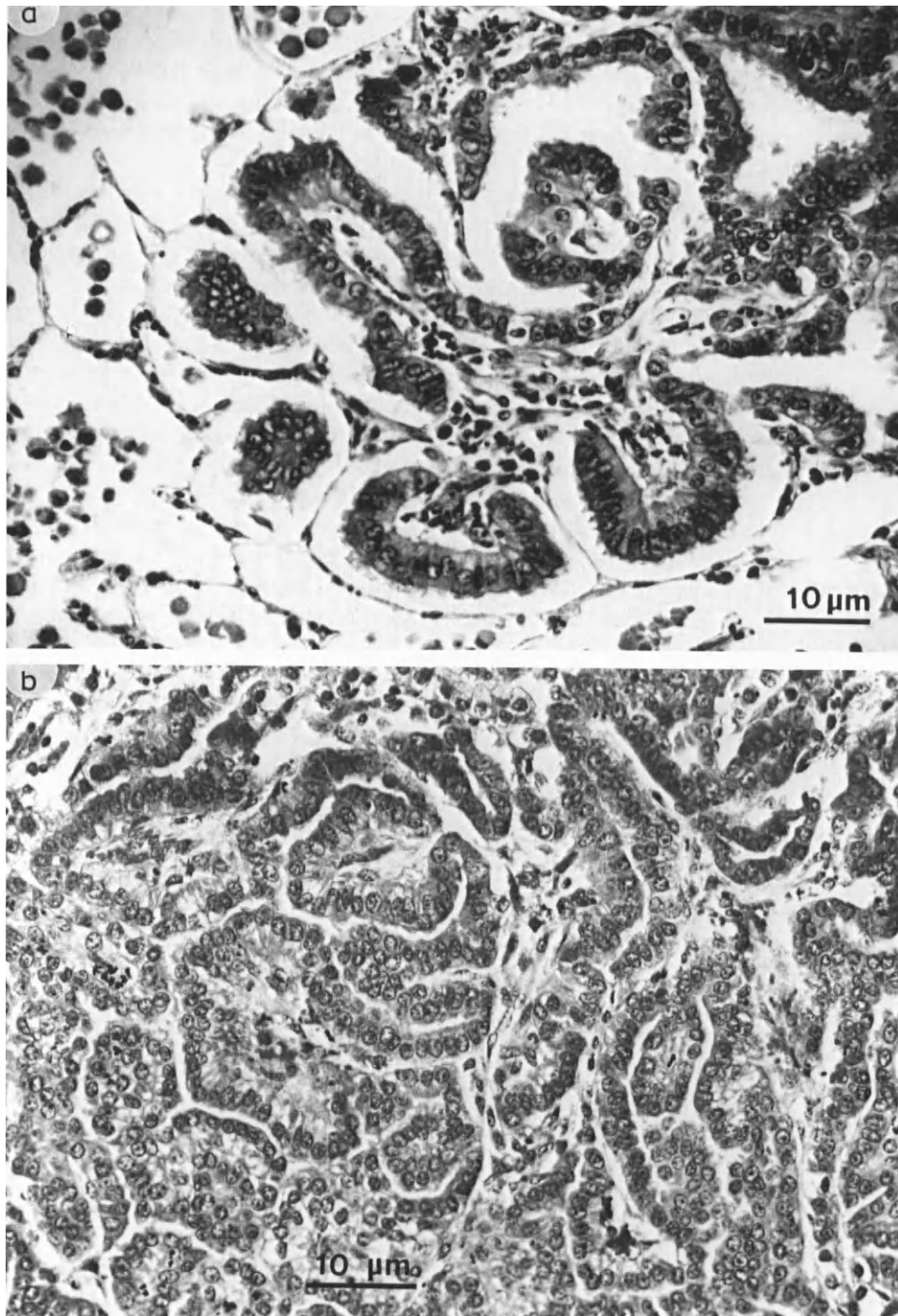
Fig. 161. Histological appearance of jaagsiekte lesions as shown in Fig. 160; note the papilliform proliferations of neoplastic epithelial cells filling and obliterating the alveolar lumen; (a) early lesion, (b) advanced lesion.

earance from electron lucent to electron dense to granules filled with mye-linoid membrane whorls. These granules are probably the source of the copious lung exudate pathognomonic for jaagsiekte (Payne and Verwoerd, 1984).

Scanning electron microscopy (Fig. 162) revealed grape-like clusters of tumor cells filling the alveolar lumen. The tumor cells are covered with abundant microvilli, distinguishing them from normal pneumocytes (Payne and Verwoerd, 1984).
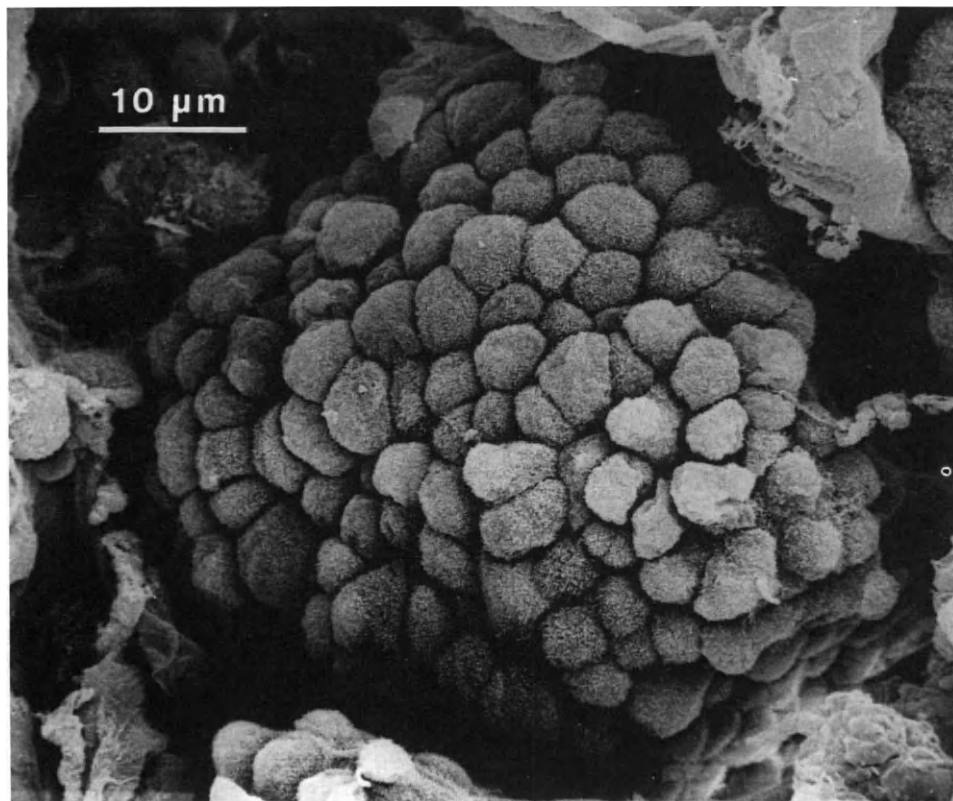
Fig. 162. Scanning electron microscopy of a cluster of proliferating tumor cells filling an alveolus; the surface of the tumor cells is covered with microvilli, in contrast to the almost smooth appearance of the normal epithelial lining (courtesy A. Payne).
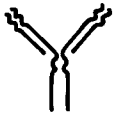
IMMUNE REACTION

There is abundant evidence of acquired resistance to jaagsiekte on both flock and national levels. It is not known whether this resistance is immunological in nature, but some form of immunity is very likely. Hod et al. (1972) claimed that hyper-7S-immunoglobulinemia is characteristic of advanced jaagsiekte and used its detection as a diagnostic tool. In our laboratory, however, a sensitive enzyme-immunoassay only rarely detected specific circulating antibodies. Even acute experimental cases secreting large amounts of virus in the lung exudate often had no detectable antibodies in their serum.

On the other hand, it has been demonstrated that even highly purified virus, derived from lung fluid, is complexed with immunoglobulins, mainly of the IgA-class (Verwoerd et al., 1983). Furthermore, it has previously been found that the lung exudate from adenomatous lungs is very rich in both IgA and IgG (Goudswaard et al., 1980). It is therefore very likely that the immune response to the jaagsiekte virus is mainly, if not exclusively, local in nature and mediated by IgA. The absence of circulating antibodies could be due to the absence of a viremia, which in turn derives from the fact that replication of the virus is localized in the lung epithelium. No evidence has so far been found for the involvement of cellular immunity.

LABORATORY DIAGNOSIS

Clinical signs such as dyspnea in the absence of a fever reaction and especially a clear viscous lung exudate are indicative of jaagsiekte. However, as

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all the symptoms are not always present and since secondary infections are common, a laboratory diagnosis is essential.

Histopathological examination is the method of choice, since it can distinguish clearly between jaagsiekte and chronic pneumonia, lung-worm infestation and maedi-visna. Histologically, the only problem for an inexperienced pathologist is to distinguish very early adenomatous lesions from epithelialization of the alveolar lining.

Early claims for a serological test probably depended on the demonstration of secondary *Mycoplasma* infections, which are very common in jaagsiekte lungs (Wandera, 1971). The apparent absence of circulating antibody has so far prevented the development of a diagnostic serum test. However, it is possible to detect both antibodies and virus in the lung exudate and lung washings by means of enzyme- or radio- immuno assays, and a diagnostic test based thereon may become feasible if the practical problem of sample collection can be surmounted. The same applies to the detection of reverse transcriptase in lung fluid.

PROPHYLAXIS AND CONTROL

At present the only prophylactic measure available is to maintain a closed flock and to avoid the introduction of new animals. In view of the long incubation period and the lack of a test sensitive enough to detect early infections or carriers, it is not yet possible to determine whether an animal is free from the virus.

Conflicting results have been obtained by different workers in attempts to immunize animals with vaccines prepared from affected lungs (Tustin, 1969). Our present knowledge about the type of immunity involved supports the view that such vaccines, especially when administered parenterally, are worthless. When the problem of cultivating the virus is solved, it may be possible to develop a vaccine suitable for stimulating the local immune response.

Control at present depends entirely on strict isolation and elimination of all animals showing symptoms. In Iceland a strict slaughter policy was succesful in eradicating the disease (Sigurdsson, 1958). In our experience, a farmer can reduce the incidence of jaagsiekte in a flock to less than 1% by immediately removing any animal suspected of having early symptoms. Infectivity of the virus is low and infection is probably limited to young lambs. By eliminating the progeny of affected ewes the incidence can therefore be reduced further.

Retroviruses are generally very unstable outside the host cell, and even though JSRV is probably stabilized by being coated with antibody, it cannot survive exposure and desiccation for long. It should therefore be a relatively easy virus to eradicate once a sensitive test to detect carriers becomes available.

REFERENCES

Dawson, M., Venables, C. and Jenkins, C.E., 1985. Experimental infection of a natural case of sheep pulmonary adenomatosis with maedi–visna virus. Vet. Rec., 116: 588–589

De Villiers, E.M. and Verwoerd, D.W., 1980. Presence of *Herpesvirus ovis* DNA sequences in cellular DNA from sheeps lungs affected with jaagsiekte (pulmonary adenomatosis). Onderstepoort J. Vet. Res., 47: 109–112.

Dungal, N., Gislason, G. and Taylor, E.L., 1938. Epizootic adenomatosis in the lungs of sheep — comparisons with jaagsiekte, verminous pneumonia and progessive pneumonia. J. Comp. Pathol. Therap., 51: 46–68.

Goudswaard, J., Verdouw- Chamalaun, C.V.M. and Noordzij, A., 1980. Quantitation of immunoglobulins in ovine sera and secretions by laser nephelometry. Vet. Immunol. Immunopathol., 1(2): 163–177.

Herring, A.J., Sharp, J.M., Scott, F.M.M. and Angus, K.W., 1983. Further evidence for a retrovirus as the aetiological agent of sheep pulmonary adenomatosis (jaagsiekte). Vet. Microbiol., 8: 237-249.

Hod, I., Perk, K., Nobel, T.A. and Klopfer, U., 1972. Lung carcinoma of sheep (jaagsiekte). III Lymph node, blood and immunoglobulin. J. Nat. Cancer Inst., 48: 487-507.

Houwers, D.J. and Terpstra, C., 1984. Sheep pulmonary adenomatosis. Vet. Rec., 114: 23.

Hunter, A. and Munro, R., 1983. The diagnosis, occurrence and distribution of sheep pulmonary adenomatosis in Scotland 1975-1981. Br. Vet. J., 139: 153-159.

Irving, S.G., Perk, K., Hod, I., Gazit, A., Yaniv, A., Zimber, A. and Tal, M., 1984. Isolation and characterization of a novel retrovirus from sheep affected by pulmonary carcinoma. Virology, 134: 244-248.

Malmquist, W.A., Krauss, H.H., Moulton, J.E. and Wandera, J.G., 1972. Morphologic study of virus-infected lung cell cultures from sheep pulmonary adenomatosis (jaagsiekte). Lab. Invest., 26: 528-533.

Markson, L.M., Spence, J.B. and Dawson, M., 1983. Investigations of a flock heavily infected with maedi-visna virus. Vet. Rec., 112: 267-271.

Martin, W.B., Scott, M.M., Sharp, G.M., Angus, K.W. and Norval, M., 1976. Experimental production of sheep pulmonary adenomatosis (jaagsiekte). Nature (London), 264: 1976-1977.

Pálsson, P.A., 1976. Maedi and visna in sheep. In: R.H. Kimberlin (Editor), Slow Virus Diseases of Animals and Man. North-Holland, Amsterdam, pp. 17-58.

Payne, A.L. and Verwoerd, D.W., 1984. A scanning and transmission electron microscopy study of jaagsiekte lesions. Onderstepoort J. Vet. Res., 51: 1-13.

Payne, A.L., Verwoerd, D.W. and Garnett, H.M., 1983. The morphology and morphogenesis of jaagsiekte retrovirus. Onderstepoort J. Vet. Res., 50: 317-322.

Payne, A.L., York, D.F. De Villiers, E.M., Verwoerd, D.W., Quérat, G., Barban, V., Sauze, N. and Vigne, R., 1986. Isolation and identification of a South African Lentivirus from jaagsiekte lungs. Onderstepoort J. Vet. Res., 53: 55-62.

Perk, K., 1982. Slow virus infections of ovine lung. Adv. Vet. Sci. Comp. Med., 26: 267-287.

Perk, K., Hod, I. and Nobel, T.A., 1971. Pulmonary adenomatosis of sheep (jaagsiekte) I Ultrastructure of the tumor. J. Nat. Cancer Inst., 46: 525-537.

Perk, K., Michalides, R., Spiegelman, S. and Schlom, J., 1974. Biochemical and morphologic evidence for the presence of an RNA tumor virus in pulmonary carcinoma of sheep (jaagsiekte). J. Nat. Cancer Inst., 53: 131-135.

Sharp, J.M. and Herring, A.J., 1983. Sheep pulmonary adenomatosis; demonstration of a protein which cross reacts with the major core proteins of Mason-Pfizer monkey virus and mouse mammary tumor virus. J. Gen. Virol., 64: 2323-2327.

Sharp, J.M., Angus, K.W., Jassim, F.A. and Scott, F.M.M., 1986. Experimental transmission of sheep pulmonary adenomatosis to a goat. Vet. Rec., 119: 245.

Sigurdsson, B., 1958. Adenomatosis of sheep lungs. Experimental transmission. Arch. Ges. Virusforsch., 8: 51-58.

Snyder, S.P., Demartini, J.E., Ameghino, E. and Caletti, E., 1983. Coexistence of pulmonary adenomatosis and progressive pneumonia in sheep in the central sierra of Peru. Am. J. Vet. Res., 44: 1334-1338.

Tustin, R.C., 1969. Ovine jaagsiekte. J. S. Afr. Vet. Med. Assoc., 40: 3-23.

Tustin, R.C., Williamson, A., York, D.F. and Verwoerd, D.W., 1988. Experimental transmission of jaagsiekte (ovine pulmonary adenomatosis) to goats. Onderstepoort J. Vet. Res., 55: 27-32.

Verwoerd, D.W., Meyer-Scharrer, E. and Du Plessis, J.L., 1977. Transplantation of cultured jaagsiekte (sheep pulmonary adenomatosis) cells into athymic nude mice. Onderstepoort J. Vet. Res., 44: 271-274.

Verwoerd, D.W., Williamson, A.L. and De Villiers, E.M., 1980. Aetiology of jaagsiekte: Transmission by means of subcellular fractions and evidence for the involvement of a retrovirus. Onderstepoort J. Vet. Res. 47: 275-280.

Verwoerd, D.W., Payne, A.L., Myer, M.M. and York, D.F., 1983. Isolation and preliminary characterization of the jaagsiekte retrovirus (JSRV). Onderstepoort J. Vet. Res., 50: 309-316.

Verwoerd, D.W., Tustin, R.C. and Payne, A.L., 1985. Jaagsiekte: An infectious pulmonary adenomatosis of sheep. In: G. Olsen, S. Krakowka and J.R. Blakeslee (Editors), Comparative Pathobiology of Viral Diseases. CRC Press, Boca Raton, FL, pp. 53-76.

Wandera, J.G., 1971. Sheep pulmonary adenomatosis (jaagsiekte). Adv. Vet. Sci. Comp. Med., 15: 251-283.

Bunyaviridae

The family name comes from Bunyamwera, a place in Uganda where the type species, Bunyamwera virus, was isolated.

Bunyaviruses are spherical or oval particles, 90–100 nm in diameter; they possess a membrane envelope with projections. The projections are glycoproteins, designated G1 and G2.

The genome consists of three molecules of a single-stranded noninfectious RNA, designated according to their molecular weights L (large), M (medium) and S (small), which are surrounded by a nucleocapsid protein (N). A minor large protein (L) is probably a transcriptase.

Bunyaviruses replicate in the cytoplasm and mature by budding into smooth-surfaced vesicles in the Golgi region or nearby.

The family comprises over 200 viruses which are subdivided into five genera: *Bunyavirus, Phlebovirus, Nairovirus, Uukuvirus* and *Hantavirus*. The genera *Bunyavirus* and *Nairovirus* are organized into various serogroups. The members of each serogroup are closely related antigenically but exhibit weak serologic relationships with members of other serogroups within the same genus (previously called "supergroup"). The genera *Phlebovirus, Uukuvirus* and *Hantavirus* at present each contain a single serogroup. Molecular analyses showed differences between selected members of the five genera especially with regard to a unique nucleotide sequence at the 3′ end of each of the three RNA segments; this sequence is common to members of a genus (Bishop et al., 1980; Schmaljohn et al., 1985).

Bunyaviruses, with the exception of hantaviruses, are transmitted by arthropods. It is surprising that among so many bunyaviruses there are only a few known to occur in ruminants, namely Akabane, Rift Valley fever and Nairobi sheep disease virus; each of these represents a genus and serogroup (Table 30).

Akabane virus was found associated with epizootic bovine congenital arthrogryposis and hydranencephaly in Japan, Australia and Israel. [Arthrogry-

TABLE 30

Bunyaviridae: Subdivision into genera and serogroups (modified from Matthews, 1982)

Genus	Number of viruses	Number of serogroups	Virus in ruminants	
			Name	Serogroup
Bunyavirus	145	16	Akabane[a]	Simbu
Phlebovirus	30	1	Rift Valley fever	Sandfly fever
Nairovirus	27	6	Nairobi sheep disease	Nairobi sheep disease[b]
Uukuvirus	7	1		
Hantavirus	numerous	3[c]		
Possible members of the family	22	4		

[a] In Australia also Aino, Peaton and Tinaroo.
[b] Related to the type species Crimean–Congo hemorrhagic fever (Casals and Tignor, 1980).
[c] Described as "serotypes".

posis: persistent flexure or contrature of a joint. Hydranencephaly: complete or almost complete absence of cerebral hemispheres, the space they normally occupy being filled with cerebrospinal fluid.] Vectors are *Culicoides* (a genus of biting flies) and mosquitoes.

Rift Valley fever virus (RVFV) causes abortion and death in pregnant and newborn sheep, goats and cattle; the infection is restricted to Africa. During outbreaks in Egypt (Meegan et al., 1979; Swartz et al., 1980), abortion and focal hepatonecrosis were the dominant signs of disease. It may infect man (high mortality rates in Egypt). The virus occurs also in buffalos, camels and ante-lopes. Vectors are mosquitoes, but contact infections probably happen, too.

Nairobi sheep disease virus (NSDV) causes hermorrhagic gastroenteritis in sheep and goats with involvement of the female genital tract and with high mortality; the condition is restricted to Africa. Ticks serve as vectors.

REFERENCES

Bishop, D.H.L. et al., 1980. Bunyaviridae. Intervirology, 14: 125–143.

Casals, J. and Tignor, C.H., 1980. The *Nairovirus* genus: Serological relationships. Intervirology, 14: 144–147.

Matthews, R.E.F., 1982. Classification and nomenclature of viruses. Bunyavirida. Intervirology, 17(1–3): 115–118.

Meegan, J.M., Hoogstraal, H. and Moussa, M.I., 1979. An epizootic of Rift Valley fever in Egypt 1977. Vet. Rec., 105: 124–125.

Schmaljohn, C.S. et al., 1985. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. Science, 227: 1041–1044.

Swartz, T.A., et al., (Editors), 1980. Rift Valley Fever. Karger, Basel.

Chapter 43

Akabane Virus

Y. INABA and M. MATUMOTO

INTRODUCTION

Akabane virus, a member of the Simbu group of bunyaviruses, has been shown to cause outbreaks of abnormal deliveries in Japanese cattle, such as abortions, stillbirths, premature births and calf deformities referred to as congenital arthrogryposis–hydranencephaly (AH) syndrome (Miura et al., 1974; Kurogi et al., 1975, 1976, 1977a). Seasonal occurrence of congenital AH syndrome in cattle, sheep and goats has also been reported in Australia and Israel. In both countries the etiologic role of Akabane virus is now accepted (Kalmar et al., 1975; Hartley et al., 1975, 1977; Parsonson et al., 1975, 1977; Della-Porta et al., 1977; Kurogi et al., 1977b; Narita et al., 1978; Hashiguchi et al., 1979).

Inaba et al. (1975) proposed to designate the congenital AH syndrome in cattle, sheep and goats as "Akabane disease". However, this term now seems inappropriate because studies in Australia (Coverdale et al., 1979; I.M. Parsonson, personal communication, 1978) have indicated that other viruses (Aino, Peaton and Tinaroo) belonging to the Simbu group of the family Bunyaviridae may also be involved in the syndrome.

The disease has been reviewed by Matumoto and Inaba (1980), Inaba and Matumoto (1981) and Porterfield and Della-Porta (1981).

VIRUS PROPERTIES

Akabane virus was originally isolated from mosquitoes, *Aedes vexans* and *Culex tritaeniorhynchus*, in Japan in summer 1959. "Akabane" is the name of the village where the virus was first isolated (Matsuyama et al., 1960; Oya et al., 1961). Subsequent serological studies could classify Akabane virus in the Simbu group (Takahashi et al., 1978), one of the serological groups in the family Bunyaviridae (Porterfield et al., 1976).

Virus particles are roughly spherical, variable in size, 70–130 nm in diameter, and most have a ragged, closely adherent envelope with 9 nm peplomers when examined by negative staining EM (Fig. 163; Takahashi et al., 1978; Ito et al., 1979).

Akabane virus is readily filtered through membrane filters of 200- or 100-nm pore size, but not through 50-nm filters (Doherty et al., 1972; Takahashi et al., 1978). The virus is readily inactivated by ether, chloroform (Doherty et al., 1972; Takahashi et al., 1978) and sodium deoxycholate, but is not precipitated by protamine sulfate (Oya et al., 1961; Takahashi et al., 1978). The virus is very acid labile, being rapidly inactivated at pH 3, and is readily inactivated by
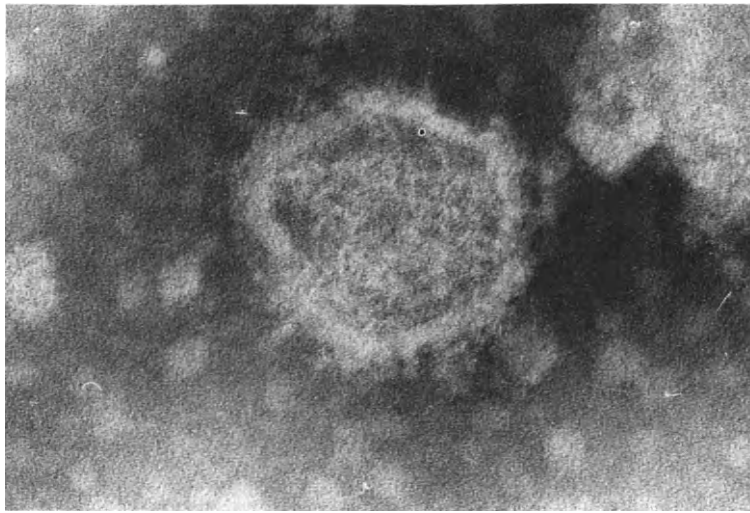
Fig. 163. Electron micrograph of negatively stained Akabane virus, × 30 000.

trypsin (Takahashi et al., 1978); it is also very heat labile, losing about 0.3 log of infectivity per hour at 37°C (Takahashi et al., 1978).

The virus contains three size classes of single-stranded RNA with molecular weights of 2.15 × 10^6 (large, L), 1.5 × 10^6 (medium, M), and 0.48 × 10^6 (small, S) (Pattnaik and Abraham, 1983) and four proteins, two glycoproteins (G1, G2), the nucleoprotein (N) and probably a large protein (L). There appear to be five proteins in virus-infected cells : L, G1, G2, N and one smaller nonstructural protein (Porterfield and Della-Porta, 1980; Della-Porta et al., 1981).

The hemagglutination by Akabane virus is shown to be dependent on the pH as well as the NaCl molarity of the diluent; the hemagglutinin titer is significantly improved by increasing the NaCl molarity to $0.4 M$ and standardizing the pH to 6.0–6.2 (Goto et al., 1976). Akabane virus gives similar titers with duck and goose erythrocytes and somewhat higher titers with pigeon erythrocytes, but no reaction with erythrocytes from cattle, sheep, guinea-pigs, day-old-chickens and humans. Hemagglutination is specifically inhibited by antisera (Goto et al., 1976, 1978). Analysis by CsCl equilibrium density gradient centrifugation indicates the hemagglutinating activity to be structurally associated with the virion (Takahashi et al., 1978).

Akabane virus also lyses pigeon erythrocytes at 37°C (Goto et al., 1979). The hemolytic activity of a virus suspension is enhanced by repeated freezing and thawing, whereas its hemagglutinin titer remains unchanged. Also hemolysis is dependent on the sodium chloride molarity and the pH of the diluent. Unlike the hemagglutination, it is markedly affected by the incubation temperature. The hemolytic activity is structurally associated with the virus particle. Scanning electron microscopy of pigeon erythrocytes undergoing hemolysis reveals the appearance of depressed areas with holes on the cell surface. The hemolytic activity is specifically inhibited by antisera to Akabane virus (Goto et al., 1979).

Akabane virus can be readily propagated with CPE in primary cultures and continuous cell lines. The primary cultures include those of bovine kidney, testicle and thyroid, bovine embryonic skin, swine kidney and testicle, guinea-pig kidney, hamster kidney and chick embryo cells (Oya et al., 1961; Kurogi et al., 1976). The continuous cell lines include Vero from African green monkey kidney (Hartley et al., 1975; Kurogi et al., 1975; Takahashi et al., 1978); HmLu-1 from hamster lung (Kurogi et al., 1976; 1977c; Takahashi et al., 1978); BHK21 from baby hamster kidney (Hartley et al., 1975; Kurogi et al., 1976); ESK (Miura et al., 1974), PK-15 (Kurogi et al., 1976) and MVPK-1 (Andersen and Campbell, 1978) from swine kidney; BEK-1 and MDBK from bovine embryo kidney (Kuro-

gi et al., 1976); STR from bovine thymus (Kurogi et al., 1976); RK-13 from rabbit kidney (Kurogi et al., 1976). Of the cell cultures tested, HmLu-1 cells are most sensitive and almost as sensitive as the intracerebral inoculation of suckling mice for primary isolation from clinical materials (Kurogi et al., 1976). Akabane virus forms plaques in monolayers of MVPK-1 (Andersen and Campbell, 1978), HmLu-1 and other cells (Kurogi et al., 1976, 1979a). Akabane virus was reported to multiply but induce no cytopathic effect in *Aedes albopictus* cells (Hoffmann and St. George, 1985).

The mouse is the most commonly used experimental animal for Akabane virus since the early study of Oya et al. (1961). The virus readily infects adult mice and induces fatal encephalitis after intracerebral injection, but not by extraneural routes. In contrast, suckling mice are highly susceptible by extraneural as well as intracerebral routes (Kurogi et al., 1978a; Nakajima et al., 1979). When Akabane virus is inoculated intravenously into pregnant mice, virus is not recovered from the placenta and fetal tissues, and virus has no effect on litter size or survival of young to 1 week of age (Andersen and Campbell, 1978).

Transplacental infection is produced by inoculation of pregnant hamsters (Andersen and Campbell, 1978). High titers of virus are detected first in the placenta and later in the fetus. Virus can be readily recovered from blood, lung, spleen and liver of both pregnant and nonpregnant hamsters, but it reaches higher titers and persists longer in the placenta and fetus. Litter size and survival of the newborn to 1 week of age are decreased by inoculation of pregnant hamsters (Andersen and Campbell, 1978).

Akabane virus is propagated in the chicken embryo by yolk sack inoculation. Inoculated embryos show dwarfism, cerebral defects, hydranencephalus, deformed legs and toes and arthrogryposis (Ikeda and Yonaiyama, 1978; Miah and Spradbrow, 1978; McPhee et al., 1984).

Cross CF tests revealed Akabane virus to be antigenically related to the Simbu group viruses, being more closely related to Aino, Simbu and Sathuperi viruses than to Oropouche, Ingvavuma and Manzanilla viruses (Takahashi et al., 1968). However, the neutralization, hemolysis-inhibition and HI tests seem to be more specific, since they show no cross-reaction between Akabane and Aino virus (Goto et al., 1976, 1979; Miura et al., 1978). Recently a low-titer, two-way cross-reaction was shown between Akabane and Tinaroo viruses by the neutralization test (Cybinski, 1984). Metselaar and Robin (1976) isolated a virus (MP496) in Kenya from *Anopheles funestus* mosquitoes that was considered as a strain or variant of Akabane virus; there was a slight one-way difference in the neutralization test, while the CF test could not differentiate the isolate from Akabane virus.

EPIZOOTIOLOGY

Akabane virus is known to cause sporadic epizootics of developmental deformities in cattle, sheep and goats. These epizootics have been observed in Australia, Israel, Japan and Turkey (Matumoto and Inaba, 1980; Inaba and Matumoto, 1981; Porterfield and Della-Porta, 1981; R.F. Sellers, personal communication, 1980).

Similar outbreaks now presumed to have been caused by Akabane virus were recorded in 1949–50, 1959–60 and 1966 in Japan (Inaba and Matumoto, 1981), and in 1937, 1951, 1955, 1960, 1964, 1968 and 1969 in Australia (Porterfield and Della-Porta, 1981). Those outbreaks resembled the 1972–74 outbreaks in Japan and the 1974 outbreaks in Australia, respectively, in epidemiological, clinical and pathological features, suggesting the same etiology.

There have been diseases suspected to be a congenital AH syndrome of viral

etiology in Argentina (J.A. Villa, personal communication, 1978), Rhodesia (Rudert et al., 1978; Shimshony, 1978) and South Africa (B. Barnard, personal communication, 1980).

Akabane virus was first isolated in Japan from *Aedes vexans* and *Culex tritaeniorhynchus* mosquitoes in 1959 (Oya et al., 1961) and later in Australia from biting midges, *Culicoides brevitarsis* (Doherty et al., 1972; St. George et al., 1978). Recently the virus was isolated from *Culicoides oxystoma* in Japan (Kurogi et al., 1987). During the outbreaks in Japan in 1972–1974 (Omori et al., 1974; Kurogi et al., 1975), the virus was isolated from naturally infected bovine fetuses and from the blood of sentinel cows (Kurogi et al., 1976). The virus was also recovered from the blood of sentinel cattle (St. George et al., 1977) and sheep (Della-Porta et al., 1977).

In Africa, Akabane virus has also been isolated from mosquitoes and culicoides. Metselaar and Robin (1976) isolated the virus from the mosquito *Anopheles funestus* collected in Kenya. In 1970, Akabane virus was isolated from culicoides caught in South Africa (Theodoridis et al., 1979).

A high incidence of neutralizing antibody to Akabane virus was shown in Japanese cattle (Kurogi et al., 1975). Other species found to have antibody were horses, goats and sheep, but none was found in chickens, pigs and man (Furuya et al., 1980). Oya (1971, 1972) reported antibody in pigs and monkeys in Indonesia, in monkeys in Malaya and in the Philippines, in pigs in Taiwan, and in horses in Thailand. Antibody has been found in sera collected upon arrival from cattle imported to Japan from Australia (27/48, 56%), Indonesia (11/13, 85%), Korea (11/50, 22%), but no antibody was found in cattle, horses, pigs and sheep imported from Canada, England, France, New Zealand, the USA and Venezuela (Y. Inaba et al., unpublished data, 1977). Antibody has been reported in cattle and sheep in Cyprus (Sellers and Herniman, 1981), in cattle, sheep and goats in Israel (Kalmar et al., 1975) and in cattle, sheep, goats, horses and camels in the Arabian Peninsula (Al-Busaidy et al., 1988). In Kenya antibody to Akabane virus was detected in cattle, sheep, goats, camels and horses, and in wild zebras and a whole range of wild ruminant species (Davies and Jasset, 1985). In Australia, serological evidence indicates that Akabane virus infects cattle, sheep, horses, buffaloes and camels, but not wallabies, kangaroos, rats, chickens, ducks and man (Doherty et al., 1972; Della-Porta et al., 1976; Cybinski et al., 1978).

These results of virus isolation and serological tests, although fragmentary, indicate a wide distribution of Akabane virus among cattle and other domestic animals in many southeast Asian countries, the Arabian peninsula, the Middle East and Africa.

The biting midge *Culicoides brevitarsis* is the only insect from which Akacorrelation of antibody in cattle with the geographical distribution of *C. brevitarsis*. The development of Akabane antibody coincides with the periods when *C. brevitarsis* is detected. These findings suggest that the midge may be a principal vector of Akabane virus in Australia (Della-Porta et al., 1976; Cybinski et al., 1978). *C. brevitarsis* feeds also on sheep and horses, and antibody to Akabane virus has been found in these species, giving further support to the theory that *C. brevitarsis* is a principal vector (Doherty, 1972). The failure to demonstrate antibodies in chickens, ducks, wallabies and man may be due to the failure of the suspected vector *C. brevitarsis* to attack them or to the failure of the virus to multiply in these species (Cybinski et al., 1978).

In the far north of Australia *C. brevitarsis* adults are active throughout the year, and Akabane virus seems to survive in transmission cycles involving cattle, sheep and horses as vertebrate hosts, and *C. brevitarsis* as vector. The breeding (in cow dung) and feeding habits (restricted to cattle, sheep and horses) of the vector make this cycle likely (Doherty, 1972). However, in
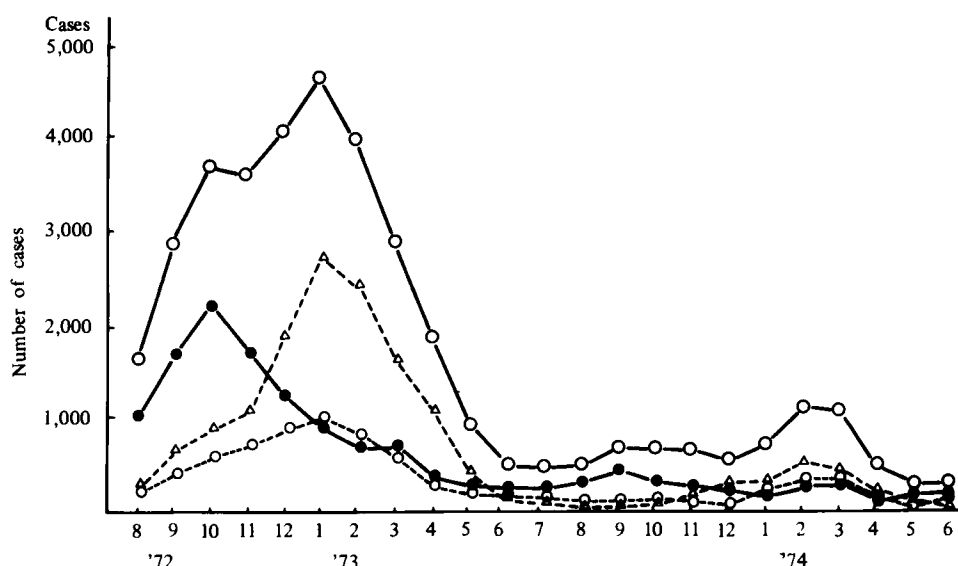
Fig. 164. Monthly numbers of reported cases; (O—O) total, (●—●) abortion and premature birth, (Δ——Δ) arthrogryposis and hydranencephaly, (O————O) stillbirth (Bureau of Animal Industry, Ministry of Agriculture, Forestry and Fishery, Tokyo).

southern Australia there is little, if any, activity of *C. brevitarsis* in the winter. This precludes continuous active transmission of Akabane virus, and an explanation of the apparent endemicity of the virus through the winter is awaited. It may be explained by assuming repetitive annual arrivals of infected vertebrates — or more probably arthropods — from truly endemic areas further north.

Akabane virus has been isolated from *Aedes vexans, Culex tritaeniorhynchus* (Oya et al., 1961) and *Culicoides oxystoma* (Kurogi et al., 1987) in Japan, and from *Anopheles funestus* mosquitoes in Kenya (Metselaar and Robin, 1976). In Japan the geographical distribution and seasonal occurrence of the disease, as well as active virus transmission in the summer suggest the involvement of vectors (Kurogi et al., 1975). However, information on the vector(s) is lacking and the mechanism of transmission and survival of the virus in nature awaits elucidation. In South Africa, Akabane virus was isolated from culicoides (Theodoridis et al., 1979).

In Japan the disease has a seasonal and geographical distribution. Dairy and beef cattle were affected likewise in the 1972–73 outbreak. Prevalence of abortion was first recognized in southern district in August, somewhat later in central district in September. As shown in Fig. 164, the monthly number of reported cases of abortion and premature birth increased rapidly in August and September, reached a peak in October, and then gradually declined. On the other hand, the number of cases of AH syndrome showed a gradual rise in the early months of the outbreak and a sharp rise in December, reaching a peak in January 1973. Stillbirths showed a gradual increase and decline with a peak in January 1973. The outbreak subsided in May 1973 (Kurogi et al., 1975; Matumoto and Inaba, 1980; Inaba and Matumoto, 1981). The epizootic recurred in 1973–74. It resembled the preceding outbreak in the geographical distribution, although it was more localized and tended to spare the areas severely affected by the previous outbreak and to move to adjoining areas. The dams affected in the last outbreak were apparently unaffected in this one (Kurogi et al., 1975). A small outbreak was observed in 1974–75, cases being recognized in the prefectures bordering the Sea of Japan as well as in the western parts of the country. The number of the reported cases in these epizootics, 1972–75, was 42 000 (37% abortion and premature birth, 22% stillbirth, and 41% congenital

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AH syndrome), and the direct and indirect economic losses were in excess of US$ 20 × 10⁶ (Matumoto and Inaba, 1980; Inaba and Matumoto, 1981). During the 1974 epizootic in Australia losses of calves affected with the AH syndrome were in excess of 5000 cases and if abortions and stillbirths were included, based on the Japanese experience, could have been as high as 15 000 animals (Snowdon, 1979).

In the 1974 epizootic in New South Wales, Australia, Shepherd et al. (1978) recorded the clinical and pathological lesions distribution and incidence in an attempt to determine an epidemiological pattern. The probable period of infection correlated well with the presence of *C. brevitarsis* in the epidemic area; the distribution and incidence of neurologic cases likewise correlated with the expected geographical and climatic distribution of *C. brevitarsis* from an endemic area into nonendemic areas. The possibility that infected midges may be moved by the wind has been considered (Shepherd et al., 1978; Al-Busaidy et al., 1988); wind-borne spread has been proposed for bluetongue (Sellers et al., 1978), African horse sickness (Sellers et al., 1977) and bovine ephemeral fever (Morgan and Murray, 1969; Murray, 1970). About 20% of the animals in the endemic area of northern Australia did not have antibody to Akabane virus. A number of these susceptible animals could become infected and produce the sporadic and isolated cases which have been observed in this area (Hartley et al., 1977; Shepherd et al., 1978). Supporting this view, antibody to Akabane virus was shown to develop in cattle in northern Australia throughout the year (Cybinski et al., 1978).

The only outbreak of this disease reported in Israel occurred during 1969–70; of all dairy calves born during the epizootic 2–4% suffered from AH syndrome. In severely affected areas the syndrome was encountered in most herds, but its incidence within a herd was relatively low. In severely affected herds the number of abnormal calves amounted to 30% of all calvings during the peak months of the epizootic. In the epizootic areas arthrogryposis occurred between November and February, with peak incidence in December, whereas hydranencephaly occurred between November and June, with peak incidence in April (Markusfeld and Mayer, 1971).

PATHOGENESIS

The disease is caused by an in utero infection resulting in abortion, premature birth, stillbirth and congenital AH syndrome.

Pregnant cows, ewes and goats infected with Akabane virus do not present any clinical signs. Although the site of initial infection is not known, viremia seems to be a constant feature (Kurogi et al., 1975, 1977a, b; St. George et al., 1978; Parsonson et al., 1975, 1977, 1981a,b, 1985). These findings, together with infection of the placenta in pregnant animals, suggest that the virus may reach the fetus through hematogenous infection of the placenta (Parsonson et al., 1975, 1977; Kurogi et al., 1976; Hashiguchi et al., 1979). However, Akabane virus does not produce a persistent infection of the fetus as is observed in bluetongue (Luedke et al., 1977; Gibbs et al., 1979). Data on precolostral sera from newborn calves and sera from their mothers in the epidemic areas suggest that Akabane virus may invade the fetus in about 33% of the infected pregnant cows (Kurogi et al., 1975).

