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Review article

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Abstract – Border disease (BD) is a congenital virus disease of sheep and goats first reported in 1959 from the border region of England and Wales. BD virus (BDV) is a pestivirus in the genus Flaviviridae and is closely related to classical swine fever virus and bovine virus diarrhoea virus (BVDV). Nearly all isolates of BDV are non-cytopathogenic (ncp) in cell culture. There are no defined serotypes but pestiviruses isolated from sheep exhibit considerable antigenic diversity and three distinct antigenic groups have been identified. Distribution of the virus is world-wide. Prevalence rates vary in sheep from 5 to 50 % between countries and from region-to-region within countries. The disease in goats is rare and characterized by abortion. Clinical signs in sheep include barren ewes, abortions, stillbirths and the birth of small weak lambs. Affected lambs can show tremor, abnormal body conformation and hairy fleeces (so-called 'hairy-shaker' or 'fuzzy' lambs). Vertical transmission plays an important role in the epidemiology of the disease. Infection of fetuses can result in the birth of persistently infected (PI) lambs. These PI lambs are viraemic, antibody negative and constantly excrete virus. The virus spreads from sheep to sheep with PI animals being the most potent source of infection. Apparently healthy PI sheep resulting from congenital infection can be identified by direct detection of viral antigen or viral RNA in leukocytes or by isolation of ncp virus from blood or serum in laboratory cell cultures. Isolation of virus is unreliable in lambs younger than 2 months old that have received colostral antibody. The isolation of virus from tissues of aborted or stillborn lambs is difficult but tissues from PI sheep contain easily detectable levels of virus. To detect the growth of virus in cell cultures it is essential to use an immune-labelling method. Acute infection is usually subclinical and viraemia is transient and difficult to detect. Sheep may also be infected following close contact with cattle excreting the closely related BVDV. © Inra/Elsevier, Paris

border disease / border disease virus / pestivirus / sheep / goat

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Résumé – Border disease ou hypomyélinogenèse congénitale chez le mouton et la chèvre. La « *border disease* » (BD) est une maladie virale congénitale qui sévit chez le mouton et la chèvre. Elle a été décrite pour la première fois en 1959 à la frontière (en anglais, *border*, d'où le nom de la maladie, NDLR) entre l'Angleterre et le Pays de Galles. Le virus de la BD (VBD) est un pestivirus du genre Flaviviridae qui est fortement apparenté au virus de la peste porcine classique et au virus de la diarrhée bovine virale (VDBV). Presque tous les isolats de VBD sont non-cytopathogènes (ncp) en culture cellulaire. Il n'y a pas de sérotype défini, mais il existe une diversité antigénique considérable entre les différents pestivirus isolés du mouton, et trois groupes antigéniques distincts ont été identifiés. Le virus se retrouve dans le monde entier. Les taux de prévalence chez le mouton varient de 5 à 50 % selon le pays et même d'une région à l'autre dans un même pays. La maladie chez la chèvre est rare et se caractérise par un avortement. Les signes cliniques chez le mouton incluent stérilité, avortements, mortinatalité, et naissance d'agneaux petits et faibles. Les agneaux affectés peuvent avoir des tremblements, une constitution physique anormale, et un pelage très fourni. La transmission verticale joue un rôle important dans l'épidémiologie de la maladie. L'infection de fœtus peut entraîner la naissance d'agneaux infectés de manière persistante (IP). Ces agneaux IP sont virémiques, séronégatifs, et excrètent constamment le virus. Le virus se répand d'un mouton à l'autre, les animaux IP étant la source d'infection la plus importante. Les moutons IP, apparemment sains, mais infectés in utero, peuvent être identifiés par la détection directe d'antigène viral, ou d'ARN viral dans les leucocytes, ou par l'isolement de virus ncp à partir du sang ou du sérum dans les cultures cellulaires de laboratoire. L'isolement du virus n'est pas fiable chez les agneaux de moins de 2 mois qui ont reçu les anticorps du colostrum. L'isolement du virus à partir de tissus d'agneaux avortés ou mort-nés est difficile, mais les tissus de moutons IP contiennent des niveaux de virus facilement détectables. Afin de détecter la croissance du virus dans les cultures cellulaires, il est essentiel d'utiliser une technique d'immuno-marquage. L'infection aiguë est généralement subclinique, et la virémie est transitoire et difficile à détecter. Les moutons peuvent également être infectés après un contact avec des bovins excrétant le virus, apparenté, de la diarrhée bovine virale. © Inra/Elsevier, Paris

border disease / **hypomyélinogenèse congénitale / pestivirus / mouton / chèvre**