Histological examination of the bovine fetus from which Akabane virus was isolated reveal pathological changes of encephalomyelitis and polymyositis of skeletal muscles. IF staining demonstrates viral antigen in cells of skeletal muscle and cerebral tissue. Virus has been recovered from the brain, cerebral fluid, spinal cord, skeletal muscles and placenta of a naturally infected bovine

fetus at high titers (Kurogi et al., 1976). These observations, together with those obtained after experimental infection of pregnant dams (Kurogi et al., 1977a, b; Hashiguchi et al., 1979) indicate that the primary pathological lesions in infected fetuses are encephalomyelitis and polymyositis. Primary lesions in the fetus may soon result in abortion, premature birth or stillbirth. Of fetuses surviving the infection some may gradually develop brain lesions such as hydrocephalus, spongiform lesions and marked reduction in the number of motor neurons in the spinal anterior horn (Blood, 1956; Whittem, 1957; Nobel et al., 1971; Konno et al., 1975; Moriguchi et al., 1976; Hatley et al., 1977). The development of these secondary lesions depends upon the severity and the distribution of the primary damage in the CNS and the gestational stage when the infection occurs.

Arthrogryposis may ensue from the damage in the CNS (Blood, 1956; Whittem, 1957; Nobel et al., 1971; Konno et al., 1975; Kurogi et al., 1975; 1976, 1977a, b; Moriguchi et al., 1976; Hartley et al., 1977; Parsonson et al., 1977; Hashiguchi et al., 1979). These fetuses may be born at term or prematurely, but in some stillbirth may be the result. This sequence of events explains the contrasting time course in an outbreak for cases of abortion and premature birth, of stillbirth, and of congenital AH syndrome (see Fig. 164) and also the fact that some CNS inflammatory lesions were found, particularly during the early phase of the outbreaks (Kurogi et al., 1975).

Polymyositis was observed in naturally and experimentally infected fetuses, suggesting that it may be the cause of the congenital deformities and muscular changes observed in natural cases; alternatively, the changes could be sequelae of the CNS involvement (Kurogi et al., 1977a).

Further virological and histological investigations are needed to fully understand the pathogenesis of this disease.
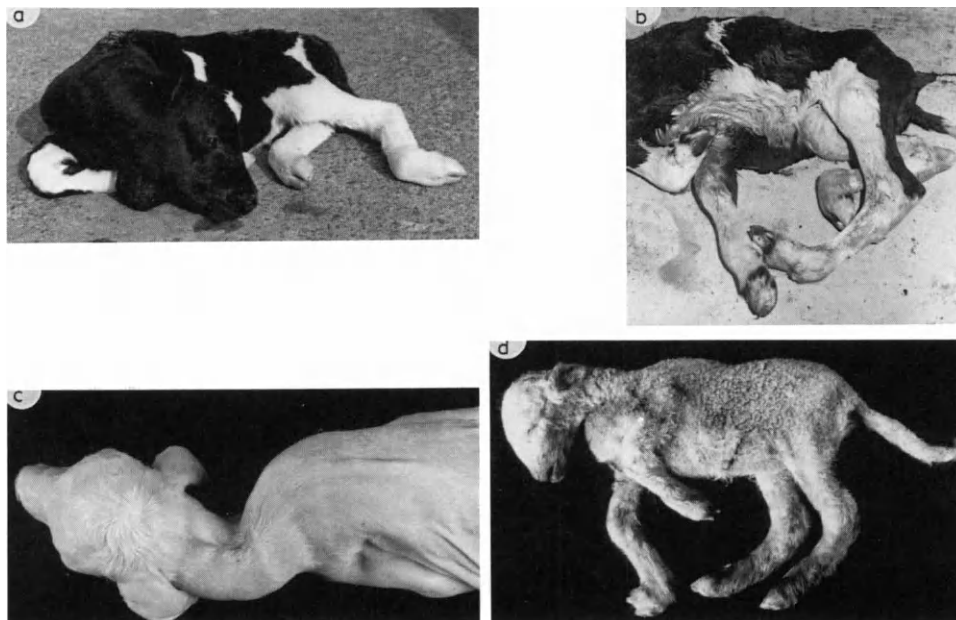
Fig. 165. (a,b) Arthrogrypotic newborn calf with severe involments of fore- and hind-limbs and torticollis; (c) torticollis in a fetus from a pregnant goat inoculated with Akabane virus; (d) congenital deformity in a lamb from a pregnant ewe inoculated with Akabane virus, arthrogryposis (courtesy of Dr. S. Inui, Hokkaido Branch Laboratory, National Institute of Animal Health, Sapporo, Hokkaido, Japan).

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DISEASE SIGNS

An epizootic in cattle may be first noticed by the increased incidence of abortions and premature births in late summer or autumn, followed some months later by the birth of incoordinated calves, calves with mild to severe arthrogryposis and sometimes cervical scoliosis, torticollis and kyphosis, and calves with hydranencephaly, sometimes associated with arthrogryposis.

No clinical abnormalities have been seen in dams during pregnancy.

Arthrogryposis is characterized by flexion or extension of various joints (Fig. 165a–d) and is seen in approximately 30–50% of affected calves (Blood, 1956; Whittem, 1957; Markusfeld and Mayer, 1971; Nobel et al., 1971; Kurogi et al., 1975; Shepherd et al., 1978). The affected joint cannot be straightened even by the application of force. Any joint may be affected, the carpal and tarsal joints being involved in most cases. The fore limbs are more often involved than the hind limbs. Most arthrogrypotic calves are unable to stand or walk by themselves.

The birth of most arthrogrypotic calves is associated with dystocia, necessitating embryotomy or caesarian section, and affected calves are usually slaughtered or die shortly after birth.

Calves with hydranencephaly due to Akabane virus resemble in appearance and behavior calves congenitally deformed by bluetongue virus (Nobel et al., 1971; Hartley and Wanner, 1974; Shepherd et al., 1978). Calves born with hydranencephaly may survive for several months if hand reared, but they never thrive. The calves are mature, but are underweight at birth. They also show various signs: blindness, nystagmus, deafness, dulness, slow suckling, paralysis and incoordination (Blood, 1956; Markusfeld and Mayer, 1971).
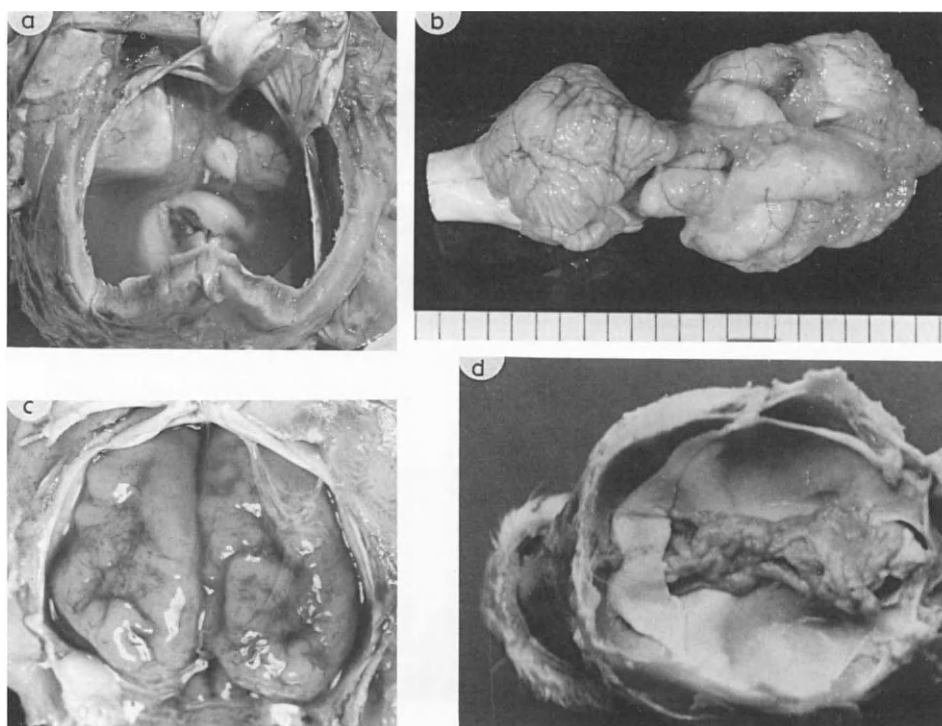
Fig. 166. (a) Dorsal view of the content of the cranial cavity of a calf with hydranencephaly; (b) cerebral defect of a newborn calf; (c) congenital deformity in a fetus from a pregnant goat inoculated with Akabane virus, hydranencephaly; (d) congenital deformity in a fetus from a pregnant goat inoculated with Akabane virus, hydranencephaly (courtesy of Dr. S. Inui, Hokkaido Branch Laboratory, National Institute of Animal Health, Sapporo, Hokkaido, Japan).

PATHOLOGY

Arthrogryposis and hydranencephaly are seen as the main gross lesions of Akabane virus infection. Cervical scoliosis, torticollis and kyphosis are also seen (see Fig. 165). Lesions differ in severity and in their detailed histopathology. Experimental infection at different gestational stages resulted in deformities: between 76 and 96 days of gestation for cattle, and between 30 and 46 days of gestation for sheep (Kurogi et al., 1977a; Parsonson et al., 1977, 1981c, d; Narita et al., 1978; Hashiguchi et al., 1979).

Arthrogrypotic calves show mainly restriction of movement of limb joints and in some cases neck joints. Joint surfaces are normal, and the bones are unaffected. In the affected limbs the muscles are usually smaller in bulk and paler in color than normal (Blood, 1956; Whittem, 1957; Bonner et al., 1961; Markusfeld and Mayer, 1971; Hartley and Haughey, 1974; Hartley and Wanner, 1974; Konno et al., 1975; Hartley et al., 1977).

Microscopically there is a severe loss of myelinated fibers in the lateral and ventral funiculi of the spinal cord, together with the loss of ventral horn neurons and of nerve fibers in ventral spinal nerves. However, white matter is unaffected (Whittem, 1957; Konno et al., 1975; Hartley et al., 1977).

In hydranencephalic calves the cerebral cortex is represented by a thin shell of edematous brain tissue, filled with fluid in the cystically dilated lateral ventricles (Fig. 166). The meninges, especially the pachymeninges, are thickened. In most cases the mesencephalon and metencephalon are intact. The cerebellum in most cases appears normal. Aplasia of the cerebellum has not been seen (Blood, 1956; Whittem, 1957; Konno et al., 1975; Hartley et al., 1977).

Early in the outbreaks calves which are incoordinate or unable to stand at birth are found, but no gross pathological lesions are seen in these animals. Microscopically there is a mild to moderate nonsuppurative acute encephalomyelitis, most evident in grey matter of the mid and posterior brain (Konno et al., 1975; Hartley et al., 1977).
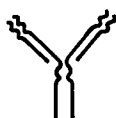
IMMUNE REACTION

In studies with Akabane virus the neutralization, HI, hemolysis–inhibition, CF, AGID and IF tests have been utilized (Matumoto and Inaba, 1980; Inaba and Matumoto, 1981; Porterfield and Della-Porta, 1981).

The neutralization test in Vero cell cultures by the microtiter method has been shown to be particularly useful for testing large numbers of sera (Hartley et al., 1975; Kurogi et al., 1975). The HI antibody titer was found to be closely correlated with the neutralizing antibody (Goto et al., 1978) and hemolysis–inhibiting antibody titer (Goto et al., 1979).

The CF test has principally been used for comparison of Simbu group viruses (Doherty et al., 1972). The neutralization test and the HI and hemolysis–inhibition tests are more specific, since they showed no cross-rection between Akabane and Aino viruses (Goto et al., 1976; Miura et al., 1978; Goto et al., 1979).

The AGID test has been shown to detect antibodies formed against other Simbu group viruses as well as the CF test (Porterfield and Della-Porta, 1981). The IF method has been used for detection of antigen in brain tissue as well as skeletal muscular tissue of naturally infected fetuses (Kurogi et al., 1976).

Animals infected with Akabane virus rapidly develop neutralizing, HI and hemolysis–inhibiting antibodies (Goto et al., 1978, 1979; Parsonson et al., 1981a,b,c,d). In cattle, these antibodies can be detected between 7 and 14 days following infection, and they are initially sensitive to 2-mercaptoethanol, indicating their IgM nature (Goto et al., 1978). Significant levels of neutralizing

antibody persist in cattle for at least 2 years following infection (Porterfield and Della-Porta, 1981). There is a biphasic antibody response, probably representing the switch from IgM to IgG (Hashiguchi et al., 1979; Parsonson et al., 1981a).

Fetal calves, lambs and kids infected in utero often develop virus neutralizing antibodies (Parsonson et al., 1977; Kurogi et al., 1977a,b; Hashiguchi et al., 1979). Fetal calves develped antibodies to Akabane virus between 70–96 days of gestation (Kurogi et al., 1977a) and fetal lambs (Parsonson et al., 1977; Hashiguchi et al., 1979) and fetal kids (Kurogi et al., 1977b) between 30–115 days of gestation.

Normal calves, lambs and kids aquire maternal antibodies by ingesting colostrum. In lambs, colostral neutralizing antibody declined to become virtually undetectable after 4 months of age. In calves there is a similar pattern, although the neutralizing antibodies remain at detectable levels for up to 6 months of age (Porterfield and Della-Porta, 1981).

LABORATORY DIAGNOSIS

For the isolation of Akabane virus, clinical material is inoculated into suckling mice by the intracerebral route (Oya et al., 1961; Doherty et al., 1972; Kurogi et al., 1976, 1978a), or into cultures of primary baby hamster kidney cells or continuous hamster cell lines like HmLu-1 and BHK-21 (Kurogi et al., 1976; Della-Porta et al., 1977; St. George et al., 1977). The most useful tissues for attempts to isolate the virus are the placentomes and fetal membranes, but also other tissues from aborted fetuses are likely to yeild isolates (Kurogi et al., 1976; Della-Porta et al., 1977). However, it is unlikely that virus will be isolated from deformed full-term offspring because of the serum neutralizing antibodies formed in the fetus. Fresh specimens from aborted fetuses occurring in late summer and early autumn give a better chance of successful virus isolation. Some inoculated mice die from encephalitis but others recover after showing mild clinical illness; serial passages can be readily made in suckling mice. In inoculated cell cultures Akabane virus replicates with CPE.

Comparison of the tissue culture isolation system with suckling mice indicated that mice are more sensitive. However, HmLu-1, Vero and BHK-21 cell lines are almost as sensitive as mice. Antigens of Akabane virus can be demonstrated in cerebral or muscular tissues of aborted fetuses by the IF technique (Kurogi et al., 1976).

For diagnosis by serology, the neutralization test has proved most useful. Serum samples from the dam and its fetus, and a presuckling sample of serum from the affected newborn animal should be collected for neutralization test. In cattle, sheep and goats transfer of maternal antibodies through the placenta to the fetus does not occur, and the newborn acquires maternal antibodies by ingesting colostrum. On the other hand, the fetus develops the ability to produce antibody upon antigenic stimulation early in gestation. Therefore the presence of antibody in precolostral or fetal serum indicates an intrauterine infection with Akabane virus or an antigenically related virus (Miura et al., 1974; Kurogi et al., 1975; Hartley et al., 1975, 1977; Coverdale et al., 1978).

There is serological evidence that Aino virus, another member of the Simbu group, may be associated with congenital bovine AH syndrome (Miura et al., 1974; Kurogi et al., 1975; Coverdale et al., 1978, 1979; Cybinski and St. George, 1978). There are some serological and experimental data that Tinaroo and Peaton viruses of the Simbu serogroup may also be associated with in utero infections (Porterfield and Della-Porta, 1981; I.M. Parsonson, personal communication, 1978).

PROPHYLAXIS AND CONTROL

There are two main approaches to the prophylaxis of Akabane virus infection. One is aimed at the vector and the other at protection of individual animals by vaccination. The former method includes the elimination of breeding sites and/or the direct attack on the adult arthropods by use of insecticides. It may have a temporary effect and help to interrupt the cycle for 1 or 2 weeks; the avoidance of exposure to vectors by screening of houses or by use of insect repellents is equally possible. These vector control measures are generally impractical, and knowledge concerning the vector is still inadequate.

The main method of prophylaxis is vaccination. A formalin-inactivated, aluminum phosphate gel-adsorbed vaccine has been developed with Akabane virus propagated in hamster lung cell cultures (Kurogi et al., 1978b). It induces neutralizing antibody after intramuscular inoculation in cattle as well as in goats, guinea pigs and mice. Antibody response of cattle after one dose is poor both in antibody level and seroconversion rate. However, a second dose given 4 or more weeks later induces a rapid and high-titered antibody formation in nearly all the animals. The antibody levels decline, but a booster dose given one year later again provokes a rapid response. Considering this pattern together with the seasonal incidence of the disease initial immunization should be made with two doses followed by a booster injection in late spring or early summer, immediately before the epizootic season. The vaccination prevents the development of viremia and infection of the fetus in pregnant cattle and goats challenged with virulent Akabane virus; it exerts few side effects in cattle. Cows vaccinated during pregnancy gave birth to healthy calves at term. The vaccine is licensed for general use in Japan. Kirkland and Barry (1985) reported that a β-propiolacton-inactivated vaccine was effective in the prevention of bovine Akabane disease in Australia.

A live virus vaccine has been developed with the attenuated strain of Akabane virus obtained by serial passage at 30°C in hamster lung cell cultures (Kurogi et al., 1979a,b). The vaccine, which has been found innocuous in calves and pregnant cows inoculated by i.c., i.v. and s.c. route, does not produce pyrexia, leukopenia and viremia but induces neutralizing antibody when inoculated into calves and pregnant cows. No virus has been recovered from various organs and fetuses of the vaccinated animals. Significant levels of neutralizing antibody persist in cows for at least 6 months following vaccination. The vaccine virus cannot be passaged serially in calves by intravenous inoculation of blood. However, the vaccine induces viremia in pregnant ewes and causes intrauterine infection at a very high frequency (Hashiguchi et al., 1981). In pregnant cows vaccination by the subcutaneous route prevented infection of the fetus upon challenge with virulent Akabane virus. Cows vaccinated during pregnancy gave birth to healthy calves at term. No adverse effects of vaccination on milk production was shown. Lyophilized vaccine was stored at 4°C without loss of infectivity for at least one year. In view of these findings the vaccine is currently being made commercially available in Japan for prophylaxis of the disease in cattle.

REFERENCES

Al-Busaidy, S.M., Mellor, P.S. and Taylor, W.P., 1988. Prevalence of neutralizing antibodies to Akabane virus in the Arabian Peninsula. Vet. Microbiol., 17: 141–149.

Andersen, A.A. and Campbell, C.H., 1978. Experimental placental transfer of Akabane virus in the hamster. Am. J. Vet. Res., 339: 301–304.

Blood, D.C., 1956. Arthrogryposis and hydranencephaly in newborn calves. Aust. Vet. J., 32: 125–131.

Bonner, R.B., Mylrea, P.J. and Doyle, B.J., 1961. Arthrogryposis and hydranencephaly in calves. Aust. Vet. J., 37: 160–161.

Coverdale, O.R., Cybinski, D.H. and St. George, T.D., 1978. Congenital abnormalities in calves associated with Akabane virus and Aino virus. Aust. Vet. J., 54: 151–152.

Coverdale, O.R., Cybinski, D.H. and St. George, T.D., 1979. A study of the involvement of three Simbu group arboviruses in bovine congenital arthrogryposis and hydranencephaly in the New England area of New South Wales. In: T.D. St. George and E.L. French (Editors), Proceedings of the 2nd Symposium on Arbovirus Research in Australia. CSIRO and Queensland Institute of Medical Research, pp. 130–139.

Cybinski, D.H., 1984. Douglas and Tinaroo viruses: two Simbu group arboviruses infecting *Culicoides brevitarsis* and livestock in Australia. Aust. J. Biol. Sci., 37: 91–97.

Cybinski, D.H. and St. George, T.D., 1978. A survey of antibody to Aino virus in cattle and other species in Australia. Aust. Vet. J., 54: 371–373.

Cybinski, D.H., St. George, T.D. and Paull, N.I., 1978. Antibodies to Akabane virus in Australia. Aust. Vet. J., 54: 1–3.

Davies, F.G. and Jesset, D.M., 1985. A study of the host range and distribution of antibody to Akabane virus (genus *Bunyavirus*, family Bunyaviridae) in Kenya. J. Hyg. Camb., 95: 191–196.

Della-Porta, A.J., Murray, M.D. and Cybinski, D.H., 1976. Congenital bovine epizootic arthrogryposis and hydranencephaly in Australia. Distribution of antibodies to Akabane virus in Australian cattle after the 1974 epizootic. Aust. Vet. J., 52: 496–501.

Della-Porta, A.J., O'Halloran, M.L., Parsonson, I.M., Snowdon, W.A., Murray, M.D., Hartley, W.J. and Haughey, K.J., 1977. Akabane disease: Isolation of the virus from naturally infected ovine foetuses. Aust. Vet. J., 53: 51–52.

Della-Porta, A.J., Parsonson, I.M., McPhee, D.A., Snowdon, W.A., Standfast, H.A., St. George, T.D. and Cybinski, D.H., 1981. Biochemical studies of Akabane virus isolates and a possible structural relationship to virulence. Dev. Cell Biol., 7: 103–110.

Doherty, R.L., 1972. Arboviruses of Australia. Aust. Vet. J., 48: 172–180.

Doherty, R.L., Carley, J.G., Standfast, H.A., Dyce, A.L. and Snowdon, W.A., 1972. Virus strains isolated from arthropods during an epizootic of bovine ephemeral fever in Queensland. Aust. Vet. J., 48: 81–86.

Furuya, Y., Shoji, H., Inaba, Y. and Matumoto, M., 1980. Antibody to Akabane virus in horses, sheep and goats in Japan. Vet. Microbiol., 5: 239–242.

Gibbs, E.P.J., Lawman, M.J.P. and Herniman, K.A.J., 1979. Preliminary observations on transplacental infection of bluetongue in sheep — a possible overwintering mechanism. Res. Vet. Sci., 27: 118–120.

Goto, Y., Inaba, Y., Kurogi, H., Takahashi, E., Sato, K., Omori, T., Hanaki, T., Sazawa, H. and Matumoto, M., 1976. Improved hemagglutination of Simbu group arboviruses with higher sodium chloride molarity diluent. Vet. Microbiol., 1: 449–458.

Goto, Y., Inaba, Y., Miura, Y., Kurogi, H., Takahashi, E., Sato, K., Omori, T., Hanaki, T., Sazawa, H. and Matumoto, M., 1978. Hemagglutination–inhibition test applied to the study of Akabane virus infection in domestic animals. Vet. Microbiol., 3: 89–99.

Goto, Y., Inaba, Y., Tamura, Y., Hanaki, T., Sazawa, H. and Matumoto, M., 1979. Hemolytic activity of Akabane virus. Vet. microbiol., 4: 261–278.

Hartley, W.J. and Haughey, K.G., 1974. An outbreak of microcephaly in lambs in New South Wales. Aust. Vet. J., 50: 55–58.

Hartley, W.J. and Wanner, R.A., 1974. Bovine congenital arthrogryposis in New South Wales. Aust. Vet. J., 50: 185–188.

Hartley, W.J., Wanner, R.A., Della-Porta, A.J. and Snowdon, W.A., 1975. Serological evidence for the association of Akabane virus with epizootic bovine congenital arthrogryposis and hydranencephaly syndromes in New South Wales. Aust. Vet. J., 51: 103–104.

Hartley, W.J., DeSaram, W.G., Della-Porta, A.J., Snowdon, W.A. and Shepherd, N.C., 1977. Pathology of congenital bovine epizootic arthrogryposis and hydranencephaly and its relationship to Akabane virus. Aust. Vet. J., 53: 319–325.

Hashiguchi, Y., Nanba, K. and Kumagai, T., 1979. Congenital abnormalities in newborn lambs following Akabane virus infection in pregnant ewes. Natl. Inst. Anim. Health Q. (Jpn.), 19: 1–11.

Hashiguchi, Y., Murakami, Y. and Nanba, K., 1981. Response of pregnant ewes inoculated with Akabane disease live virus vaccine. Natl. Inst. Anim. Health Q. (Jpn.), 21: 113–120.

Hoffmann, D. and St. George, T.D., 1985. Growth of epizootic hemorrhagic disease, Akabane, and ephemeral fever viruses in *Aedes albopictus* cells maintained at various temperatures. Aust. J. Biol. Sci., 38: 183–188.

Ikeda, S. and Yonaiyama, K., 1978. Deformities of chick embryos in experimental Akabane virus infection. Natl. Inst. Anim. Health Q. (Jpn.), 18: 89–96.

Inaba, Y. and Matumoto, M., 1981. Congenital arthrogryposis–hydranencephaly syndrome. In: E.P.J. Gibbs (Editor), Virus Diseases of Food Animals. Academic Press, London, pp. 653–671.

Inaba, Y., Kurogi, H. and Omori, T., 1975. Akabane disease: Epizootic abortion, premature birth, stillbirth and congenital arthrogryposis–hydranencephaly in cattle, sheep and goats caused by Akabane virus. Aust. Vet. J., 51: 584–585.

Ito, Y., Kurogi, H., Goto, Y., Inaba, Y. and Omori, T., 1979. Electron microscopy of Akabane virus. Acta Virol., 23: 198–202.

Kalmar, E., Peleg, B.A. and Savir, D., 1975. Arthrogryposis–hydranencephaly syndrome in newborn cattle, sheep and goats. Serological survey for antibodies against the Akabane virus. Refu. Vet., 32: 37–54.

Kirkland, P.D. and Barry, R.D., 1985. The epidemiology and control of Akabane disease. In: A.J. Della-Porta (Editor), Veterinary Viral Diseases, Their Significance in South-East Asia and the Western Pacific, Academic Press, London, pp. 430–433.

Konno, S., Moriwaki, M., Nakagawa, M., Uchimura, M., Kamimiyata, M. and Tojinbara, K., 1975, Congenital abnormality of calves with arthrogryposis and hydranencephaly in Japan in 1972–1973. Natl. Inst. Anim. Health Q. (Jpn.), 15: 52–53.

Kurogi, H., Inaba, Y., Goto, Y., Miura, Y., Takahashi, H., Sato, K., Omori, T. and Matumoto, M., 1975. Serologic evidence for etiologic role of Akabane virus in epizootic abortion-arthrogryposis–hydranencephaly in cattle in Japan, 1972–1974. Arch. Virol., 47: 71–83.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Omori, T., Miura, Y., Goto, Y., Fujiwara, Y., Hatano, Y., Kodama, K., Fukuyama, S., Sasaki, N. and Matumoto, M., 1976. Epizootic congenital arthrogryposis–hydranencephaly syndrome in cattle: Isolation of Akabane virus from affected fetuses. Arch. Virol., 51: 67–74.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Satoda, K., Goto, Y., Omori, T. and Matumoto, M., 1977a. Congenital abnormalities in newborn calves after inoculation of pregnant cows with Akabane virus. Infect. Immun., 17: 338–343.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Goto, Y. and Omori, T., 1977b. Experimental infection of pregnant goats with Akabane virus. Natl. Inst. Anim. Health Q. (Jpn.), 17: 1–9.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Satoda, K. and Omori, T., 1977c. Development of Akabane virus and its immunogen in HmLu-1 cell culture. Natl. Inst. Anim. Health Q. (Jpn.), 17: 27–28.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Akashi, H., Satoda, J. and Omori, T., 1978a. Pathogenicity of different strains of Akabane virus for mice. Natl. Inst. Anim. Health Q. (Jpn.), 18: 1–7.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Goto, Y., Satoda, K., Omori, T. and Hatakeyama, H., 1978b. Development of inactivated vaccine for Akabane disease. Natl. Inst. Anim. Health Q. (Jpn.), 18: 97–108.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Akashi, H., Satoda, K. and Omori, T., 1979a. An attenuated strain of Akabane virus: A candidate for live virus vaccine. Natl. Inst. Anim. Health Q. (Jpn.), 19: 12–22.

Kurogi, H., Inaba, Y., Akashi, H., Takahashi, E., Sato, K., Satoda, K., Sugimoto, C., Hatakeyama, H. and Omori, T., 1979b. Immune response of various animals to Akabane disease live virus vaccine. Natl. Inst. Anim. Health Q. (Jpn.), 19: 23–31.

Kurogi, H., Akiba, Y., Inaba, Y. and Matumoto, M., 1987. Isolation of Akabane virus from the biting midge, *Culicoides oxystoma* in Japan. Vet. Microbiol., 15: 243–248.

Luedke, A.J., Jochim, M.M. and Jones, R.H., 1977. Bluetongue in cattle: Effects of *Culicoides varipennis* transmitted bluetongue virus on pregnant heifers and their calves. Am. J. Vet. Res., 38: 1687–1695.

Markusfeld, O. and Mayer, E., 1971. An arthrogryposis and hydranencephaly syndrome in calves in Israel, 1969/70. Epidemiological and clinical aspects. Refu. Vet., 28: 51–61.

Matsuyama, T., Oya, A., Ogata, T., Kobayashi, I., Nakamura, T., Takahashi, M. and Kitaoka, M., 1960. Isolation of arborviruses from mosquitoes collected at live-stock pens in Gumma Prefecture in 1959. Jpn. J. Med. Sci. Biol., 13: 191–198.

Matumoto, M. and Inaba, Y., 1980. Akabane disease and Akabane virus. Kitasato Arch. Exp. Med., 53: 1–21.

McPhee, D.A., Parsonson, I.M., Della-Porta, A.J. and Jarrett, R.G., 1984. Teratogenicity of Australian Simbu serogroup and some other Bunyaviridae viruses: the embryonated chicken egg as a model. Infect. Immun., 43: 413–420.

Metselaar, D. and Robin, Y., 1976. Akabane virus isolated in Kenya. Vet. Rec., 99: 86.

Miah, A.H. and Spradbrow, P.B., 1978. The growth of Akabane virus in chicken embryos. Res. Vet. Sci., 25: 253–254.

Miura, Y., Hayashi, S., Ishihara, T., Inaba, Y., Omori, T. and Matumoto, M., 1974. Neutralizing antibody against Akabane virus in precolostral sera from calves with congenital arthrogryposis–hydranencephaly syndrome. Arch. Ges. Virusforsch., 46: 377–380.

Miura, Y., Inaba, Y., Goto, Y., Takahashi, E., Kurogi, H., Hayashi, S., Omori, T., and Matumoto, M., 1978. Serological comparison of Aino and Samford viruses in Simbu group of Bunyaviruses. Microbiol. Immunol., 22: 651–654.

Morgan, I. and Murray, M.D., 1969. The occurrence of ephemeral fever of cattle in Victoria in 1968. Aus. Vet. J., 45: 271–274.

Moriguchi, R., Izawa, H. and Soekawa, M., 1976. A pathological study on calves with arthrogryposis and hydranencephaly. Zentralbl. Veterinärmed. B, 23: 190–199.

Murray, M.D., 1970. The spread of ephemeral fever of cattle during the 1967–68 epizootic in Australia. Aust. Vet. J., 46: 77–82.

Nakajima, Y., Takahashi, E. and Konno, S., 1979. Encephalomyelitis in mice experimentally infected with Akabane virus. Natl. Inst. Anim. Health Q. (Jpn.), 19: 47–52.

Narita, M., Inui, S. and Hashiguchi, Y., 1978. The pathogenesis of congenital encephalopathies in sheep experimentally induced by Akabane virus. J. Comp. Pathol., 89: 229–240.

Nobel, T.A., Klopfer, U. and Neuman, F., 1971. Pathology of an arthrogryposis–hydranencephaly syndrome in domestic ruminants in Israel, 1969/70. Refu. Vet., 28: 144–151.

Omori, T., Inaba, Y., Kurogi, H., Miura, Y., Nobuto, K., Ohashi, Y. and Matumoto, M., 1974. Viral abortion, arthrogryposis–hydranencephaly syndrome in cattle in Japan, 1972–1974. Bull. Off. Int. Epizoot., 81: 477–458.

Oya, A., 1971, 1972. Annual Reports WHO Regional Reference Center for Arbovirus. Natl. Inst. Health, Tokyo, Japan.

Oya, A., Okubo, T., Ogata, T., Kobayashi, I. and Matsuyama, T., 1961. Akabane, a new arbovirus isolated in Japan. Jpn. J. Med. Sci. Biol., 14: 101–108.

Parsonson, I.M., Della-Porta, A.J., Snowdon, W.A. and Murray, M.D., 1975. Congenital abnormalities in foetal lambs after inoculation of pregnant ewes with Akabane virus. Aus. Vet. J., 51: 585–586.

Parsonson, I.M., Della-Porta, A.J. and Snowdon, W.A., 1977. Congenital abnormalities in newborn lambs after infection of pregnant sheep with Akabane virus. Infect. Immun., 15: 254–262.

Parsonson, I.M., Della-Porta, A.J., Snowdon, W.A. and O'Halloran, M.L., 1981a. Experimental infection of bulls with Akabane virus. Res. Vet. Sci., 31: 157–160.

Parsonson, I.M., Della-Porta, A.J., Snowdon, W.A. and O'Halloran, M.L., 1981b. The consequences of infection of cattle with Akabane virus at the time of insemination. J. Comp. Pathol., 91: 611–619.

Parsonson, I.M., Della-Porta, A.J., O'Halloran, M.L., Snowdon, W.A., Fahey, K.J. and Standfast, H.A., 1981c. Akabane virus infection in the pregnant ewe. 1. Growth of virus in the foetus and the development of the foetal immune response. Vet. Microbiol., 6: 197–207.

Parsonson, I.M., Della-Porta, A.J. and Snowdon, W.A., 1981d. Akabane virus infection in the pregnant ewe. 2. Pathology of the foetus. Vet. Microbiol., 6: 209–224.

Parsonson, I.M., Della-Porta, A.J. and Selleck, P.W., 1985. Pathology of Akabane virus in the ovine foetus: immunofluorescence and histological findings. In: A.J. Della-Porta (Editor), Veterinary Viral Diseases, Their Significance in South-East Asia and the Western Pacific. Academic Press, London pp. 439–442.

Pattnaik, A.K. and Abraham, G., 1983. Identification of four complementary RNA species in Akabane virus-infected cells. J. Virol., 47: 452–462.

Porterfield, J.S. and Della-Porta, A.J., 1981. Bunyaviridae: infections and diagnosis. In: E. Kurstak and C. Kurstak (Editors), Comparative Diagnosis of Viral Diseases, Academic Press, New York, Vol. 4, pp. 479–504.

Porterfield, J.S., Casals, J., Chumakov, M.P., Gaidamovich, S. Ya., Hannoun, C., Holmes, I.H., Horzinek, M.C., Mussgay, M., Okar-Blom, N. and Russel, P.K., 1976. Bunyaviruses and Bunyaviridae. Intervirology, 6: 13–24.

Rudert, C.P., Lawrence, J.A., Foggin, C. and Barlow, R.M., 1978. A rigid lamb syndrome in sheep in Rhodesia. Vet. Rec., 102: 374–377.

Sellers, R.F. and Herniman, K.A.J. 1981. Neutralizing antibodies to Akabane virus in ruminants in Cyprus. Trop. Anim. Health Prod., 13: 57–60.

Sellers, R.F., Pedgley, D.E., and Tucker, M.R., 1977. Possible spread of African horse sickness on the wind. J. Hyg., 79: 279–298.

Sellers, R.F., Pedgley, D.E. and Tucker, M.R., 1978. Possible windborne spread of bluetongue to Portugal, June–July 1956. J. Hyg., 81: 189–196.

Shepherd, N.C., Gee, C.D., Jessep, T., Timmins, G., Carroll, S.N. and Bonner, R.B., 1978. Congenital bovine epizootic arthrogryposis and hydranencephaly. Aust. Vet. J., 54: 171–177.

Shimshony, A., 1978. Rigid lamb syndrome. Vet. Rec., 103: 409.

St. George, T.D., Cybinski, D. and Paull, N.I., 1977. The isolation of Akabane virus from a normal bull. Aust. Vet. J., 54: 249.

St. George, T.D., Standfast, H.A. and Cybinski, D.H., 1978. Isolation of Akabane virus from sentinel cattle and *Culicoides brevitarsis*. Aust. J. Vet. J., 54: 558–561.

Snowdon, W.A., 1979. Some arthropod-borne viruses infecting livestock in animal. Proceedings of a Symposium on Tropical Agriculture Research. Trop. Agric. Res. Serie, Trop. Agric. Res. Center, Ministry of Agriculture and Forestry, Nishigahara, Kita-ku, Tokyo, No. 13, pp. 131–139.

Takahashi, E., Inaba, Y., Kurogi, H., Sato, K., Goto, Y., Ito, Y., Omori, T. and Matumoto, M., 1978. Physicochemical properties of Akabane virus, a member of the Simbu arbovirus group of the family Bunyaviridae. Vet. Microbiol., 3: 45–54.

Takahashi, K., Oya, A., Ogada, T., Matsuo, R., Kuma, M. and Noguchi, H., 1968. Aino virus, a new member of Simbu group of arbovirus from mosquitoes in Japan. Jpn. J. Med. Sci. Biol., 21: 95–101.

Theodoridis, A., Neville, E.M., Els, H.J. and Boshoff, S.T., 1979. Viruses isolated from *Culicoides* midges in South Africa during unsuccessful attempts to isolate bovine ephemeral fever virus. Onderstepoort J. Vet. Res., 46: 191–198.

Whittem, J.H., 1957. Congenital abnormalities in calves: Arthrogryposis and hydranencephaly. J. Pathol. Bacteriol., 73: 375–387.

Chapter 44

Rift Valley Fever Virus

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INTRODUCTION

The virus causing Rift Valley Fever (RVF) was first isolated in 1931 from sheep brought into the Rift Valley region of Kenya (Daubney et al., 1931). The disease pattern indicated an arthropod vector, but mosquito transmission was not proven until 1949 (Smithburn et al., 1949). The 1951 South African outbreak demonstrated that the disease was not limited to a unique geographic region (Alexander, 1951). RVF since has been described throughout most of sub-Saharan Africa and recently the Nile Valley. This outbreak caused extensive human disease in the southeastern delta region of Egypt in 1977; RVF reached the Mediterranean coast of Egypt in 1978 (Hoogstraal et al., 1979). RVF has not been reported to date outside the African continent. Several review articles can serve as entrees into the extensive literature (Weiss, 1957; Easterday, 1965; Peters and Meegan, 1981; WHO/FAO Working Group on Rift Valley Fever, 1982; Shimshony and Barzilai, 1983; Lupton and Peters, 1984).

VIRUS PROPERTIES

Several physical and chemical properties are common in more than 200 viruses in the Bunyaviridae. Physical properties of the virion include a spherical shape bounded by a host-cell-derived membrane with virus-coded glycoprotein spikes, a diameter of 85–100 nm, and a buoyant density of 1.21 g/ml for the virion and 1.29 g/ml for the virion nucleoprotein core. When viewed in thin section by transmission electron microscopy, Bunyaviridae have a morphogenesis consistent with helical symmetry of the nucleoprotein core (Fig. 167). Virions are formed by budding through the smooth endoplasmic reticulum with many virions seen in Golgi cisternae which communicate with the extracellular environment. Virion formation appears to start with a small crescent-shaped region of the smooth membrane acquiring virus glycoprotein, to which the nucleocapsid attaches. Assembly proceeds as virions are enclosed by smooth membranes, traverse the cytoplasm, and are extruded from the cell (Smith and Pifat, 1982).

Detection of differences among strains of RVF virus (RVFV) requires the sensitivity of RNA fingerprinting. All isolates examined have been essentially identical using routine serologic tests. The Egyptian 1977 isolates, however, were more pathogenic in rats (Peters and Anderson, 1981) than earlier isolates. A Central and West African virus, Singa, previously considered an ungrouped *Bunyavirus*, has been shown to be a strain of RVFV (Meegan et al., 1983).

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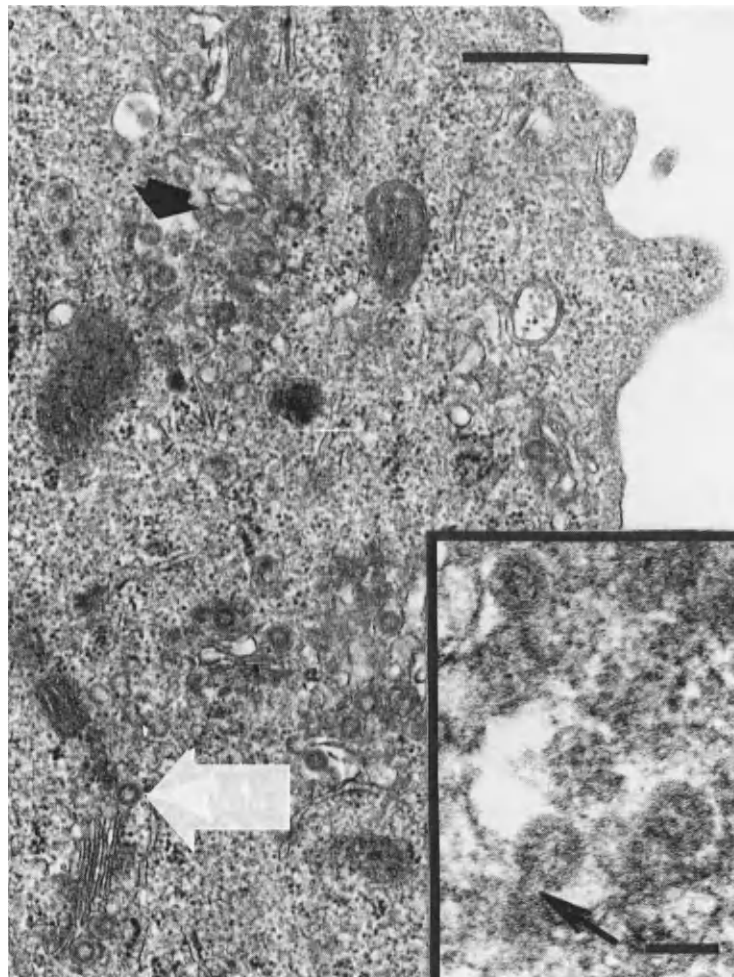
Fig. 167. Areas of developing RVF virions in the cytoplasm of a Vero cell, a continuous cell line derived from African Green monkey kidney. The plasma membrane is visible at the upper right and has no associated virions. Virions are developing around internal smooth membranes; the broad arrow indicates a virion that has almost completely budded into a smooth membrane bounded vacuole. The insert shows a higher magnification of the budded virion and the continuity of the smooth membrane and the stalk still attaching the bud to the membrane, indicated by the thin arrow; virions are lying free in the vacuole; a small virion is lying in the cisterna of a Golgi-like stack of smooth membrane bound vesicles; bar = 1 μm.

Neurotropic strains have been produced in the laboratory, and the Lunyo strain may be a naturally occurring neurotropic strain.

Virion structure does not completely explain the observed stability. Like most enveloped virions, RVFV is sensitive to lipid solvents, detergents, and low pH. However, at neutral or alkaline pH and in the presence of protein, such as in blood or serum, the virus has an extraordinary stability, and retains infectivity for up to 4 months at 4°C. Maintaining virus specimens below 0°C resulted in the retention of infectivity for 8 years (Easterday, 1965). In serum, infectivity is retained for up to 3 h at 56°C (Mims and Mason, 1956). Clearly, sera must be considered infectious after the usual 30–60-min heat treatment for complement inactivation prior to CF and plaque-reduction neutralization testing. Likewise, cold acetone extraction of sera for HI testing is not effective in eliminating infectious virus, since 12 h are required in acetone at −30°C for inactivation. Formalin does inactivate the virus, even at the low concentrations used for the human vaccine: a 0.3% final concentration for 72 h at 37°C.

Betapropiolactone (0.1% final concentration) also can be used for inactivation according to the procedure of Casals (1979).

It must be stressed that RVFV is relatively stable in aerosols, with a biological half-life in excess of 77 min at 25°C and 30% RH. Humans have been infected by aerosols of blood created when RVFV-infected sheep were slaughtered (Hoogstraal et al., 1979). In the past, RVF laboratory infections were common. Therefore, it is important that only vaccinated individuals handle material suspected to contain RVFV and that they carry out all manipulations in biological safety cabinets designed to contain aerosols.

Cleaning of RVFV-contaminated surfaces can be accomplished with strong household bleach, either sodium or calcium hypochlorite. Residual chlorine should be tested before using the solution and should exceed 5000 ppm. Both disinfectants are rendered ineffective by large amounts of organic materials; therefore, blood and serum spills must be treated several times.

EPIZOOTIOLOGY

The host range of RVFV, as identified by seroconversion, is more extensive than the host range defined by severe disease. In Africa, disease seems limited to domestic ruminants, with imported European animals being more severely affected than native African breeds. Outbreaks among cattle and sheep are characterized by widespread abortion and rapidly fatal neonatal disease. Fulminant neonatal disease may be the first indication of RVF in areas where abortion rates are high due to other agents such as *Brucella* or bluetongue virus. Generally, disease progression and its severity are inversely proportional to age. While adult sheep and cattle may suffer at least 10–30% mortality, animals less than 7 days old may have fatality rates approaching 100%. Goats are more variable than sheep or cattle with respect to severe disease, and the incidence of seroconversion following an epizootic is much lower. Domestic Asian buffalo were extensively involved in the 1977 Egyptian epizootic; the clinical disease resembled that observed in cattle.

Other domestic animals are relatively resistant. Equines develop antibody in the absence of disease. Camels, like Equidae, can be infected but show no clinical signs. RVF was circumstantially linked to abortions in camels in Kenya (Scott et al., 1963; Davies et al., 1985), although virus was not isolated. Laboratory infection of pregnant rabbits resulted in abortion in the absence of other clinical signs. Mice and hamsters die from fulminant liver necrosis. Guinea pigs and most rat strains do not show illness. Pigs can be infected only with massive doses of virus and show no disease. Both wild and domestic fowl are refractory to infection.

Reservoirs for RVFV are unidentified even though extensive searches have been made in Kenya, Zimbabwe, and South Africa. In sub-Saharan Africa, RVF epizootics do not appear to spread gradually from enzootic foci; rather, RVF epizootics occur simultaneously over areas covering hundreds of kilometers during years of exceptionally heavy rains. Annually, transmission to sentinel animals occurs in certain enzootic areas which are recognized in the wet coastal forest regions of Kenya, Mozambique, and eastern South Africa. When RVF is introduced into a new region, such as Egypt, gradual spread occurs that is characteristic of arthropod-borne disease. Furthermore, transovarial transmission in arthropods occurs among viruses of other genera of the Bunyaviridae, which suggests that a similar mechanism might exist with RVFV.

If the RVF reservoir were not vertebrates but transovarially infected *Aedes* mosquitoes, the epizootic pattern observed in sub-Saharan Africa would be expected. As higher than normal rains flood low lying areas, water would reach

eggs from previous years, and larvae would emerge and mature into infected adults. These infected mosquitoes then could initiate a virus-amplifying cycle in vertebrates, which other genera of mosquitoes would transform into an epizootic. RVFV has been isolated from unfed female and male adult *Culex linneatopennis* reared from larvae taken from artificially flooded grassland depressions in Kenya (Linthicum et al., 1985). However, proof of transovarial transmission is not complete since RVFV isolations from eggs and larvae have not yet been made.

Mosquitoes of many species of the genera *Aedes, Anopheles, Culex, Eretmapodites,* and *Mansonia* can transmit RVFV under field conditions. Additionally, North American mosquitoes of the genera *Aedes, Anopheles,* and *Culex* have been shown to transmit RVF under laboratory conditions (Gargan et al., 1984). In laboratory tests *Culex pipiens*, an important RVFV vector in Egypt, preferred to feed on feverish animals. Such behaviour in the field would assure blood meals of maximal virus content (Turell et al., 1984). Also, *Culex pipiens* shows increasing vector competence as laboratory holding temperatures are raised (Turell et al., 1985), which might explain the observed lull in RVF transmissions during the winter months in Egypt even though *Culex pipiens* numbers remained high.

The broad range of possible vertebrate amplifying hosts plus the numerous potential invertebrate vectors would suggest that RVFV introduction anywhere in the world could be followed by an epizootic involving multiple mosquito species. Arthropods also might transmit RVFV by strictly mechanical means. The high viremia common in RVF-infected animals is sufficient to contaminate the mouth parts of most biting arthropods, resulting in mechanical spread of the virus (Hoch et al., 1985).

Other arthropods caught in field situations have been found to contain RVFV, but biologic transmission has not been established after ingestion of blood meals containing infectious virus. *Hyalomma dromedarii* and *Rhipicephalus sangineus* ticks, fed as larvae, did not transmit or even retain RVFV transtadially when tested (Sabry et al., 1984). *Phlebotomous* and *Lutzomyia* sand flies did not become infective after natural feeding on viremic hosts; however, the virus was transmitted if the gut barrier was circumvented by intrathoracic inoculation (Hoch et al., 1984). Likewise, midges and black flies have not been shown to transmit RVFV (Shimshony and Barzilai, 1983).

Blood and serum from RVFV-infected animals are important infectious sources for human disease. Slaughterhouse workers, farmers, veterinarians, and laboratory technicians are the principal at-risk occupations in an RVF epizootic. Transmission of RVFV by consumption of milk or meat from infected animals does not appear common.

Geographic distribution of RVFV outbreaks is shown in Fig. 168. RVF is endemic in virtually all sub-Saharan Africa. Potential for spread into Asia exists, since only Egypt and Israel have had active vaccination programs.

Recent reports from Senegal and Mauritania, and the Institute Pasteur de Dakar, indicate a major epizootic of RVF occurred in 1987. In early October 1987 an increase in human febrile disease and the appearance of an often fatal hemorrhagic disease were noted at the Rosso hospital and surrounding area of the Senegal river. The virus has been isolated and identified from over 400 human specimens. Current estimates indicate a higher than previously documented human mortality rate with an estimate of 200 probable RVF fatalities occurring during the October–January period. The number of reported cases decreased in early 1988 concomitant with the decline in mosquito populations caused by the onset of dry weather. Retrospective serological studies to detect human and animal IgM antibody indicate an extensive outbreak. Local domestic animal species were heavily involved, and there are anecdotal reports of
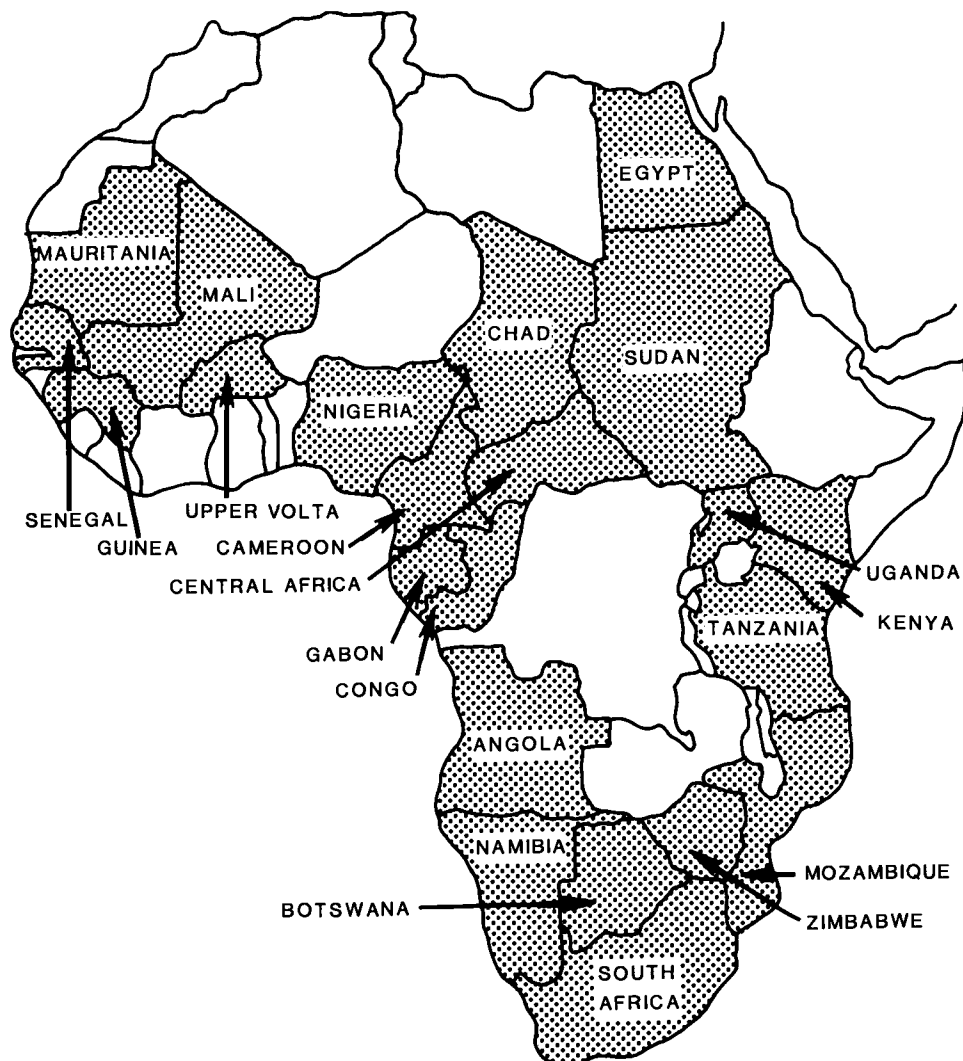
Fig. 168. Map of Africa showing reported RVF outbreaks.

high mortality in young lambs; abortion in sheep approached 90% in affected flocks.

No isolates have been made from arthropods. Previous isolations and low levels of antibody in domestic animals in West Africa had indicated RVFV had been circulating in an endemic state in this area before 1987. There is speculation that major environmental changes brought about by the 1986–87 damming of the Senegal River near Rosso might have provided new conditions conducive to epizootic RVFV transmission.

PATHOGENESIS

Arthropod transmission is recognized as the principal mode of disease spread, although infections following contact with infected tissues or inhalation of aerosols may occur. Regardless of the portal of entry, the liver is the primary site of viral replication. Studies in inbred, susceptible Wistar-Furth rats showed viral replication was restricted to certain organs: most renal glomeruli and some tubules, foci of adrenal cortical cells, most areas of the spleen except the T-dependent periarteriolar sheaths, some littoral macrophages in lymph nodes, scattered small vessel walls, and essentially every cell

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in the liver. Viremia of 10^7–10^8 PFU/ml and liver titers of $10^{6.5}$–10^7 PFU/g are attained within 24 h after experimental infection (Peters and Anderson, 1981). Sequential determinations have not been reported for ruminants, but similar events would be expected. In the pregnant animal, there is a predilection of the virus for the placentomes via the hematogenous route. Rapid fetal death due to direct viral infection rather than to generalized placentitis is believed to be the principal cause of abortion. There is rapid progress from mild hepatocellular changes to massive necrosis. Hemorrhagic diathesis, reduced prothrombin levels, and prolonged clotting times have been observed in laboratory-infected animals, suggesting that disseminated intravascular coagulation may play a role in the disease process. Yet, definitive experimental data are lacking (Coetzer, 1982).

DISEASE SIGNS

Sheep and cattle are the primary ruminant species affected by RVFV, with goats being involved to a lesser extent. Clinical signs observed vary considerably and are related to the species and age of the animals involved. Typically, younger animals are the most susceptible. Disease among animals can be divided into peracute, acute, subacute, and inapparent forms.

Sheep
Disease is characterized by a short incubation period, a definite but short febrile period, and signs indicative of liver necrosis. The peracute form most commonly occurs in lambs less than 7 days old. In experimentally infected animals, an incubation period of approximately 12 h preceeds onset of a febrile (40–42°C) episode that terminates in death within 24–48 h. Inappetence, listlessness and weakness are the only signs observed prior to death. Mortality ranges from 90 to 100%. The acute disease form occurs with older lambs and occasionally adult sheep. Fever (40–41°C) is accompanied by variable clinical signs that may include vomiting, rapid pulse, unsteady gait, and hemorrhagic diarrhea. The oral mucosa may be hyperemic and the conjuctivae injected (Coetzer, 1977; Erasmus and Coetzer, 1981). Although there have been reports of catarrhal stomatitis with erosions of the lips, tongue, and mucosa of the cheeks combined with necrosis of the udder or scrotum, these signs likely were caused by concomitant infections with other agents. Death may occur in 1–4 days in 10–30% of older lambs and adult sheep. The mortality rate increases markedly if the general health of the flock is reduced. Subacute RVF, the common form in adult sheep, is evidenced by anorexia, general weakness, a febrile response of 24–96-h duration, and a low mortality rate. Only in adult sheep does inapparent infection occur, with a slight febrile response being the only sign. Abortions in pregnant ewes are common, with an incidence of 95–100%. Although abortions are common and the fetal pathology sequence well defined (Coetzer, 1977), the placental degeneration is not well documented. The infected placenta will be retained in a significant proportion of the ewes, and secondary uterine infections may develop. Genetic differences among breeds of sheep, similar to those observed in inbred rats, may determine why some adult sheep develop lethal infections while others only encounter mild, inapparent disease.

Cattle
Abortions or infertility have been the principal signs of RVF in cattle. Other overt signs are infrequent, but they include pyrexia, salivation, fetid diarrhea, and occasionally decreased milk production. Infection of calves results in a

febrile response, labored breathing, and death. Mortality rate is 10–70% among calves and 5–10% among adults. Incidence of abortions ranges from 80 to 100%.

Other ruminants

Field reports show abortions to be extensive among infected goats, camels and both Asian and African buffalos. Disease in goats is analogous to the disease in sheep. Generally, goats are more resistant.

Humans

Four distinct disease patterns have been recognized in humans (Peters and Meegan, 1981). All infected patients initially experience a febrile episode characterized by acute onset, severe myalgia, headache, and retro-orbital pain. Recovery typically occurs within 4–7 days; however, a few individuals will develop one of three types of secondary complications. In some a hemorrhagic syndrome occurs 2–4 days after the febrile episode, and is evidenced by jaundice, hematemesis, melana, and petechiae. This hemorrhagic form has been observed in the preponderance of fatal cases. A second complication is meningo-encephalitis diagnosed 5–15 days after onset of fever; clinical signs include disorientation, hallucinations and vertigo that can progress to coma and death. The third complication is ocular disease characterized by macular retinal exudate that causes loss of central vision. Eye involvement, when seen, occurs about 15 days after onset of fever; it persists for a prolonged time, sometimes leading to permanent visual impairment.

Clinical pathology data were obtained from experimentally infected domestic and laboratory animals. A leukopenia developed which was most severe in younger animals. The lowest leukocyte concentrations were detected 3–4 days after infection, corresponding to maximal viremia and temperature responses. Leukocytosis often was evident in the later stages of disease. Liver function abberations were demonstrated by different enzyme analyses. Clotting time was increased in mice. Elevated SGOT, GGT and LDH values are common. Virus may be isolated from plasma when the serum fraction is negative.

PATHOLOGY

Focal liver necrosis is the most consistent gross pathologic finding in all species affected. In newborn lambs, the necrotic foci are scattered throughout the parenchyma and may be associated with petechial subcapsular hemorrhages. As the disease progresses, the grey to pale-yellow necrotic areas enlarge to 1–2 mm in diameter, often resulting in complete destruction of the normal hepatic architecture. The liver is friable, irregularly congested, and may take on a mottled brown to yellow color. Other changes include subcutaneous, serosal, and visceral hemorrhages, mild to moderate icterus, hemorrhagic enteritis and abomastitis, generalized lymphadenopathy and, rarely, hemoperitoneum. In older lambs and adult sheep, the hapatic lesions are usually less severe than in the newborn lamb and consist of scattered necrotic foci with varying degrees of visceral and serosal hemorrhages. Those cases with marked hemorrhages in the liver also may present coagulated blood in the lumen of the gallbladder. Gross pathology of the liver is illustrated in Fig. 169. Lesions in calves older than one week, adult cattle, and goats are similar to those in sheep but generally less severe (Coetzer and Ishak, 1982).

Microscopically, the liver lesions are similar in all species but vary in severity with the susceptibility of the host. In newborn lambs, whole lobules throughout the liver are nearly destroyed, whereas in older animals the necrosis is usually confined to focal areas within individual lobules. The
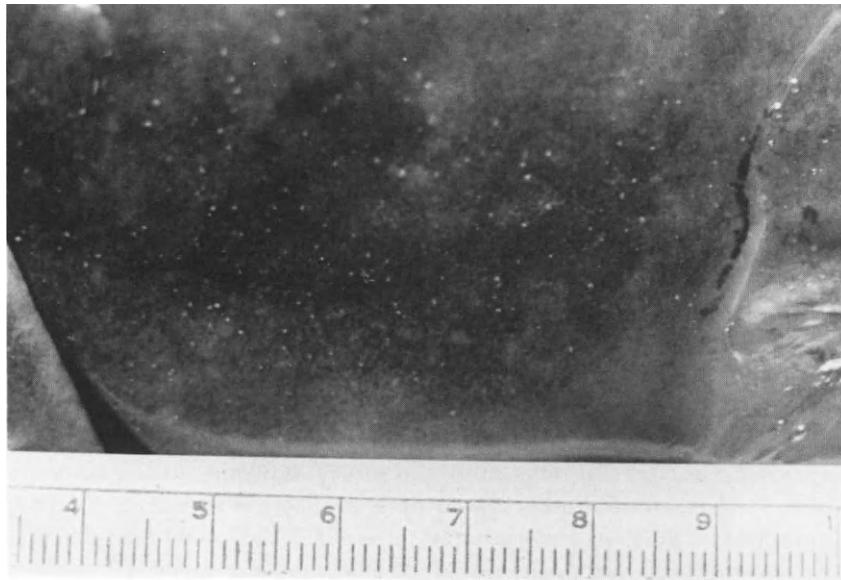
Fig. 169. Liver lobe of a bovine fetus infected with RVFV; note the multifocal white areas of necrosis (courtesty of the Armed Forces Institute of Pathology, Washington, DC).

microscopic changes in the liver of newborn lambs are considered diagnostic-ally characteristic for RVF. Fig. 170 shows microscopic pathology of the liver. The changes are characterized by massive diffuse necrosis of hepatocytes associated with semi-demarcated foci of primary coagulative necrosis. In acute hepatic lesions, hemorrhage is widespread with virtually no inflammatory response. Eosinophilic intranuclear inclusion bodies may be seen in degenerat-ing and necrotic hepatocytes. Other lesions described include acidophilic bodies seen throughout the parenchyma, generalized necrosis of lymphocytes
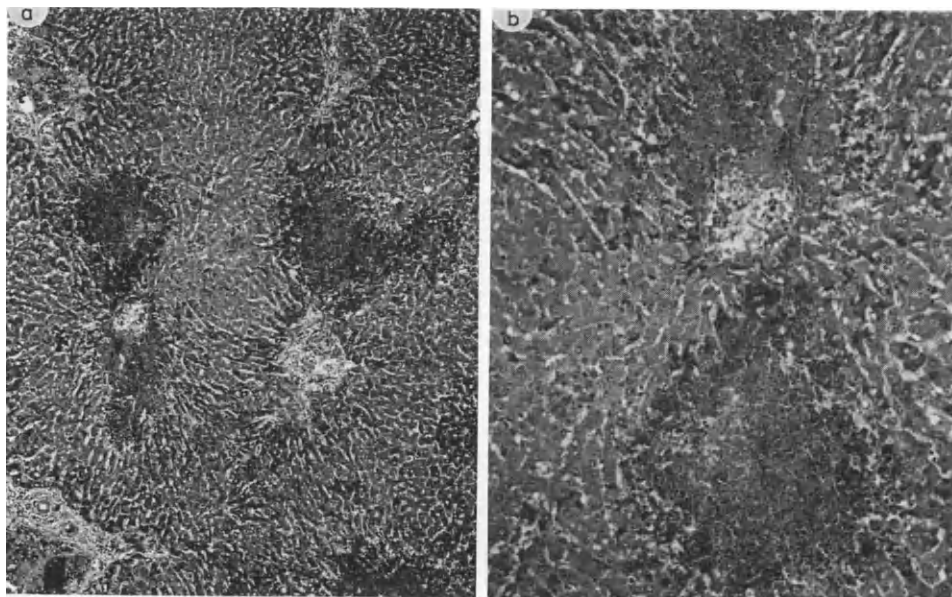
Fig. 170. (a) Photomicrograph of a liver section from a lamb with RVF showing paracentral and centrilobular necrosis; the distributional pattern of hepatocellular necrosis is distinctly circum-scribed in this lamb. (b) Higher magnification of paracentral and centrilobular necrosis; the necrosis is coagulative, involving hepatocytes and associated cells; there is a loss of the hepatic cord and sinusoidal pattern (courtesy of the Armed Forces Institute of Pathology, Washington, DC).

in the lymph nodes and spleen, and hyalinization, pyknosis, and karyorrhexis of the cellular elements in the kidney glomeruli (Erasmus and Coetzer, 1981).

In adult sheep, the necrotic areas remain focal and are infiltrated by poly-morphonuclear leukocytes and histiocytes. In these foci, sinusoids become distended with erythrocytes, and focal, but widespread, hemorrhages develop. Focal necrosis and hemorrhages are frequently observed in the adrenal cortex, lymph nodes and spleen. Gastrointestinal lesions vary from mild mucocatarrhal enteritis to extensive hemorrhagic gastroenteritis.

Placental pathology has not been described adequately. In aborted fetuses, the pathologic alterations observed resemble those seen in severe disease of lambs. It would appear that abortions are related to fetal infection rather than to placental inflammation.
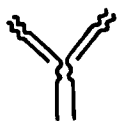
IMMUNE REACTION

The humoral response to infection with RVFV has been studied extensively in naturally infected humans and in experimentally infected sheep and cattle. The patterns appear similar. After an incubation period of 1–4 days and a viremic period of 1–4 days, antibodies measured in neutralization, HI, CF, IF or ELISA tests rise rapidly. In experimentally infected sheep, neutralizing antibodies first appear at day 10 p.i. and reach peak titers by day 20. Preliminary studies using an ELISA to measure sheep antibodies showed the IgM response to precede the IgG response by approximately 24 h. The time course of decay of the IgM response has not yet been studied in any domestic animal. Naturally infected humans develop antibodies between 7 and 10 days after onset of clinical symptoms. The human IgG and IgM responses appear to rise closely in time, but the IgM antibodies start to quickly decline in titer after peaking at 1 month. Titers of IgM are very low (but measurable) at 6 and 12 months after infection. The IgG titers remain high; neutralizing, HI, IF and ELISA antibodies have been measured in one human 47 years after infection. Field observations have shown that infected domestic animals also appear to have a long-lasting immunity. In humans and a limited number of sheep and cattle studied, antibody titers measured by virus neutralization, HI and ELISA remained high, while antibodies measured by CF and IF assays decreased to low titers 1—2 years after infection. Laboratory studies have documented earlier field observations and show that lambs up to at least 3 months of age are protected by maternal antibodies. Aspects of CMI with RVFV infection have not been studied in detail in any animal.

Domestic animals, in general, develop higher titer antibodies after immunization with live-attenuated vaccines than after inoculation with killed vaccines. Available vaccines, however, induce much lower titer antibodies than those measured after natural infection. Live vaccines induce antibodies detectable in all tests, whereas killed vaccines induce low-titer antibodies, usually only measurable in plaque-reduction virus neutralization, ELISA and HI tests. In challenge studies, even low postimmunization neutralization titers of 10 appeared sufficient to protect a majority of sheep and cattle from clinical disease. Titers of 20 or greater are totally protective. Antibody titers, after immunization with any killed vaccine, decay quickly and booster immunizations are required. Passively transferred antibodies by themselves are sufficient to protect experimentally inoculated mice.

In humans, there has been speculation that the encephalitic and ocular clinical RVF syndromes have an immunopathological component. These diseases occur after the febrile (viremic) period during early convalescence, when serum antibodies are developing. Antibodies have been detected in cere-

brospinal fluid of patients with RVF encephalitis. The cerebrospinal fluid contains IgG and IgM antibodies which appear to be locally synthesized and could play a role in the pathology of the disease. Laboratory models for encephalitic disease have been developed in certain strains of rats, and future studies should yield new insights into immune pathogenetic aspects of RVFV infection in both humans and animals. Preliminary studies in rodent models show that aerosol exposure to RVFV may produce encephalitis in a portion of the vaccinated animals that remain asymptomatic, if challenged by any other route.

LABORATORY DIAGNOSIS

Originally RVF was thought to be geographically limited to sub-Saharan Africa; therefore, RVF usually was not considered in the diagnosis of disease outbreaks outside this area. The epizootic in Egypt, combined with increased surveillance efforts, established that the virus exists in a wide variety of ecological zones across most countries in Africa. The large number of possible amplifying hosts and the many mosquito vectors of RVFV have made its potential spread to other countries a distinct threat. Additionally, air transfer of animals has made rapid transport of infected animals during the brief RVF incubation period possible. Table 31 is a listing of observations which suggest that RVF should be considered in the differential diagnosis of an unusual disease outbreak.

There are several reference laboratories that will assist in the diagnosis of RVF. They also will supply reference antigens and antibody and will provide consultants to help establish diagnostic tests or investigate an outbreak (Shope et al., 1982). When a presumptive diagnosis of RVF is made, national authorities immediately should notify the Office International des Epizooties (OIE), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). These organizations have established joint working groups to provide assistance in the event of an outbreak of RVF or the threat of an epizootic.

If RVFV is suspected as the causative agent of an outbreak, the collection of samples for laboratory diagnosis should be performed cautiously to prevent aerosol or contact infection. Recommendations for the safe collection, packaging and transportation of virologic specimens have been published by the World Health Organization (Madeley, 1977).

TABLE 31

Observations suggestive of a RVF outbreak

1.	High abortion rates (possibly approaching 100%) in ewes and cows, but lower rates in goats; abortions possibly increased in other ruminants, including camels and water buffaloes.
2.	Increased mortality (possibly approaching 100%) in lambs and calves less than 7 days of age; lower rates of disease and mortality in older animals and other species of ruminants.
3.	Extensive liver lesions on gross examination.
4.	Association with season of high density of biting arthropods.
5.	Potential route of introduction from Africa or the Mediterranean area.
6.	Human febrile disease, especially prevalent among people with close contact with animals (veterinarians, farmers, abattoir workers, etc.); if an extensive epidemic occurs, a small percentage of infected humans may develop encephalitis, blinding retinitis, or a fatal hemorrhagic disease.

The virus circulates to high titer in most susceptible animals, and virus isolation usually is successful if diagnostic samples are transported on wet ice. Whole blood or serum collected at the peak of pyrexia, and specimens of liver, placenta and fetus have been the most common material used for virus isolation during past epizootics. Samples from liver, brain, kidney, heart and spleen should be collected in 10% buffered formalin for histological examination. Ideally paired sera are needed for serological diagnosis: one collected during the acute phase of illness and one convalescent sample obtained 14–28 days after onset of symptoms. Humans should not be overlooked as a source of diagnostic material, and, if possible, samples of suspected mosquito vectors could be collected for virus isolation studies.

In the laboratory, several techniques have been used for diagnosis. Histopathologic examination can yield the first clues that the disease is RVF. Examination will reveal characteristic liver necrosis in all susceptible animals, with the degree of pathology being related to species and age of the animal involved.

A definitive diagnosis of RVF is accomplished only by isolation and identification of the virus or by serological demonstration of a 4-fold or greater rise in specific, neutralizing antibody titer between acute and convalescent sera. If virus isolation is to be attempted, laboratory workers should be immunized to protect against a potentially fatal laboratory infection. Such studies should be limited to insect-proof, high-containment laboratories.

The virus can be isolated in a number of common cell culture systems, including VERO, BHK-21, and many primary cells of avian, mouse, bovine, hamster, monkey or sheep origin. Cytopathogenic changes are visible in 2–4 days and advance quickly to complete destruction of the cell monolayer. Susceptible laboratory animals for isolation are suckling mice, adult mice, hamsters and 1–3-day-old lambs. Isolation should always include adult mice, and hamsters if possible, because RVFV is one of the few viruses that will kill adult mice and hamsters within 1–4 days after inoculation by the intraperitoneal route. Engorged hemorrhagic livers are seen in most mice and hamsters at necropsy. Typical necrosis can be found when sections of these livers are examined microscopically.

Viral isolates can be identified definitively as RVFV in mouse or plaque-reduction neutralization tests using reference polyclonal or monoclonal antiserum (available through the WHO). If these tests cannot be performed, a presumptive diagnosis can be made by preparing antigens from the unknown virus and testing it for positive reactions with reference RVFV antiserum in HI, CF or IF tests. Antigens for HI or CF tests are best made from suckling mouse brain. Infected cells can be fixed and stained for immunofluorescence identification studies.

Other rapid diagnostic methods attempt to demonstrate viral antigen in field specimens. Gel diffusion has demonstrated antigen in infected specimens, but the test is very insensitive. IF, ELISA and RIA techniques, utilizing reference antibodies, have proved successful in limited studies to data, although these procedures must be considered experimental techniques at present.

The following serological tests commonly have been used to demonstrate RVFV antibody in domestic animals and humans: CF, AGID, HI, mouse and plaque neutralization, IF and ELISA. These assays follow standard procedures commonly utilized in veterinary and public health laboratories of most countries. In the HI test, European domestic goose erythrocytes are the cells of choice. Male geese should be used since estrogens may interfere with viral hemagglutination. Cells from 1-day-old chicks may be used but give a much lower viral antigen titer. In some of these tests, various low-titer, nonspecific cross-reactions do occur between the RVFV antigens and antibodies developed

in response to infection with other viruses in the phlebotomus fever serogroup. These nonspecific cross-reactions are minimal in the plaque-reduction VN test which is the preferred confirmatory test. The distribution of cross-reacting phleboviruses currently is limited to the Mediterranean area, Africa, and Central and South America. Their role as disease agents in animals is not well studied; however, few have been isolated from domestic animals, and none have been implicated in major epizootics. In a diagnostic setting where the presence of other infecting phleboviruses seems unlikely, a seroconversion or a high convalescent anti-RVFV antibody titer measured in any of the above-listed serological tests would be strong presumptive evidence of RVF.

Measurement of IgM antibodies has proved useful in establishing the diagnosis in humans when a single convalescent serum sample was the only material available. Our laboratory currently is studying the IgM response in sheep to determine the time of appearance and disappearance of this antibody to aid in the development of rapid diagnostic assays.

The diagnosis of RVF must be differentiated from a number of other diseases with similar clinical findings. The following should be included in the differential diagnosis: enterotoxemia of sheep, bluetongue, ephemeral fever, Wesselbron and Middleburg virus infection. Other potentially confusing diseases include: brucellosis, vibriosis, trichomoniasis, Nairobi sheep disease, ovine enzootic abortion, and bracken or arsenic poisoning. Heavy contamination of pasture with anticoagulant rodenticides can also produce an outbreak of hemorrhage and abortion with numbers approaching those seen in RVF disease (Feinsod et al., 1986). Definitive diagnosis requires virus isolation or serology. Some preliminary findings might suggest RVF; for example, death of adult mice is not seen with enterotoxemia and bluetongue, and clinical disease in pigs, horses, and rabbits is seen with Wesselbron but not with RVF.

PROPHYLAXIS AND CONTROL

Immunization of susceptible animals is the most effective mechanism for the control of RVF (Bres, 1981). All livestock in enzootic areas should be immunized; annual immunization of livestock in nonenzootic areas is justified only in the face of an epizootic. Due to multiple vector species living in diverse habitats and potential insecticide resistance, vector control in enzootic areas is expensive and may be only moderately successful. Mosquito and biting fly control measures, if implemented, should incorporate the use of larvicides to increase effectiveness. Movement of livestock during an epizootic should be regulated to prevent extension of the disease to areas which are RVFV-free. Sick animals should not be slaughtered, to reduce the risk of aerosol infection from infectious blood and other body fluids. Stockmen should be cautioned to prevent mechanical transmission of the virus by changing needles between animals when administrating injectable materials. Veterinarians, stockmen, and abbatoir workers in enzootic areas are at high risk and should be immunized. The WHO/FAO has made specific recommendations for measures to be taken to control an epizootic (Shope et al., 1982).

Two types of veterinary vaccines are currently used to immunize sheep and cattle in Africa (Assad et al., 1983). The modified live virus (Smithburn strain) vaccine is inexpensive, can be produced in large amounts in a short time, is stable when properly lyophilized and is highly effective. This vaccine produces a solid and long-lasting immunity to RVF. A single inoculation may induce protection as early as 6–7 days after injection, and the offspring of vaccinates may be protected for up to 5 months of age. Even though about half of the sheep vaccinated develop vaccine viremia, arthropod transmission to and virus am-

plification in unvaccinated susceptible sheep are not proven. The vaccine is teratogenic and abortogenic and should not be used during pregnancy. Fetal loss under field conditions in South Africa, however, has been minimal only because timing of vaccination avoids pregnancy. Reversion of the vaccine to virulence or reassortment of the vaccine virus with other *Phleboviruses* to produce a reassortant virus with increased virulence is a remote theoretical possibility that must be considered.

Formalin-inactivated vaccines have been shown to be effective when multiple inoculations are given. Long-lasting immunity does not develop; therefore yearly booster immunizations are needed. Two doses of an experimental inactivated vaccine produced in the USA were required to protect pregnant ewes from viremia and fetal damage on virulent virus challenge. Inactivated vaccines are more costly to produce and generally have poor stability because they cannot be lyophilized due to the presence of an adjuvant. Even though a possibility exists of residual live virus escaping inactivation, inactivated vaccines would be more appropriate than current modified live products for use in livestock or zoo animals moving from enzootic areas to areas free of the disease.

Convalescent sera have been used to confer passive protection to sheep, laboratory rodents, and non-human primates. In 1–3-day-old lambs, 10–30 ml of convalescent serum administered intravenously or intraperitoneally was effective, even after signs of illness were detectable. A number of antiviral drugs are effective against RVFV in vitro, and good results have been obtained using Ribavirin® in experimental animals. Human leukocyte interferon has been used experimentally to protect RVFV-infected rhesus monkeys from viremia and liver damage.

Recently, two experimentally modified live RVF vaccine candidates have been developed. One candidate, developed in Egypt, is a minute plaque variant of RVFV (Moussa et al., 1986). The second candidate, an intensively mutagenized RVFV strain developed in the USA, has greatly reduced pathogenicity for adult mice and hamsters, yet is immunogenic (Caplen et al., 1985). Only limited preliminary data are available on these preparations.

Chemotherapy research of RVF disease in laboratory rodents and monkeys has shown that ribavirin and poly IC acting as an interferon inducer are effective treatments (Peters et al., 1986). However, neither treatment has been tested in ruminants.

REFERENCES

Alexander, R.A., 1951. Rift Valley fever in the Union. J. S. Afr. Vet Med. Assoc., 22: 105–109.

Assad, F., Davies, F.G., Eddy, G.A., El Karamany, R., Meegan, J.M., Ozawa, Y., Shimshony, A., Shope, R.E., Walker, J. and Yedloutschnig, R.J., 1983. The use of veterinary vaccines for prevention and control of Rift Valley fever: Memorandum from a WHO/FAO meeting. Bull. WHO, 61: 261–268.

Bres, P., 1981. Prevention of spread of Rift Valley fever from Africa. In: T.A. Swartz, M.A. Klingberg and N. Goldblum (Editors), Rift Valley Fever, Contributions to Epidemiology and Biostatistics, 3: 178–190.

Caplen, H., Peters, C.J. and Bishop, D.H.L., 1985. Mutagen directed attenuation of Rift Valley fever as a method for vaccine development. J. Gen. Virol., 66: 2271–2277.

Casals, J., 1979. Arenaviruses. In: E.H. Lennette and N.J. Schmidt (Editors), Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. American Public Health Association, Washington, DC, pp. 815–842.

Coetzer, J.A., 1977. The pathology of Rift Valley fever. I. Lesions occurring in natural cases in new-born lambs. Onderstepoort J. Vet. Res., 44: 205–212.

Coetzer, J.A., 1982. The pathology of Rift Valley fever. II. Lesions occurring in field cases in adult cattle, calves and aborted foetuses. Onderstepoort J. Vet. Res., 49: 11–17.

Coetzer, J.A. and Ishak, K.G., 1982. Sequential development of the liver lesions in new-born lambs infected with Rift Valley fever virus. I. Macroscopic and microscopic pathology. Onderstepoort J. Vet. Res., 49: 103–108.

Daubney, R., Hudson, J.R. and Garrham, P.C., 1931. Enzootic hepatitis or Rift Valley fever: An undescribed disease of sheep, cattle and man from East Africa. J. Pathol. Bacteriol., 34: 545–579.

Davies, F.G., Koros, J. and Mbugua, H., 1985. Rift Valley fever virus in Kenya: The presence of antibody to the virus in camels. J. Hyg. (Lond.), 94(2): 241–244.

Easterday, B.C., 1965. Rift Valley fever. Adv. Vet. Sci., 10: 65–127.

Erasmus, B.J. and Coetzer, J.A.W., 1981. The symptomatology and pathology of Rift Valley fever in domestic animals. In: T.A. Swartz, M.A. Klingberg and N. Goldblum (Editors), Rift Valley Fever. Contributions to Epidemiology and Biostatistics, 3: 77–82.

Feinsod, F.M., Allan, I., Ragai, A. and Saah, A.J., 1986. Rodenticide-induced signs simulating Rift Valley fever in sheep and goats in Egypt. Vet. Rec., 118(10): 270–272.

Gargan, T.P., Clark, G.G., Dohm, D.J. and Bailey, C.L., 1984. Experimental transmission of Rift Valley fever virus by North American mosquitoes. Abstr. 33rd Annu. Meet. Am. Soc. Trop. Med. Hyg., p. 210.

Hoch, A.L., Turell, M.J. and Bailey, C.L., 1984. Replication of Rift Valley fever virus in the sandfly *Lutzomyia longipalpis*. Am. J. Trop. Med. Hyg., 33: 295–299.

Hoch, A.L., Gargan, T.P., II and Bailey, C.L., 1985. Mechanical transmission of Rift Valley fever virus by hematophagous diptera. Am. J. Trop. Med. Hyg., 34: 188–193.

Hoogstraal, H., Meegan, J.M., Khalil, G.M. and Adham, F.K., 1979. The Rift Valley fever epizootic in Egypt 1977–78. 2. Ecological and entomological studies. Trans. R. Soc. Trop. Med. Hyg., 73: 624–629.

Linthicum, K.J., Davies, F.G., Kairo, A. and Bailey, C.L., 1985. Rift Valley fever virus (family Bunyaviridae, genus *Phlebovirus*). Isolations from Diptera collected during an inter-epizootic period in Kenya. J. Hyg. (Lond.), 95(1): 197–209.

Lupton, H.W. and Peters, C.J., 1984. Rift Valley fever. Proc. U.S. Anim. Health Assoc., 87: 279–290.

Madeley, C.R., 1977. Guide to the Collection and Transportation of Virological Specimens. World Health Organization, Geneva.

Meegan, J.M., Hoogstraal, H. and Moussa, M.I., 1979. An epizootic of Rift Valley fever in Egypt in 1977. Vet. Rec., 105: 124–125.

Meegan, J.M., Digoutte, J.P., Peters, C.J. and Shope, R.E., 1983. Monoclonal antibodies to identify Zinga virus as Rift Valley fever virus. Lancet, 1: 641.

Mims, C.A.C. and Mason, P.J., 1956. Rift Valley fever virus in mice. V. Properties of the hemagglutinin present in infective serum. Br. J. Exp. Pathol., 37: 423–433.

Moussa, M.I., Abdel Wahab, K.S.E. and Wood, O.L., 1986. Experimental infection and protection of lambs with a minute plaque varient of Rift Valley fever virus (ZH501). Am. J. Trop. Med. Hyg., 35(3): 660–662.

Peters, C.J. and Anderson, G.W., Jr., 1981. Pathogenesis of Rift Valley fever. In: T.A. Swartz, M.A. Klingberg and H. Goldblum (Editors), Rift Valley Fever. Contributions to Epidemiology and Biostatistics, 3: 21–41.

Peters, C.J. and Meegan, J.M., 1981. Rift Valley fever. In: G. Beran (Editor), CRC Handbook Series in Zoonosis. CRC Press, Boca Raton, FL, pp. 403–420.

Peters, C.J., Reynolds, J.A., Slone, T.W., Jones, D.E. and Stephen, E.L., 1986. Prophylaxis of Rift Valley fever with antiviral drugs, immune serum, an interferon inducer, and a macrophage activator. Antiviral Res., 6: 285–297.

Sabry, M., Hoogstraal, H., Wood, O.L. and Moussa, M.I., 1984. An experimental study of Rift Valley fever virus in common Egyptian Ixodid ticks. J. Egypt. Public Health Assoc., 58: 345–349.

Scott, G.R., Coackley, W., Roach, R.W. and Cowdy, N.R., 1963. Rift Valley fever in camels. J. Pathol. Bacteriol., 86: 229–231.