Plan

1. INTRODUCTION

Border disease in sheep and goats is caused by the pestivirus, border disease virus (BDV). BDV spreads naturally by the oro-nasal route and by vertical transmission. It is a cause of congenital disease in sheep and goats but can also cause acute and persistent infections. This paper will principally describe BDV infection in sheep. Infection in goats is rare and will only be described where it is known to differ from infection in sheep. Comprehensive reviews of the elucidation of the cause and pathology of the disease have been published (Barlow and Patterson, 1982; Terpstra 1985). Major advances since then have been in the molecular structure of the virus and the relationships between sheep isolates and pestiviruses from other species (Nettleton and Entrican, 1995; Paton, 1995; Thiel et al., 1996).

2. VIRUS PROPERTIES

The genus pestivirus within the family Flaviviridae has been divided into classical swine fever virus (CSFV), bovine virus diarrhoea virus (BVDV) and border disease virus (BDV). The viruses were named after the diseases from which they were first isolated and traditionally pestiviruses isolated from pigs have been termed CSFV, those from cattle BVDV and those from sheep BDV. It is now known that cross-infection between species occurs readily and viruses have consequently been grouped more according to their reac-

tivity with monoclonal antibodies and to their nucleotide sequences at selected genomic regions. Viruses have also been tested by cross-neutralization experiments with a view to identifying the best viruses for candidate vaccines.

Pestiviruses are enveloped, spherical particles approximately 50 nm in diameter. The pestivirus genome is a positive single-stranded RNA molecule, approximately 12.5 kb long. There is a single open reading frame (ORF) flanked by a 5'-non-coding region (5'-NC) of 356–385 bases and a 3'-non-coding region (3'-NC) of 223–228 bases (figure 1). The ORF encodes proteins composed of 4 000 amino acids processed by viral and cellular enzymes. Of the proteins within the ORF the first protein is a non-structural autoprotease N^{pro} followed by the structural C nucleocapsid protein and glycoproteins, E^{rns}, E1 and E2. Of the glycoproteins, E2 is the immunodominant major envelope protein. The remaining proteins are non-structural of which NS2-3 has attracted most interest owing to its role in cytopathogenity of pestivirus isolates. Virtually all pestivirus isolates from sheep and goats are non-cytopathic in cell culture. Two cytopathic sheep isolates have been described, however, and in both these it has been shown that they contain insertions of cellular sequence within the NS2-3 encoding region which results in its cleavage to NS2 and NS3 (Becher et al., 1996). This is analogous to the similar process in BVDV viruses which is associated with the development of mucosal
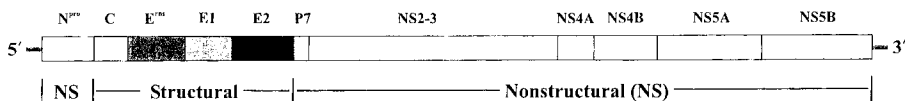
Figure 1. Diagrammatic representation of final protein products of a non-cytopathic pestivirus. In cells infected with a cytopathic pestivirus NS2-3 is detectable as are its products NS2 and the conserved, immunodominant NS3.

disease in cattle. Such cattle are persistently infected with non-cytopathic (NCP) BVDV following in-utero infection. Mutation of the persisting virus RNA in the region coding for NS2-3 can result in overproduction of NS3 which is correlated with the development of mucosal disease.

From studies with monoclonal antibodies and phylogenetic analysis of genomic sequences from the 5'-NC, N^{pro}, C and E2 coding regions a consensus is emerging that there are four principal genotypes of pestiviruses: BVDV-1, BVDV-2, BDV and CSFV (Paton et al., 1994; Becher et al., 1995; Tijssen et al., 1996). Two pestiviruses, one from a deer and one from a giraffe, do not fit into any of these groups and constitute separate virus types (Van Rijn et al., 1997).