Shimshony, A. and Barzilai, R., 1983. Rift Valley fever. Adv. Vet. Sci. Comp. Med., 27: 347–425.

Shope, R.E., Peters, C.J. and Davies, F.G., 1982. The spread of Rift Valley fever and approaches to its control. Bull. WHO, 60: 299–304.

Smith, J.F. and Pifat, D.Y., 1982. Morphogenesis of sandfly viruses (Bunyaviridae family). Virology, 121: 61–81.

Smithburn, K.C., Haddow, A.J. and Lumsden, W.H.R., 1949. Rift Valley fever transmission virus by mosquitoes. Br. J. Exp. Pathol., 30: 35–47.

Turrell, M.J., Bailey, C.L. and Rossi, C.A., 1984. Increased mosquito feeding on Rift Valley fever virus-infected lambs. Am. J. Trop. Med. Hyg., 33: 1232–1238.

Turrell, M.J., Rossi, C.A. and Bailey, C.L., 1985. Effect of extrinsic incubation temperature on the ability of *Aedes taeniorhychus* and *Culex pipiens* to transmit Rift Valley fever virus. Am. J. Trop. Med. Hyg., 34(6): 1211–1218.

Weiss, K.E., 1957. Rift Valley fever — a review. Bull. Epizoot. Dis. Afr., 5: 431–458.

WHO/FAO Working Group on Rift Valley Fever, 1982. Rift Valley Fever: An Emerging Human and Animal Problem. WHO Offset Publication No. 63, Geneva, 69 pp.

Chapter 45

Nairobi Sheep Disease Virus

C. TERPSTRA

INTRODUCTION

Nairobi sheep disease (NSD) is a noncontagious tick-transmitted virus disease of small ruminants, characterized by fever, hemorrhagic gastroenteritis, abortion of pregnant animals and a high mortality, especially in sheep. The disease was first observed in the vicinity of Nairobi among sheep and goats originating from dry areas in the northern and southern parts of Kenya and was reported by Montgomery (1917). NSD is known to occur in East and Central Africa, but from antibody surveys the virus is suspected to be present as far north as Ethiopia and Somalia and as far southward as Botswana and the Mozambique coastal plain.

VIRUS PROPERTIES

NSD virus (NSDV) has been shown antigenically closely related to Ganjam virus, the causative agent of a tick-borne infection of sheep and goats in India, and more distantly related to Dugbe virus, responsible for a tick-transmitted infection of cattle in Nigeria (Davies et al., 1978).

NSDV has all the characteristics of a *Bunyavirus* (Terpstra, 1983). It is sensitive to lipid solvents and detergents, and is rapidly inactivated at high and low pH values. Even in the range of optimal stability (pH 7.4–8.0) and with 2% serum, the virus is easily inactivated with a half-life period of 6.8 days at 0°C and 1.5 h at 37°C. The stability increases in the presence of higher protein concentrations. Sterile citrated whole blood remains infective for 66 days at 5°C, 33 days at 15–18°C, 42 h at 37°C and 1.5 h at 50°C, but infectivity is destroyed after 5 min at 60°C (Montgomery, 1917).

The virus replicates in vitro in primary or secondary cell cultures derived from sheep testis, sheep kidney, goat testis and goat kidney, and in continuous cell lines of BHK and the vector tick *Rhipicephalus appendiculatus* (Coackley and Pini, 1965; Howarth and Terpstra, 1965; Munderloh, 1977). Infected cells usually develop a CPE, while staining with H&E reveals typical eosinophilic, intracytoplasmic inclusion bodies (Coackley and Pini, 1965). The polar and bipolar inclusions, which also can be observed by immunofluorescence, were found to contain large clusters of polyribosomes when examined by EM (Terpstra and Vreeswijk, unpublished results, 1983).

NSDV produces a fatal infection in infant mice, inoculated by intracerebral or intraperitoneal routes, but adult mice are only susceptible by the intracerebral route (Weinbren et al., 1958). The virulence of the virus rapidly decreases after serial passage in infant mouse brains. Terpstra (1969) reported a statistic-

ally significant difference in duration of illness and mortality between Persian fat-tailed sheep infected with the fifth mouse brain passage and those infected with the progeny of the fifth brain passage after repassage through ticks.

The mouse-brain-attenuated strain could successfully be propagated in embryonated hen eggs and in day-old guinea pigs following intracerebral inoculation.

EPIZOOTIOLOGY

Sheep and goats are the natural vertebrate hosts of NSDV. Cattle, buffalo, horses and pigs are refractory to infection. Although naturally occurring fatal infections have been reported in blue duikers (*Cephalophus caerulus*) and viremia followed by an immune response has been demonstrated in experimentally infected field rats (*Arvicanthis abyssinicus nubilans*), antibody studies have not lent support to the assumption that small antilopes or wild rodents are involved in the maintenance cycle of NSDV (Davies, 1978a). Antibodies have been found in human sera, and accidental laboratory acquired infections accompanied by fever, joint aches and general malaise have been reported.

Ticks, mainly of the genus *Rhipicephalus*, serve as invertebrate hosts of the virus. The common vector of NSDV is the brown tick (*R. appendiculatus*), but other species, including *R. pulchellus*, *R. evertsi* and *R. simus* and the bont tick *Amblyomma variegatum*, can transmit the virus. *R. appendiculatus* is by far the most efficient vector and the only one in which transovarian transmission has been demonstrated. For egg transmission, the adult female needs a viremic blood meal (Davies and Mwakima, 1982). Within the same generation, all stages subsequent to the one which has sucked infected blood remain infective, irrespective whether an instar has fed on an immune, susceptible or non-susceptible host. The virus only will be passed on to the next tick generation if the host develops a viremia before the infecting adult female has completed its meal. Lewis (1946) studied the survival of NSDV in *R. appendiculatus*. Unfed larvae were able to infect a sheep 6 months after hatching, while unfed nymphae and adults remained infective for 12 and 28 months, respectively.

The distribution of NSDV antibodies in sheep and goats in Kenya and Uganda roughly corresponds with the distribution of *R. appendiculatus* (Terpstra, 1969; Davies, 1978b). Most sheep and goats in areas enzootic for NSD have antibody to the virus and only incidental losses are observed. The high frequency of antibody in different age groups presumably reflects a high exposure rate and infection at an early age, when the lambs are still protected by maternal immunity. Outbreaks of NSD either may arise as a result of movement of susceptible animals into enzootic areas or as a result of an incursion of infected ticks into NSD-free flocks or areas. The latter situation may occur in years with excessive or prolonged rains, which result in vegetational and microclimatic changes favorable for an extension in the range of *R. appendiculatus*. Under these circumstances infected ticks may be introduced into NSD-free dry zones with movements of stock or wild ruminants and cause an epizootic among local sheep and goats in the area. Outbreaks may also result from incursions of ticks in NSD-free flocks following a breakdown in tick control measures.

Experimental infection and natural challenge by infective ticks has revealed no difference in susceptibility between local Persian fat-tailed and European breeds of sheep, although the latter stand a better chance of survival (Terpstra, 1969). The 50% infective dose of the fully virulent Entebbe strain of NSDV was found close to 0.01 mouse LD-50 for both local and exotic breeds.

The mortality in the field has been reported as high as 70–90% for local breeds and 30% for exotics and cross-breds (Montgomery, 1917).

Goats are regarded less susceptible than sheep, but the mortality of indigenous breeds may rise to 90%.

PATHOGENESIS

Mugera and Chema (1967) studied the pathogenesis of the disease following intravenous inoculation, but nothing at all is known about the sequence of events after vector exposure. In all probability, the virus reaches the target organs with the blood stream directly from the location of the infecting bite, although an initial replication in the regional lymph node cannot be excluded. The virus replicates to high titers in liver, lung, spleen and other organs of the reticuloendothelial system and appears to have preference for the vascular endothelium. The primary lesions consist of endothelial swelling, edema and necrosis of the capillary walls in the mucosae of the abomasum, small and large intestine, gall bladder and female genital tract. The vascular lesions are accompanied with dilatation of the capillary vessels, hemorrhages and catarrhal inflammation in the superficial layers of the mucosae and are followed by coagulation necrosis and desquamation of the epithelium. Edema of the submucosae is also much in evidence.

Vascular involvement also has been observed in lung, heart and kidneys. Congestion of pulmonary capillaries occasionally leads to hemorrhages and serous exudate in the alveoli, which may be aggravated by the granular degeneration, fragmentation and necrosis of muscle fibres in the myocardium. The kidney lesions are the most severe and consist of a glomerulotubular nephritis with hemorrhages, degeneration of tubular epithelium and tubuli filled with hyalin and cellular casts as early as 48 h p.i. A fatty infiltration and hydropic degeneration of liver cells around the central veins has been observed at 72 h p.i.

DISEASE SIGNS

Signs of disease usually appear 4–6 days after application of infected ticks, but this period can considerably be extended when the ticks do not readily feed. The experimentally obtained incubation period is in agreement with observation on natural outbreaks, which occurred 5–6 days after animals had been transferred to paddocks infested with *R. appendiculatus*.

In case of needle infection, the incubation period normally varies from 1 to 4 days, with a maximum of 6 days, depending on the virus dose, route of infection, virulence of the strain and individual resistance of the animals. Clinical signs start with a steep rise of the body temperature above 41°C. The fever persists for 1–7 days and may peak up to 42°C. The febrile response is accompanied by leukopenia and viremia, but virus can no longer be isolated from the blood 24 h after the temperature has returned to normal. In acute and peracute fatal cases the temperature rapidly drops below normal, whereas in less acute cases animals die 2–5 days after the temperature starts to fall. Physical signs of illness, characterized by diarrhea, usually appear 1–3 days after the onset of fever. The diarrhea worsens during the following days. The feces become watery and fetid and often contain mucus. Sometimes almost unchanged blood is passed. The animals show dullness and inappetence, stand with drooping head while straining continuously and sometimes moan at each expiration. In about half the cases a mucopurulent, often blood-tinged nasal discharge can be observed. Pregnant ewes frequently abort.

The clinical picture of the disease in goats is similar to that in sheep, but the symptoms are less severe.

PATHOLOGY

The hindquarters, soiled with feces or sometimes blood and feces are the most striking on external examination of the carcass. There is often conjunctivitis and dried crusts may be seen around the nostrils as a result of nasal discharge. The skin may be dehydrated, especially after a long period of scouring.

The post-mortem picture is that of a hemorrhagic septicemia, accompanied by a catarrhal or hemorrhagic inflammation of the gastrointestinal and female genital tract. The most prominent changes are found in the gastrointestinal tract. The lesions consist of multiple hemorrhages of different size and are mainly located on the longitudinal folds of the abomasum mucosa, the distal part of the ileum and ileocecal valve, the cecum and the ascending part of the ansa spiralis. The hemorrhages in the mucosa of cecum and colon frequently are arranged in longitudinal bands, and in severe cases lines of ecchymosis extend from the top of the cecum along the entire colon up to the rectum (Fig. 171). These striations in cecum and/or colon are the most outstanding and often the only post-mortem sign, but occasionally they may be absent. The small intestine is often inflamed and contains a little watery fluid, whereas the contents of colon and rectum, if present, are liquid.

Hemorrhages may also be found under the serosa of cecum, colon and gall bladder, under the renal capsule, in the mucosae of the female genital tract, especially after abortion, and in the lower parts of the respiratory tract. The mucosa of the nasal cavity often shows catarrhal inflammation, is congested and dark red in color. The heart is mostly pale and flaccid, with petechiae on the epicardium and ecchymoses on the endocardium, and unclotted blood inside.

As regards the hemopoietic system, spleen and superficial lymph nodes are usually slightly swollen, but the mesenteric glands may be grossly enlarged with lymph exuding from the cut surface. The marrow of the long bones frequently has a gelatinous, bright cherry-colored appearance.
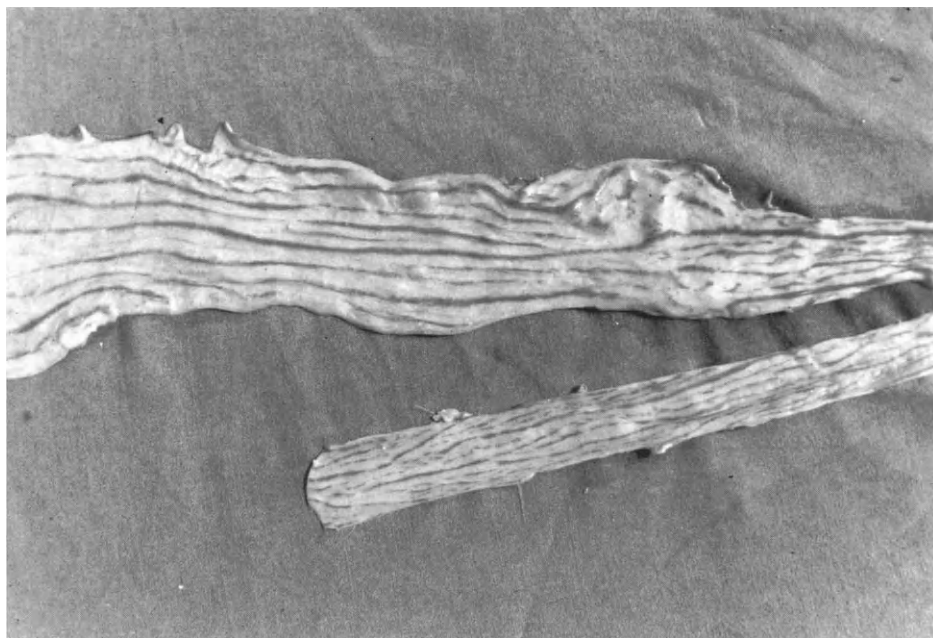
Fig. 171. Hemorrhagic striations in mucosae of cecum and colon of a sheep fatally infected with NSDV.

Glomerulo-tubular nephritis, myocardial degeneration and coagulative necrosis in the mucosa of the gall bladder are consistent microscopical lesions.
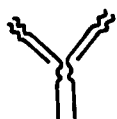
IMMUNE RESPONSE

Animals that survive an infection develop antibodies to NSDV, which can be demonstrated by CF, neutralization, AGID, indirect IF and indirect HA tests (Terpstra, 1969; Davies et al., 1976). Complement-fixing antibody can be detected for only 6–9 months and neutralization titers are low and insignificant. With the other three tests antibodies appear within a couple of days after the body temperature has returned to normal and persist for at least 15 months. An ELISA for detection of NSDV antibody has been described (Munz et al., 1983), but its application in the field has as yet to be assessed.

LABORATORY DIAGNOSIS

The clinical signs and post-mortem lesions are usually sufficiently distinct to make a tentative diagnosis of NSD. The picture, however, is seldom complete and laboratory investigations are necessary to permit beyond doubt differentiation from rinderpest, peste-des-petits-ruminants, Rift Valley fever and heartwater.

Laboratory methods are aimed at the detection of the virus or virus-specific antigens, or at detecting antibodies against the virus. Virus multiplies to high titers in lung, liver, spleen and mesenteric lymph nodes and also can be recovered from whole blood or serum during the febrile stage of disease. Samples for virus isolation should be sent on ice without preservatives and reach the laboratory by the quickest possible means. NSDV replicates in different cell cultures and in infant mouse brains. On primary isolation in cell cultures, however, the virus may not cause a CPE. Hence, immunofluorescent methods are extremely useful for the detection and rapid identification of isolates (Davies et al., 1977). Primary isolation of the virus in infant mouse brain and identification either by IF or CF tests is a slightly more sensitive method and suitable for laboratories without cell culture facilities. Taking into consideration the sensitivity of cell cultures and mice in comparison with that of the natural host, there is no doubt that inoculation of a susceptible sheep with suspensions of infected organs or blood is by far the most sensitive method for the isolation of NSDV.

Viral antigen can be detected by immunodiffusion in spleen, lung and mesenteric lymph nodes of sheep that have suffered an acute fatal infection, using NSDV-specific hyperimmune serum as a source of antibody (Terpstra, 1969). The method is simple and rapid and can be applied in field laboratories or diagnostic centres without facilities for both cell culture and immunofluorescence.

Paired serum samples collected during the febrile phase and convalescence can be examined for a rise of specific antibody.

PROPHYLAXIS AND CONTROL

Being a tick-transmitted disease, control and prophylaxis of NSD should be sought in vector control and vaccination. *R. appendiculatus* is also the principal vector for East Coast fever and consequently NSD has been controlled or even eradicated in highly productive areas as an additional gain of the tick

control programmes for East Coast fever. Such programmes are costly, and experience has shown that unless pursued rigidly and systematically together with quarantine measures for livestock and checks on movement of game animals, the ticks will return and with them the tick-borne diseases.

Two types of vacine—a modified live virus (MLV) attenuated by serial mouse brain passage (Terpstra, 1969) and an inactivated oil-adjuvant vaccine (Davies et al., 1974)—have been used on experimental base. Although a single dose of MLV vaccine rapidly induces immunity, the strain has lost some immunogenicity and annual revaccination is recommended for sheep to provide full protection. The MLV strain causes a viremia, but reversion to virulence is theoretical as the strain is no longer transmitted by *R. appendiculatus*. On the other hand, two doses of the inactivated vaccine are required for optimal stimulation of antibody production, and the duration of immunity appears to be limited.

The incidental losses from NSD in enzootic areas do not warrant the application of either tick control measures or prophylactic vaccination. Control of NSD by vaccination, therefore, should be directed toward individual animals or groups at risk, notably sheep and goats moved from clean into enzootic areas and flocks of NSD-free farms or in areas infested by infective ticks.

REFERENCES

Coackley, W. and Pini, A., 1965. The effect of Nairobi sheep disease virus on tissue culture systems. J. Pathol. Bacteriol., 90: 672–675.

Davies, F.G., 1978a. A survey of Nairobi sheep disease antibody in sheep and goats, wild ruminants and rodents within Kenya. J. Hyg. (Camb.), 81: 251–258.

Davies, F.G., 1978b. Nairobi sheep disease in Kenya. The isolation of virus from sheep and goats, ticks and possible maintenance hosts. J. Hyg. (Camb.), 81: 259–265.

Davies, F.G. and Mwakima, F., 1982. Qualitative studies of the transmission of Nairobi sheep disease virus by *Rhipicephalus appendiculatus* (Ixodoidea, Ixodidae). J. Comp. Pathol., 92: 15–20.

Davies, F.G., Mungai, J. and Shaw, T., 1974. A Nairobi sheep disease vaccine. Vet. Rec., 94: 128.

Davies, F.G., Jessett, D.M. and Otieno, S., 1976. The antibody response of sheep following infection with Nairobi sheep disease virus. J. Comp. Pathol., 86: 497–502.

Davies, F.G., Mungai, J.N. and Taylor, M., 1977. The diagnosis of Nairobi sheep disease. Trop. Anim. Health Prod., 9: 75–80.

Davies, F.G., Casals, J., Jessett, D.M. and Ochieng, P., 1978. The serological relationships of Nairobi sheep disease virus. J. Comp. Pathol., 88: 519–523.

Howarth, J.A. and Terpstra, C., 1965. The propagation of Nairobi sheep disease virus in tissue culture. J. Comp. Pathol., 75: 437–441.

Lewis, E.A., 1946. Nairobi sheep disease. The survival of the virus in the tick *Rhipicephalus appendiculatus*. Parasitology, 37: 55–59.

Montgomery, R.E., 1917. On a tick-borne gastro-enteritis of sheep and goats occurring in British East Africa. J. Comp. Pathol. Ther., 30: 28–58.

Mugera, G.M. and Chema, S., 1967. Nairobi sheep disease: A study of its pathogenesis in sheep, goats and suckling mice. Bull. Epizoot. Dis. Afr., 15: 337–354.

Munderloh, U., 1977. Die Nairobi sheep disease—Eine synoptische Darstelling der Erkrankung sowie Untersuchungen zum Verhalten des Erregers in vivo und in vitro. Vet. Med. Diss., University of München, 76 pp.

Munz, E., Reimann, M. and Meier, K., 1983. An enzyme-linked immunosorbent assay (Elisa) for the detection of antibodies to Nairobi sheep disease virus in comparison with an indirect immunofluorescent and hemagglutination test. Zentralbl. Veterinärmed. B, 30: 473–479.

Terpstra, C., 1969. Nairobi sheep disease. Studies on virus properties, epizootiology and vaccination in Uganda. Thesis, University of Utrecht, 172pp.

Terpstra, C., 1983. Physical and biological properties of Nairobi sheep disease virus. Vet. Microbiol., 8: 531–541.

Weinbren, M.P., Gourlay, R.N., Lumsden, W.H.R. and Weinbren, B.M., 1958. An epizootic of Nairobi sheep disease in Uganda. J. Comp. Pathol. Ther., 68: 174–187.

Picornaviridae

This family of smallest RNA viruses (from Italian picolo = small) contains the genera *Enterovirus, Cardiovirus* and *Aphthovirus* with more than 230 types.

Virions are nonenveloped, roughly spherical nucleocapsids 22–30 nm in diameter with a T = 1 type of icosahedral symmetry. Electron microscopically the surface is almost featureless. The capsid is constructed from 60 subunits, each consisting of one molecule of each of the four major capsid polypeptides.

The picornavirion contains one molecule of infectious single-stranded RNA of positive polarity with a mol.wt. of about 2.5×10^6. A covalently linked protein VPg is present at the 5' terminus of the RNA. Four major polypeptides participate in the construction of the capsid.

Virion buoyant density depends mainly on the genus; enteroviruses and cardioviruses band at 1.33–1.34 g/cm³, whereas rhinoviruses possess a density of 1.38–1.42 and aphthoviruses of 1.43–1.45 g/cm³ in CsCl. Sedimentation coefficients are between 140 and 165 S.

Replication is entirely in the cytoplasm, in close association with membranes. Partially double-stranded replicative intermediates are formed, both strands of which are used as templates. Functional proteins are produced by posttranslational cleavage from large precursor polyproteins.

During picornavirus replication, the cellular metabolism is arrested and the cells (in vitro) soon show signs of degeneration; they are then smaller, rounded, refractory, and pyknotic. The release of the virus progeny from the cell is facilitated by this degeneration, which results in cytolysis.

Picornaviruses are either very resistant or very sensitive to pH changes. The former fall into the genera *Enterovirus* and *Cardiovirus*, and the latter into the genera *Aphthovirus* and *Rhinovirus*. Foot-and-mouth disease (FMD) viruses form the genus Aphthovirus. Bovine enteroviruses (BEV) and bovine rhinoviruses have been isolated either from healthy cattle or from cases of acute enteric and/or respiratory disease. Their importance is mainly for differential diagnosis following cultivation from samples sent to the laboratory; their cytopathogenicity (in vitro) resembles that of an aphthovirus.

Chapter 46

Foot-and-Mouth Disease Virus

J.A. MANN and R.F. SELLERS

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly infectious disease of cattle, sheep, goats and pigs as well as of a number of wild animals, including buffalo, deer, other wild ungulates and hedgehogs. Man is occasionally infected. The disease is characterized by the formation of vesicles on the feet and in and around the mouth and, in young animals, by sudden death due to heart failure.
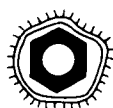
VIRUS PROPERTIES

FMD virus (FMDV) is stable between pH 7 and 9. Outside these pH values inactivation occurs. A number of chemical substances inactivate the virus at the acid and alkaline ranges of pH. These include phosphoric, sulphuric, citric, acetic and formic acids, and sodium carbonate, sodium metasilicate and sodium hydroxide on the alkaline side (Sellers, 1968). In the field 4% sodium carbonate, 1% sodium hydroxide and 1% sodium metasilicate have been used. Acids are usually formulated with detergents to improve wettability, and a number of proprietary products are available. Other disinfectants or chemicals such as formalin (vapor or solution), glutaraldehyde and peracetic acid have been used. Phenolic compounds and quaternary ammonium compounds have little effect. In the absence of organic matter, oxidizing agents, e.g. hypochlorites, will inactivate the virus.

Heat, UV irradiation and gamma irradiation have been used to render animal products free of FMDV. Once rigor mortis has set in, the production of lactic acid leads to its inactivation. The effect of heat is variable, depending on the time, temperature and the extent to which the product is protected. Overall, in treatment of contaminated fluids 90°C is the temperature aimed at. Milk, however, may be treated at lower temperatures, as can some meat products.

Seven serotypes of FMDV are recognized: O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1. There is no cross-immunity between types, although an animal which has recovered from six types may be immune to the seventh. Within the types, a number of subtypes have been described. These are based on differences found as a result of vaccinating animals, since cattle vaccinated with a vaccine of one subtype are not necessarily protected against disease caused by another subtype.

CF, SN, RIA and ELISA techniques have been used to differentiate strains. However, subtyping (Pereira, 1977) is looked at from two points of view, namely (1) the relation of the field strain to vaccines available for protection and (2) the source of the virus causing the outbreak. Investigations under (1) attempt to

group strains together, whereas the detective work required under (2) serves to look at small differences.

For results on cross-protection by available vaccines, strains are studied mainly by SN and cross-protection tests and to a lesser extent by CF, RIA and ELISA.

For detective work on the origin, field strains are examined by a number of tests in addition to those mentioned before, namely, PAGE and electrofocusing for examining the structural and nonstructural polypeptides, and RNA ribonuclease T_1 mapping for analyzing the genome. Nucleotide and amino acid sequencing are also used (Samuel et al., 1988).

Interpretation of tests for deciding which vaccine to use is probably easier than interpretation of the "fingerprinting" results, because of the variety and the variation of field strains of virus.

EPIZOOTIOLOGY

FMD affects cloven-hoofed animals. Among domestic animals cattle, sheep, goats and pigs are included; among wild ruminants, African buffalo, deer (roe, muntjac, impala and kudu), warthogs and bush pigs are natural hosts. Elephants, coypu, agouti and hedgehogs are also affected.

FMD also affects man but not to any great extent (Hyslop, 1973); only 37 authentic cases have been described. People became infected by handling infective animals or materials in abattoirs, on farms, in vaccine production plants or in laboratories. In other instances they drank infected milk or breathed in virus aerosols. In some patients the first symptoms noted were fever, sore throat, headache or general malaise; vesicles subsequently appeared on hands or feet and in the mouth. In others a vesicle appeared at a local site, followed by fever and a generalization of vesicles. People do not appear to be infectious to other people. Healing is uneventful, although recurrence of lesions without presence of virus has been described. There is a record of a worker in a Waldmann type vaccine laboratory being infected twice, first with type C, then with type O. Type O was responsible in 23 cases, type A in one and type C in six cases of infection in man.

FMD has been reported over the years from most countries of the world. Strains of types O, A and C occur in South America and in Europe (including Turkey and the USSR), types O, A, C and Asia 1 in the Near East, O, A, C and Asia 1 in Central and Southeast Asia, O, A and C in North America, O, A, C, SAT 1 and SAT 2 in West and Central Africa, O, A, C, SAT 1, SAT 2 and SAT 3 in East and southern Africa (Pereira, 1981). At present, North and Central America have been free for a number of years, as have Norway, Sweden and Ireland. The disease has never occurred in Iceland. Australia and Japan have been free since 1871 and 1908, respectively, and the disease has never been reported from other parts of Australasia.

The main effect of the disease is due to its high contagiousness. Economic losses result from the loss of milk production delay in the animals' reaching maturity for marketing, loss of draught power in bullocks, abortions in pregnant animals and deaths in calves, lambs or kids. In addition, disease or fear of disease disrupts trade in animals or animal products such as meat, offal, dried milk or products such as semen and embryos.

FMD can spread by direct contact, by animal products, meat, milk, semen, embryos, by the airborne route or by mechanical means such as on people and wild animals and birds, vehicles and fomites (Hyslop, 1970; Sellers, 1971).

Animals affected with FMD give out virus in their breath, in their secretions and excretions and from the skin.

Affected animals or those incubating the disease harbor FMDV in their tissues, milk, semen or ova. Virus present in muscle is inactivated by lactic acid developing after slaughter. In offal and bone marrow, no pH changes take place and these products can lead to infection of animals.

Semen is infective before bulls show lesions, and thus artificial insemination can spread disease. Virus is present in milk to high titer just before and at the time of appearance of lesions; if such milk is fed to calves and piglets they can become infected (Burrows et al., 1971).

Cattle, sheep and goats infected with FMD excrete virus as an aerosol. The amounts vary from 10^3 to more than 10^8 infectious units per day. Most of the airborne particles are 6 μm or more in diameter. Normally they will fall to the ground, but as a result of turbulences with suitable winds FMDV will be spread as a plume. With relative humidities greater than 60%, airborne virus will survive many hours. Such humidities occur frequently at night when atmospheric conditions are stable. Most spread over land is within 10 km of the source. However, where particles are lifted by wind through valleys and over hills, spread may cover longer distances. Also over the sea spread is further under certain atmospheric conditions since dispersion of the virus is less pronounced. This feature has been observed in spread from northern Germany to Denmark, from Denmark to Sweden and from France to the south coast of England (Gloster et al., 1982; Donaldson et al., 1982).

Of the animals at risk, cattle are more likely to become infected than sheep, goats or pigs, partly because they are better samplers (the respiratory rate of cattle is 100 l/min, that of sheep and pigs is 5–10 l/min) and partly because less virus is required to infect cattle (10^1–10^2 infectious units). Large herds of cattle are more at risk than individual animals or small herds.

Airborne spread is mainly found where large numbers of animals, especially pigs, are infected, where conditions are suitable for minimal dispersion of the virus aerosol plume and where there are large concentrations of susceptible stock downwind. Such conditions are found mainly in northern Europe. Elsewhere, spread is more likely at night and during periods when closer contact occurs, e.g. at waterholes.

People who have been in contact with affected animals can carry virus on their person, in the nose or, in the case of veterinarians, on their instruments. Vehicles that have carried infected animals may expose subsequent loads to the risk of infection. Nonsusceptible domestic animals, wild animals and birds may transport the virus mechanically. FMDV may survive on inanimate objects, in dung and slurry, and on hay and straw, for varying periods depending on the temperature and weather conditions.

Cattle, sheep, goats and pigs may be virus carriers in a number of ways. When they are infected by direct contact, they may have virus on them for the next 24 h. After the incubation period, infection may develop which is not perceived or may not give rise to overt lesions; these animals may excrete more virus. This is found especially with sheep and goats and may be observed in cattle and pigs that have been vaccinated.

In pigs, FMDV does not persist for more than a month. However, a virus may persist in the pharyngeal region of cattle, sheep and goats (post-infection carriers). Of cattle, 50% may remain carriers for 9 months and a few for 2 years or more. For 50% of sheep and goats the carrier period is 9 weeks and for some 9–11 months. No experimental evidence of transmission from such carriers has been found, but they may present a potential for virus maintenance. However, African buffaloes have been shown to transmit infection.

In some areas FMD is enzootic, in others introduction of the virus may lead to epizootic or sporadic outbreaks.

Strains vary in the severity of symptoms induced. Some strains are more virulent for cattle and others for pigs. Sheep and goats are usually rather resistant. However, the SAT 1 epidemic in the Middle East in 1962 caused considerable losses in sheep. In parts of Africa local cattle are more resistant than cattle introduced from Europe. Rapidly growing animals or high milk yielders are more susceptible.

In areas where pig husbandry is practised on a wide scale, there is danger of spread through trade or as a result of high concentrations of airborne virus being produced by intensive units.

Wildlife may play an important part in maintenance of the disease in East, Central and southern Africa (Hedger, 1981). In these areas and in other parts of the world, the virus is maintained in a cycle involving ruminants. In South America, Africa and Southeast Asia these include cattle and buffalo; in the Middle East sheep probably play an important role in disseminating the disease.

PATHOGENESIS

The natural route of infection in FMD is via the respiratory tract. Virus entry and multiplication probably take place in the pharyngeal area (Burrows et al., 1971). Soon after infection, virus spreads in the lymphatic system and the blood to infect many tissues and organs. Large amounts of virus are present in secretions and excretions, often before clinical signs become apparent (Burrows, 1968; Burrows et al., 1981). There is some evidence that infection can also take place in the lungs (Sutmöller and McVicar, 1976). It is argued that virus associated with very small airborne particles can reach the alveolar spaces and be carried directly into the blood stream by macrophages. Virus which enters in this manner is then deposited in undetermined target tissues in which multiplication occurs.

Virus titers in the animal decline as levels of neutralizing antibody increase. A significant proportion of cattle become "carriers" following infection (post-infection carriers). In these animals virus can be recovered from the pharynx by means of a probang cup for up to 2.5 years after infection (Hedger, 1968). The epidemiological importance of post-infection carriers in FMD is not clear, but it is a potential mechanism for virus maintenance.

DISEASE SIGNS

Following an incubation period, usually between 2 and 8 days, clinical signs become evident. The disease is characterized by fever, depression, anorexia and the appearance of vesicles in the mouth and on the feet. In lactating animals there is an acute drop in milk production. Vesicles filled with straw-colored fluid are found on the tongue, dental pad, gums and lips. Lesions may also be present at the nares and on the skin of the muzzle. On the feet, vesicles are most prominent at the bulbs of the heel, along the interdigital cleft and, to a lesser extent, along the coronary bands. Lesions may be present on the teats and occasionally on the surface of the udder in affected cows and heifers.

Morbidity is high, up to 100%, but mortality is very low except in calves, where sudden death may occur in a significant proportion. Calves which die suddenly with FMD show no outward signs of infection. The principal lesion found at post-mortem is an acute viral myocarditis, characterized by an excess of fluid in the pericardium and degeneration of the cardiac muscle. The pale

areas of degeneration are often striated and give rise to an appearance referred to as "tiger heart".

Adult cattle become lame and sometimes salivate excessively. The shifting of weight from one foot to another and "smacking" of lips are clear signs of pain associated with the feet and mouth.

Lesions on the tongue rupture within a few hours, revealing extensive bright red ulcers which sometimes bleed when first formed. However, even very severe lesions in the mouth heal quickly, and within a few days animals may be eating normally. Ruptured vesicles on the feet usually become infected, which prolongs the healing process, and in many cases chronic lameness and permanent damage to the structure of the hoof ensues. Other serious sequelae are permanent loss of full milk production and infertility.

Chronic illness is often associated with FMD but its causes, other than secondary bacterial infection, are not defined. Some abnormalities have been attributed to pathological effects on the endocrine system and some nonspecific degenerative changes in these organs have been reported. A "panting" syndrome has been found in cattle in South America and in India, possibly due to an effect on the myocardium.

The clinical signs of FMD and vesicular stomatitis are indistinguishable. The range of species involved may be of some value in differential diagnosis. For example, horses as well as cattle can be affected by vesicular stomatitis.

Conditions which give rise to erosive lesions in the mouth, such as BPS and MD, can be confused with FMD. While the former should be distinguishable by the papular nature of the lesions, sporadic cases of MD may show severe vesication of the tongue. During outbreaks, less serious conditions such as foul-of-the-foot, actinobacillosis and actinomycosis may be mistaken for FMD. Sudden death in calves might be attributed to causes other than FMD.

PATHOLOGY

Gross changes are confined to areas of vesicle formation, principally the tongue, lips, gums, dental pad, nares, the skin of the muzzle, the bulbs of the heel and interdigital cleft. The udder and teats may be involved in cows and heifers. At post-mortem inspection, gross lesions may also be found on the pillars of the rumen.

At the height of infection, vesicles may be found at varying stages of development. Large vesicles filled with clear straw-colored fluid will usually rupture at about 24 h. As the epithelium becomes detached, bright red ulcers are revealed. Such lesions may involve a large proportion of the surface of the tongue. Fragments of necrotic epithelium usually remain attached to lesions for some days. Within 4–5 days of the onset of disease granulation tissue is evident, especially on the tongue and other parts of the mouth.

TABLE 32

A guide to the ageing of lesions

Fresh unruptured vesicles on tongue and/or feet	24–48 h
Ruptured vesicles partially covered with ragged but intact epithelium; exposed bright red ulcers	2–3 days
Epithelium necrotic and caseous; granulation tissue obvious on tongue or mouth parts	4–5 days
Epithelium lost; healing process under way in mouth; old and new horn clearly separated on hoof	7+ days

Determination of the age of lesions is useful in epidemiological investigations; Table 32 provides a general guide.

In general terms, the development of microscopic lesions is similar in all locations on the body. The cells most affected are those in the stratum spinosum in the deeper layers of the epidermis. These swell, become eosinophilic and show what has been described as "ballooning degeneration" (Gailiunas et al., 1964). The intercellular bridges break down, allowing fluid and debris to accumulate and form microvesicles. Microvesicles filled with edema fluid, cell debris and infiltrated with leukocytes coalesce to form macrovesicles. It is important to note that the stratum germinatium remains largely intact and is seldom breached in FMD. This allows rapid healing, since new epithelium is generated at many centres over the whole surface of the ulcers. The underlying dermis is infiltrated by inflammatory cells and is intensely hyperemic.
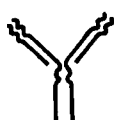
IMMUNE REACTION

The antibody response in cattle following infection with FMDV is similar to that following inoculation with a foreign protein. Antibodies can be detected from about 4 days after infection. This early antibody reaches peak levels in 7-14 days and declines to undetectable amounts within about 30 days (Brown et al., 1964). Early antibodies are of the IgM class of immunoglobulin and will neutralize and precipitate both homologous and heterologous virus. Later during the infectious process antibodies belonging to the IgG class can be detected. These arise at about 10-14 days and reach a peak at about 28 days. The antibodies which occur later will neutralize and precipitate virus and fix complement, although they are much more type-specific than those belonging to the IgM class (Skinner, 1953).

Convalescent cattle remain totally immune to homologous virus for 3-4 months. After this, degrees of partial immunity can be demonstrated. For example, Cunliffe (1964) challenged animals which had recovered about 1 year earlier and found that, while local lesions could be produced at the sites of inoculation, no generalized infection occurred and no other signs of disease were observed. Further studies indicated that an immune state can persist for at least 4.5 years. The rate at which immunity declines depends upon age, level of nutrition, physiological state and breed of the animals.

It is possible to immunize cattle passively with whole blood or serum from recovered animals. In fact, this was sometimes used as a method of protection before vaccines became available. Antibody is present in the colostrum of convalescent and immunized cows (Schneider, 1955), and calves which suckle immune dams rapidly acquire serum antibody (Van Bekkum, 1966). This can interfere with vaccination for 3-6 months and afford protection against challenge for up to 6 weeks.

Immunity following vaccination is similar to that after natural infection but its duration is somewhat shorter. A single inoculation of inactivated vaccine will protect against experimental challenge for 3-6 months (Fogedby et al., 1952). The duration of immunity is affected by the type of adjuvant used in the vaccine formulation. The more recently developed oil emulsion vaccines protect for rather longer periods. It is usual to vaccinate at least annually in order to maintain a herd immunity which will withstand field challenge.

LABORATORY DIAGNOSIS

A vesicular condition appearing in cattle and involving other species of

cloven-hoofed animals, i.e. sheep and pigs, might be tentatively diagnosed as FMD. Where a single animal or single species is involved, laboratory confirmation must be sought.

The commonly used test for FMDV is the identification of complement fixing antigen in vesicle fluid or epithelial material. The success and rapidity of this test depend largely on the quality of the material submitted. Vesicle fluid can be collected from unruptured lesions with a needle and syringe. Clean pieces of epithelium weighing at least 1 g are most suitable for diagnostic purposes. ELISA is used in many laboratories (Ferris and Dawson, 1988).

If a sample fails to type in the CF test or ELISA, it is passaged in tissue culture. Bovine thyroid or kidney cells are most commonly used. When gross CPE has developed, the tissue culture fluid is used in the CF test or ELISA. If no CPE is observed, then at least one blind passage should be carried out. Occasionally animals, e.g. cattle, sheep, pigs or mice, may be used in the process of identification.

The sequence of events in the laboratory diagnosis of FMDV is shown in the diagram below.

Specimen of epithelium and/or vesicle from field case

CF test or ELISA against seven types of FMDV

Result in 2.5 h

or

Result in 18–24 h

If no result, passage in bovine thyroid or bovine kidney cell cultures (IB-RS-2 pig kidney cells: pigs only)

Gross CPE: test by CF/ELISA

No CPE: further passage in cell cultures

Results passed on to control authority
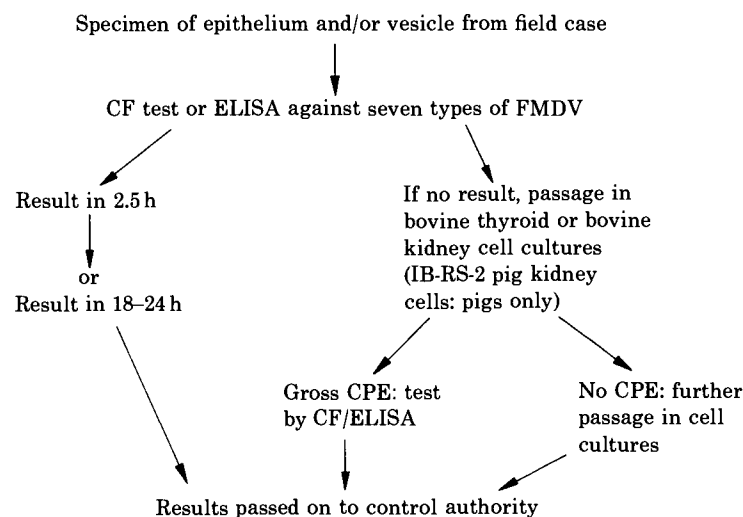
PROPHYLAXIS AND CONTROL

FMDV is spread by direct contact between affected and susceptible animals, by meat, milk, semen or ova, by the airborne route or by virus contaminating people, domestic or wild animals, birds, vehicles or fomites. The aim of control measures is to break this cycle of spread.

Control of movement of livestock is one of the most effective measures. Lactic acid produced in meat should be sufficient to inactivate the virus, but offal and tissues where there is no change in pH must be heated to inactivate the virus. Milk should also be treated by heat. People should be discouraged from visiting infected farms and, where handling of animals has to be done, the person should be disinfected before and afterwards. Vehicles should be cleaned and disinfected between carrying animals. Such means of spread are controllable.

However, it is difficult to control virus diffusion by the airborne route or by wild animals or birds. Here the aim should be to minimize the amounts of virus that are likely to be produced by affected animals (especially pigs) or to be

available for contamination of wild animals. Access of wild animals or birds to domestic species should be prevented.

Besides attempting to block spread, the aim is to reduce the amount of virus produced by the animals. This is done by slaughter (stamping out), vaccination or a combination of both.