While CSF viruses are predominantly restricted to pigs, examples of the other three genotypes have all been recovered from sheep (Vilcek et al., 1997). Examination of three goat isolates has shown them to be BVDV-1 types (Becher et al., 1997).

The complete genomic sequence of the American BDV reference strain, BD-31, has recently been published (Ridpath and Bolin, 1997). When compared with the available complete genomic sequences of other pestiviruses the predicted amino-acid sequence identity varied from 71 (BVDV-1) to 78 % (CSFV). Phylogenetic analysis segregated the pestiviruses into two branches, one containing BD31 and the CSFV strains and one containing BVDV-1 and BVDV-2 strains. The regions of highest sequence identity were in the 5'-NC and the non-structural virus polypeptide NS3. Comparison of the BD31 sequence with incomplete sequences from nine other BDVs showed close homology with five including the Australian reference strain X818 and the UK Moredun reference strain. Of the remaining four viruses, three were more similar to viruses from the BVDV-2 geno-

type and one (R2727) was more similar to viruses from the BVDV-1 genotype.

There are relatively few reports of cross-neutralization experiments involving pestiviruses from sheep and goats. Nevertheless four principal serological groups have been identified (Wensvoort et al., 1989), which correlate with the monoclonal antibody and genotype groupings (Paton et al., 1995). As with the genotyping results the giraffe and deer isolates are serologically distinct from the other four groups (Dekker et al., 1995).

Due to the paucity of cross-neutralization results with sheep pestiviruses we selected ten non-cytopathic isolates from different regions of the UK and compared them with a cattle pestivirus. The source of the viruses is shown in *table I*. All viruses were plaque-purified three times and pairs of pestivirus-naïve lambs were infected intranasally with 5×10^7 $TCID_{50}$ of each virus. Serum was collected from each lamb 10 weeks later and tested in micro neutralization tests against approximately 100 $TCID_{50}$ of all the viruses. All the lambs seroconverted, except one infected with R2727, and produced neutralizing antibody titres between 256 and 2880 against their homologous virus. The geometric mean titres of neutralizing antibody produced by pairs of sheep are shown in *table II*. It can be seen that the BD viruses are serologically related but there is a spectrum of antigenic cross-reactivity, with two clusters of more strongly related strains. Thus G1480, JH2816, A1870, L991 and G2048 (The Moredun BDV group) appear to be closely related to each other but distinct from the second group containing D861, B1056 and Weybridge (The Weybridge BDV group). The two strains G1305 and R2727 do not immediately fall into either group. The coefficient of antigenic similarity (R value) for the 11 viruses was determined as described previously (Howard et al., 1987; Nettleton, 1987). The closer the value of R is to

Table I. Designation and origin of pestivirus strains selected for cross-neutralization studies in sheep.

Strain	Isolation	Origin
Moredun(G1480) Reference strain	1976	Scotland-Lothian
Weybridge Reference strain	1977	England (Harkness et al., 1977)
A1870	1980	Wales-Aberystwyth
B1056	1980	Scotland-Borders (Vantsis et al., 1980)
D861	1983	England-Cumbria
G1305	1986	Scotland-Grampian (Nettleton, 1987)
G2048	1986	Scotland-Strathclyde (Bonniwell et al., 1987)
JH2816	1985	England (Brockman et al., 1988)
L991	1988	England-Cheshire
R2727	1988	England (Brockman et al., 1988)
KY1203	1984	England (Howard et al., 1987)

100 the more closely related are the strains. Values of $R \geq 25$ depict a less than four-fold difference between strains which is not significantly different within the conditions of the neutralization tests. A value of $R < 5$ signifies a > 20-fold reciprocal difference between the homologous and heterologous titres, and has been used to define virus serotypes.

The R values shown in *table III* emphasize further the two groups of serologically distinguishable BDV isolates, the one group related to Moredun BDV and the other to the Weybridge and BVD viruses. These antigenic differences correlate well with genotyping results since all the Moredun BDV related isolates type as true BDVs whereas representatives of the other group all fit into what is now known as the BVDV-1 genogroup (Becher et al., 1994; Vilcek et al., 1994). These results together with recent genotyping studies on a total of 38 UK sheep isolates show that 23 (60 %) are true BD viruses and

10 (26 %) belong to the BVDV-1 group. A further five isolates (13 %) belong to the BVDV-2 group (Vilcek et al., 1997).