Slaughter of infected animal stops the production of virus, and slaughter of animals in direct or indirect contact with the source of infection breaks the infective chain. This is an economic method only in countries where disease incidence is low. In other countries, it may not be economically or socially acceptable and there vaccination is carried out (Boldrini, 1978).

Although attenuated vaccines have been used in the past, they have not been found satisfactory; the present vaccines contain inactivated virus. Three main methods of virus production are used: (1) growth in surviving fragments of tongue epithelium (Frenkel method), (2) growth in roller bottles containing monolayers of calf kidney or BHK 21 cells, (3) growth in suspension cultures of BHK 21 cells. In addition, virus can be grown in IB-RS-2 cells (Mowat et al., 1978).

The virus so produced is inactivated with aziridines (AEI, BEI). Outbreaks of disease have been associated with the use of formalin for inactivation and it is therefore no longer used to the same extent. Adjuvants most commonly used for cattle and sheep vaccines contain aluminium hydroxide and saponin. In South America oil adjuvants are advocated.

The choice of strains of FMDV to use in the vaccine is important. Such a strain should grow to high titer, should produce high amounts of immunogenic material, and should be genetically stable. The strain should also protect against as wide a spectrum as possible of strains within the type.

Once made, the vaccine is tested for immunity and potency. Potency is expressed, for example, in median protective doses (PD_{50}), and the minimum level laid down is 3–7 PD_{50}.

Vaccines may be monovalent, bivalent, trivalent or polyvalent. There appears to be no interference between the antigens of the different types. The first vaccination leads to immunity in ruminants for about 3–6 months. Subsequent vaccinations may give protection for a year in cattle but only about 6 months in sheep. The dosage for sheep is one-third of that for cattle.

A number of vaccination schemes are practised, depending on the weight of infection likely to be encountered. Where the greatest risk of infection is likely, ruminants are vaccinated three times a year; with a medium risk, animals are vaccinated twice a year. In countries where the weight of infection is low, ruminants are vaccinated twice the first year and subsequently annually. Maternal antibody may last for 3–6 months and can interfere with immunization. However, to avoid a gap in protection, countries recommend first vaccination at ages ranging from 2–6 months.

Vaccination is usually confined to cattle; for example, in Europe 46% of cattle are vaccinated as compared with 3.42% of sheep and 2.76% of pigs. Cattle are regarded as the animals most likely to be affected and to suffer economic loss through failure to produce milk or be fattened. Owing to the vaccination programme, in some countries there may be times of the year when only about 20% of the animals in an area are protected.

The timing of vaccination depends on the husbandry of the country. Where there is a rainy and dry season, vaccination should be done at the beginning of the dry season, since movement of animals is restricted during the rainy season and spread of disease may occur once movements start in the dry season.

When an outbreak occurs in a country that vaccinates, controls on movement may be established. In addition, there may be stamping out of the animals on the farm and ring vaccination around.

In some countries vaccine is kept as a reserve in order to help control outbreaks. In other countries vaccination is limited to frontiers where disease may occur.

It should be realized that present vaccines do not protect cattle completely until three vaccinations have been given. Before that time, local multiplication of virus may occur in the pharyngeal area or possibly at an abrasion on the skin. Thus, if one wishes to reduce infection, the other control measures have to be enforced.

Some of the vaccines, on introduction, have caused allergic or post-vaccinal reactions; these have been caused by antibiotics or proteins in the vaccine.

With the advent of recombinant DNA techniques, new vaccines may be introduced (Bachrach et al., 1983); these are genetically expressed VP1 vaccines or synthetic peptides incorporating the major antigenic site on the VP1 (peptides 141–160), or peptides biosynthesized in cells from the appropriate incorporated oligonucleotides. Such vaccines are at present in the developmental stage. They are safe and stable but they still have to be evaluated for efficiency, for duration of immunity and for immunogenic ability against a range of strains likely to be met in the field.

REFERENCES

Bachrach, H.L., Callis, J.J., Brown, F. and Strohmaier, K., 1983. Achievements in genetic engineering and their influence on the control and prevention of animal disease. Paper presented at 51st General Session, Off. Int. Epizoot., Paris, pp. 1–30.

Boldrini, G.M., 1978. Vaccination and the control of foot-and-mouth disease. Vet. Rec., 102: 194–198.

Brown, F., Cartwright, B. and Newman, J.F.E., 1964. Further studies on the early antibodies in the sera of cattle and guinea pigs infected with FMDV. J. Immun., 93: 397–402.

Burrows, R., 1968. Excretion of foot-and-mouth disease virus prior to development of lesions. Vet. Rec., 82: 387–388.

Burrows, R., Mann, J.A., Greig, A., Chapman, W.G. and Goodrige, D., 1971. The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. J. Hyg. (Camb.), 69: 307–321.

Burrows, R., Mann, J.A., Garland, A.J.M., Greig, A. and Goodridge, D., 1981. The pathogenesis of natural and simulated natural foot-and-mouth disease in cattle. J. Comp. Pathol., 91: 599–609.

Cunliffe, H.R., 1964. Observations on the duration of immunity in cattle after experimental infection with FMDV. Cornell Vet., 54: 501–510.

Donaldson, A.I., Gloster, J., Harvey, L.D.J. and Deans, D.H., 1982. Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981. Vet. Rec., 110: 53–57.

Ferris, N.P. and Dawson, M., 1988. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases. Vet. Microbiol., 16: 201–209.

Fogedby, E.G., Malmquist, W.A., Osteen, O.L. and Johnson, M.L., 1952. Influence of various amounts of aluminium hydroxide on the immunity produced by foot-and-mouth disease vaccine. Nord. Vet. Med., 4: 745–754.

Gailiunas, P., Cottral, G.E. and Seibold, H.R., 1964. Teat lesions in heifers experimentally infected with FMDV. Am. J. Vet. Res., 25: 1062–1069.

Gloster, J., Sellers, R.F. and Donaldson, A.I., 1982. Long distance transport of foot-and-mouth disease virus over the sea. Vet. Rec., 110: 47–52.

Hedger, R.S., 1968. The isolation and characterisation of FMDV from clinically normal herds of cattle in Botswana. J. Hyg. (Camb.), 66: 27–36.

Hedger, R.S., 1981. Foot-and-mouth disease. In: J.W. Davis, L.H. Karstad and D.O. Trainer (Editors), Infectious Diseases of Wild Mammals. Iowa State University Press, Ames, IA, 2nd edn., pp. 87–96.

Hyslop, N. St G., 1970. The epizootiology and epidemiology of foot-and-mouth disease. Adv. Vet. Sci. Comp. Med., 14: 261–307.

Hyslop, N. St G., 1973. Transmission of the virus of foot-and-mouth disease between animals and man. Bull. World Health Org., 49: 577–585.

Mowat, G.N., Garland, A.J.M. and Spier, R.E., 1978. The development of foot-and-mouth disease vaccines. Vet. Rec., 102: 190–193.

Pereira, H.G., 1977. Subtyping of foot-and-mouth disease virus. Dev. Biol. Stand., 35: 167–174.

Pereira, H.G., 1981. Foot-and-mouth disease. In: E.P.J. Gibbs (Editor), Virus Diseases of Food Animals. Academic Press, London, Vol. 2, pp. 333–363.

Samuel, A.R., Knowles, N.J. and Kitching, R.P., 1988. Serological and biochemical analysis of some recent type A foot-and-mouth disease virus isolates from the Middle East. Epidemiol. Inf., 101: 577–590.

Schneider, B., 1955. Transmission of FMD antibodies from the dam to the new born calf. Monatsh. Tierheilk., 7: 137–143.

Sellers, R.F., 1968. The inactivation of foot-and-mouth disease virus by chemicals and disinfectants. Vet. Rec., 83: 504–506.

Sellers, R.F., 1971. Quantitative aspects of the spread of foot-and-mouth disease. Vet. Bull., 41: 431–449.

Skinner, H.H., 1953. One week old white mice as test animals in FMD research. Proc. 15th Int. Vet. Congr., Stockholm, Part 1, p. 195.

Sutmöller, P. and McVicar, J.W., 1976. Pathogenesis of foot-and-mouth disease: the lung as an additional portal of entry of the virus. J. Hyg. (Camb.), 77: 235–243.

Van Bekkum, J.G., 1966. The influence of FMD vaccination of the mother on the levels of neutralizing antibody in her young. Bull. Off. Int. Epizoot., 65: 439–442.

Chapter 47

Bovine Enteroviruses

N.J. KNOWLES and J.A. MANN

INTRODUCTION

Bovine enteroviruses (BEV) have been isolated both from normal cattle and from cattle with enteric, respiratory and reproductive disease problems.
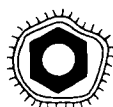
VIRUS PROPERTIES

BEV are members of the genus *Enterovirus* in the family Picornaviridae and are characterized as naked, icosahedral viruses having a ribonucleic acid genome and a diameter of 28 nm. They are stable over a wide pH range and are stabilized against thermal inactivation by divalent cations.

Numerous antigenic comparisons of BEV have been made and as many as ten serological types described. However, two major studies have established (by VN and CF tests) that there are probably only two serotypes (La Placa et al., 1965; Knowles and Barnett, 1985). Within these two types many antigenic variants exist, the differences between type 2 strains being generally greater than between type 1 strains. There exist within both types "broad" and "specific" antigenic variants. Virus strains belonging to type 1 have been found to agglutinate mammalian erythrocytes (rhesus monkey and sheep) (La Placa et al., 1965; Knowles and Barnett, 1985). Guinea pig erythrocytes are agglutinated by strains of both serotypes (Knowles and Barnett, 1985). The growth of type 2 strains in tissue culture is inhibited by low concentrations of 2-(α-hydroxy-benzyl)-benzimidazole (HBB) (Portolani et al., 1968). Higher concentrations of both HBB and another inhibitor of virus replication, guanidine hydrochloride, inhibit the growth of most BEV (Adair et al., 1987).

Most BEV strains grow readily in primary kidney cells derived from cattle, pigs, sheep, goats, guinea pigs, rabbits, monkeys and humans. They also grow in the established cell lines IB-RS-2, BHK 21, HeLa, Vero, Ehrlich ascites tumor cells (mouse) and L cells (mouse). They can also be cultivated in embryonating chicken eggs and cells derived from chick embryos. Some strains are pathogenic for suckling mice. Abortion in pregnant guinea pigs has been observed following experimental infection.

The physico-chemical properties of enteroviruses renders them relatively resistant to many farm disinfectants, especially those based on acids or alkalis. The presence of organic matter also hinders effective disinfection. The best results can be obtained by the use of combinations of acids, iodophors and detergents together with very efficient cleansing schedules.

Sterilization of animal products can be accomplished by heat treatment. The acidity which develops during rigor mortis is insufficient to inactivate en-

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terovirus; thus, refrigeration of contaminated meat products will preserve the virus indefinitely unless they are processed at some previous stage by heat (at least 60°C for 1 h).

EPIZOOTIOLOGY

BEV belonging to serotype 1 have been isolated from domestic cattle (*Bos taurus*), water buffalo (*Bubalus bubalis*; Mehrotra, 1973; Urakawa and Shingu, 1987), sheep (*Ovis aries*; Jain and Batra, 1985; Sharma et al., 1986), goats (*Capra hircus*; Jain and Batra, 1985), Sika deer (*Cervus nippon*; Urakawa and Shingu, 1987), a dog (*Canis familiaris*; R.N. Srivastava and N.J. Knowles, unpublished data, 1986), free-living African buffalo (*Syncerus caffer*) and impala (*Aepyceros melampus*; Hamblin et al., 1985); however, viruses belonging to serotype 2 have only been isolated from domestic cattle. Neutralizing antibodies have been detected in humans, monkeys, cattle, horses, pigs, goats, sheep, dogs, rabbits, guinea pigs and fowl (Moscovici et al., 1961; McFerran, 1962; Yamada, 1965).

Enteroviruses have a global distribution and have been isolated from feces and pharyngeal washings of healthy and diseased cattle. Latent virus has also been found in cultures of many tissues and organs.

While little is known concerning reservoir hosts and vectors, it can be assumed that the most common means of spread is by contact with infected animals or animal products.

Since it is not possible to relate the presence of enterovirus with specific disease syndromes, it is difficult to determine speed of spread, incidence or economic implications. However, the world-wide distribution indicates facility of spread and the conditions to which the virus has been linked—diarrhea (Wilner, 1969), abortion (Moll and Finlayson, 1957) and respiratory disease (Moll and Ulrich, 1963; Stott et al., 1980)—can be of considerable economic importance.

PATHOGENESIS

Enteroviruses generally infect by the oral route, thus gaining access to the alimentary canal. This is almost certainly the manner in which BEV gain access to the host. The resistant nature of the virus allows passage unaffected along the digestive tract. Most enterovirus infections in man and animals are subclinical and this is probably also the case in BEV. In these instances virus passes to a local lymph node, producing a low-level antibody response, and progresses no further. In clinical cases more widespread infection occurs in a number of target organs, giving rise to symptoms as described below.

DISEASE SIGNS

The clinical syndromes most frequently associated with enterovirus infection are abortion, stillbirth, infertility and neonatal death. Enteritis and respiratory disease have also been attributed to enterovirus infection.

PATHOLOGY

There are no pathological signs which could be considered pathognomonic.
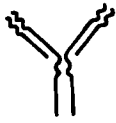
IMMUNE REACTION

Antibodies, usually IgM, may be present in the blood of bovine fetuses. Since antibodies do not cross the bovine placenta, the presence of fetal antibody indicates intrauterine infection.

The sera of adult animals can contain antibodies to a number of variants. These antibodies will be of the IgG class. A study of the profile of fetal and maternal antibody may indicate infection of the fetus, since in many cases the maternal serum will contain antibodies to three–five more variants than the fetal serum (Dunne et al., 1974).

The pathogenicity of BEV for cattle, monkeys or man is not clear, but neutralizing antibodies have been found in all these species (Moscovici et al., 1961).

LABORATORY DIAGNOSIS

BEV may be isolated from feces, esophageal–pharyngeal scrapings, vaginal mucus, semen, blood, placenta, fetuses and fetal fluids, lungs, salivary gland, small intestine, lymph node, hard palate, nasal swab, epithelium, abomasum, large intestine, rectum, liver, kidney, pancreas and spleen. They grow readily on primary bovine cells (kidney, testis, thyroid).

Many other mammalian cells with support their growth in vitro. BEV have been reported to infect mice, exhibiting lesions similar to those produced by the human coxsackievirus (Adair et al., 1987). Some BEV strains can cause abortion in pregnant guinea pigs (Moll, 1964).

Tests for antigen detection include CF and HA, while neutralization and ID tests are used for antibody detection.

Antigenic variants exist within the two serological types and may be so diverse as to be considered distinct serotypes. However, there are also "broad spectrum" strains linking many unrelated variants. Recently these "broad spectrum" strains have been proposed as prototypes for the production of reference antisera (Knowles and Barnett, 1985). The host in which reference antisera are raised may be important. Chickens are said to produce "broad spectrum" antibodies and rabbits produce specific antibodies, while guinea pigs provide antisera of an intermediate nature.

The rapid identification of BEV by serological techniques is necessary where their isolation may confuse the diagnosis of more important diseases, e.g. FMD and vesicular stomatitis.
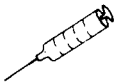
PROPHYLAXIS AND CONTROL

Since it is difficult to associate enterovirus infection with specific disease conditions, vaccination has not been attempted. However, with only two serotypes the use of "broad" variants for vaccines might prove useful. The importance of good animal husbandry cannot be emphasized too much in the control of nonspecific viral conditions, together with the isolation of infected animals and effective cleansing and disinfection.

REFERENCES

Adair, B.M., Kennedy, S., McKillop, E.R., McNulty, M.S. and McFerran, J.B., 1987. Bovine, porcine and ovine picornaviruses: identification of viruses with properties similar to human coxsackieviruses. Arch. Virol., 97: 49–59.

Dunne, H.W., Huang, C.M. and Whei Jun Lin, 1974. Bovine enteroviruses in the calf. An attempt at serologic biologic, and pathologic classification. J. Am. Vet. Med. Assoc., 164: 290–294.

Hamblin, C., Knowles, N.J. and Hedger, R.S., 1985. The isolation and identification of bovid enteroviruses from free-living wild animals in Botswana. Vet. Rec., 116: 238-239.

Jain, N.C. and Batra, S.K., 1985. Isolation and characterization of ovine enteroviruses. Ind. J. Virol., 1: 17–25.

Knowles, N.J. and Barnett, I.T.R., 1985. A serological classification of bovine enteroviruses. Arch. Virol., 83: 141–155.

La Placa, M., Portolani, M. and Lamieri, C., 1965. The basis for a classification of bovine enteroviruses. Antigenic characters studied with chicken immune sera and rhesus monkey erythrocytes agglutinating activity. Arch. Ges. Virusforsch., 17: 98–115.

McFerran, J.B., 1962. Bovine enteroviruses. Ann. N.Y. Acad. Sci., 101: 436–443.

Mehrotra, M.L., 1973. Isolation and characterization of cytopathogenic viral agents resembling enteroviruses of buffalo. Indian J. Anim. Sci., 43: 624–628.

Moll, T., 1964. Abortion and stillbirth of guinea pigs resulting from experimental exposure to bovine enteric virus. Am. J. Vet. Res., 25: 1757–1762.

Moll, T. and Finlayson, A.V., 1957. Isolation of cytopathogenic viral agent from the faeces of cattle. Science, 126: 401–402.

Moll, T. and Ulrich, M.I., 1963. Biologic characteristics of certain bovine enteric viruses. Am. J. Vet. Res., 24: 545–550.

Moscovici, C., La Placa, M., Maisel, J. and Kempe, C.H., 1961. Studies of bovine enteroviruses. Am. J. Vet. Res., 22: 852–863.

Portolani, M., Palenzona, A.M., Bernardini, A. and La Placa, M., 1968. Bovine enteroviruses: Apparent correlation between antigenic characters and capacity to grow in the presence of small concentrations of 2-(α-hydroxybenzyl)-benzimidazole. Arch. Ges. Virusforsch., 24: 428–432.

Sharma, A.K., Knowles, N.J., Prasad, S. and Srivastava, R.N., 1986. Preliminary studies on a sheep enterovirus. Ind. J. Virol., 2: 86–88.

Stott, E.J., Thomas, L.H., Collins, A.P., Crouch, S., Jebbett, J., Smith, G.S., Luther, P.D. and Caswell, R., 1980. A survey of virus infections of the respiratory tract of cattle and their association with disease. J. Hyg. Camb., 85: 257–270.

Urakawa, T. and Shingu, M., 1987. Studies on the classification of bovine enteroviruses. Microbiol. Immunol., 31: 771–778.

Wilner, B.I., 1969. Classification of the Major Groups of Human and Other Animal Viruses. Burgess Publishing Company, Minneapolis, MN, pp. 32–39.

Yamada, S., 1965. Studies on bovine enteroviruses. IV. Neutralizing antibodies in the Kyushu District. Jpn. J. Vet. Sci., 27: 317–323.

Chapter 48

Bovine Rhinoviruses

R.F. SELLERS

Bovine rhinoviruses have been isolated both from normal cattle and from cattle with clinical signs of respiratory disease.

Bovine rhinovirus is a member of the family Picornaviridae. Like human and equine rhinoviruses, it is 25–31 nm in diameter with icosahedral symmetry. It is resistant to ether and chloroform and is unstable at pH 6.0 and lower values; it is not stabilized by $MgCl_2$ at 50°C for 60 min.

Three serotypes have been described. Serotype 1 contains strains Sd-1, 181-V (Germany), C-07, VC-96 and FSI-43 (USA), RS3X (UK) and M47 and Chitose (Japan); serotype 2 contains EC-11 (UK) (Ide and Darbyshire, 1972; Shimizu et al., 1974; Kurogi et al., 1975; Lupton et al., 1980); serotype 3 contains H-1 (Japan) (Yamashita et al., 1985).

Because of its acid lability, bovine rhinovirus is inactivated by chemicals and disinfectants used for foot-and-mouth disease (Sellers, 1968).

Bovine rhinovirus has been isolated from cattle in the FRG, USA, England, Japan and Sudan and no doubt its distribution is world-wide in bovid species. Antibodies are found among all ages but disease is usually described in young animals. It spreads by direct contact, especially where cattle are kept in large numbers and where movement and introduction of fresh animals are frequent. It is associated with other bovine respiratory viruses, especially parainfluenza 3 virus, respiratory syncytial virus and bovine enterovirus. The degree of morbidity caused by the virus is not known.

Disease signs associated with isolation of bovine rhinovirus include fever, depression, inappetence, lachrymation and conjunctivitis, nasal discharge, cough and difficulty in breathing.

In experimental infection of colostrum-deprived calves, only focal or interstitial pneumonia has been found at necropsy. Animals develop antibodies after infection. Local IgA antibodies are probably present in natural secretions.

Laboratory diagnosis is essentially a differential diagnosis to establish the cause of the respiratory illness. Such illness could be due to, for example, bovine respiratory syncytial virus, parainfluenza 3, bovine enteroviruses or adenoviruses as well as chlamydial and bacterial agents.

Isolation of bovine rhinoviruses is carried out from nasal swabs in tissue cultures of bovine origin, e.g. calf kidney primary cultures or calf kidney cell lines. Cells are incubated at 30–33°C and rotated. The virus produces a CPE in 3–9 days.

Samples of serum taken during the acute and convalescent phases can be tested in neutralization tests to demonstrate a rise in antibody.

Infection with bovine rhinoviruses is widespread. There are no vaccines. Control must therefore, as with other respiratory diseases, be based on sound husbandry.

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REFERENCES

Ide, P.R. and Darbyshire, J.H., 1972. Studies with a rhinovirus of bovine origin. III. The pathogenesis and pathology of infection with strain RS 3X in calves. Arch. Ges. Virusforsch., 36: 335–342.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Goto, Y. and Omori, T., 1975. Serological differentiation of bovine rhinoviruses. Nat. Inst. Anim. Health Q., 15: 201–202.

Lupton, H.N., Smith, M.H. and Frey, M.L., 1980. Identification and characterization of a bovine rhinovirus isolated from Iowa cattle with acute respiratory tract disease. Am. J. Vet. Res., 41: 1029–1034.

Sellers, R.F., 1968. Inactivation of foot-and-mouth disease virus by chemicals and disinfectants. Vet. Rec., 83: 504–506.

Shimizu, Y., Narita, M. and Murase, N., 1974. Isolation of bovine rhinovirus from calves with respiratory disease. Nat. Inst. Anim. Health Q., 14: 35–41.

Yamashita, H., Akashi, H. and Inaba, Y., 1985. Isolation of a new serotype of bovine rhinovirus from cattle. Arch. Virol., 83: 113–116.

Chapter 49

Calicivirus-Like Agents

G.N. WOODE

INTRODUCTION

Small viruses have been found in diarrheic feces of humans, calves and piglets which have a superficial morphologic resemblance to the members of the Caliciviridae. However, these have not been characterized or grown in cell culture and have not been shown to possess the properties of the Caliciviridae (Matthews, 1982).

The term was first used to describe virus-like particles in human feces (reviewed by Madeley, 1979), and similar particles were subsequently identified in the feces of lambs by Snodgrass and Gray (1977), of calves by Woode and Bridger (1978) and of pigs by Bridger (1980).

VIRUS PROPERTIES

Bovine calicivirus-like agents have not been characterized chemically. When negatively stained with 2% potassium phosphotungstate, fecal particles have a reticulate appearance with capsomeres projecting from their outer edges. Occasionally particles are penetrated by stain and show an outer rim, 7–8 nm wide. The particles occur singularly or in groups containing as many as several hundred virions. Within these groups small particles about 18 nm
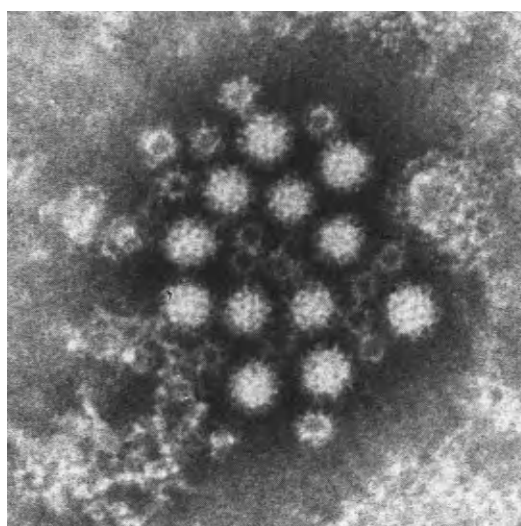
Fig. 172. Negatively stained fecal preparation of bovine calicivirus-like agent (Newbury agent) (courtesy J. Med. Microbiol.).

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diameter are frequently and characteristically observed. The mean diameter of complete particles is 33 nm (Fig. 172; Woode and Bridger, 1978). The particles are similar but not identical to the description of calicivirus-like particles from humans (Madeley, 1979) and to members of the Caliciviridae (Smith et al., 1983).

Serological tests, other than IEM, have not been developed. Two-way cross-protection experiments in gnotobiotic calves have confirmed the presence of at least two antigenically different strains and probably more (Woode and Bridger, 1978; Bridger et al., 1984). Antiserum to Newbury agent was tested against a bovine calicivirus (Smith et al., 1983) and 17 other serotypes of calicivirus (12 serotypes of San Miguel sea lion virus, a vaccine strain of feline calicivirus and caliciviruses isolated from a gorilla, a rattlesnake, a mink and a walrus); no antigenic relationship with any one of these caliciviruses was detected (A.W. Smith, personal communication, 1983).

EPIZOOTIOLOGY

Calicivirus-like agents were identified in fecal samples of three herds with a history of calf diarrhea in England. All initial isolates were contaminated by astrovirus. In one herd other pathogens were not identified as frequent causes of the scour, but in two herds infection with coronavirus or rotavirus were common (Woode and Bridger, 1978). The authors consider that infections with these agents are probably common, as it is not necessary to search diligently for infected herds, once diagnostic methods have been developed. From a survey of calf diarrhea in southern England 6 of 18 outbreaks were associated with calicivirus-like agents (Bridger et al., 1984). However, the calicivirus-like agent infections of calves have been reported only from one group in England and this may be the result of the difficulty of identifying the viruses in fecal samples by routine examination (Bridger et al., 1984). Recently a calicivirus-like agent has been identified with calf diarrhea in the USA (G.N. Woode, unpublished data, 1983). A calicivirus (Tillamook strain) was isolated from calves with respiratory disease; this virus caused vesicular lesions in experimentally infected pigs. Although this virus was not neutralized by 18 calicivirus typing antisera, it has the typical morphology and is probably a member of the vesicular exanthema group of swine viruses (Smith et al., 1983).

Experimentally the fecal–oral route of transmission is successful, with diarrhea commencing 24–72 h p.i. Under farm conditions, infections appear to spread in a similar manner, following direct or indirect contact with fecal material.

PATHOGENESIS

Calicivirus-like agents can infect orally, and virus is found in the feces 24–72 h p.i. There is no report of involvement of organs other than the small intestine. Excretion of detectable virus is limited to a few days.

DISEASE SIGNS

Gnotobiotic calves 1 to more than 40 days of age when orally fed the virus become infected and excrete the virus. Calves less than 8 days of age develop mild diarrhea characterized by a change in color of the feces (yellow) and some softening. Calves aged more than 16 days may develop profuse yellow diarrhea and some calves excrete fresh blood. Anorexia may persist for 1–2 days. There

is considerable variability between calves in the severity of diarrhea, anorexia, watery nature of the stools and fecal output. Dry matter content is a poor indicator of disease severity and may vary from 5 to 30%. When anorexia is more severe, diarrhea and fecal output is reduced. There may be virus strain differences in virulence for gnotobiotic calves. In general, virus particles are detectable in the feces a few hours before diarrhea commences, or their presence coincides with the onset of diarrhea and may be detectable for a further 1–4 days. Usually few particles are excreted and they can be difficult to find (Woode and Bridger, 1978; Hall et al., 1982, 1984; Bridger et al., 1984). D-xylose absorption is a good measure of the clinical effect of infection and may be reduced by as much as 100% 1 h after feeding (Woode and Bridger, 1978) but can show variability amongst individual calves with reductions ranging from 43 to 96% (calicivirus SVR-1) or 18 to 32% (calicivirus SRV-2) of preinfection levels (Bridger et al., 1984).

PATHOLOGY

There are no macroscopic lesions seen, although some calves will have blood in the rectal contents. Lesions occur mainly in the anterior 33% of the small intestine, characterized by villus atrophy and reduced lactase activity in the jejunum. There is swelling of epithelial cells with shortening or loss of the microvilli. Infected cells degenerate and villus atrophy develops, with mild inflammatory response and crypt hyperplasia. Virus has not been observed in the cells involved in the lesion by transmission EM and immunofluorescence is not observed with convalescent serum (Woode et al., 1978; Hall et al., 1982; Bridger et al., 1984), but a faint reaction is observed by the immunoperoxidase method (Hall et al., 1984). Loss of activity of β-galactosidase occurs throughout the small intestine.
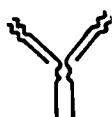
IMMUNE REACTION

Convalescent serum has IEM activity. Tissue cultures inoculated with virus preparations show no sign of infection and do not immunofluoresce; affected tissues also fail to show immunofluorescence, although there is a faint reaction by the immunoperoxidase method.

LABORATORY DIAGNOSIS

EM of fecal or intestinal contents, with minimal dilution, pelleted through 20–40% sucrose and stained with potassium phosphotungstate, is the most commonly used diagnostic method. IEM can increase the isolation rate, but only for those strains antigenically related. Immunoperoxidase staining of sections of the small intestine has been described. At least two and possibly more isolates of bovine calicivirus-like agents are unrelated antigenically as judged by cross-protection studies. It is probable that many serotypes exist (Woode and Bridger, 1978; Smith et al., 1983; Bridger et al., 1984).

PROPHYLAXIS AND CONTROL

There are no known control methods, and the importance of these viruses in the calf scour syndrome is not known. However, they were isolated from one

herd which had a significant incidence of calf diarrhea and death, but from which rotavirus and coronavirus were rarely isolated. Further studies on the importance of these agents is required. The viruses survive for months and probably years in feces at 4°C. As the syndrome appears to be one of malabsorption, supportive electrolyte therapy is recommended.

REFERENCES

Bridger, J.C., 1980. Detection by electron microscopy of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. Vet. Rec., 107: 532–533.

Bridger, J.C., Hall, G.A. and Brown, J.F., 1984. Characterization of a calici-like virus (Newbury agent) found in association with astrovirus in bovine diarrhea. Infect. Immun., 43: 133–138.

Hall, G.A., Bridger, J.C. and Brooker, B.E., 1982. Enteropathology of gnotobiotic calves infected with a calicivirus-like (Newbury) agent. Proceedings XII World Veterinary Congress on Diseases of Cattle, the Netherlands, Volume 1, pp. 217–221.

Hall, G.A., Bridger, J.C., Brooker, B.E., Parson, K.R. and Ormerod, E., 1984. Lesions of gnotobiotic calves experimentally infected with a calicivirus-like (Newbury) agent. Vet. Pathol., 21: 208–215.

Madeley, C.R., 1979. Comparison of the features of astroviruses and caliciviruses seen in samples of feces by electron microscopy. J. Infect. Dis., 139: 519–523.

Matthews, R.E.F., 1982. Classification and nomenclature of viruses. Intervirology, 17: 133–134.

Smith, A.W., Mattson, D.E., Skilling, D.E. and Schmitz, J.A., 1983. Isolation and partial characterization of a calicivirus from calves. Am. J. Vet. Res., 44: 851–855.

Snodgrass, D.R. and Gray, E.W., 1977. Detection and transmission of 30 nm virus particles (astroviruses) in faeces of lambs with diarrhoea. Arch. Virol., 55: 287–291.

Woode, G.N. and Bridger, J.C., 1978. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. J. Med. Microbiol., 11: 441–452.

Chapter 50

Astroviruses

G.N. WOODE

INTRODUCTION

Astrovirus particles were first observed in the diarrheic feces of children (reviewed by Madeley, 1979). Astroviruses are small round virus particles 28–30 nm in diameter, with a round unbroken edge and a characteristic six-pointed star with a white center and surface hollows that are triangular. Intestinal astrovirus infections were subsequently reported in lambs (Snodgrass and Gray, 1977), in calves (Woode and Bridger, 1978), in piglets (Bridger, 1980), in turkeys (McNulty et al., 1980), in red deer (Tzipori et al., 1981) and hepatitis of ducklings (Gough et al., 1984).
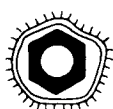
VIRUS PROPERTIES

Bovine, human and red deer astroviruses infect primary kidney cell cultures, infected cells being identified by IF test (Woode and Bridger, 1978; Woode et al., 1984). The addition of trypsin to the media permitted the adaptation of human and bovine astroviruses to cell culture (Lee and Kurtz, 1981; Aroonprasert et al., 1989). The morphology of each astrovirus isolate is similar (Fig. 173).

Herring et al. (1981) reported on the purification and characterization from gut contents of ovine astrovirus. The virus has a density in $CsCl_2$ of 1.35–1.37 g/ml and a single-stranded RNA genome with an S value of 34 and a poly (A) tract. There are two major capsid polypeptides with a similar mol. wt. of approximately 3.3 × 10^4. They conclude that astroviruses must be considered a separate group with a polypeptide composition intermediate between the Picornaviridae and Caliciviridae. In contrast, the human astrovirus has four polypeptides, suggesting a classification in the Picornaviridae. It is stable at pH 3 and in chloroform (Kurtz and Lee, 1987; Aroonprasert et al., 1989).

Astroviruses isolated from children, calves, lambs, piglets and red deer do not share common IF antigens (Lee and Kurtz, 1977; Woode and Bridger, 1978; Snodgrass et al., 1979; Bridger, 1980; Tzipori et al., 1981). The recent isolates of bovine astrovirus in the USA share IF antigens and cross-react with antiserum to the UK astrovirus isolated by Woode and Bridger (1978) (Fig. 174). However, the two USA isolates and the UK isolate do not share neutralizing antigens, which suggests that there are at least three serotypes (Woode et al., 1985). At least four serotypes of human astrovirus exist (Kurtz and Lee, 1984).
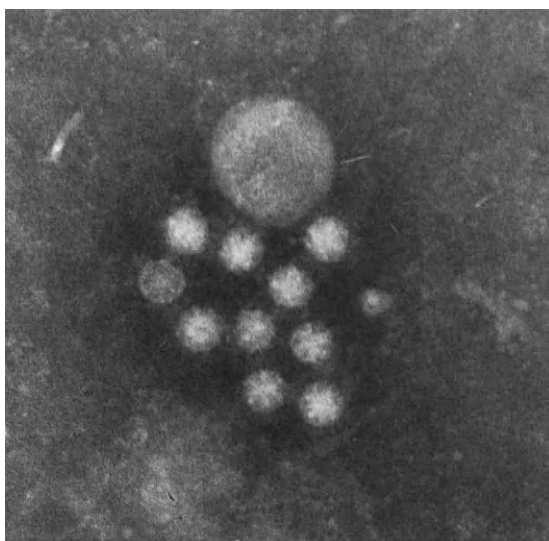
Fig. 173. Negatively stained fecal preparation of bovine astrovirus (courtesy J. Med. Microbiol.).

ASTROVIRUSES IN ACUTE ENTERITIS OF CALVES

Astroviruses have been isolated from diarrheic calves in association with calicivirus-like agent, coronavirus and rotavirus in England (Woode and Bridger, 1978; Bridger et al., 1984) and with rotavirus, coronavirus and *Cryptosporidium* in the USA (Woode et al., 1984, 1985). In the opinion of these authors astrovirus does not cause a clinical syndrome in calves, and for this reason the virus has not been studied extensively. However, studies in the USA have shown that although gnotobiotic calves infected with astrovirus do not develop clinical illness, cytopathic changes are observed in the dome epithelium of the Peyer's patches (Woode et al., 1984, 1985). Experimentally, infection is transmissible by the fecal–oral route. Virus infectivity persists several years at 4°C in fecal contents and at least 6 months as a 25% suspension of feces in phosphate buffered saline. Serological surveys for bovine astrovirus infection in the
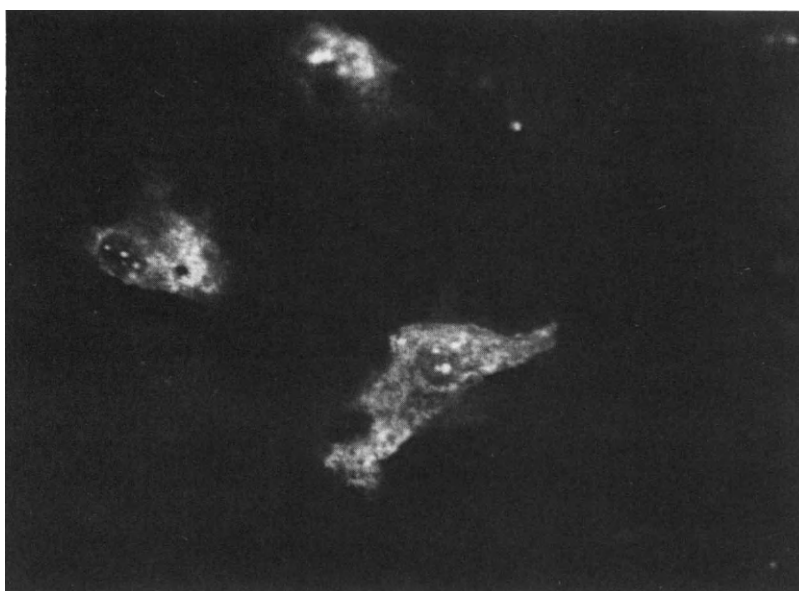
Fig. 174. Immunofluorescence in primary calf kidney cells infected with bovine astrovirus (courtesy J. Med. Microbiol.).

UK and USA reported 27% and 31% of sera, respectively, to be antibody positive (Woode and Bridger, 1978; Woode et al., 1984, 1985). In the USA 28 of 1060 (2.6%) calf diarrhea samples were positive for astrovirus. The low rate of isolation may reflect the subclinical nature of most infections, with isolation being a matter of chance.

Virus enters by the oral route and is excreted in the feces 24–72 h p.i. Studies with two different bovine astrovirus serotypes reported infection in the M cells of the dome epithelium in the Peyer's patch area of the ileum, by IF and by observation of virions in degenerating epithelium by transmission EM (Woode et al., 1984, 1985). Subsequent studies showed astrovirus infection in dome epithelium throughout the small intestine. Animals usually excrete virus for at least 5–7 days and some remain infected and excrete virus in feces for at least 3 weeks p.i.

No clinical signs directly attributable to astrovirus infection have been reported.

There is one study reported on the pathology (Woode et al., 1984). Only the epithelium overlying the domes of the Peyer's patches in the ileum region is involved. Most of the M cells of the dome epithelium are positive in immunofluorescence for astrovirus antigen. The tips of most domes are covered by cell debris containing swollen and degenerated epithelial cells, mononuclear cells and eosinophilic cells. Other domes are covered by flat to cuboidal epithelium. The Peyer's patches associated with infected domes show central cellular depletion and contain large cells with dark nuclei and eosinophilic cytoplasm. Ultrastructurally the virus is observed in both M cells and enteroabsorptive cells of the dome epithelium; its mean diameter is 30 nm.

Calves develop serum antibodies to astrovirus which can be demonstrated by IF and neutralization tests and by IEM.

Because of the small size and few particles produced, diagnosis by EM is difficult. Only primary calf kidney cell cultures can be infected by the virus, and immunofluorescent cells are demonstrable 24 h p.i. This method can be used for serology, both immunofluorescence and neutralization, and for titrating excreted virus (Woode et al., 1985). Calves excrete up to 10^5–10^7 infectious units per gram of feces for several days.

No methods of control of infection have been reported. The resistance of the virus enhances its ability to persist in the cattle population.

ASTROVIRUS IN ACUTE ENTERITIS OF LAMBS

Astrovirus infections in lambs have been studied by only one group, in Scotland. Data on geographical distribution of the virus are lacking, and nothing is known on the role of reservoir hosts, persistence in animals or how the virus spreads. It probably persists in the sheep population by virtue of its resistance to environmental factors when in feces and possibly by reinfection of the adult population. The virus was isolated from 8 to 17 lambs and probably spread by the fecal–oral route. Experimentally there is an incubation period of 2–3 days before virus is excreted in the feces, and this coincides approximately with the onset of diarrhea or softening of the feces (Snodgrass and Gray, 1977).

Under experimental conditions the fecal–oral route of infection occurs. Virus has been detected only in the epithelial cells of the small intestine (Snodgrass et al., 1979). The virus is excreted for at least 9 days p.i. (Snodgrass and Gray, 1977).

Experimental infections of 2-day-old gnotobiotic lambs cause mild diarrhea after an incubation period of 48 h; recovery occurs within 4–5 days (Snodgrass et al., 1979).

Astrovirus infection is observed by IF test in the mature villus epithelial cells and in subepithelial macrophages of the small intestine (Snodgrass et al., 1979). Infected cells are scattered irregularly in the apical 50% of the villi and are best observed in lambs killed early p.i. These cells are cuboidal in appearance and virus-containing inclusion bodies may be seen in the cytoplasm.

Partial villus atrophy occurs at 38–45 hours p.i. in the midgut region and at 38–120 hours p.i. in the ileum. There is crypt hyperplasia in the anterior, mid and distal small intestine. The effects are most marked in the ileum. The lactase levels in the tissues are consistently reduced in the midgut region but not in the proximal or distal tissues. Complete healing may occur within 5 days of infection.

Ultrastructural studies show crystalline arrays of virus particles, 29 nm, in the cytoplasm; virus particles are also observed in apical pits, in tubules and in secondary lysosomes. Virus particles may be seen aligning along microvilli, and sloughing cells show degenerate microvilli (Gray et al., 1980).

Convalescent sera contain antibodies demonstrable by IF test and specific for lamb astrovirus.

The diagnostic method of choice is EM. The fecal contents are diluted to 20% in distilled water, the suspension allowed to settle and a drop stained with 1% potassium phosphotungstate pH 7.0.

ASTROVIRUSES IN ACUTE ENTERITIS OF RED DEER

There is one report of the infection (Tzipori et al., 1981). The virus was observed in the feces of artificially reared red deer fawns suffering from diarrhea attributable to cryptosporidial infection. Particles with the typical morphology of astrovirus were seen (28–30 nm in diameter). Convalescent sera reacted in IF tests with infected cells in culture, but not with tissues infected with lamb rotavirus. The role of astrovirus in the outbreak of diarrhea is uncertain and there is no evidence available concerning its pathogenicity.