The relevance of all these typing results to vaccine development requires further work. In particular, there is a dearth of cross-protection studies. The only cross-protection test published used field brain material to infect and challenge pregnant ewes. The viruses recovered from these field outbreaks were G1480 (Moredun reference strain) a true BD virus and B1056 a BVDV-1 isolate. In that thorough experiment 12 pregnant ewes previously exposed to BVDV-1 were challenged intramuscularly with heterologous BD virus on the 54th day of gestation; 11 ewes (92 %) had diseased lambs. A further 11 pregnant ewes previously exposed to BDV were similarly challenged with the heterologous BVDV-1 strain; one ewe aborted and five had diseased progeny, i.e. 55 % of the ewes had diseased lambs. In contrast, similar sized groups of

Table II. Geometric mean titres of neutralizing antibody produced by pairs of sheep against each of 10 BD viruses and 1 BVD virus (KY1203). Titres for the R2727 antiserum are from a single sheep.

Antiserum	G1480	JH2816	A1870	L991	G2048	G1305	R2727	D861	B1056	Wey	KY1203
Antigen											
G1480	1 214	255	256	256	128	128	180	27	27	7	23
JH2816	304	724	430	360	128	76	90	27	16	9	13
A1870	607	304	2 036	256	255	128	256	11	152	27	38
L991	512	304	215	1 018	181	90	128	44	54	19	38
G2048	1 448	512	430	360	859	255	90	22	38	27	32
G1305	128	54	54	107	76	362	32	11	16	8	16
R2727	724	152	64	215	27	215	2 048	304	362	256	152
D861	360	107	76	90	128	107	1 024	1 024	429	256	429
B1056	27	16	8	19	6	5	180	64	512	45	255
Wey	107	64	32	90	23	27	512	215	215	430	255
KY1203	360	96	45	215	54	46	512	859	607	256	1 018

Table III. Coefficient of antigenic similarity (R) for BDV isolates and BVD virus KY1203.

Antigen	Antiserum G1480	JH2816	A1870	L991	G2048	G1305	R2727	D861	B1056	Wey	KY1203
G1480	100										
JH2816	30	100									
A1870	25	30	100								
L991	33	39	16	100							
G2048	42	32	25	27	100						
G1305	19	13	10	16	25	100					
R2727	23	10	6	11	4	10	100				
D861	9	6	2	6	6	8	39	100			
B1056	3	3	3	4	2	2	27	23	100		
Wey	4	4	3	6	4	4	39	35	21	100	
KY1203	8	4	3	9	4	4	19	59	54	39	100

immune ewes all had normal lambs when challenged with the homologous virus to which they had been previously exposed (Vantsis et al., 1980).

This result could be expected given the R values of the two viruses in *table III* and would imply that any BD vaccine should contain at least one representative from the BDV and BVDV-1 groups. The protection between BVDV-2 and these other two sheep-infecting groups will need to be studied.

3. CLINICAL DISEASE

3.1 Acute infections

Healthy newborn and adult sheep exposed to BDV experience only mild or inapparent disease. Slight fever and a mild leucopaenia are associated with a short-lived viraemia detectable between days 4 and 11 post-infection after which serum neutralizing antibody appears.

Occasional BDV isolates have been shown to produce high fever, profound and prolonged leucopaenia, anorexia, conjunctivitis, nasal discharge, dyspnoea and diarrhoea, and 50 % mortality in young lambs. One such isolate was recovered from a severe epidemic of Aveyron disease among milk sheep in France in 1984 (Chappuis et al., 1986). A second such isolate was a BDV contaminant of a live CSFV vaccine (Wensvoort and Terpstra, 1988).

3.2. Fetal infection

The main clinical signs of BD are seen following the infection of pregnant ewes. While the initial maternal infection is subclinical or mild the consequences for the fetus are serious. Fetal death may occur at any stage of pregnancy but is commoner in fetuses infected early in gestation. Small dead fetuses may be resorbed or their abortion pass unnoticed since the ewes continue to feed well and show no sign of discomfort. As lambing time approaches, the abortion of larger fetuses, stillbirths and the premature births of small, weak lambs will be seen. The infection of pregnant goats produces severe placentitis and high death rates among kid fetuses (Barlow and Patterson, 1982).

During lambing, an excessive number of barren ewes will become apparent but it is the diseased live lambs that present the main clinical features characteristic of BD. The clinical signs exhibited by BD lambs are very variable and depend on the breed of sheep, the virulence of the virus and the time at which infection was introduced into the flock (Barlow and Patterson, 1982; Bonniwell et al., 1987; Roeder et al., 1987). Affected lambs are usually small and weak, many being unable to stand. Nervous signs and fleece changes are often apparent. The nervous symptoms of BD are its most characteristic feature. The tremor can vary from violent rhythmic contractions of the muscles of the hind legs and back to barely detectable fine trembling of the head, ears and tail. Fleece abnormalities are most obvious in smooth-coated breeds which have hairy fleeces especially on the neck and back. Abnormal brown or black pigmentation of the fleece may also be seen in BD-affected lambs.

With careful nursing a proportion of BD lambs can be reared although deaths may occur at any age. The nervous symptoms gradually decline and can have disappeared by 3 to 6 months of age. Weakness, swaying of the hind-quarters together with fine trembling of the head may reappear at times of stress. Affected lambs often grow slowly and under normal field conditions many will die before or around weaning time. Occasionally this is the first presenting sign of disease when losses at lambing time have been low and no lambs

with obvious symptoms of BD have been born.

Some fetal infections occurring around mid-gestation can result in lambs with severe nervous signs, locomotor disturbances and abnormal skeletons. Such lambs have lesions of cerebellar hypoplasia and dysplasia, hydranencephaly and porencephaly resulting from necrotizing inflammation. The severe destructive lesions appear to be immune mediated, and lambs with such lesions frequently have high concentrations of serum antibody to BDV. Most lambs infected in late gestation are normal and healthy and are born free of virus but with BDV antibody. Some such lambs, however, can be stillborn or weak and many die in early life.

3.3. Persistent viraemia

When fetuses are infected before they have any immune system and survive they are born with a persistent viraemia. The ovine fetus can first respond to an antigenic stimulus between approximately 60 and 85 d of its 150-d gestation period. In fetuses infected before the onset of immune competence viral replication is uncontrolled and 50 % fetal death is common. In lambs surviving infection in early gestation, virus is widespread in all organs. Such lambs appear to be tolerant to the virus and have a persistent infection usually for life. A precolostral blood sample will be virus positive and antibody negative. Typically, there is no inflammatory reaction and the most characteristic pathological changes are in the central nervous system (CNS) and skin. At all levels in the CNS there is a deficiency of myelin. This may be slight in lambs with mild or no symptoms but is severe in lambs with pronounced nervous symptoms. In the skin there is an increased size of primary wool follicles and fewer secondary wool follicles causing the hairy or coarse fleece.

Persistently viraemic sheep can be diagnosed by virus isolation/detection in a blood sample. Viraemia is readily detectable at any time except in the first 2 months of life when virus is masked by colostral antibody and in animals older than 4 years old some of which develop low levels of anti-BDV antibody (Nettleton et al., 1992). Although virus detection in blood during an acute infection is difficult, persistent viraemia should be confirmed by retesting animals after an interval of at least 3 weeks.

Some viraemic sheep survive to sexual maturity and are used for breeding. Lambs born to these infected dams are always persistently viraemic. Persistently viraemic sheep are a continual source of infectious virus to other animals and their identification is a major factor in any control programme. Sheep being traded should be screened for the absence of BDV viraemia.

Rams that are persistently infected (PI) usually have poor quality, highly infective semen and reduced fertility. All rams used for breeding should be screened for persistent BDV infection on a blood sample. Semen samples can also be screened for virus but they are much less satisfactory than blood due to their toxicity for cell cultures.

3.4. Late-onset disease in persistently viraemic sheep

Some persistently infected sheep housed apart from other animals spontaneously develop intractable scour, wasting, excessive ocular and nasal discharges sometimes with respiratory distress. At necropsy such sheep have gross thickening of the distal ileum, caecum and colon resulting from focal hyperplastic enteropathy. Cytopathic BDV can be recovered from the gut of these lambs. With no obvious outside source of cytopathic virus it

is most likely that such virus originates from the lamb's own virus pool. Other persistently infected sheep in the group do not develop the disease. This syndrome, which has also been suspected in occasional field outbreaks of BD, has several similarities with bovine mucosal disease (Gardiner et al., 1983; Nettleton et al., 1992; Monies and Simpson, 1997).