CONCLUSION

Little is known of the importance of astroviruses in causing diarrhea in humans and animals. Lamb astrovirus causes widespread lesions in enterocytes, but frequency of infection in lambs has not been studied. There is doubt about their role in humans (Bishop, 1982). In calves, although they do not produce diarrhea, the dome epithelial lesion may affect the gut local immune response and the ability of the animal to control other enteric infections. These viruses are probably widespread in nature, with perhaps each animal species possessing a population unrelated antigenically to astroviruses of other species, and different serotypes being common for each species. Whether the various astroviruses belong to one family of viruses is yet to be determined, as they are only related in their morphology.

REFERENCES

Aroonprasert, D., Fagerland, J.A., Kelso, N.E., Zheng, S. and Woode, G.N., 1989. Cultivation and partial characterization of bovine astrovirus. Vet. Microbiol., 19: 113–125.
Bishop, R.F., 1982. Other small virus-like particles in humans. A.J. Tyrrel and A.Z. Kapikian (Editors), Virus Infections of the Gastrointestinal Tract. Marcel Dekker, New York, pp. 195–209.

Bridger, J.C., 1980. Detection by electron microscopy of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. Vet. Rec., 107: 532–533.

Bridger, J.C., Hall, G.A. and Brown, J.F., 1984. Characterization of a calici-like virus (Newbury agent) found in association with astrovirus in bovine diarrhea. Infect. Immun., 43: 133–138.

Gough, C.F., Collins, M.S., Borland, E. and Keymer, I.F., 1984. Astrovirus-like particles associated with hepatitis in ducklings. Vet. Rec., 114: 3905.

Gray, E.W., Angus, K.W. and Snodgrass, D.R., 1980. Ultrastructure of the small intestine in astrovirus infected lambs. J. Gen. Virol., 49: 71–82.

Herring, A.J., Gray, E.W. and Snodgrass, D.R., 1981. Purification and characterization of ovine astrovirus. J. Gen. Virol., 53: 47–55.

Kurtz, J.B. and Lee, T.W., 1984. Human astrovirus serotypes. Lancet, 2: 1405.

Kurtz, J.B. and Lee, T.W., 1987. Astroviruses: human and animal. In: Novel Diarrhoea Viruses. CIBA Foundation Symposium 128, pp. 92–101.

Lee, T.W. and Kurtz, J.B., 1977. Astroviruses detected by immunofluorescence. Lancet, 2: 406.

Lee, T.W. and Kurtz, J.B., 1981. Serial propagation of astrovirus in tissue culture with the aid of trypsin. J. Gen. Virol., 57: 421–424.

Madeley, C.R., 1979. Comparison of the features of astroviruses and caliciviruses seen in samples of feces by electron microscopy. J. Infect. Dis., 139: 519–523.

McNulty, M.S., Curran, W.L. and Mcferran, J.B., 1980. Detection of astroviruses in turkey faeces by direct electron microscopy. Vet. Rec., 106: 561.

Snodgrass, D.R. and Gray, E.W., 1977. Detection and transmission of 30 nm virus particles (astroviruses) in faeces of lambs with diarrhoea. Arch. Virol., 55: 287–291.

Snodgrass, D.R., Angus, K.W., Gray, E.W., Menzies, J.D. and Paul, G., 1979. Pathogenesis of diarrhoea caused by astrovirus infections in lambs. Arch. Virol., 60: 217–226.

Tzipori, S., Menzies, J.D. and Gray, E.W., 1981. Detection of astrovirus in the faeces of red deer. Vet. Rec., 108: 286.

Woode, G.N. and Bridger, J.C., 1978. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. J. Med. Microbiol., 11: 441–452.

Woode, G.N., Pohlenz, J.F., Gourley, N.E.K. and Fagerland, J.A., 1984. Astrovirus and Breda virus infection of dome cell epithelium of bovine ileum. J. Clin. Microbiol., 19: 623–630.

Woode, G.N., Gourley, N.E.K., Pohlenz, J.F., Liebler, E.M., Mathews, S.L. and Hutchinson, M.P., 1985. Serotypes of bovine astrovirus. J. Clin. Microbiol., 22: 668–670.

Chapter 51

Borna Disease in Sheep

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INTRODUCTION

Borna Disease (BAD) has a long history; it has been known in Europe for more than 250 years (Zwick, 1939). This disease is a naturally occurring enzootic nonpurulent meningoencephalomyelitis of horses and sheep in Germany and Switzerland (Heinig, 1969; Ludwig and Thein, 1977; Gosztonyi and Ludwig, 1984a; Metzler et al., 1976) and has not been recognized with certainty in other countries.

The causitive agent was identified as a virus (Zwick et al., 1926; Ernst and Hahn, 1926). Clinical pictures were first described in 1813 as "Hitzige Kopfkrankheit der Pferde" and the name derives from Borna, a town in Sachsen, where the disease caused severe outbreaks in the horse population. The disease has been grouped with the slow virus infections because of biological properties of the agent and the clinical manifestations. All available data indicate that the agent belongs to the conventional enveloped viruses (Ludwig and Becht, 1977).

BAD has stimulated a wide interest in clinicians, neuropathologists, virologists and immunologists because of its model character for other slow and persistent virus infections of the CNS (Ludwig et al., 1973; Mayr and Danner, 1974; Danner, 1977; Hirano et al., 1983; Kao et al., 1984; Gosztonyi and Ludwig, 1984b; Narayan et al., 1984; Ludwig et al., 1985). This chapter deals mainly with BAD in sheep. Early knowledge about BAD and adaptation of the virus to the rabbit stems from the 1920s (Zwick and Seifried, 1925; Ernst and Hahn, 1926; Nicolau and Galloway, 1928). The histopathological studies by Seifried and Spatz (1930) and Pette and Környey (1935) analyzed the inflammatory reaction in the brain. Growth in tissue culture (Mayr and Danner, 1972; Ludwig et al., 1973) as well as information on the immune response (Ludwig et al., 1977; Gierend and Ludwig, 1981; Kao et al., 1984) were essential for further virus and antigen characterization.
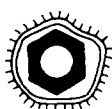
VIRUS PROPERTIES

Physical and chemical characteristics

Filtration experiments have shown that BAD virus (BADV) passes collodium membrane filters and appears to have a size between 85 and 128 nm (Elford and Galloway, 1933; Danner and Mayr, 1979). Ultrahistological examinations of BADV-infected cells could not demonstrate any virus particles

although a majority of cells was positive for antigen. Moreover, in naturally as well as experimentally infected animals, defined particles have not yet been found indicative of virion structures (Gelderblom et al., unpublished data; Gosztonyi and Ludwig, 1984a).

The most significant findings in experimental BAD of rabbits and rats were large nuclear bodies in nerve cells and astrocytes, with a central granular structure and a fibrillar capsule (Anzil and Blinzinger, 1972; Blinzinger and Anzil, 1973). Nuclei of neurons in naturally infected horses also contain nuclear bodies. They are 54–90 nm in diameter (Bestetti, 1976). Based on comparative light and electron microscopic examinations, nuclear bodies were identified as the Joest–Degen inclusion bodies. In the cytoplasm of horse brain cells, small aggregates of fine filaments with a diameter of the individual filaments between 4 and 5 nm have been described (Gosztonyi and Ludwig, 1984a).

BADV has not yet been classified (Matthews, 1982). In comparison with other viruses little is known about its physicochemical properties.

There is some evidence that the genetic material of BADV consists of RNA: (1) From persistently infected green monkey kidney cells ^{3}H-uridine-labelled particles could be isolated while banded at 1.2 g/ml in sucrose gradients. No label was detectable when ^{3}H-thymidine was used in the persistently infected cultures (Ludwig and Becht, 1977); (2) from the staining behaviour of intracellular virus material with acridine orange with and without previous nuclease treatment some evidence was obtained that single-stranded RNA is present in the inclusions (Danner and Mayr, 1979; Weber, 1980); (3) virus multiplication in cell cultures was suppressed by 6-azauridine, a substance interfering mainly with the replication of RNA viruses. IVDR or BVDR, known to interfere with the replication of DNA viruses, did not suppress BADV growth in tissue culture, as demonstrated by antigen expansion in foci (H. Ludwig and Leiskau, unpublished data).

Until now, BADV can only be assayed by infectivity. In CsCl a density of 1.16 g/ml was reported (Danner and Mayr, 1979); we could not reproduce these results (H. Ludwig and G. Pauli, unpublished data). Our data obtained with cell-associated and salt-released virus gave prominent infectivity bands at 1.18 and 1.22 g/ml, respectively, when sucrose was applied for gradient centrifugation (Pauli and Ludwig, 1985).

BADV was inactivated by UV light at the same rate as conventional viruses, e.g. vesicular stomatitis virus, with more than $3 \log/54 \, \text{erg/mm}^2/\text{s}$ (Danner and Mayr, 1979). A similar quick infectivity loss had been reported by Nicolau and Galloway (1928) and Heinig (1955/56, 1969).

BADV is labile to treatment with chloroform, ether and acetone (Zwick et al., 1927; Heinig, 1969; Danner and Mayr, 1979; Ludwig et al., 1985), indicating that it is enveloped. The effect of formaldehyde on BADV has not been defined, although this chemical has been used for many years to produce an inactivated vaccine. Virus infectivity is lost in a short time when chlorine containing disinfectants or formaldehyde are applied to virus preparations.

BADV is rather sensitive to heat. Infectivity is destroyed completely after 30 min at 56°C. At 37°C BADV lost its infectivity completely after 2 days, whereas it remained infectious for at least 3 months at + 4°C (Danner and Mayr, 1979; Herzog and Rott, 1980). At − 70°C and − 20°C virus can be kept for months and years with an insignificant titer reduction, especially when brain suspensions are frozen (M. Kao and H. Ludwig, unpublished data). Under vacuum at 4°C, brain suspension remained infectious over years (Zwick and Witte, 1931).

The infectivity of BADV was completely destroyed at pH 3.0 within 30 min

at 4°C; at pH 7.0 no infectivity was lost under these conditions (Heinig, 1955; Danner and Mayr, 1979).

The known BADV-specific antigens are the soluble antigen (S-antigen), antigens involved in virus neutralization, antigens on the plasma membrane which are not yet identified (Ludwig et al., 1984) and a 14.5 kD protein recently isolated from infected brains (Ludwig et al., 1985). The S-antigen was first demonstrated by Von Sprockhoff and Nitzschke (1955) and could be extracted from brains of naturally as well as experimentally infected animals. It is also present in persistently infected cells (Ludwig et al., 1973; Pauli and Ludwig, 1981). The S-antigen consists of two components, a 42 kD and a 22 kD protein (Ludwig et al., 1977). Experimentally induced antibodies do not neutralize the virus and give no protection against disease (Pauli and Ludwig, 1981). In vitro or in vivo this antigen appears first in the nucleus and later on diffusely spread in the cytoplasm of tissue culture cells or neurons (Ludwig et al., 1985). It has been successfully quantitated using CF tests (Nitzschke, 1957; Ludwig et al., 1973; Danner et al., 1978), direct and indirect IF tests (Wagner et al., 1968; Shadduck et al., 1970; Krey et al., 1979), direct or indirect precipitation techniques (Ludwig et al., 1977; Gierend and Ludwig, 1981; Gierent, 1982) and by peroxidase–antiperoxidase techniques like ELISA or the biotin–avidin technique (Gosztonyi and Ludwig, 1984a; Kao et al., 1984; Pauli et al., 1984a, b).

Evidence for the presence of another class of antigens comes from neutralization experiments. We first found that BADV can be neutralized by a variety of sera from naturally and experimentally infected animals (Ludwig et al., 1981; Hirano et al., 1983; Gosztonyi and Ludwig, 1984a; Kao et al., 1984); the virus is obtained from brain or tissue culture preparations. Some of the sera reached titers of 5000 when a sensitive focus reduction test combined with an immunostaining technique was used (Kao et al., 1984; Pauli et al., 1984a; Kao, 1985; Ludwig et al., 1985).

Recently, a protein with a mol. wt. of 14.5 kD could be isolated from the brain of infected rats, mice and chickens; the extraction procedure involved nonionic detergents. This protein was demonstrable when high virus titers (more than 10^5 focus-forming units per ml) were present in the brains. Control brains never showed a protein of this size. The function of this protein is unclear. Under natural and experimental infection, no antibodies were produced against it. We were able to induce antibodies which recognize the protein in immunoblotting. These antibodies did not neutralize BADV, nor did they cross-react with the S-antigen described above (Schädler et al., 1985).

Cultivation

The virus is passaged by i.c. inoculation or kept in persistently infected cell lines. Until now, virus can only be monitored by infectivity titration, either in the animal or in tissue culture (Danner et al., 1978; Hirano et al., 1983; Kao et al., 1984; Kao, 1985). BADV was regarded as being cell-bound, but treatment of infected cells with increased salt concentrations in the medium released the virus.

The host spectrum for BADV is very broad. In vitro virus replicates in epithelial and fibroblastoid cells and especially in neurons. In vivo antigen has only been found in neurons (Danner et al., 1978; Herzog and Rott, 1980; Tsukamoto et al., 1982; Tsukamoto and Ludwig, 1983; Kao et al., 1984; Pauli and Ludwig, 1985). In vitro and in vivo no alterations such as cell fusion, transformation or lysis can be observed after BADV infection.

TABLE 33

Summary of the reports on natural and experimental infection of sheep with BADV

Origin of virus (infection)	Inoculation (route)	Clinical signs	Incubation period (days)	Disease period (days)	Transmission to rabbits Virus[a]	Histol. lesions[b]	Reference
Horse (natural)	sheep (subdur.)	+	62–77	6–10	+	ND	Beck and Frohböse (1926)
Sheep (experimental)	sheep (i.c.)	+	ND	ND	ND	ND	
Horse (natural)	sheep (i.c.)	−	ND	ND	ND	ND	Zwick et al. (1927)
Rabbit (experimental)	lamb (i.c.)	+	54–60	ND	ND	+	
ND	sheep (i.c.)	−	ND	ND	ND	+	Matthias (1954)
ND	sheep (i.c.) (n = 35)	+ (13/35)	ND	ND	+ (11/35)	+ (21/35)	Matthias (1958)
	sheep (i.c.) (n = 175)	+ (94/175)	ND	ND	+ (90/175)	+ (94/175)	
	sheep (i.v.)	+	ND	ND	ND	ND	
ND	lambs (i.n.) (n = 2)	+	48	ND	+[c]	ND	Heinig (1964)
Sheep (natural)	rabbits (i.c.)	+	37–59	ND	+	+	Metzler et al. (1976)
Rabbit	sheep (i.c.)	−	ND	ND	+[d]	ND	H. Ludwig and M. Kao (unpublished data)
Strain V	sheep (i.c.)	−	ND	ND	−	ND	

[a] Infectious virus assayed in rabbits.
[b] Perivascular infiltrates or Joest-Degen intranuclear inclusions were regarded diagnostic for BAD.
[c] Virus was present in secretions of the nose.
[d] Infectious virus assayed in tissue culture.
ND = no data.

EPIZOOTIOLOGY

Reports on the epizootiology of BADV are contradictory. The facts are that disease occurred only in some areas in Germany, especially in Saxony, Thuringia, Württemberg, Bavaria and Hesse. The character of the disease in sheep and in horses is mostly enzootic (Ihlenburg, 1957). Horses show disease sporadically, whereas in sheep most of the animals become sick. Up to 20% of the animals from a herd can be afflicted. The reports uniformly state that enzootics are limited to special areas and do not move into other places. Furthermore, the percentages of infected animals breeding over longer time periods remain approximately the same, meaning that the enzootics did not die out. There is no correlation between BAD cases in horses and sheep over the years; the peak of the disease incidence is at end of spring and in the summer months. Sheep of all age groups acquire the disease.

PATHOGENESIS

There is not much information on the pathogenesis in sheep, since experimental infection was not easy to perform. The pathogenetic mechanisms, however, are thought to be the same in sheep, horses and experimentally infected animals like rabbits. The portal of entry for the virus is not known; it can, however, be assumed that infection occurs via the olfactory or nasopharyngeal path (Gosztonyi et al., 1985). The few experimental infections reported show that some investigators could infect sheep intracerebrally and the animals became sick, whereas others — and this is our own experience, too — showed that i.c. infection did not induce BAD. A general experience is that sheep do not show clinical signs, although carrying and excreting the virus, since by inoculation of rabbits with excretions of these sheep or brain material infectious virus was demonstrable. In our experiments two sheep were infected i.c. with the rabbit adapted strain V. The animals showed no signs of disease. In one animal sacrificed $1\frac{1}{2}$ year p.i. virus could be demonstrated by tissue culture assays to be present in the brain as well as in the retina (H. Ludwig and M. Kao, unpublished data). The conflicting results in the literature might reflect the potency of BADV for adaptation. Probably only sheep-adapted virus strains cause disease, whereas strains from naturally infected horses or from experimentally infected animals are not virulent for the sheep. The data on natural and experimental infections of sheep are listed in Table 33. In some cases disease could be induced in sheep by i.c. and by i.v. inoculation, whereas i.n. inoculation was not as reliable and induced disease only in a few cases. Heinig (1964) is the only author to show that BADV is present in nasal secretions. His experimental infections revealed also that only half of the animals carried virus when sheep brain was assayed for infectivity in the rabbit.

BADV does not always cause disease in sheep. This information came independently from several reports, raising the question whether sheep and not horses might be the natural host for this virus. Also, from the older literature it becomes evident that horses in farms had BAD, whereas in the sheep flocks only a few animals appeared to be sick. The sheep is the host species for scrapie and visna (Kimberlin, 1984), which represent classical CNS diseases, as well as for bacterial infections like listeriosis, or parasitic infections like coenurus cerebralis. It is possible that through the extensive breeding and the poor conditions at which sheep are often kept this species has developed a high resistance against overt disease caused by a variety of agents. With some of them the incubation period cannot be defined, and once clinical symptoms are seen, the disease has characteristics of a slow infection. It is certainly of

interest that sheep are the natural host of unconventional agents like that of scrapie as well as of conventional viruses like MVV (Sigurdsson, 1954) and BADV and that all these infect the CNS.

DISEASE SIGNS

BAD in sheep shows the symptoms of a nonpurulent encephalomyelitis. The incubation period could never be clearly defined but usually lies between 4 weeks and several months. The major symptoms have been summarized by Heinig (1969). Behavioural alterations in some sheep of a herd are the first signs. They separate from the herd, show an impaired sensorium, some are apathetic when the watchdog or the shepherd calls them. They interrupt eating without reason. Sometimes hyperesthesia is noted in the hind part of the body. In the course of the disease often complete inappetence and disturbances of the sensorium become a prominent clinical symptom. Sheep stand apathetic in a dark corner of the stable and lean their head against the wall; they grind their teeth, walk around in circles and show paralysis of the laryngopharynx. Disturbances of the visual system are rare (Hiepe, 1958/59, 1960; Ihlenburg, 1959, 1962; Klaus, 1959), but anomalies in the pupillae without alterations of the eye background can be diagnosed. The animals are reluctant to run and show atactic movements. Finally they lie down and cannot be forced to stand up again. If the symptoms are less severe the sheep may show only sudden collapsing or restlessness, and eventually disturbances of the sensorium. In some cases recovery has been described (Ihlenburg, 1959; Primer, 1925). Temperature, pulse and breathing are usually physiological. The incubation period is unknown; however, once the symptoms start the disease lasts for 1 or 2 weeks and lethality reaches approximately 90%. BAD in sheep occurs mostly during spring, with a peak in April and May.

For differential diagnosis, a variety of bacterial infections like listeriosis, *Clostridium tetani* infection as well as MVV and scrapie must be taken into account (Kimberlin, 1984). Furthermore, parasitic infections like coenurus cerebralis are clinically relevant.

PATHOLOGY

Macroscopically, no characteristic changes can be seen in organs and in the brain of sheep with BAD. Occasionally a prominent appearance of the brain vessels is obvious. Histologically, infiltrations with lymphocytes can be observed in the meninges and in the brain. Like in other BADV-infected animals perivascular infiltrates are a characteristic feature also in sheep. They are prominent in the grey matter of the brain. Predilection sites are the brain stem and hippocampus (Beck, 1925, 1926). Beck and Frohböse (1926) first studied the microscopic lesions in infected sheep and emphasized their resemblance to those found in brains of diseased horses. In neurons of the Ammonshorn they described the characteristic oxyphilic intranuclear Joest–Degen inclusions (Joest and Degen, 1909; Joest, 1911). Neuropathologic reports always emphasize the similarity of the pathologic changes in sheep with those reported in the horse and other animals (Dahme et al., 1979). Prominent neuronal degeneration with neuronophagia can be observed. The presence of intranuclear inclusion bodies is not stated in all reports. Metzler et al. (1976) described massive glia proliferations without affliction of the cerebellum and the medulla; a nonpurulent meningencephalomyelitis was obvious in all diseased animals. In contrast to intensive studies on the distribution of virus-specific

antigen in the brain of naturally infected horses and experimentally infected animals (Ludwig et al., 1985), nothing similar is known for the infected sheep brain.
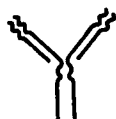
IMMUNE REACTION

The immune response to BADV has been defined not only in natural infections of the horse (Ludwig and Thein, 1977; Gosztonyi and Ludwig, 1984a), but also in experimentally infected rabbits (Ludwig et al., 1977; Roggendorf et al., 1983), rats (Hirano et al., 1983), mice (Kao et al., 1984), tupaias, hamsters and chickens (Anzil et al., 1973; Gosztonyi et al., 1983; Ludwig et al., 1985). Besides antibodies recognizing the S-antigen, those which neutralize the virus have been detected. Concerning the cellular immune response, only in the rhesus monkey could T-cells be demonstrated to react with BADV-infected tissue culture cells (Stitz et al., 1980). Further evidence for the influence of the cellular immune response of the disease pattern comes from studies in rabbits (Gierend and Ludwig, 1981), rats (Hirano et al., 1983; Narayan et al., 1983) and mice (Kao et al., 1984).

For sheep little information is available. Perivascular infiltrates known from horses and other animals were also reported for sheep. They appear to be pathognomonic for the disease (Zwick et al., 1927; Matthias, 1958; Heinig, 1964). Antibodies were reported in only a few cases of natural outbreak in Switzerland. Antibody titers of 5000 were measured in some sheep by an indirect IF test (Metzler et al., 1976). In contrast to our experience with naturally and experimentally infected animals (Ludwig et al., 1985), these authors did not report neutralizing antibodies.

LABORATORY DIAGNOSIS

The diagnosis of BAD intra vitam is based on the clinical symptoms, although a differentiation from other virus infections of the CNS (pseudorabies, rabies, visna, scrapie, etc.) or bacterial infections (listeriosis, tetanus, etc.) often is difficult. Therefore, laboratory diagnosis is essential. This can be performed by virus isolation in tissue culture or by infection of rabbits or newborn rats (Pauli et al., 1984; Kao, 1985). Rabbits show disease in 3–4 weeks and usually die. Highly sensitive is the newborn rat. It replicates virus to titers of 10^5–10^6 per ml. In one case we could isolate BADV from an experimentally infected but not diseased sheep by inoculation of retina material into rats (Ludwig and Kao, unpublished).

Infection is demonstrated by the presence of BADV-specific antigen in cultured cells which can be stained by the IF test. The replication of BADV in the newborn rat is also demonstrated by the presence of virus-specific antigen in cultured tissue.

The blood and cerebrospinal fluid of suspect sheep should be tested for specific antibodies. In one report, antibodies could be demonstrated in the serum (Metzler et al., 1976). Anti-S-antigen antibodies have been reported in naturally infected horses (Ludwig and Thein, 1977). It is not known whether sera or CSFs of infected sheep neutralize BADV as shown for the horse (Ludwig et al., 1985).

The laboratory diagnosis should be supported by histological examination of the brain and a search for perivascular infiltrates and Joest–Degen inclusion bodies. The most convincing and quick diagnosis is by demonstration of BADV-specific antigen in different brain areas (Wagner et al., 1968; Shadduck et al., 1970).

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PROPHYLAXIS AND CONTROL

When BAD has been diagnosed clinically it should be assumed that more animals are infected than those showing clinical signs. The positive ones should be slaughtered and others placed in isolation. Hygienic measures are always indicated to eliminate the virus in secretions and excretions. Formaldehyde or any disinfectant acting on enveloped viruses are recommended for use. At present, no vaccine can be recommended. In areas where BAD was endemic in horses, vaccination was practiced with questionable results. Although BAD no longer appears to be an economical problem for sheep breeding, it is important to be alert and to examine animals with BAD-like symptoms, especially in areas where the disease was endemic.

REFERENCES

Anzil, A.P. and Blinzinger, K., 1972. Electron microscopic studies of rabbit central and peripheral nervous system in experimental Borna disease. Acta Neuropathol., 22: 305–318.

Anzil, A.P., Blinzinger, K. and Mayr, A., 1973. Persistent Borna Virus infection in adult hamsters. Arch. Ges. Virusforsch., 40: 52–57.

Beck, A., 1925. Beitrag zur enzootischen Encephalitis des Schafes. Z. Infektionskr. Haustiere, Berlin, 28: 99–109.

Beck, A., 1926. Die enzootische Encephalitis des Schafes. Dtsch. Tierärztl. Wochenschr., 34: 764/ Tierärztl. Rundsch., 32: 801.

Beck, A. and Frohböse, H., 1926. Die enzootische Encephalitis des Schafes. Vergleichende experimentelle Untersuchungen über die seuchenhafte Gehirnrückenmarksentzüdung der Pferde und Schafe. Arch. Wiss. Prakt. Tierheilk., 54: 84–110.

Bestetti, G., 1976. Mikroskopische und ultrastrukturelle Untersuchungen an Joest-Degen'schen Einschlusskörperchen bei spontaner Borna Krankheit des Pferdes. Schweiz. Arch. Tierheilk., 118: 493–498.

Blinzinger, K. and Anzil, A.P., 1973. Large granular nuclear bodies (karyosphaeridia) in experimental Borna virus infection. J. Comp. Pathol., 83: 589–596 (1973).

Dahme, E., Schröder, B. and Danner, K., 1979. Licht- und elektronenmikroskopische Bewertung des Reaktionsmusters und der intranukleären Einschlusskörperchen bei der Borna-Encephalitis des Pferdes. Vortag bei der 28. Tagung der europäischen Gessellschaft für Veterinärpathologie, Stuttgart, 4–5 June, 1979.

Danner, K., 1977. Borna disease: patterns of infection. In: V. ter Meulen and M. Katz (Editors), Slow Virus Infections of the Central Nervous System. Springer Verlag, Heidelberg, Berlin, pp. 84–88.

Danner, K. and Mayr, A., 1979. In vitro studies on Borna virus. II. Properties of the virus. Arch. Virol., 61: 261–271.

Danner, K., Heubeck, D. and Mayr, A., 1978. In vitro studies on Borna virus. I. The use of cell cultures for the demonstration, titration and production of Borna viruses. Arch. Virol., 57: 63–75.

Elford, W.J. and Galloway, I.A., 1935. Filtration of the virus of Borna disease through graded collodion membranes. Br. J. Exp. Pathol., 14: 196–205.

Ernst, W. and Hahn, H., 1926. Übertragbarkeit der seuchenhaften Gehirn-Rückenmarksentzündung des Pferdes (Bornasche Krankheit) auf kleine Versuchstiere (Kaninchen und Meerschweinchen). Münch. Tierärztl. Wochenschr., 77: 46–47.

Gierend, M., 1982. Zur Pathogenese der Bornaschen Krankheit: Untersuchungen über die zelluläre Immunantwort, die immunsuppressive Behandlung und die Elektronencephalographie (EEG). Vet Med. Dissertation, Berlin.

Gierend, M. and Ludwig, H., 1981. Influence of immunosuppressive treatment on Borna disease in rabbits. Arch. Virol., 67: 217–228.

Gosztonyi, G. and Ludwig, H., 1984a. Borna disease of horses: An immunohistological and virological study of naturally infected animals. Acta Neuropathol. (Berlin), 64: 213–221.

Gosztonyi, G. and Ludwig, H., 1984b. Neurotransmitter receptors and viral neurotropism. Neuropsychiatr. Clin., 3: 107–114.

Gosztonyi, G., Leiskau, T. and Ludwig, H., 1983. The significance of the Borna disease virus infection for the non-mammal, the chicken. Zentralbl. Bakteriol. Mikrobiol. Hyg. Orig. A., 255: 170.

Gosztonyi, G., Kao, M. and Ludwig, H., 1985. Neural Propagation of Borna Disease Virus in mice following intranasal inoculation. Tagung der Sektion Virologie der deutschen Gesellschaft für Hygiene und Mikrobiologie, Erlangen, 27–30 March, 1985.

Heinig, A., 1955. Die pH-Resistenz des Virus der Bornaschen Krankheit der Pferde. Arch. Exp. Vet. Med., 9: 517–521.

Heinig, A., 1955/56. Die Einwirkung des UV-Lichtes auf das Virus der Bornaschen Krankheit der Pferde und Schafe. Wiss. Z. Ernst-Moritz-Arndt-Univ., Greifwald, 5: 199–203.

Heinig, A., 1964. Zur experimentellen Infektion von Pferden und Schafen mit dem Virus der Bornaschen Krankheit (BK). Arch. Exp. Vet. Med., 18: 753–766.

Heinig, A., 1969. Die Bornasche Krankheit der Pferde und Schafe. In: H. Röhrer (Editor), Handbuch der Virusinfektionen bei Tieren. Gustav Fischer Verlag, Jena, Vol. 4, pp. 83–148.

Herzog, S. and Rott, R., 1980. Replication of Borna disease virus in cell cultures. Med. Microbiol. Immunol., 168: 153–158.

Hiepe, T., 1958/59. Die Bornasche Krankheit: Klinische-diagnostische Untersuchungen an Pferden und Schafen mit besonderer Berücksichtigung des Liquor cerebrospinalis. Wiss. Z. Univ. Leipzig, 8: 263–338.

Hiepe, T., 1960. Die Bedeutung der Liquor Untersuchungen für die Neurodiagnostik bei Pferd und Schaf. Zentralbl. Veterinärmed., 7: 152–159.

Hirano, N., Kao, M. and Ludwig, H., 1983. Persistent, tolerant or subacute infection in Borna Disease virus infected rats. J. Gen. Virol., 64: 1521–1530.

Ihlenburg, H., 1957. Zur Epidemiologie, Diagnose und Behandlung der Bornaschen Krankheit. Arch. Exp. Vet. Med., 11: 835–861.

Ihlenburg, H., 1959. Beitrag zum klinischen Bild der Bornaschen Krankheit des Schafes. Monatsh. Veterinärmed., 14: 503–506.

Ihlenburg, H., 1962. Experimentelle Prüfung der Empfänglichkeit der Ziege für das Virus der Bornaschen Krankheit. Monatsh. Veterinärmed., 17: 337–341.

Joest, E., 1911. Weitere Untersuchungen über die seuchenartige Gehirn-Rückenmarksentzündung (Bornasche Krankheit) des Pferdes mit besonderer Berücksichtigung des Infekionsweges und der Kerneinschlüsse. Z. Infektionskr. Haustiere, 10: 293–320.

Joest, E. and Degen, K., 1909. Über eigentümliche Kerneinschlüsse der Ganglienzellen bei der enzootischen Gehirn-Rückenmarksentzündung der Pferde. Z. Infektionskr. Haustiere, 6: 348–356.

Kao, M., 1985. Die Pathogenese der Borna Krankheit bei der Ratte. Ein Modell für Persistente Infektionen und subakute/akute Krankheiten des Zentralnervensystems und für die Fettsucht (Obesity syndrome). Vet. Med. Diss., Berlin.

Kao, M., Ludwig, H. and Gosztoyni, G., 1984. Adaptation of Borna disease virus to the mouse. J. Gen. Virol., 65: 1845–1849.

Kimberlin, R.H., 1984. Slow viruses: conventional and unconventional. In: F. Brown and G. Wilson (Editors), Toply and Wilson's Principles of Bacteriology, Virology and Immunity. Edward Arnold, London, Vol. 4, pp. 487–510.

Klaus, H., 1959. Untersuchungen über das Serum Eisen bei bornakranken Schafen und Kaninchen. Arch. Exp. Veterinärmed., 13: 298–307.

Krey, H.F., Ludwig, H. and Boschek, C.B., 1979. Multifocal retinopathy in Borna disease virus infected rabbits. Am. J. Ophthalmol., 87: 157–164.

Ludwig, H. and Thein, P., 1977. Demonstration of specific antibodies in the central nervous system of horses naturally infected with Borna disease virus. Med. Microbiol. Immunol., 163: 215–226.

Ludwig, H. and Becht, H., 1977. Borna disease — a summary of our present knowledge. In: V. ter Meulen and M. Katz (Editors), Slow Virus Infections of the Central Nervous System. Springer Verlag, New York, pp. 75–83.

Ludwig, H., Becht, H. and Groh, L., 1973. Borna disease (BD), a slow virus infection — biological properties of the virus. Med. Microbiol. Immunol., 158: 275–289.

Ludwig, H., Koester, V., Pauli, G. and Rott, R., 1977. The cerebrospinal fluid of rabbits infected with Borna disease virus. Arch. Virol., 55: 209–223.

Ludwig, H., Krey, H.F. and Roggendorf, W., 1981. Neurological symptoms in Borna disease virus infected animals. Wiss. Film u.A. 1257, Excerpta Medica, Amsterdam.

Ludwig, H., Pauli, G. and Gierend, M., 1984. Nachweis von Oberflächenantigenen auf Borna Virus infizierten Zellen. Berl. Münch. Tierärztl. Wochenschr., 97: 47–51.

Ludwig, H., Kraft, W. Kao, M., Gosztonyi, G., Dahme, E. and Krey, H.F., 1985. Die Borna Krankheit bei natürlich und experimentell infizierten Tieren: Ihre Bedeutung für Forschung und Praxis. Tierärztl. Praxis, 13: 421–453.

Matthews, R.E.F., 1982. Classification and nomenclature of viruses. Intervirology, 17: 1–200.

Matthias, D., 1954. Der Nachweis von latent infizierten Pferden, Schafen und Rindern und deren Bedeutung als Virusreservoir bei der Bornaschen Krankheit. Arch. Exp. Vet. Med., 8: 506–511.

Matthias, D., 1958. Weitere Untersuchungen zur Bornaschen Krankheit der Pferde und Schafe, Arch. Exp. Vet. Med., 12: 920–947.

Mayr, A. and Danner, K., 1972. In vitro-Kultivierung von Borna-Virus über Gehirn-Explantate infizierter Tiere. Zentralbl. Veterinärmed. B, 19: 785–800.

Mayr, A. and Danner, K., 1974. Persistent infections caused by Borna Virus. Infection, 2: 64–69.

Metzler, A., Frei, U. and Danner, K., 1976. Virologisch gesicherter Ausbruch der Bornaschen Krankheit in einer Schafherde der Schweiz. Schweiz. Arch. Tierheilkd., 118: 483–492.

Narayan, O., Herzog, S., Freese, K., Scheefers, H., and Rott, R., 1983. Pathogenesis of Borna disease in rats: Immune-mediated viral ophthalmoencephalopathy causing blindness and behavioral abnormalities. J. Infect., 148: 305–315.

Nicolau, S. and Galloway, I.A., 1928. Borna disease and enzootic encephalomyelitis of sheep and goats. Med. Res. Counc. Spec. Report Series No. 121, H.M. Stationery Office, London, pp. 7–90.

Nitzschke, E., 1957. Über den Nachweis eines komplementbindenden Antigens und komplementbindender Antikörper bei weissen Ratten, Meerschweinchen und Pferden, die mit dem Virus der Bornaschen Krankheit infiziert waren. Zentralbl. Veterinärmed., 3: 289–296.

Pauli, G. and Ludwig, H., 1981. Some biochemical and biological properties of the major Borna disease virus specific antigen. Vth International Congress of Virology, Strassbourg, France, 2–7 August, 1981, p. 121.

Pauli, G. and Ludwig, H., 1985. Increase of virus yields and release of Borna disease virus from persistently infected cells. Virus Res., 2: 29–33.

Pauli, G., Grunmach, J. and Ludwig, H., 1984a. Focus-Immunoassay for Borna disease virus-specific antigens. Zentralbl. Veterinärmed. B, 31: 552–557.

Pauli, G., Gregersen, J.-P. and Ludwig, H., 1984b. Plaque/focus immunoassay: a simple method for detecting antiviral monoclonal or other antibodies and viral antigens in cells. J. Immunol. Methods, 74: 337–344.

Pette, H. and Környey, St., 1935. Über die Pathogenese und die Pathologie der Bornaschen Krankheit im Tierexperiment. Dtsch. Z. Nervenheilkd., 136: 20–65.

Priemer, B., 1925. Gibt es beim Schaf eine der Bornerschen Krankheit des Pferdes analoge Erkrankung? Vet. Med. Diss., Leipzig.

Roggendorf, W., Sasaki, S. and Ludwig, H., 1983. Light microscopic and immunhistologic investigations on the brain of Borna disease virus infected rabbits. Neuropathol. Appl. Neurobiol., 9: 287–296.

Schädler, R., Diringes, H. and Ludwig, H., 1985. Isolation and characterization of a 14 500 molecular weight protein from brains and tissue cultures persistently infected with Borna disease virus. J. Gen. Virol., 66: 2479–2484.

Seifried, O. and Spatz, H., 1930. Die Ausbreitung der encephalitischen Reaktion bei der Bornaschen Krankheit der Pferde und deren Beziehung zu der Encephalitis epidemica, der Heine-Medinischen Krankheit und der Lyssa des Menschen. Eine vergleichend-pathologische Studie. Z. Neurol. Psychiatr., 124: 317–382.

Shadduck, J.A., Danner, K. and Dahme, E., 1970. Fluoreszenzserologische Untersuchungen über Auftreten und Lokalisation von Borna-Virusantigen in Gehirnen experimentell infizierter Kaninchen. Zentralbl. Veterinärmed. B, 17: 453–459.

Sigurdsson, B., 1954. Observation on the slow infection of the sheep. Br. Vet. J., 110: 341–354.

Stitz, L., Krey, H.F. and Ludwig, H., 1980. Borna disease in rhesus monkeys as a model for uevo-cerebral symptoms. J. Med. Virol., 6: 333–340.

Tsukamoto, T. and Ludwig, H., 1983. Long term cultures of neural retina and pigment epithelium from newborn rabbits. Z. Naturforsch., 38c: 141–145.

Tsukamoto, T., Pauli, G., Leiskau, T. and Ludwig, H., 1981. Borna virus infection in embryonic nerve cells and in cultures of non-neuronal origin. 38. Tagung der DGHM, Göttingen 5–8 October, 1981.

Von Sprockhoff, H. and Nitzschke, E., 1955. Untersuchungen über das komplementbindende Antigen in Gehirnen Bornavirus-infizierter Kaninchen. 1. Mitteilung: Nachweis eines löslichen Antigens. Zentralbl. Veterinärmed., 2: 185–192.

Wagner, K., Ludwig, H. and Paulsen, J., 1968. Fluoreszenzserologischer Nachweis von Borna-Virus Antigen. Berl. Münch. Tierärztl. Wochenschr., 81: 395–396.

Weber, D., 1980. Versuche zur Bestimmung des Nukleinsäuretyps und zum Nachweis von reverser Transkriptase bei Borna-Virus. Vet. Med. Diss., München.

Zwick, W., 1926. Über die ansteckende Gehirn-Rückenmarksentzündung (Kopfkrankheit, Bornasche Krankheit) der Pferde. Dtsch. Tierärztl. Wochenschr., 34: 763–764.

Zwick, W., 1939. Bornasche Krankheit und Encephalomyelitis der Tiere. In: E. Gildenmeister, E. Haagen and O. Waldmann (Editors), Handbuch der Viruskrankheiten. Gustav Fischer Verlag, Jena, pp. 252–354.

Zwick, W. and Seifried, O., 1925. Übertragbarkeit der seuchenhaften Gehirn-Rückenmarksentzündung des Pferdes (Bornaschen Krankheit) auf kleine Versuchstiere (Kaninchen). Berl. Tierärztl. Wochenschr., 41: 129–132.

Zwick, W. and Witte, J., 1931. Über die Widerstandsfähigkeit des Virus der Bornaschen Krankheit gegen Trocknung und über Schutzimpfungsversuche mit getrockneter virushaltiger Gehirnsubstanz. Berl. Tierärztl. Wochenschr., 47: 33–35.

Zwick, W., Seifried, O. and Witte, J., 1927. Weitere Untersuchungen über die seuchenharte Gehirn-Rückenmarksentzündung der Pferde (Bornasche Krankheit). Z. Infektionskr. Haustiere, 32: 150–179.

Unconventional Infectious Agents

SCRAPIE

The term "unconventional viruses" was applied by Gajdusek (1977) to agents of transmissible slow diseases characterized by a subacute spongiform encephalopathy in sheep and goats (scrapie), man (Kuru and Creutzfeldt–Jakob disease) and mink. These diseases thus form a nosological entity. Kuru (which means shivering, trembling) is a fatal neurological condition occurring in the Fore tribe of cannibals in New Guinea, while Creutzfeldt–Jakob disease is a fatal presenile dementia of midadult life that is not geographically restricted.

The central enigma is the nature of the agent of scrapie (for review see Carp et al., 1985). The only criterion of its presence is infectivity leading to disease. The transmissibility to mice and hamsters has greatly facilitated research, which is otherwise hampered by long incubation periods (60–200 days or more). Infectivity is highly resistant to inactivation by the usual chemical and physical sterilizing agents, e.g. formaldehyde, heat, ionizing radiation, etc.

Infectivity appears to be bound to a protein of 26–30 k and the agent is thus not a viroid. In brain and spleen EM revealed filamentous structures called "scrapie-associated fibrils" which are probably a product of infection and not the agent itself.

Remarkable is the lack of humoral and CMI responses. Unresolved is at present the question of relationship between scrapie and similar diseases in man and mink.

BORNA DISEASE

We deliberately also placed the agent of Borna disease among "unconventional viruses". Borna disease is a transmissible nonpurulent meningoencephalomyelitis occurring mainly in horses and sheep in certain parts of Germany (Borna is the name of a city and district in Saxony). Remarkable is that the agent of Borna disease, although easily cultivable and immunologically fully active, could never be visualized.

REFERENCES

Carp, R.I., Merz, P.A., Kascak, R.J., Merz, G.S. and Wisniewski, H.M., 1985. Nature of the scrapie agent: Current status of facts and hypotheses. J. Gen. Virol., 66: 1357–1368.
Gajdusek, D.C., 1977. Unconventional viruses and the origin and disappearance of kuru. Science, 197: 943–960.

Chapter 52

Scrapie Agent

R.M. BARLOW

INTRODUCTION

The literature on scrapie and similar subacute spongiform encephalopathies in man and animals (Kuru, Creutzfeldt–Jakob disease and transmissible mink encephalopathy) is redolent with the variety of etiological and pathogenetic concepts which have been advanced; it is also the graveyard of many beliefs and false hopes concerning the nature of the causal agents. Experimental work in the natural host is hampered by the very long incubation period of scrapie with the attendant problems of defining the initial scrapie status of the sheep and controlling lateral spread of infection. These constraints have encouraged the use of laboratory animals for work on the biology of scrapie and the nature of the causal agent. Several species of laboratory rodent are susceptible; most advances have come from work in hamsters and genetically defined inbred lines of mice. It is not the intention here to review this literature, as there are already several volumes which trace the development of work with the so-called "slow virus" diseases (Thormar, 1971; Kimberlin, 1976; Prusiner and Hadlow, 1979; Tyrrell, 1979). The purpose is to present a concise account of scrapie in sheep, drawing on the results obtained from laboratory animals only when these illuminate aspects of the disease in its natural host.

PROPERTIES OF THE AGENT

Infectivity is retained by a 50-nm filtrate of infected brain tissue homogenates and has been associated with membrane fragments in the microsome fraction. The mol. wt. has been assessed at between 15×10^4 and 15×10^5. Several reports have recorded the presence of 35-nm virus-like particles in post-synaptic processes of nerve cells in both natural and mouse-adapted scrapie (Bignami and Parry, 1971; Baringer et al., 1981), but it is uncertain whether these particles represent the scrapie agent or merely result from the disorders caused by it.

Scrapie agents vary in resistance to heat inactivation, but all show unusual resilience to a wide variety of chemical and physical procedures. They withstand boiling, exposure to formaldehyde for long periods, histological processing procedures, exposure to UV light and ionizing radiations to an extent unprecedented by conventional animal viruses.

Scrapie agent has been likened to the viroid of potato spindle tuber disease (PSTV), a naked RNA molecule of 5×10^4 mol. wt. which must rely upon the biosynthetic mechanisms of the host for its replication. PSTV has a heat and UV light resistance approaching that of scrapie, but is inactivated by ribonu-

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clease and other chemical treatments which the scrapie agent resists. Scrapie to a large extent also resists treatment with DNase but is inactivated by proteases. These properties distinguish the agent from conventional viruses and viroids and have led to the startling suggestion that it might be some novel kind of infectious protein. It has been designated "prion" (Prusiner, 1982) and has been purified. An oligonucleotide probe complementary to the mRNA sequence of this prion protein has been synthesized (Chesebro et al., 1985).

The DNA clone from scrapie-infected mouse brain hybridized to an mRNA found in both normal and scrapie-infected brain. Thus, this mRNA is not uniquely associated with scrapie activity, which suggests that prion protein may be a normal component of mouse and hamster brain. Though the amount of prion protein mRNA is similar in both normal and scrapie brain there is a higher content of this protein in the diseased brain (Oesch et al., 1985). The latter has been shown to differ from the normally encoded protein in two respects: it is partially resistant to proteinase K, an enzyme which completely destroys the closely related protein in normal brain, and it can polymerize into rods and filaments of amyloid (Bendheim et al., 1984). Thus, whilst prion protein differs from prion protein-related protein in some molecular or configurational way and is very closely associated with the scrapie infectious particle, it is unlikely to be the actual etiological agent. A more plausible suggestion is that a small scrapie-specific nucleic acid is embedded within a tightly packed host-derived protein that is essential for transfection. This has been termed a "virino" (Kimberlin, 1982). Such an hypothesis can accommodate the presently established facts concerning the agent of scrapie. Furthermore it has been found recently that scrapie-associated fibrils (see below) form the core of tubulofilamentous particles with an outer coat of single-stranded DNA. When the purified DNA was treated with mung bean nuclease a 17 kilobase band was left intact which retained infectivity at low titer (Narang, 1989). These results are compatible with scrapie agent being a single-stranded DNA virus.

At present "isolation" and characterization of the scrapie agent is dependent upon bioassay in inbred lines of mice of defined genotype. Different strains of scrapie agent have been identified on the basis of their incubation period characteristics and the patterns of neuropathological change.

These strains can be classified into three groups according to the stability of these characteristics on subpassage in mice. Class I agents are completely stable, class II agents can be made to change their characteristics gradually by passage in a different mouse genotype and class III agents are unstable, the above characteristics being liable to spontaneous changes.

The extreme resistance of scrapie agents to inactivation by physical and chemical treatments, and the protracted nature of the biological procedures necessary to test for residual activity create unique problems for disinfection and sterilization. Complete incineration is probably the most satisfactory method of destruction. Disposable instruments and utensils which can be burned after use should be used for the manipulation of tissues wherever possible. For instruments such as boning knives, saws and bone forceps, washing in detergent solutions followed by soaking in 5% sodium hypochlorite solution is recommended where autoclaving for at least 1 h at 121°C is impracticable. In our laboratory we have used two such autoclave cycles at 132°C, interspersed with overnight immersion in hot phenol. This treatment is hard on instruments and may appear excessively severe. However, as resistance of scrapie to heat inactivation varies with agent strain (Dickinson and Taylor, 1978) and with respect to chemical inactivation may also change at different times during the incubation period of a single strain (Kimberlin, 1979), it is

probably sound practice to use a sequence of powerful inactivating agents for sterilization purposes.

EPIZOOTIOLOGY

Scrapie is a naturally occurring disease of sheep and goats which has been known in Europe for at least 250 years. A similar disorder has been described in mule deer (*Odocoileus hemionus hemionus*) and blacktailed deer (*Odocoileus hemionus columbianus*) in discontinuous contact with domestic and other wild ruminants in wildlife facilities in Colorado and Wyoming (Williams and Young, 1980). It is also thought that the pathologically similar transmissible encephalopathy of mink may be scrapie acquired by ingestion of offals from infected sheep. Experimentally, scrapie has been transmitted by the inoculation of brain tissue homogenates from affected animals into mice, rats, voles, syrian hamsters, gerbils and New World monkeys.

Scrapie is endemic in Europe and has been recorded in northern Africa, Asia, India and North and South America, where it has been considered as being largely caused by the importation of sheep from Britain. The disease has been imported also into South Africa, Australia and New Zealand, but following eradication by slaughter of imported stock and all their progeny, these countries at present appear to be free of the disease.

Observations of natural outbreaks in the USA have indicated that the disease spreads from flock to flock by the movement of infected but apparently normal animals. There was no evidence that the disease spread to adjacent flocks in the absence of such movements or that vectors or other host species were involved in the spread to sheep and goats.

Attempts to demonstrate scrapie agents in the excretions and secretions of affected animals have been largely negative, though on one occasion mice inoculated with *Haemonchus contortus* or with fetal cotyledon from affected goats developed scrapie (Hourrigan et al., 1979).

In rida, the Icelandic name for scrapie, it is considered that ingestion of water and feed contaminated by infected flock-mates is an important method of spread. In northern Iceland rida has reappeared 2-4 years after an area left without sheep for 3 years was restocked with healthy lambs from areas where the disease was unknown. This experience suggests an indirect spread of the disease or a long persistence of the causal agent in the environment.

In countries in which scrapie is endemic, the incidence is highest in certain breeds, but within breeds the prevalence may fluctuate over a period of years. Clinical scrapie is most common in sheep 2-5 years of age with a peak incidence at 3 years, though occasionally it occurs in much younger animals. The disease has a familial pattern, attributable to a mainly maternal mode of transmission. Though vertical transmission has been suspected, there is no direct evidence that transmission occurs in utero. However, fetal membranes are infected and neonates may acquire the infection directly or as a result of contamination of the environment.

Clinical scrapie is probably always fatal, although the course of the disease may vary from as little as a week to several months. This variation may be related to the strain of scrapie agent involved and the genotype of host. However, host genotype/agent strain interactions are better documented in experimental systems in inbred lines of mice and govern mainly the incubation period and the types and distribution of pathological changes in the brain. These interactions can be manipulated within wide limits and to the extent that incubation period may exceed the median life span of the mice.

In sheep also, susceptibility to scrapie is subject to a degree of genetic control. Sheep have been selected for increased or decreased susceptibility to scrapie induced by subcutaneous injection of a defined challenge. In the systems which have been most thoroughly investigated susceptibility appears to be controlled by a single gene, the dominant allele of which confers increased susceptibility. However, there is evidence that scrapie agent may still replicate in "resistant" sheep, but only after a long delay. Thus "resistance" may sometimes be merely an extension of the incubation period. Furthermore sheep which are "resistant" to one strain of scrapie may be susceptible to infection with another strain.

For the commercial farmer the economic consequences of scrapie may be bearable if he can dispose of potentially infected stock at an earlier age than that at which clinical signs are expected. However, for the pedigree breeder scrapie presents serious problems; selection of breeding stock according to performance of progeny is seriously compromised or impossible, and his product has greatly diminished marketability. Furthermore livestock imports from countries in which scrapie occurs may be prohibited.
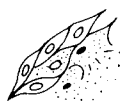
PATHOGENESIS

Epidemiologically maternal transmission is the common mode of spread, and it is considered that primary infection with scrapie occurs via the alimentary tract either prenatally from agent in the amniotic fluid or from a contaminated environment (Hadlow et al., 1982). By mouse inoculation, scrapie agent was first detected in the tonsils, retropharyngeal and mesenteric lymph nodes of clinically normal 10–14-month-old Suffolk lambs from a flock experiencing a high incidence of natural scrapie. In the CNS it was first detected at 25 months of age, and clinical signs were seen in sheep with high concentrations of agent in brain at 34–57 months of age.

These observations accord with well-documented pathogenetic mechanisms in experimental mice (Dickinson and Outram, 1979). Following intraperitoneal inoculation there is a "zero phase" during which the agent cannot be recovered. The length of the zero phase varies according to the genotype of mouse and the strain of agent, but thereafter the agent replicates in lymphoreticular tissues and reaches a plateau titer in spleen of about 10^4 mouse LD_{50}. At about the time this plateau is reached the agent can also be recovered from the brain. Clinical signs appear when titers in the brain reach about the same level as in the spleen, but in the brain the titer continues to rise until death supervenes. Thus the patterns of agent replication in spleen and brain are similar in host/agent combinations of differing zero phase length. The incubation period of scrapie is extended in mice inoculated at 1 day old (when the lymphoreticular system is immature) in splenectomized mice and in mice given large doses of corticosteroids. However, reduction of the incubation period of scrapie in mice was observed following general stimulation of the immune system by a single injection of the methanol extraction residue of BCG (Kimberlin and Cunnington, 1978). Thus the lymphoreticular system (LRS) is important to peripheral sequences of scrapie pathogenesis, but pathological changes have not yet been detected in the LRS and the identity and properties of the cells concerned are unknown. It seems that the LRS contains a number of genotypically specified scrapie replication sites, but since, in a given strain of mouse, the inoculation of a long-incubation-period strain of scrapie can block a subsequently inoculated short-incubation-period strain, such replication sites are probably restricted in number. Furthermore, passage of a given strain of scrapie agent in a "long-incubation-period mouse genotype" does not affect
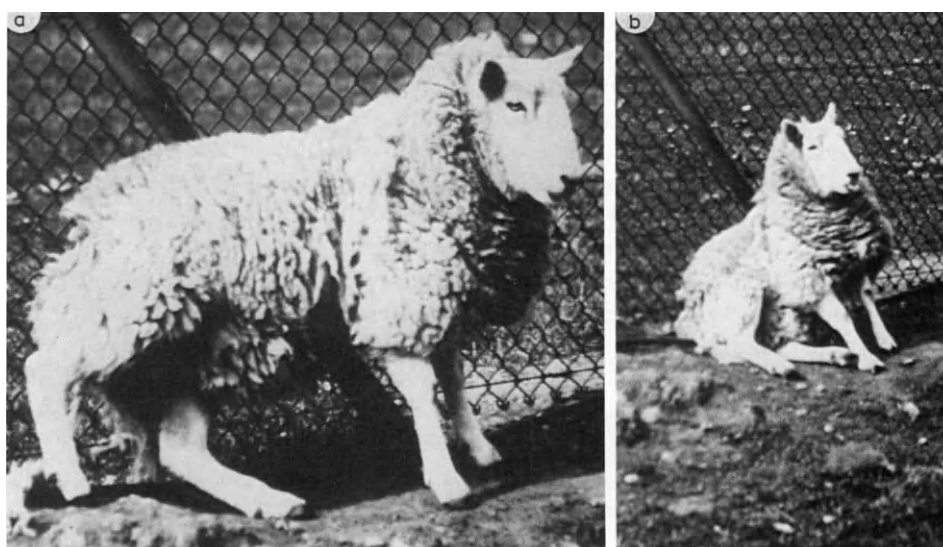
Fig. 175. Cheviot sheep exhibiting vigorous pruritus (a) followed by collapse of the hind quarters (b).

its incubation period subsequently in a short-incubation-period mouse genotype, and therefore agent replication is considered to be a host permitted rather than a host adapted phenomenon.

DISEASE SIGNS

Changes in temperament, and in sensory and locomotor function are the principal clinical effects of scrapie, but the relative involvement of each of the three features varies according to flock.

Changes in temperament vary from lassitude and stupor to hyperexitability with trembling and to collapse if the animal is stimulated.

Sensory disturbance is most commonly manifested by pruritus (Fig. 175). The sheep rubs its hind quarters and flanks on any firmly fixed object and may bite at any accessible part of its body. The rubbing and biting cause wool loss and
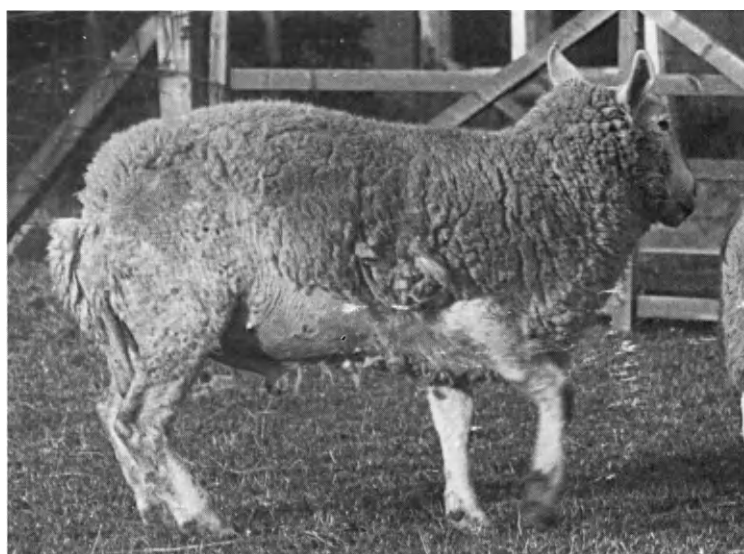
Fig. 176. Scrapie-affected Cheviot sheep showing wool loss and cutaneous abrasions due to intense pruritus.

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superficial abrasion of the skin (Fig. 176). Whilst rubbing, the affected animal may raise its head and make exaggerated nibbling movements with the lips and tongue. This "nibbling reflex" can often be elicited also by applying deep massage to the muscles of the back.

Locomotor disturbance is insidious in onset and associated at first with hypermetria and incoordination, progressing to weakness and ataxia. In later stages the animal may be unable to rise without assistance but will hobble about for some time thereafter.

Though some scrapie-affected sheep become obese, emaciation is more common, especially in the terminal phases. Once clinical signs have appeared they are almost invariably progressive until death.

There are no laboratory tests that can be applied in the live animal to support the diagnosis of scrapie. In some cases it may be helpful to examine skin scrapings to eliminate ectoparasites as a possible alternative cause of pruritus.

PATHOLOGY

The significant pathological changes in scrapie are microscopic and confined to the CNS. The characteristic changes are vacuolation, degeneration and necrosis of nerve cells, spongy vacuolar transformation of the neuropil, and astrocytic hypertrophy with hyperplasia of astrocytic fibres (Fig. 177). The lesions are noninflammatory and nondemyelinating. There is considerable variation in location, extent and severity of these changes, much of which is associated with the breed of sheep and may possibly be related to strain of agent. In one experimental situation in Cheviot sheep the lesions were virtually absent in a proportion of animals even in clinically typical terminal scrapie cases.
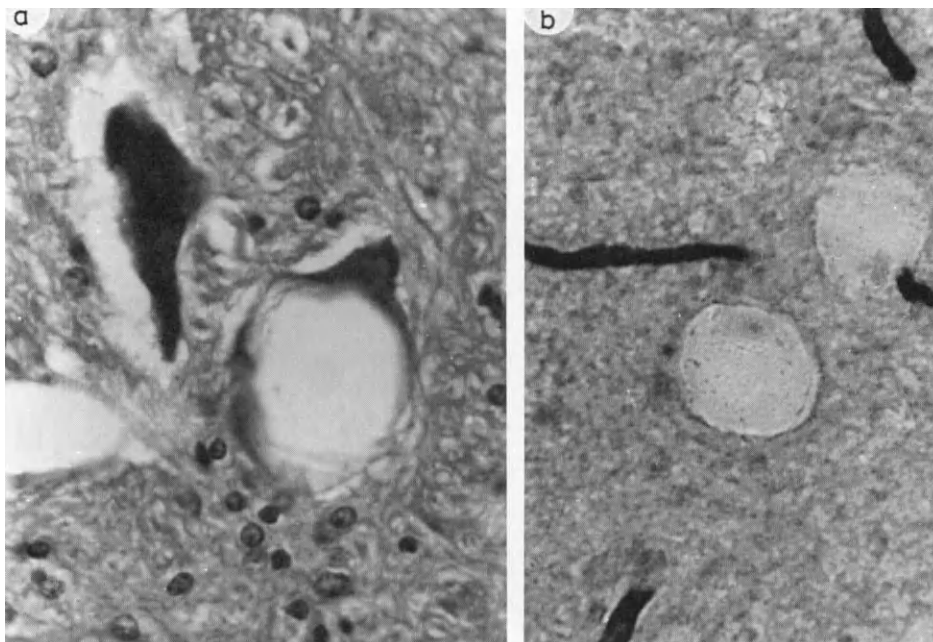
Fig. 177. (a) A vacuolated nerve cell in the brain stem of a scrapie-affected sheep; an adjacent neuron is hyperchromatic and shrunken; H&E, × 350. (b) Vacuolated neurons in brain stem of scrapie-affected sheep, showing loss of golgi apparatus; frozen section lead capture method for thiamine pyrophosphatase, × 300.
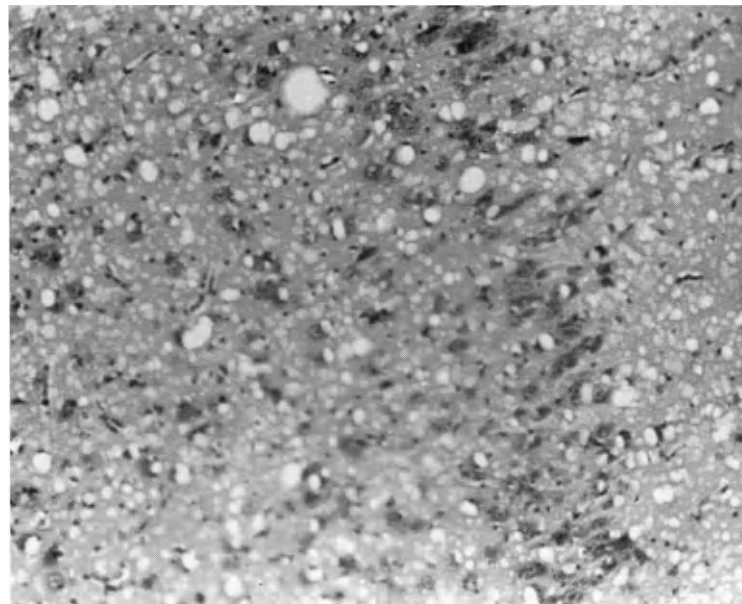
Fig. 178. Amygdaloid area of brain of scrapie-affected sheep; note the spongy vacuolar transformation of the neuropil (status spongiosis) and the prevalence of glial nuclei lying in pairs or small clusters; H & E, × 160.

The most severe lesions are usually found in the raphe and nuclei of the medulla, but in some instances the midbrain, diencephalon and paraterminal body are more severely involved. The cerebral cortex and hippocampus are never as severely affected as in mice with some mouse-passaged strains. Attempts to correlate clinical signs and neuropathology have been unrewarding, although frequently in very obese subjects the thalamus and hypothalamus are extensively involved.

Recently cerebrovascular amyloid deposits have been reported to occur in a proportion of scrapie-affected sheep (Gilmour et al., 1986). This material bears some resemblance to the cerebral amyloidosis occurring in some forms of experimental murine scrapie (Bruce et al., 1976), though the latter forms as plaques in the neuropil without an obvious close relationship to blood vessels and usually contains argyrophilic elements (Fig. 178).

EM of negatively stained subcellular fractions of brain from scrapie-affected animals has consistently revealed fibrils with a characteristic morphology which are absent from similar preparations of normal brain. They have been termed scrapie-associated fibrils (Merz et al., 1981). They are 100–500 nm in length and are composed of either two or four beaded filaments 4–6 nm in width arranged in an helical configuration. They are immunologically identifiable with prion protein and with components of cerebral amyloid. Thus they would appear to be closely related to the etiological agent of scrapie.
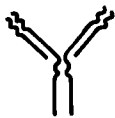
IMMUNE REACTION

Although it is evident that the LRS plays an important but incompletely understood part in the pathogenesis, there is as yet no evidence of any specific immunological reactivity in sheep or laboratory animals. In Herdwick sheep with scrapie, IgG levels were 70–90% higher than controls in a considerable proportion of clinical cases (Collis et al., 1979), but this could not be interpreted and may be a secondary effect.

In mice, some strains of scrapie agent are associated with the development

of amyloid plaques, which could represent either an abortive immunological response or a host-coded protein produced in response to the scrapie agent or to products of its activity.

A consistent response of mice to i.c. inoculation with scrapie is an increase in the weight of the spleen, the onset of which in one study in C_3H/HeJ mice was associated with reduced mitogen responsiveness (Garfin et al., 1978); however, pure T- and B-cell suspensions from scrapie-infected and control BALB/c mice showed no consistent differences in mitogen responsiveness (Kasper et al., 1982). Neonatal thymectomy did not alter the incubation period of scrapie in mice, suggesting that CMI neither contributes to the pathogenesis nor plays a significant role in the host's defence against the disease. However, incubation of scrapie brain homogenate with mouse peritoneal macrophages has been reported to extend the incubation period and has led to the suggestion that, in vitro, macrophages may inactivate the scrapie agent (Carp and Callahan, 1982).

LABORATORY DIAGNOSIS

Since in scrapie there are no specific serological responses to infection, examination of the brain of the affected animal is at present the only practical method of supporting a clinical diagnosis.

Histological examination is the method of choice, and it is important that the brain is not traumatized or autolyzed before fixation, as this may interfere with the recognition of characteristic lesions. The complete brain from a freshly killed animal should be immersed in 10–20 times its own volume of 10% formalin or formol ammonium bromide and fixation allowed to proceed for several days before transplantation to the laboratory. It is important that the brain is examined systematically, as the lesions may be restricted in their distribution and can be very mild or even inapparent.

It is a widely held view that naturally occurring scrapie in sheep and goats may be transmitted to mice. However, this is not always so, as the following example illustrates: Fraser (1983) inoculated ten inbred lines of mice (comprising almost 700 animals) with brain material from five cases of histologically confirmed Icelandic scrapie (rida) acquired from different parts of the country. In one case no evidence of transmission was obtained and in the other four successful transmissions varied from 1.4 to 27%.

Several attempts have been made to propagate scrapie agents in tissue culture. The "Chandler strain" has been shown to survive in a cell line derived from mouse brain for 100 passages (Haig and Clarke, 1971). However, agent replication appeared to be in synchrony with cell division, as titers remained low ($10^{1.1}$–$10^{3.5}$ $ID_{50}/0.05$ ml) and could be assessed only by i.c. inoculation of mice.

PROPHYLAXIS AND CONTROL

No prophylactic or therapeutic measures have been found effective against scrapie. Control measures depend upon local epidemiological knowledge, from which it is possible to decide between two options: eradication, or containment within economically acceptable limits. Eradication is feasible only where a secure environment has been previously uncontaminated and animals imported into it have been contained in the most stringent quarantine conditions. However, it is difficult to determine the likely duration of the incubation period, which may be 8–10 years and involve several generations of sheep. If

disease occurs without quarantine, contamination of the environment becomes a further unquantifiable hazard. As experience in Iceland indicates, keeping pasture free of sheep stocks for a period of years may be insufficient to eliminate infection. Containment involves the culling of all progeny of any females which become affected. Pregnant ewes which develop clinical disease should also be eliminated before lambing. The role of the ram in the spread of scrapie is less significant, but he may cause infection through contact and transmit genetic susceptibility to infection to his progeny.

REFERENCES

Baringer, R.J., Prusiner, S.B. and Wong, J.S., 1981. Scrapie associated particles in post synaptic processes. J. Neuropathol. Exp. Neurol., 40: 281–288.

Bendheim, P.E., Barry, R.A., De Armand, S.J., Stites, D.P. and Prusiner, S.B., 1984. Antibodies to a scrapie prion protein. Nature, 310: 418–421.

Bignami, A. and Parry, H.B., 1971. Aggregations of 35 nm particles associated with neuronal cytopathic changes in natural scrapie. Science (New York), 171: 389–390.

Bruce, M.E., Dickinson, A.G. and Fraser, H., 1976. Cerebral amyloidosis in scrapie affected sheep. Neuropathol. Appl. Neurobiol., 2: 471–478.

Carp, R.I. and Callahan, S.M., 1982. Effect of mouse peritoneal macrophages on scrapie infectivity during extended in vitro incubation. Intervirology, 17: 201–207.

Chesebro, B., Race, R., Wehrly, K., Nishio, J., Bloom, M., Lechner, D., Bergstrom, S., Robbins, K., Mayer, L., Keith, J.M., Garon, C. and Haase, A., 1985. Identification of scrapie prion protein specific mRNA in scrapie infected and uninfected brain. Nature, 315: 331–333.

Collis, S.C., Kimberlin, R.M. and Millson, G.C., 1979. Immunoglobulin G levels in the sera of Herdwick with natural scrapie. J. Comp. Pathol., 89: 389–396.

Dickinson, A.G. and Taylor, D.M., 1978. Resistance of scrapie agent to decontamination. N. Engl. J. Med., 299: 1413–1414.

Dickinson, A.G. and Outram, G.W., 1979. The scrapie replication site hypothesis and its implications for pathogenesis. In: S.B. Prusiner and W.J. Hadlow (Editors), Slow Transmissible Diseases of the Nervous System. Academic Press, New York, Vol. 1, pp. 13–31.

Fraser, H., 1983. A survey of primary transmission of Icelandic scrapie (Rida) to mice. In: L. Court (Editor), Unconventional Viruses and Infections of the Nervous System. Masson, Paris, pp. 34–46.

Garfin, D.E., Stites, D.P., Zitnik, L.A. and Prusiner, S.B., 1978. Suppression of polyclonal B-cell activation in scrapie infected C3H/HeJ mice. J. Immunol., 120; 1986–1990.

Gilmour, J.S., Bruce, M.E. and MacKellar, A., 1986. Cerebro-vascular amyloidosis in scrapie affected sheep. Neuropathol. Appl. Neurobiol., 12: 173–183.

Hadlow, W.J., Kennedy, R.C. and Race, R.E., 1982. Natural infection of Suffolk sheep with scrapie virus. J. Infect. Dis., 146: 657–664.

Haig, D.A. and Clarke, H.C., 1971. Multiplication of the scrapie agent. Nature, 234: 106–107.

Hourrigan, J., Klingsporn, A., Clark, W.W. and De Camp, M., 1979. Epidemiology of scrapie in the United States. In: S.B. Prusiner and W.J. Hadlow (Editors), Slow Transmissible Diseases of the Nervous System. Academic Press, New York, Vol. 1, pp. 331–356.

Kasper, K.C., Stites, D.P., Bowman, K.A., Panitch, H. and Prusiner, S.B., 1982. Immunological studies of scrapie infection. J. Neuroimmunol., 3: 187–201.

Kimberlin, R.H., 1976. Slow Virus Diseases of Animals and Man (Research Monographs Frontiers of Biology 44). North Holland/Elsevier, Amsterdam/New York, 264 pp.

Kimberlin, R.H., 1979. The biology of the scrapie agent. In: D.A.J. Tyrrell (Editor), Aspects of Slow and Persistent Virus Infections (New Perspectives in Clinical Microbiology 2). Martinus Nijhoff, The Hague, pp. 4–29.

Kimberlin, R.H., 1982. Scrapie agent: prions or virions? Nature, 297: 107–108.

Kimberlin, R.H. and Cunnington, P.F., 1978. Reduction of scrapie incubation time in mice and hamsters by a single injection of methanol extraction residue of BCG. FEBS Microbiol. Lett., 3: 169–172.

Merz, P.A., Somerville, R.A., Wisniewski, H.M. and Iqbal, K., 1981. Abnormal fibrils from scrapie infected brain. Acta Neuropathol. (Berl.), 54: 63–74.

Narang, H.K., 1989. Evidence that scrapie agent is a DNA virus. Neuropathol. Appl. Neurobiol., 15: 265 (Abstract).

Oesch, B., Westaway, D., Walchli, M., McKinley, M.P., Kent, S.B.H., Aebersold, R., Barney, R.A., Tempst, P., Teplow, D.B., Hood, L.E., Prusiner S.B. and Weissmann, C., 1985. A cellular gene encodes scrapie PrP27–30 protein. Cell, 40: 735–746.

Pruisner, S.B., 1982. Novel proteinaceous infectious particles cause scrapie. Science, 216: 136–144.

Prusiner, S.B. and Hadlow, W.J., 1979. Slow Transmissible Diseases of the Nervous System. Academic Press, New York, Vol. 1, 472 pp., and Vol. 2, 526 pp.

Thormar, H., 1971. Slow infections of the CNS. Z. Neurol., 199: 1–23; 199: 151–166.

Tyrrell, D.A.J., 1979. Aspects of Slow and Persistent Virus Infections (New Perspectives in Clinical Microbiology 2). Martinus Nijhoff, The Hague, 286 pp.

Williams, E.S. and Young, S., 1980. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. J. Wildl. Dis., 16: 89–98.

Chapter 53

Acute Respiratory Disease in Cattle

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INTRODUCTION

Acute respiratory disease in cattle is a serious problem in countries with intensive or semi-intensive systems of feeding and management. The etiology is complex and many reviews list the microorganisms incriminated. Some of the viruses which can infect the respiratory tract of cattle are summarized in Table 34.

Viruses and bacteria, together with physical stress, transport, overcrowding, irregular feeding and poor standard of hygiene as well as environmental factors (ventilation, temperature and humidity) play an important role in pathogenesis.

The disease has world-wide distribution. It affects cattle of all races and ages, tends to recur and responds poorly to preventive and therapeutic treatment. Even if the disease for the most part occurs during calfhood, adult cattle may also be involved.

TABLE 34

Simultaneous rise of antibodies to 15 different agents in the same diseased cattle[a]

P13 virus	BVDV	RSV	Adenovirus								Reovirus			*Chlamydia*
			1	2	3	4	5	6	7	8	1	2	3	
(13)	2	0	2	0	2	2	1	0	0	0	2	2	3	4
2	(11)	0	3	1	1	2	1	1	0	0	3	0	2	3
0	0	(2)	0	0	0	0	0	0	2	0	0	0	1	0
2	3	0	(9)	1	2	1	1	1	1	1	1	1	0	4
0	1	0	1	(3)	1	0	1	0	0	0	0	0	0	2
2	1	0	2	1	(6)	1	0	0	0	0	0	2	0	2
2	2	0	1	0	1	(13)	0	2	8	5	1	0	1	2
1	1	0	1	1	0	0	(8)	2	2	1	3	3	1	1
0	1	0	1	0	0	2	2	(8)	2	1	3	1	0	0
0	0	2	1	0	0	8	5	1	(12)	6	2	1	1	1
0	0	0	1	0	0	5	1	1	6	(9)	1	0	0	1
2	3	0	1	0	0	1	3	3	2	1	(21)	10	8	0
2	2	0	0	1	0	2	0	3	1	1	0	(13)	4	0
3	3	0	1	0	0	0	1	1	0	1	0	8	(11)	2
4	3	0	4	2	2	2	1	0	1	1	0	0	0	(9)

[a]Numbers of cattle with a rise of antibody to each agent; numbers in brackets indicate that a group of cattle with a rise of antibody to a certain agent was tested for antibody rise to each of the other agents.
Source: Moreno-López (1979).

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The symptomatology of acute respiratory disease is similar in different outbreaks and includes depression, inappetence, dullness, increased respiration and pulse rates in the early stages. The respiratory disorder may or may not be accompanied by diarrhea.

Acute respiratory disease in cattle is not attributable to a single etiological agent. In many instances, mixed infections with two or more agents occur, as shown in Table 34. Such mixed infections can contribute to the severity of the disease. Moreover, the microorganisms involved appear to act synergetically. Examples of synergism have been demonstrated in the USA (shipping fever, caused by parainfluenza-3 (PI3) virus and *Pasteurellae*) and Sweden (Umeå disease, caused by PI3 virus and BVDV).

It is difficult to estimate the economic losses, but in terms of mortality, loss of milk production and delayed weight gains, the acute respiratory disease is considered as one of the most costly and troublesome problems for the cattle industry.

INFECTION AND DISEASE

It is generally accepted that viruses are the primary agents and that bacteria like *Pasteurellae* may, as secondary invaders, accentuate the pathology of the respiratory tract, as in "shipping fever". Some of the viruses are circulating in the cattle population, as for instance PI3 virus, which is common in the respiratory tract and is easily transmitted. High percentages of seropositive animals were found in various countries. Serological surveys have shown that, on the contrary, BVDV was much less frequent on farms separated geographically than on farms with a close contact of animals on common pastures. Collectivization of calves with a different immunological status like that occurring on breeder farms or in large feedlots, leads to accumulation of viruses introduced by animals in the incubation or convalescence stages or by latent carriers. Under these circumstances, explosive outbreaks of respiratory and enteric disease may occur. It is unknown how stress factors during massive transport and crowding promote pathogenicity of various respiratory viruses, because it is not possible to mimic a natural outbreak by experimental infection. All too frequently only a mild disease is reproduced. Some of the viruses per se have an adverse influence on defence mechanisms. It was shown that infection with PI3 virus may initially diminish the cell-mediated immune response; also, BVDV may act on lymphocytes and neutrophils, suppressing antibody production and phagocytosis, respectively. Suppression may promote invasion of other agents, particularly through close contact and crowding in stables lacking proper ventilation. As regards diagnosis, a somewhat pathetic conclusion can be drawn: if foot-and-mouth disease virus is isolated from an animal, the disease has been identified. However, if PI3 virus or BVDV has been isolated, you have diagnosed the infection but not an "acute respiratory/enteric disease", because other viruses and also bacteria might be etiologically involved.

As diagnostic techniques become more widely applied, an increasing number of viruses has been isolated from cattle with acute respiratory disease. At the end of the 1950s, "shipping fever" was described as a disease following massive transports to or from the enormous feedlots of the USA, and PI3 virus was isolated as the first virus of a condition with a multiple viral etiology.

In Sweden, at about the same time, there were outbreaks of a severe acute respiratory–enteric syndrome on farms in the Umeå district in the northern part of the country. Local veterinarians provisionally called the syndrome "Umeå disease". Thus BVDV became the second member of a multiple etiology.

At that time, PI3 virus was suspected to be the cause of the infection in the respiratory tract (manifested by tracheitis and pneumonia), and BVDV to be the cause of infection in the intestinal tract (manifested by erosions in the oral and intestinal mucosa). Both viruses were indeed identified. However, it has to be kept in mind that the terms "parainfluenza" and "bovine viral diarrhea" are merely taxons by which these viruses are classified.

During the following decade, the first two bovine adenoviruses (types 1 and 2) were described in the USA, and subsequently a third type was identified in the UK. These are the isolates that grow in kidney cells with a hexon antigen common to all mastadenoviruses. Then Hungarian workers reported the isolation of two new bovine adenoviruses, types 4 and 5, which preferentially grow in cultured calf testicle cells. Then additional adenoviruses, types 6 through 8 were reported as "testicle-cell types" from various countries and, finally, from Bulgaria, a type 9 being a "kidney-cell type". Regarding the "testicle-cell type" adenoviruses, the proof for the presence of antigen(s) common to mastadenoviruses is equivocal at present. The types 4, 5 and 7 have been described each as a single causative agent of acute respiratory–enteric disease in Hungary and Japan, respectively.

During the 1970s the first records appeared on bovine respiratory syncytial virus (BRSV), closely related to the human respiratory syncytial virus and causing pneumonia in cattle often less than 2 years of age. BRSV was found either as a single agent or as a member of a multiple etiology (as recently reported from UK and USA) condition.

In some countries the bovid herpesvirus-1, better known as infectious bovine rhinotracheitis (IBR) virus, is a "solo" pathogen. IBRV was the first viral agent definitely shown to cause respiratory infection in cattle. Herpesvirus type 3 (malignant catarrhal fever virus), with a low and sporadic incidence, is also incriminated in the bovine respiratory–enteric disease complex.

The role of herpesvirus type 4 (prototype strain Movar 33/63) has not been elucidated. However, a respiratory illness caused by this virus in association with *Pasteurella multocida* was quite severe.

It is easy to extend this listing by adding, for example, bovine enteroviruses, rhino- and parvoviruses, coronaviruses and the reoviruses. The isolation of these viruses from apparently healthy cattle as well as from those with acute respiratory–enteric disease indicates that they are widespread. However, because they appear to be infrequently implicated in disease processes, it seems that they cause infection only under certain conditions. Bacteria such as *Chlamydia, Pasteurellae* and mycoplasmas may influence the cycles of infection with viruses.

CONTROL MEASURES

Young animals require more care than adult animals. Calves fare best when confined individually or in very small groups. This facilitates individual observation, control of diseased animals and maintenance of a higher standard of hygiene. A large number of animals maintained in close contact provides ideal conditions for the spread of disease agents via the respiratory tract. The immunological status, especially passive immunity of the animals, is important in connection with respiratory disease. Protective titers of serum antibodies have been calculated for some of the respiratory pathogens. PI3 virus infection seems to be prevented by a serum neutralizing antibody titer of 32 and IBRV infection by a titer of 2. The significance of local nasal antibodies and CMI against respiratory disease is of special importance.

With the complex etiology as that associated with respiratory infections it

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is logical that control measures are not easy. Control of the disease may be attempted by diminishing the frequency of infections and by implementing a vaccination program against the most frequent agents. Some authors have tried to suppress infections by interferon or interferon inducers, i.e. avirulent viruses known to stimulate nonspecific defence mechanisms, including interferon production; however, no efficient control procedures have become available.

The development of effective and practical vaccines for the prevention of respiratory disease is notoriously difficult, due to the multiplicity of agents which are involved. As shown in Table 34, at least 15 distinct microorganisms have been identified. Certain viruses, however, produce more severe disease than others; vaccines against them may be expected to break the chain of events leading to respiratory disease.

The multiplicity of agents necessitates the use of polyvalent vaccines; polyvalence does not appear to affect the immunogenicity of the individual agents. Although desirable, the incorporation of all potential pathogens in a single vaccine is difficult, if not impossible. The results of some limited field trials with a tetravalent vaccine have in no way been encouraging.

Finally, it must be reemphasized that a problem of this complexity is not solved by a single measure but demands that attention is given to both the infectious and environmental causes of the disease.

REFERENCE

Moreno-López, J., 1979. A serosurvey of viruses during outbreaks of acute respiratory and/or enteric disease in Swedish cattle. Zentralbl. Veterinärmed. B, 26: 634–640.

Chapter 54

Viral Gastroenteritis in Ruminants

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INTRODUCTION

Diarrhea is one of the leading causes of morbidity and mortality in infants and young animals in both developing and developed countries. It has been estimated that over 500×10^6 cases of diarrhea occur annually in humans, leading either directly or indirectly to approximately 10×10^6 deaths (Editorial, 1978). Accurate estimates of diarrhea cases in ruminants are not available, but they would appear to be at least as high as in humans on a percentage basis, thus indicating the economical importance of this disease syndrome.

Until recently, the agents responsible for most cases of nonbacterial gastroenteritis were not identified. However, since the discovery in 1969 by Mebus (Mebus et al., 1969) that a virus was present in feces of calves suffering from diarrhea, it has been proven that rotaviruses can infect calves and cause diarrhea. This discovery prompted the search for related viruses as a cause of diarrhea in other animals. As a result of these investigations, rotavirus has been found to be a major cause of nonbacterial gastroenteritis in most mammals and in fowl (Flewett and Woode, 1978; McNulty, 1978; Woode, 1982), and it is now accepted that up to 60% of nonbacterial gastroenteritis cases may be caused by rotavirus. The remaining cases of diarrhea are caused by a variety of viral agents, which may include *Coronavirus, Torovirus, Calicivirus, Parvovirus, Enterovirus, Adenovirus, Astrovirus, Minireovirus* or rota-like viruses (Table 35). However, the role and prevalence of many of these agents in causing diarrhea has not been firmly established, neither in ruminants nor in other mammals.

Although as microbiologists we try to identify a specific etiological agent as a cause of diarrhea, it must be emphasized that diarrhea is often multifactorial and that interactions of various factors with infectious agents can exacerbate the disease. These factors can be broadly grouped into immunological, environmental and nutritional. In each category there are large numbers of components that can interact and alter both the degree of diarrhea and the final outcome of the disease. For example, diarrhea is often associated with inclement weather, i.e. storms, sleet, cold, etc. This is probably associated with increased stress due to temperature fluctuations which can alter the animals' defense mechanisms and increase the probability of infection due to increased animal congregation.