4. EPIDEMIOLOGY

Border disease is widespread in Europe, Australasia and North America and has also been reported from Israel and North Africa. Antibody prevalence rates among adult sheep vary from 5 to 50 % between countries and from region-to-region within countries. The virus is not stable outside the host and its successful transmission is due to spread by PI sheep. Spread within a flock can take years in sheep reared extensively at grass but trough-feeding or other husbandry that allows close nose-to-nose contact will hasten spread. In sheep reared intensively indoors spread from PI animals occurs more readily and serious outbreaks of BD at lambing time can occur in sheep housed together in early pregnancy.

Pestivirus exchange between sheep and cattle occurs readily, and outbreaks of BD have been caused by transmission of virus from cattle to sheep (Carlsson, 1991). No report of natural disease in cattle due to spread of virus from sheep has been documented, but serial experimental exchange of virus between PI cattle and sheep has been investigated. There was a high degree of genetic stability but the expression of one or more epitopes on the E2 glycoprotein appeared to depend on the host species (Paton et al., 1997). There are no reports of BD originating from pigs but an outbreak of CSF-like disease in pigs was shown to have been caused by BDV (Roehe et al., 1992). There remains the theoretical pos-

sibility of sheep and goats developing BD from contact with other species of ruminants since at least 52 species of captive or free-living ruminants are known to be infected by pestiviruses (Nettleton, 1990).

Pestiviruses are important contaminants of modified live virus (MLV) vaccines. All MLV vaccines produced in ovine, bovine or porcine cell cultures or in media supplemented with serum from these species risk being contaminated with pestivirus. Outbreaks of BD have been associated with the use of such vaccines; sheep-pox and orf virus vaccines in sheep and an orf virus vaccine in goats (Nettleton and Entrican, 1995).

5. DIAGNOSIS

5.1. Virus isolation

Pestiviruses are notorious contaminants of laboratory cell cultures with fetal bovine serum being the commonest way it is introduced. It is essential that laboratories undertaking virus isolation have a guaranteed supply of pestivirus-free susceptible cells and fetal bovine or equivalent serum which contains no antipestivirus activity and no contaminating virus. The virus can be isolated in a number of primary or secondary ovine cell cultures (e.g. kidney, testes, lung). Ovine cell lines for BDV growth are rare and commercially unobtainable. Semi-continuous cell lines derived from fetal lamb muscle (FLM) or sheep choroid plexus can be useful but different lines vary considerably in their susceptibility to virus.

From live animals the most sensitive way to confirm pestivirus viraemia is to wash leukocytes three times in culture medium before co-cultivating them with susceptible cells for 7 d. Cells are frozen and thawed once and an aliquot passaged on to further susceptible cells grown on

flying coverslips. Three days later the cells are stained for the presence of pestivirus using an immunofluorescence (IF) or immunoperoxidase (IP) test.

From dead animals, tissues should be collected as 10 % weight by volume in virus transport medium. In the laboratory they are ground up, centrifuged to remove debris and the supernatant passed through 0.45-μm filters. Spleen, thyroid, thymus, kidney, brain, lymph nodes and gut lesions are the best organs for virus isolation.

Semen can be examined for the presence of BDV but raw semen is strongly cytotoxic and must be diluted usually at least 1:10 in culture medium. Since the major threat of BDV-infected semen is from PI rams, blood is a more reliable clinical sample than semen for identifying such animals.

5.2. ELISA for antigen detection

The first ELISA for pestivirus antigen detection was described for detecting viraemic sheep. This has now been modified into a double monoclonal antibody (mab) capture ELISA for use in sheep and cattle. Two capture mabs are bound to wells in microtitre plates and two other mabs conjugated to peroxidase serve as detector mabs (Entrican et al., 1995). The test is most commonly employed to identify persistently infected viraemic sheep using washed, detergent-lysed blood leukocytes. The sensitivity is close to that of virus isolation and it is a practical method for screening high numbers of bloods. As with virus isolation, high levels of colostral antibody can mask persistent viraemia. The ELISA test is more effective than virus isolation in the presence of antibody, but may give false negative results in viraemic lambs younger than 2 months old. The ELISA is usually not sensitive enough to detect acute BDV infections on blood samples. As well as

testing leukocytes the antigen ELISA can also be used on tissue suspensions, especially spleen, from suspected PI sheep and, as an alternative to IF and IP methods, on cell cultures.