The viruses that cause gastroenteric infections can generally be divided into two groups. For viruses of the first group, replication is restricted to the gastrointestinal tract; they induce disease as a result of direct effects only on the cells of the intestine (Fig. 179). Most of these agents enter the host directly, via the oral cavity, and pass into the gastrointestinal tract. Viruses of the

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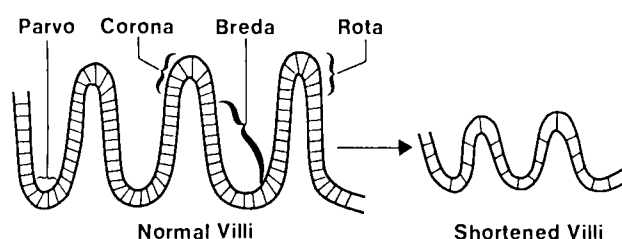
Fig. 179. Sites of infection and cell destruction by some enteric viruses.

second group enter the host via the oral cavity and replicate in the gastrointestinal tract but they do not remain localized. These viruses may spread to other target organs such as lymphoid tissue (Kahn, 1978) or even the CNS (Nathanson, 1979).

At least eleven different viruses can cause intestinal damage and diarrhea in ruminants under appropriate conditions (Table 35). All of these agents produce the most severe clinical signs during the first few weeks of life (Woode and Bridger, 1975; Little and Shadduck, 1982). However, there are reports of virus shedding associated with diarrhea in older animals as well (Von Bonsdorff et al., 1976; McNulty et al., 1978; Jones et al., 1979). In most cases the infection of older animals is subclinical, and it has been suggested that they serve as virus carriers and are a source of infection for young susceptible animals. In fact, recent reports suggest that removal of carrier animals from a herd reduces or eliminates the disease. The reason for increased severity of diarrhea in younger animals and a higher mortality is that the viruses generally cause greater villous atrophy in the younger animals.

AGENTS INVOLVED

Rotaviruses

Rotavirus-induced disease was first described by Cheever and Mueller

TABLE 35

Some enteric viruses of ruminants

Virus	Site of replication	
	Horizontal	Longitudinal
Rotavirus	enterocytes/villus tip	small intestine
Rota-like virus	enterocytes/villus tip	small intestine
Coronavirus	enterocytes/top half	small, large intestine, colon
Corona-like virus	enterocytes/top half	small intestine
Torovirus (Breda)	mid villus/crypts	small intestine, colon
Astrovirus	?	?
Calicivirus	?	?
Parvovirus	crypts, lymphoid	small, large intestine
Adenovirus	enterocytes	small intestine
Reovirus	?	?
Enterovirus	?	?

? Insufficient data available for definitive statements to be made.

(1948); later rotaviruses were established as important infectious agents in mice by Kraft (1957, 1958). However, rotavirus was not recognized as an important pathogen in domestic animals until Mebus et al. (1969) identified it as a cause of neonatal diarrhea in calves. Since then it has been demonstrated to play a major role in nonbacterial gastroenteritis in most mammalian species. In most outbreaks the disease occurs suddenly and spreads rapidly to other susceptible individuals. The reason for this rapid spread is that the concentration of virus can reach 10^{11} particles per gram of feces, which is equivalent to 10^7 infectious doses (Flewett and Woode, 1978; Woode et al., 1976a, b). Experimental inoculation of bacteria-free filtrates containing rotavirus causes diarrhea within 12–24 h in susceptible young animals. Diarrheic animals are also anorexic and can vomit. The reason for such rapid clinical signs is that in the absence of passive antibody or local acquired immunity, the virus infects the enterocytes of the villi, rapidly killing them. The replication cycle of rotavirus is approximately 12 h (Carpio et al., 1981).

Rotavirus infection is generally limited to the small intestine in calves, pigs and humans (Middleton et al., 1974; Mebus and Newman, 1977; McAdaragh et al., 1980), but antigen can be found in the colon of lambs (Snodgrass et al., 1977), pigs (Theil et al., 1978) and mice (Little and Shadduck, 1982). Viral infection occurs in the enterocytes of the upper half of the villi of the small intestine, resulting in rapid death and sloughing of the cells (see Fig. 179). With death, the villi become shortened and loose their adsorptive capacity (Woode and Crouch, 1978). The cells at the tips of the villi are responsible for production of lactase which aids in digestion of lactose. Thus the combination of reduced adsorptive capacity and reduced enzyme activity accounts for the diarrhea. Since the crypt cells are not damaged, regeneration of the enterocytes and recovery of the villi is generally rapid after the infection is overcome. Animals that recover from the disease return to normal body weight within 10–28 days after infection.

Coronaviruses

Coronaviruses can cause both respiratory and gastrointestinal infections in humans and animals (Robb and Bond, 1979). Transmissible gastroenteritis virus of swine was one of the first coronaviruses identified as a cause of diarrhea in animals (Doyle and Hutchings, 1946). Coronaviruses have also been identified as a major cause of calf diarrhea (Stair et al., 1972; Mebus, 1978; Storz et al., 1978a). Bovine coronavirus diarrhea, like rotavirus diarrhea, occurs within 15–24 h p.i. Early in infection the villous epithelial cells appear morphological normal but they contain large amounts of antigen. Since diarrhea occurs before denudation and loss of enterocytes it is postulated that it is a direct result of infection of the cell and the ensuing redirection of cellular functions from absorption to virus replication. If absorption does not occur there is accumulation of digestive fluids. As the infection proceeds cells are lost from the villi and are replaced by immature squamous to cuboidal epithelial cells which lack the enzymes required for digestion of milk. They also have a reduced absorptive capacity as is the case in all other virus infection of the gastrointestinal tract.

Other enteric viruses

Caliciviruses, astroviruses (Woode and Bridger, 1978) and parvoviruses (Storz and Bates, 1973) may be responsible for causing gastroenteritis and may account for a significant portion of the cases of diarrhea which are not caused by rotaviruses or coronaviruses. However, there are still 20–30% of diarrhea

cases for which no etiological agent has been identified. As the search continues for etiological agents other viruses are discovered. One such new agent is the Breda virus (Woode et al., 1982) which has recently been identified. In the case of Breda virus infection, the lesions at first appear similar to coronavirus infections with respect to location of lesions. However, on closer examination it becomes obvious that the disease is different, since the lesions and infected cells are visible in the lower 50% of the villi and in the crypts of the small intestine. In the colon, infected cells are present throughout the villi and in the crypts. In addition, structurally this virus is not identical to coronaviruses and is related to an equine virus described in Europe (Horzinek et al., 1984). Recent studies also suggest that other species may have antibody to this group of viruses, which has been tentatively designated as toroviruses (Horzinek et al., 1987).

Parvoviruses can infect a wide variety of animals, ranging from pets to large domestic animals (Kahn, 1978; Storz et al., 1978b). In contrast to the other viruses discussed so far, this virus family can produce systemic disease as well as enteritis. Since the virus generally replicates in rapidly dividing cells, the lesions are seen in the crypts of both the small and large intestines as well as in lymphoid tissue. Because of the replication in lymphoid tissue this disease can be more severe, especially in small animals, than other viral infections, because of interference with immune responses and damage to the crypts. However, the role of parvoviruses in diarrhea of ruminants is probably small compared to that played by the other agents.

One common feature of all of the agents described to date is that infection occurs by ingestion of the virus. Since the virus does not have to spread systemically, the incubation period is extremely short, with villus shortening and reduced fluid adsorption, dehydration and death if diarrhea is severe enough. For a differential diagnosis of the actual cause of diarrhea attempts must therefore be made at demonstrating the presence of the specific agent. However, regardless of the agent treatment will be the same.

Although these viruses have been associated with diarrhea, there are also many instances where they are present but no disease occurs. Furthermore, it is not always possible to reproduce the disease in conventional or even gnotobiotic animals to the extent that it occurs naturally. Under experimental conditions most attempts to reproduce the disease are made with a single pathogen and more importantly with plaque purified isolates. Replication of these pure populations may be restricted to very localized regions of the intestine; therefore, they do not cause sufficient damage throughout the intestine (longitudinally) to cause the damage required for disruption of intestinal function and diarrhea. Under natural conditions there may be multiple strains of a virus (Sabara et al., 1982) that infect different areas of the intestine, thus causing severe diarrhea. Another explanation for poor reproducibility of diarrhea under experimental conditions could be that avirulent strains grow in culture more rapidly than the virulent strains (Woode, 1982). If these are used to challenge animals no disease occurs. Another explanation could be related to immunity. If low levels of active or passive immunity are present this would keep the virus infection rate low so as to allow only few viruses to initiate new infections, as is often the case with persistent local infections. However, during stress the immune defense is reduced and virus shedding and diarrhea may occur. When the immune system returns to normal, diarrhea stops but virus may continue to be shed. This type of carrier state would insure the continued presence of the virus in the environment for infection of susceptible neonatal animals. Such carrier states have been demonstrated to occur both in vivo (Leece and King, 1980; Benfield et al., 1982; Crouch et al., 1985) and in vitro (Misra and Babiuk, 1980).

DIAGNOSIS

Many of the viral agents involved in causing diarrhea are not easily cultivable in vitro by conventional methods. The reason for this may be related to the virus tropism for differentiated cells. Because of the difficulty in growing enteric viruses a large variety of tests have been developed to diagnose these agents directly (Yolken, 1982). Most tests are based on the observation that there are high levels of virus particles present in the feces of diarrheic animals and humans (Woode et al., 1976a, b; Flewett and Woode, 1978; McNulty, 1978). It is therefore easy to observe virus in feces by EM techniques. However, direct observation is less efficient than if combined with serological tests, as is the case with IEM, where the virus is aggregated by specific sera and can be visualized much more easily. The availability of specific antisera and monoclonal antibodies to numerous viruses makes this a very attractive means of diagnosis. Some enteric viruses may infect cells without causing a CPE or producing infectious virus. In these cases viruses may be identified by culturing in vitro and testing for the presence of viral antigen in infected cells.

A number of points must be considered when choosing a test, including efficiency, speed, relative costs and the specific purpose for making a diagnosis (Table 36). If trained personnel and proper equipment are available then IEM, RIA and ELISA appear to be very good, with respect to specificity and speed. ELISA tests are especially useful for automation and diagnosing large numbers of samples. Furthermore, ELISA can be read by eye if readers are not available, making this test very attractive. If serotyping is also desired then many of the tests listed can be adapted if specific antisera and preferably monoclonal antibody produced against each serotype are prepared.

PATHOLOGY AND PATHOPHYSIOLOGY OF ENTERIC VIRUS INFECTIONS

In most virus infections of the gastrointestinal tract, regardless of whether the virus has a predilection for the epithelial cells at the tips of the villi or the crypts, there is shortening and occasional fusion of adjacent villi resulting in a reduced absorptive surface (Keenan et al., 1976; Leece et al., 1976; Pearson and McNulty, 1977). Infection generally begins in the proximal part of the small intestine and spreads progressively to the jejunum and ileum and

TABLE 36

Efficiency and practicality of some diagnostic techniques for detection of viruses causing gastroenteritis in animals

Method	Efficiency	Speed	Relative cost
IEM	very good	hour	high
RIA	very good	day	moderate
ELISA	very good	day	moderate–low
IF	good	hour	moderate
Culture	good	days	low–moderate
CIEP	good	hour	low
CF	poor–good	day	low
Immun. Ad. Hemagg.	average	hour–day	low
HA	poor	hour	low
AGID	poor	day	low

The practicality of IEM is good only for single samples; all other methods can be scaled up for multiple sample examination.

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sometimes to the colon (Mebus et al., 1973; Snodgrass et al., 1977). This will, however, depend on the initial infective dose, the virulence of the virus strain (Woode and Crouch, 1978), and the host's immunological status. Thus in the presence of passively acquired antibody, infection can occur but replication is limited to such an extent that either no or only mild diarrhea occurs. In rotavirus and coronavirus infections, which are limited to the cells at the tips of the villi, the absorptive cells are replaced with immature squamous to cuboidal epithelial cells. Until these cells mature their absorptive capacity and enzymatic activity is greatly reduced. Since they also appear to be relatively resistant to virus infection the disease is often self-limiting if dehydration is not so significant as to cause death (Woode, 1982; Garwes, 1982). Crypt cells are not damaged and the rate of recovery is therefore generally rapid. In contrast, after infection with viruses that replicate in the crypt cells there is a limited number of new cells available to migrate up the villi and recovery tends to take longer. It should be stressed that although the degree of villous damage may be influenced by the virulence of the virus and the immunological status of the animal, the rate of regeneration of enterocytes and enterocyte maturation may also vary with the age of the animal and the site of virus infection. Since glucose and sodium adsorption are highest in the proximal and middle part of the jejunum (Shephard et al., 1979; Bachmann and Hess, 1982), damage here would cause most severe diarrhea.

In viral infections, the mechanism of fluid loss is considered to be different from that in bacterial infections; however, the net losses may be the same. In viral infections, water is predominantly lost from the extracellular fluid due to impaired adsorption and osmotic loss primarily due to the presence of undigested lactose in the lumen rather than to active secretion (Lewis and Philips, 1972; Philips and Lewis, 1973; Tennant et al., 1978; Graham et al., 1982). However, replacement of mature adsorptive cells with immature cells, which retain some of their secretory functions, also increases the rate of secretion (Pensaert et al., 1970; Butler et al., 1974; Kerzner et al., 1977). As the virus kills the adsorptive cells there is also a loss of enzymes which are responsible for digestion of disaccharides. Furthermore, loss of differentiated villous cells diminishes glucose, sodium carrier and (Na^+-K^+)-ATPase activities, which result in a loss of sodium, potassium, chloride, bicarbonates and water. The loss of bicarbonate leads to the development of acidosis. However, acidosis also develops as a result of increased microbial activity in response to fermentation of undigested milk (Lewis and Philips, 1978), as well as the increased lactic acid production and decreased utilization in dehydrated animals (Tennant et al., 1972; Lewis et al., 1975). Acidosis can create a K^+-H^+ ion exchange across the cellular membrane and inhibit cellular functions required for maintaining normal potassium concentration with a net loss of potassium from cells. The next step that occurs is hypoglycemia due to decreased intestinal adsorption, minimal glycogen reserves in young animals, inhibited glyconogenesis and increased glycolysis (Lewis and Philips, 1978). This series of complex pathophysiological changes, if not promptly corrected, results in death.

Effective management of diarrhea requires prompt action to prevent continued loss of fluids and electrolytes. This is most economically achieved by removal of milk from the diet. This reduces the amount of undigested lactose in the lumen and therefore reduces fluid loss and acidosis. Therapy should include administration of balanced electrolyte solutions either orally or by the intravenous route. The use of intravenous fluid replacement and careful monitoring of animals could save a large percentage of severely affected animals; however, the costs are generally too high to recommend this as a standard procedure.

MIXED INFECTIONS

Severity of diarrhea is not only related to the virulence of the infectious agent and the age of the animal but also due to the presence of multiple infections. Only a minority of cases of diarrhea in animals is caused by a single virus pathogen (House, 1978). Furthermore, it has been suggested that even if a single pathogenetic virus is involved in an infection there may be heterogeneity within the pathogen (Sabara et al., 1982; Spencer et al., 1983). Therefore, if two viruses co-infect an animal and have different sites of replication, the combined effect may be much more severe than if they infected the animal individually. This may help explain why it is difficult to reproduce enteric infections in conventional calves with single plaque-purified virus isolates. Another important factor is the presence of viral–bacterial synergistic interactions. There is accumulating evidence that many bacterial infections can be more severe if combined with a virus infection (Runnels et al., 1980; Leece et al., 1982). Thus *Escherichia coli* generally produces scours only during the first few days of life. However, if an animal is infected with, for example, rotavirus or coronavirus, *E. coli* can colonize and produce a more severe disease at a later age (Fig. 180). The exact mechanisms by which this occurs is unknown; however, viruses may alter fluid transport by virtue of infecting some cells. This alteration allows the build-up of toxin by bacteria that are normally nonpathogenic. Thus the combination of toxin build-up and decreased mobility of the intestine results in diarrhea. In addition, reduced adsorption of nutrients occurs as a result of virus infection. This provides a more suitable nutritional environment for bacteria to grow, adhere and secrete more toxin.

Additionally, virus infection may reduce the rate of cell maturation. Since it is known that the physiological state of cells may alter adherence, it can be postulated that bacteria actually colonize virus-infected intestines but not normal ones. The virus infection may alter the cells in such a way as to allow direct attachment of bacteria to the viral glycoprotein expressed on their membranes or to altered host cell glycoproteins, as has been convincingly shown for virus–bacterial interactions in the respiratory tract (Sanford et al., 1978; Davison and Sanford, 1981).

Finally, some viruses induce Fc receptors on the surface of host cells. If this occurs, antibody-coated bacteria can bind via the Fc receptor and anchor to the cell, allow secretion of toxin, activation of cyclic AMP and increased fluid loss. Although herpesviruses are the only viruses reported to induce Fc receptors (Westmoreland and Watkins, 1974; Lehner et al., 1975; Costa et al., 1977), preliminary evidence suggests that bovine coronaviruses may also induce them (L.A. Babiuk, unpublished data, 1985).
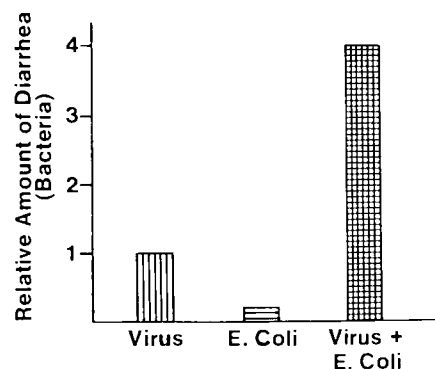
Fig. 180. Effect of the combined infection of virus plus bacteria (*E. coli*) on bacterial shedding and diarrhea.

IMMUNITY AND IMMUNOLOGICAL CONTROL OF GASTROENTERIC VIRUS INFECTIONS

A major problem with controlling gastroenteric infections in animals is the age at which the animals get the disease. Even if the adult animals are immune and transfer antibody to the young, the antibody must be present continuously in the lumen of the intestine to prevent infection, since serum antibodies are not protective (Woode et al., 1975; Snodgrass and Wells, 1976, 1978a,b). Since many mammalian species do not continue to secrete high levels of antibody in their milk after parturition, the antibody in the intestinal lumen drops rapidly and the young becomes fully susceptible even if it has acquired high levels of serum antibody. Thus, in cattle, the colostrum generally has high antibody levels to most enteric viruses, since the infection rate in adults is high. However, within 5–7 days after parturition antibody levels drop below the threshold required to neutralize virus in the lumen. This is the reason that most enteric virus infections causing neonatal diarrhea in mammals do so after 1 week of age.

The observed requirement for local immunity has stimulated the interest in immunizing the newborn. There is presently an oral vaccine on the market for use in newborn calves to provide protection against rotaviruses and coronaviruses. Unfortunately, if the colostral antibodies can protect against virulent virus they will also prevent the attachment of vaccine virus to enterocytes. Thus the supposed early nonspecific protection, possibly by interferon and the later specific immunological protection do not occur unless the animals are immunized prior to ingestion of colostrum. This is often not possible and, therefore, this vaccine has not proven to be as successful as hoped. To overcome this problem a few attempts have been made at in utero immunization but this is impractical at present under field situations (Newman et al., 1978). However, recent advances may make this approach feasible in the near future.

The most recent trend to overcome the requirement of local immunity in gastrointestinal virus infection is hyperimmunization of the dam. This results in a much higher initial level of antibody in the colostrum, which provides excellent early protection. More importantly, even though antibody levels drop they remain above a threshold level which is protective against normal virus challenge doses. The final method of providing high levels of antibody in the lumen is by feeding monoclonal antibody to the animal. This has proven to be very effective in preventing *E. coli* induced diarrhea in calves (Sherman et al., 1982). The combination of various antiviral monoclonal antibodies with anti-*E. coli* antibody should prove effective under certain situations but is probably of limited value in field situations where animals cannot be handled routinely. Furthermore, the presence of serotypes, especially in rotaviruses (Woode et al., 1983) dictates that each serotype is represented either in the vaccine or in the monoclonal antibody mixture.

The recent advances in recombinant DNA technology have great potential for helping control gastroenteric infections in animals. They are especially relevant to producing vaccines against viruses that do not replicate well in culture. Identification of the antigens involved in protection and the genes coding for them should make it feasible to produce sufficient antigen for immunization. Furthermore, it should be possible to identify the sequences involved in protection and synthesize them (Lerner, 1983) for immunization of dams during pregnancy so as to elevate colostral and milk antibodies. The problem of multiple serotypes combined with multiple agents that can cause diarrhea in ruminants emphasizes the need for inclusion of many agents in a vaccine before a great decrease in disease incidence will be seen. In this regard economical production of these vaccines is mandatory.

A final method of reducing enteric infections is by proper management. Since it is assumed that infections either occur as a result of virus shedding from small numbers of adults or from virus in the environment, animals should not be crowded into contaminated areas. Movement of young into clean environments, away from other animals, will greatly reduce the rate of infection and economic loss. Finally, if only a limited number of animals are carriers, it may be possible to eliminate these and thus break the infection cycle. However, this hypothesis is in need of proper testing.

CONCLUSIONS

Although there is a wide variety of viruses that can cause infections of the gastrointestinal tract of animals, most of them are localized in either the crypts or the enterocytes. Infections are initiated in the proximal part of the small intestine and progress sometimes to the colon. Infections result in loss of adsorptive cells, villous atrophy, fluid loss and ion imbalance. These pathophysiological events lead to anorexia, dehydration and death. Since young animals do not have large reserves of fluids and glycogen, mortality can reach 50–80% in severe outbreaks. However, removal of milk and administration of oral electrolytes can significantly reduce losses.

Effective immunization requires that local immunity is present at an early age. Oral immunization with live attenuated vaccines is difficult due to the high levels of maternal antibody in the milk during the first few days of life. To overcome this problem the present trend is to immunize the dam so as to increase the level of antibody in milk above the threshold level required to prevent infection. As more agents and serotypes involved in gastroenteritis are identified, vaccines will have to combine various pathogens and serotypes for protection. Although in some cases vaccines may be produced by conventional methods, recombinant DNA technology may aid in providing sufficient quantities of antigens to vaccinate against viruses that do not replicate well in culture. However, it will be more difficult to test the efficacy of these vaccines under field conditions than that of many other vaccines due to the difficulty in reproducing the disease and its variable incidence from year to year in herds under natural conditions.

REFERENCES

Bachmann, P.A. and Hess, R.G., 1982. Comparative aspects of pathogenesis and immunity in animals. In: D.A.J. Tyrell and A.Z. Kapikian (Editors), Viral Infections of the Gastrointestinal Tract. Marcel Dekker, New York, pp. 361–397.

Benfield, D.A., Stotz, I., Moore, R. and McAdaragh, J.P., 1982. Shedding of rotavirus in feces of sows before and after farrowing. J. Clin. Microbiol., 16: 186–190.

Butler, D.G., Gall, D.G., Kelly, M.H. and Hamilton, J.R., 1974. Transmissible gastroenteritis: Mechanisms responsible for diarrhea in acute viral enteritis in piglets. J. Clin. Invest., 53: 1335–1340.

Carpio, H.M., Babiuk, L.A., Misra, V. and Blumenthal, R.M., 1981. Bovine rotavirus cell interactions: Effect of virus infection on cellular integrity and macromolecular synthesis. Virology, 113: 36–47.

Cheever, F.S. and Mueller, J.H., 1948. Epidemic diarrheal disease of suckling mice: III The effect of strain, litter and season upon the incidence of the disease. J. Exp. Med., 88: 309–316.

Costa, J., Rabson, A.S., Lee, C. and Tralka, T.S., 1977. Immunoglobulin binding to herpesvirus-induced Fc receptors inhibits virus growth. Nature, 269: 251–252.

Crouch, C.F., Bielefeldt Ohmann, H., Watts, T.C. and Babiuk, L.A., 1985. Chronic shedding of bovine enteric coronavirus antigen–antibody complexes by clinically normal cows. J. Gen. Virol., 66: 1489–1500.

Davison, V.E. and Sanford, B.A., 1981. Adherence of *Staphylococcus aureus* to influenza A virus-infected Madin-Darby canine kidney cell cultures. Infect. Immun., 32: 118–126.

Doyle, L.P. and Hutchings, L.M., 1946. A transmissible gastroenteritis in pigs. J. Am. Vet. Med. Assoc., 108: 257–259.

Editorial, 1978. WHO Chron., 32: 369–372.

Flewett, T.H. and Woode, G.N., 1978. The rotaviruses: Brief review. Arch. Virol., 57: 1–23.

Garwes, D.J., 1982. Coronaviruses in animals. In: D.A.J. Tyrell and A.Z. Kapikian (Editors), Virus Infections of the Gastrointestinal Tract. Marcel Dekker, New York, pp. 315–359.

Graham, D.Y., Estes, M.K. and Sackman, J.W., 1982. Rotavirus induces an osmotic diarrhea in miniature swine. Gastroenterology, 82: 1072.

Horzinek, M.C., Weiss, M. and Ederveen, J., 1984. Berne virus is not "Coronavirus-like". J. Gen. Virol., 65: 645–649.

Horzinek, M.C., Flewett, T.H., Saif, L.J., Spaan, W.J.M., Weiss, M. and Woode, G.N., 1987. A new family of vertebrate viruses: Toroviridae. Intervirology, 27: 17–24.

House, J.A., 1978. Economic impact of rotavirus and other neonatal disease agents of animals. Am. J. Vet. Med. Assoc., 173: 573–576.

Jones, R.C., Hughes, C.S. and Henry, R.R., 1979. Rotavirus infection in commercial laying hens. Vet. Rec., 104: 22.

Kahn, D.E., 1978. Pathogenesis of feline panleukopenia. J. Am. Vet. Med. Assoc., 173: 628–630.

Keenan, K.P., Jervis, H.R., Marchwicki, R.H. and Binn, L.N., 1976. Intestinal infection of neonatal dogs with canine coronavirus 1–71: Studies by virological, histological histochemical and immunofluorescent techniques. Am. J. Vet. Res., 37: 247–256.

Kerzner, B., Kelly, M.H., Gall, D.G., Butler, D.G. and Hamilton, J.R., 1977. Transmissible gastroenteritis: Sodium transport and the intestinal epithelium during the course of viral enteritis. Gastroenterology, 72: 457–462.

Kraft, L.M., 1957. Studies on the etiology and transmission of epidemic diarrhea of infant mice. J. Exp. Med., 101: 743–755.

Kraft, L.M., 1958. Observations on the control and natural history of epidemic diarrhea of infant mice (EDIM). Yale J. Biol. Med., 31: 123–137.

Leece, J.G. and King, M.W., 1980. Persistent rotaviral infection producing multiple episodes of diarrhea in weanling pigs reared in isolation. In: S.D. Acres, A.J. Forman and H. Fast (Editors), Proc. 3rd Int. Symp. on Neonatal Diarrhea, Univ. of Saskatchewan, Saskatoon, pp. 21–36.

Leece, J.G., King, M.W. and Mock, R., 1976. Reovirus-like agent associated with fatal diarrhea in neonatal pigs. Infect. Immun., 14: 816–825.

Leece, J.G., Balsbaugh, R.K., Clare, D. and King, M.W., 1982. Rotavirus and hemolytic enteropathogenic *Escherichia coli* in weanling diarrhea of pigs. J. Clin. Microbiol., 16: 715–723.

Lehner, T., Wilton, J.M.A. and Shillitoe, E.J., 1975. Immunological basis for latency, recurrences and putative oncogenicity of Herpes simplex virus. Lancet, 2: 60–62.

Lerner, R.A., 1983. Synthetic vaccines. Sci. Am., 248: 66–84.

Lewis, L.D. and Philips, R.W., 1972. Water and electrolyte losses in neonatal calves with acute diarrhea — A complete balance study. Cornell Vet., 62: 596–607.

Lewis, L.D. and Philips, R.W., 1978. Pathophysiological changes due to Coronavirus-induced diarrhea in the calf. J. Am. Vet. Med. Assoc., 173: 636–642.

Lewis, L.D., Phillips, R.W. and Elliott, E.D., 1975. Changes in plasma glucose and lactate concentrations and enzyme activities in the neonatal calf with diarrhea. Am. J. Vet. Res., 36: 413–416.

Little, L.M. and Shadduck, J.A., 1982. Pathogenesis of rotavirus infection in mice. Infect. Immun., 38: 755–763.

McAdaragh, J.P., Bergeland, M.E., Meyers, R.C., Johnshoy, M.W., Stotz, I.J., Benfield, D.A. and Hammer, R., 1980. Pathogenesis of rotavirus enteritis in gnotobiotic pigs: a microscopic study. Am. J. Vet. Res., 41: 1572–1581.

McNulty, M.S., 1978. Rotaviruses. J. Gen. Virol., 40: 1–18.

McNulty, M.S., Allan, G.M. and Stuart, J.C., 1978. Rotavirus infection in avian species. Vet. Rec., 103: 319–320.

Mebus, C.A., 1978. Pathogenesis of coronaviral infection in calves. J. Am. Vet. Med. Assoc., 173: 631–632.

Mebus, C.A. and Newman, L.E., 1977. Scanning electron, light and immunofluorescent microscopy of intestine of gnotobiotic calf infected with reovirus-like agent. Am. J. Vet. Res., 38: 553–558.

Mebus, C.A., Underdahl, N.R., Rhodes, M.B. and Twiehaus, M.J., 1969. Calf diarrhea (scours): Reproduced with a virus from a field outbreak. College of Agriculture, Univ. Nebraska. Lincoln, NE, Agric. Exp. Stn., Res. Bull. No. 233.

Mebus, C.A., Stair, E.L., Rhodes, M.B. and Tweihaus, M.J., 1973. Pathology of neonatal calf diarrhea induced by a coronavirus-like agent. Vet. Pathol., 10: 45–64.

Middleton, P.J., Szymanski, M.T., Abbott, G.D., Bortolussi, R. and Hamilton, J.R., 1974. Orbivirus acute gastroenteritis of infancy. Lancet, I: 1241–1244.

Misra, V. and Babiuk, L.A., 1980. Possible mechanisms of rotavirus persistence. In: B.N. Fields, R.

Jaenisch and C.F. Fox (Editors), Animal Virus Genetics. Academic Press, New York, pp. 727–738.

Nathanson, N. and Martin, J.R., 1979. The epidemiology of poliomyelitis. Enigmas surrounding its appearance, epidemicity and disappearance. Am. J. Epidemiol., 110: 672–692.

Newman, L.E., Whitehair, C.K. and Mullaney, T.P., 1978. Immune response of the bovine fetus to in utero vaccination with bovine coronavirus. In: S.D. Acres (Editor), Proc. 2nd Int. Symp. Neonatal Diarrhea, Univ. of Saskatchewan, Saskatoon, pp. 457–464.

Pearson, G.R. and McNulty, M.S., 1977. Pathological changes in the small intestine of neonatal pigs infected with a pig Reovirus-like agent (Rotavirus). J. Comp. Pathol., 87: 363–373.

Pensaert, M., Haelterman, E.O. and Hinsman, E.J., 1970. Transmissible gastroenteritis of swine: Virus–intestinal cell interactions. Arch. Ges. Virusforsch., 31: 335–351.

Philips, R.W. and Lewis, L.D., 1973. Viral induced changes in intestinal transport and resultant body fluid alterations in neonatal calves. Ann. Rech. Vet., 4: 87–98.

Robb, J.A. and Bond, C.W., 1979. The Coronaviridae. In: H. Fraenkel-Conrat and R.R. Wagner (Editors), Comprehensive Virology, Plenum Press, New York pp. 193–248.

Runnels, L.P., Moon, H.W., Whipp, S.C., Mathews, P.J. and Woode, G.N., 1980. Interaction of Rotavirus and Enterotoxigenic *Escherichia coli* (ETEC) in gnotobiotic calves. In: S.D. Acres, A.J. Forman and H. Fast (Editors), Proc. 3rd Int. Symp. Neonatal Diarrhea, Univ. of Saskatchewan, Saskatoon, pp. 343–358.

Sabara, M., Deregt, D., Babiuk, L.A. and Misra, V., 1982. Genetic heterogeneity within individual bovine rotavirus isolates. J. Virol., 44: 813–822.

Sanford, B.A., Shelokov, A. and Ramsay, M.A., 1978. Bacterial adherence to virus infected cells: A cell culture model of bacterial superinfection. J. Infect. Dis., 137: 176–181.

Shephard, R.W., Butler, D.G., Cutz, E., Gall, D.G. and Hamilton, J.R., 1979. The mucosal lesion in viral enteritis. Gastroenterology, 76: 770–781.

Sherman, D.M., Acres, S.D., Sadowski, P.L. and Muscoplat, C.C., 1982. Protection of calves against fatal enteropathogenic colibacillosis by orally administered K99 — Specific monoclonal antibody Conf. Res. Workers Anim. Dis., Abst. No. 240.

Snodgrass, D.R. and Wells, P.W., 1976. Rotavirus infection in lambs: Studies on passive protection. Arch. Virol., 52: 201–205.

Snodgrass, D.R. and Wells, P.W., 1978a. The immunoprophylaxis of rotavirus infections in lambs. Vet. Rec., 102: 146–148.

Snodgrass, D.R. and Wells, P.W., 1978b. Passive immunity in rotavirus infections. J. Am. Vet. Med. Assoc., 173: 565–569.

Snodgrass, D.R., Angus, K.W. and Gray, E.W., 1977. Rotavirus infection in lambs: Pathogenesis and pathology. Arch. Virol., 55: 263–274.

Spencer, G., Avendano, L.F. and Garcia, B., 1983. Analysis of human rotavirus mixed electropherotypes. Infect. Immun., 39: 569–574.

Stair, E.L., Rhodes, M.B., White, R.G. and Mebus, C.A., 1972. Neonatal calf diarrhea: Purification and electron microscopy of a coronavirus-like agent. Am. J. Vet. Res., 33: 1147–1156.

Storz, J. and Bates, R.C., 1973. Parvovirus infections in calves. J. Am. Vet. Med. Assoc., 163: 884–886.

Storz, J., Doughri, A.M. and Hajer, I., 1978a. Coronavirus morphogenesis and ultrastructural changes in intestinal infections of calves. J. Am. Vet. Med. Assoc., 173: 633–635.

Storz, J., Leary, J.J., Carlson, J.H. and Bates, R.C., 1978b. Parvovirus associated with diarrhea in calves. J. Am. Vet. Med. Assoc., 173: 624–627.

Tennant, B., Harrold, D. and Reina-Guerra, M., 1972. Physiological and metabolic factors in the pathogenesis of neonatal enteric infections in calves. J. Am. Vet. Med. Assoc., 161: 993–1007.

Tennant, B., Ward, D.E., Braun, R.K., Hunt, E.L. and Baldwin, B.H., 1978. Clinical management and control of neonatal enteric infections of calves. J. Am. Vet. Med. Assoc., 173: 654–661.

Theil, K.W., Bohl, E.H., Cross, R.F., Kohler, E.M. and Agnes, A.G., 1978. Pathogenesis of porcine rotavirus infection in experimentally inoculated gnotobiotic pigs. Am. J. Vet. Res., 39: 213–220.

Von Bonsdorff, C.H., Hovi, T., Makela, P., Hovi, L. and Tevalvoto-Aarnio, M., 1976. Rotavirus associated with acute gastroenteritis in adults. Lancet, 2: 423.

Westmoreland, D. and Watkins, J.J., 1974. The IgG receptor induced by Herpes simplex virus: Studies using radio-iodinated IgG. J. Gen. Virol., 24: 167–178.

Woode, G.N., 1982. Rotaviruses in animals. In: D.A.J. Tyrell and A.Z. Kapikian (Editors), Virus Infections of the Gastrointestinal Tract. Marcel Dekker, New York, pp. 295–311.

Woode, G.N. and Bridger, J.C., 1975. Viral enteritis in calves. Vet. Rec., 96: 85–88.

Woode, G.N. and Bridger, J.C., 1978. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. J. Med. Microbiol., 11: 441–452.

Woode, G.N. and Crouch, C.F., 1978. Naturally occurring and experimentally induced rotaviral infections of domestic and laboratory animals. J. Am. Vet. Med. Assoc., 173: 522–526.

Woode, G.N., Jones, J. and Bridger, J., 1975. Levels of colostral antibodies against neonatal calf diarrhoea virus. Vet. Rec., 97: 148–149.

Woode, G.N., Bridger, J., Hall, G.A., Jones, J.M. and Jackson, G., 1976a. The isolation of reovirus-like agents (rotavirus) from acute gastroenteritis in piglets. J. Med. Microbiol., 9: 203–209.

Woode, G.N., Bridger, J.C., Jones, J.M., Flewett, T.J., Bryden, A.S., Davies, H.A. and White, G.B.B., 1976b. Morphological and antigenic relationships between viruses (Rotaviruses) from acute gastroenteritis of children, calves, piglets, mice and foals. Infect. Immun., 14: 804–810.

Woode, G.N., Reed, D.E., Runnels, P.L., Herrig, M.A. and Hill, H.T., 1982. Studies with an unclassified virus isolated from diarrheic calves. Vet. Microbiol., 7: 221–240.

Woode, G.N., Kelso, N.E., Simpson, T.F., Gaul, S.K., Evans, L.E. and Babiuk, L.A., 1983. Antigenic relationships among some bovine rotaviruses, serum neutralization and cross protection in gnotobiotic calves. J. Clin. Microbiol., 18: 358–364.

Yolken, R.H., 1982. Enzyme immunoassays for detecting human rotavirus. In: D.A.J. Tyrell and A.Z. Kapikian (Editors), Virus Infections of the Gastrointestinal Tract. Marcel Dekker, New York, pp. 51–87.

Subject Index

Editors' Addendum

Bovine Spongiform Encephalopathy

Bovine spongiform encephalopathy (BSE) of domestic adult cattle is a new form of progressive degenerative encephalopathy occurring on farms throughout Great Britain. The disease was first described by Wells et al. (1987), who found the clinical signs, neuropathology and the presence of fibrils in affected brains as reminiscent of scrapie in sheep. The agent of BSE could be transmitted with brain homogenates from sick cattle to mice, which fell ill 292–342 days after intracerebral inoculation showing scrapie-like signs and lesions (Fraser et al., 1988). BSE is thus a transmissible disease and its agent is scrapie-like. Changes in offal rendering towards low-temperature systems over the last 10 years have favored the survival and spread of scrapie agent in ovine meat and bone meal as supplements of foodstuff for cattle (Morgan, 1988). The epidemic of BSE had thus an extended common source, in which all infected animals were index cases (Wilesmith et al., 1988). The scrapie-like agent is probably ingested within the first 6 months of life, and the infection is then followed by a 4–5-year period before clinical signs appear (Winter et al., 1989). The data from extensive studies showed that BSE itself is not simply inherited but that an individual inheritance of susceptibility is most probable (Wijeratne and Curnow, 1990).

After an insiduous onset, the disease signs are similar to those of scrapie in sheep, i.e. behavioral disorder, gait ataxia, paresis, loss of body weight and, occasionally, pruritus. The disease has a progressive course, necessitating slaughter in 1–6 months (Wells et al., 1987; Wilesmith et al., 1988). At histopathological examination, the principal lesion consisted of bilaterally symmetrical degenerative changes in certain brain stem grey matter locations in the form of neuronal vacuolation (Wells et al., 1987).

Electron microscopy of appropriately processed, negatively stained brain homogenates revealed the presence of scrapie-associated fibrils (SAF), considered unique for slow progressive encephalopathies caused by "unconventional viruses" (Wells et al., 1987; Hope et al., 1988). It is well-known that SAF are aggregates of a protein that can partially be degraded by proteinase K to result in a resistant form of 27–30 k. The protein is called SAF protein or prion protein (PrP). In a normal brain, there is the isoform of PrP which does not aggregate to fibrils and is completely degraded by proteinase K. Antisera raised in rabbits against the PrP from scrapie in sheep or mice react with the PrP in immunoblotting assays, but not with the completely degraded isoform from normal brain. Since such antisera reacted positively also with the PrP from a cow terminally affected with BSE, they should be used in post-mortem routine laboratory testing for scrapie and BSE (Farquhar et al., 1989).

BSE is a notifiable disease. Its agent belongs to "unconventional viruses" known to be extremely resistant to inactivation by standard decontamination

procedures. Since BSE is obviously a consequence of feeding cattle with scrapie-infected supplements of foodstuff, the avoidance of such supplements appears to be the major prophylactic measure (reviewed by Taylor, 1989). It remains to be seen whether "the recently introduced (July 1988) suspension of the inclusion of ruminant-derived animal protein in ruminant feedstuffs (Order 1988)" will result in reduced incidence of BSE (Wilesmith et al., 1988).

REFERENCES

Farquhar, C.F., Somerville, R.A. and Ritchie, L.A., 1989. Post-mortem immunodiagnosis of scrapie and bovine spongiform encephalopathy. J. Virol. Meth., 24: 215–222.

Fraser, H., McConnell, I., Wells, G.A.H. and Dawson, M., 1988. Transmission of bovine spongiform encephalopathy to mice. Vet. Rec., 123: 472.

Hope, J., Reckie, L.J.D., Hunter, N., Multhaup, G., Beyreuther, K., White, H., Scott, A.C., Stack, M.J., Dawson, M. and Wells, G.A.H., 1988. Fibrils from brains of cows with new cattle disease contain scrapie-associated protein. Nature, 336: 390–392.

Morgan, K.L., 1988. Bovine spongiform encephalopathy: time to take scrapie seriously. Vet. Rec., 122: 445–446.

Taylor, D.M., 1989. Scrapie agent decontamination: implications for bovine spongiform encephalopathy. Vet. Rec. 124: 291–292.

Wells, G.A.H., Scott, A.C., Johnson, C.T., Gunning, R.F., Hancock, R.D., Jeffrey, M., Dawson, M. and Bradley, R., 1987. A novel progressive spongiform encephalopahty in cattle. Vet. Rec., 121: 419–420.

Wijeratne, W.V.S. and Curnow, R.N., 1990. A study of the inheritance of susceptibility to bovine spongiform encephalopathy. Vet. Rec., 126: 5–8.

Wilesmith, J.W., Wells, G.A.H., Cranwell, M.P. and Ryan, J.B.M., 1988. Bovine spongiform encephalopathy: epidemiological studies. Vet. Rec., 123: 638–644.

Winter, M.H., Aldridge, B.M., Scott, P.R. and Clarke, M., 1989. Occurrence of 14 cases of bovine spongiform encephalopathy in a closed dairy herd. Br. Vet. J., 145: 191–194.