Several pestivirus ELISA methods have been published and a number of commercial kits are now available for detecting BVDV in cattle. While some of these may be suitable for use in sheep further evaluation is required.

5.3 Nucleic acid detection

Ovine pestiviruses can be detected by the reverse transcriptase-polymerase chain reaction (RT-PCR) using primers which also detect pestiviruses from other species (Vilcek et al., 1994). While RT-PCR has not yet been evaluated for diagnostic purposes in sheep it is likely to be of future value. The detection of viral RNA in fetal tissues may yet be an important application since other methods are insensitive. The exquisite sensitivity of RT-PCR makes it a valuable tool for detecting low level virus contaminations as in cell culture constituents or vaccines (Sandvik et al., 1997).

5.4. Serological tests

Antibody to BDV can be detected in sheep sera using virus neutralization (VN) or an ELISA. Control positive and negative reference sera must be included in every test. These should give results within predetermined limits for the test to be considered valid. Single sera can be tested to determine the prevalence of BDV in a flock, region or country. For diagnosis, however, acute and convalescent sera are the best samples for confirming acute BDV infection. Bleeds from one animal should always be tested alongside each other on the same plate.

The choice of virus for use in the VN test is difficult due to the antigenic diversity among pestiviruses. Reference strains of cytopathic BD virus, e.g. Moredun or cytopathic BVD viruses, e.g. Oregon C24V or NADL can be used. No single strain is ideal. Account should be taken of the locally predominant genotype isolated from sheep. Local strains should be tested to see which gives the highest antibody titre with a range of positive sheep sera (Brockman et al., 1988). The VN can also be performed with an NCP strain with an IP staining system being used for the readout.

A monoclonal antibody capture ELISA for measuring BDV antibodies has been described. Two pan pestivirus mabs detecting different epitopes on the immunodominant non-structural protein NS 2-3 are used to capture detergent-lysed cell culture-grown antigen. The results correlate qualitatively with the VN test (Fenton et al., 1991).

6. CONTROL

The control of BDV in a sheep flock has two essential requirements: the identification of PI sheep and the prevention of infection of susceptible pregnant ewes especially during the first half of gestation.

The control of BDV in infected flocks is difficult and will depend on the requirements of farmers in relation to their farming methods. In a flock which has recently had a sporadic outbreak of BD, the entire lamb crop and the sheep that introduced infection must be removed to slaughter before the start of the next breeding season. In endemically infected flocks of high commercial value antibody testing of different aged sheep will identify immune and susceptible groups and further blood testing can be used to identify antibody negative, virus positive PI sheep. In other

flocks the identification and disposal of PI sheep may not be practicable. In which case the level of flock immunity can be raised by deliberately exposing breeding stock to known PI lambs outwith the breeding season. The rate of virus spread will be increased by close herding indoors for at least 3 weeks.

There is currently only one available commercial vaccine for the control of BDV. This is a killed adjuvanted vaccine which contains representative strains of BD and BVD-1 viruses (Brun et al., 1993). It should be administered to young animals before they reach breeding age in order to maximize their immunity during early pregnancy. Annual booster doses may be required.

To prevent introduction of BD into a flock, only home-bred replacement females should be used. New rams should be blood tested for virus before purchase or in quarantine after arrival on the farm.

If new ewes have to be bought they should also be blood tested to detect any PI virus carriers. In the absence of blood testing, and as an aid to control all infections of breeding, newly purchased ewes should always be mated and kept separate from the rest of the flock until lambing time. Because of the risk of infection of sheep from PI cattle it is essential that pregnant ewes are never mixed with cattle.

7. CONCLUSION

The control of BD remains problematical owing to the efficient spread of the virus by PI carrier-sheep and limited information on cross-protection afforded by different virus genotypes. Further vaccine development is required with candidate vaccines being tested for efficacy in pregnant sheep. This approach has now been adopted for efficacy testing of BVD vaccines in cattle (Brownlie et al., 1995). As our knowledge of pestivirus strains from

ruminants increases it should be possible to develop pestivirus vaccines which protect both sheep and cattle.

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