REVIEW

RUMINANT PESTIVIRUSES

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SUMMARY

The ruminant pestiviruses, bovine virus diarrhoea virus (BVDV) and border disease virus (BDV) are highly successful and important pathogens which infect ruminant species worldwide. Although the serological relationships among ruminant pestiviruses require further clarification, there is growing evidence for two antigenic groups, one of which predominates in cattle and one in sheep. The success of pestiviruses stems from the ability of the non-cytopathic (NCP) biotype of the virus to cross the placenta and establish a persistent infection (PI) in the developing foetus. This biotype should be regarded as the 'normal' biotype with the cytopathic (CP) biotype being an abnormal virus that is usually isolated only from PI animals dying from mucosal disease. Recent molecular evidence points to CP viruses arising from their NCP counterparts by recombination events that include the insertion of host RNA and/or the duplication of viral RNA sequences. However, the biological mechanism through which CP viruses kill cells remains unknown. Virtually all CP and NCP viruses cause only mild, transient clinical symptoms in healthy adult animals and stimulate a protective immune response. Despite the urgent requirement for a safe, effective vaccine, there is still no commercial vaccine that has been shown to immunize dams so that foetal infection is prevented. In the absence of an effective vaccine, reliable diagnostic techniques are essential to implement effective control measures. There is now a range of monoclonal antibody-based enzyme-linked immunosorbent assays for identifying PI or convalescent animals. These tests are specific, rapid, sensitive and reliable but may themselves become redundant as they are superceded by ever-increasing molecular biology-based techniques.

KEYWORDS: Bovine virus diarrhoea; border disease; pestivirus; virus persistence; congenital infection.

INTRODUCTION

There are three pestiviruses. Hog cholera virus (HCV) (also called European or classical swine fever virus), bovine virus diarrhoea virus (BVDV) (also called mucosal disease virus) and border disease virus (BDV). They were named after the important diseases from which they were first isolated; specifically a systemic haemorrhagic disease of pigs in the USA, an enteric disease of cattle in the USA and a congenital disease of sheep in the border region between England and Wales. The viruses are classified in the virus family Flaviviridae along with the flaviviruses and human hepatitis C virus (Wengler, 1991).

Traditionally, pestiviruses isolated from pigs have been termed HCV, those from cattle BVDV and those from sheep BDV, but cross-infection between species can be achieved experimentally and has been demonstrated in field infections (Terpstra & Wensvoort, 1988; Carllson, 1991). As new information emerges, particularly that provided by monoclonal antibodies (mabs) and genome sequence data, virulent strains of HCV can be differentiated readily from BVDV and BDV isolates, which are not so easily distinguishable and are referred to increasingly as ruminant pestiviruses.

Internationally, the threat to pig production by HCV represents the most serious pestivirus infection of domestic livestock. The virus is highly infectious and spreads rapidly, causing an acute systemic haemorrhagic disease with high morbidity and mortality. The impact of such disease requires prompt control and in many countries the disease has been eradicated by slaughter or controlled by vaccination (see review by Terpstra, 1991). In contrast, the ruminant pestiviruses rarely cause such dramatic disease but nevertheless are important widespread pathogens in their natural hosts. The success of the ruminant pestiviruses is due to their ability to cross the placenta, invade the foetus and set up a persistent infection (PI) which continues into post-natal life. These PI animals excrete virus continuously and spread infection wherever they go; sometimes for years.

This review will focus on the pivotal role of congenital infection with particular emphasis on recent molecular information which has allowed partial elucidation of how virus persistence is established, maintained and can lead ultimately to dramatic disease in the virus carrier itself. Modern approaches to diagnosis and possible ways forward for the control of pestivirus infections will also be highlighted.

VIRUS PROPERTIES

Ruminant pestiviruses, particularly BVDV, are unusual among viruses in that there are two biotypes. These are designated non-cytopathic (NCP) or cytopathic (CP) depending on their effect on cultured cells. The NCP biotype is by far the most important and should be considered the genuine pestivirus. This biotype only has been reliably shown to cross the placenta; invade the foetus and set up the persistent infection so crucial for successful virus spread. NCP pestiviruses are the cause of a wide range of congenital, enteric and other diseases. By contrast, CP virus is usually associated only with mucosal disease (MD); a severe and invariably fatally disease now known to occur only in PI animals. Because animals dying of MD yield both CP and NCP viruses, so-called 'pairs' of biologically cloned CP and NCP

viruses from a single animal have proved useful in elucidating the origin of CP biotypes. The CP virus appears to arise by mutation from NCP virus within the PI animals. Although they signify a 'dead-end' for the spread of virus, CP viruses have, until recently, attracted much more attention than NCP isolates because they are recovered from cases of dramatic disease and their growth in cell cultures is more easily detected and monitored.
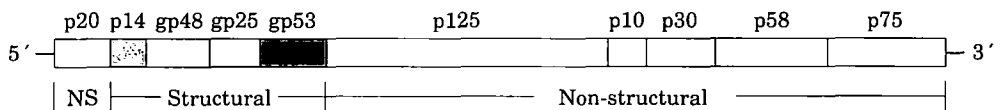
Virus structure

Pestiviruses are enveloped, spherical particles approximately 50 nm in diameter containing single-stranded positive sense RNA. The genomes of three BVD viruses have been molecularly cloned and sequenced. The CP strains Osloss (Renard *et al.*, 1985) and NADL (Collett *et al.*, 1988) have genomes 12.5 kb in length, whereas, the only NCP virus sequenced (strain SD-1) has a genome 12.3 kb long (Deng & Brock, 1992). The discrepancy in length is due to insertions of host cellular RNA sequences in the CP Osloss and NADL strains.

The BVDV RNA consists of a single open reading frame (ORF) which in the SD-1 strain is capable of encoding a polyprotein of 3898 amino acid residues, and in BVDV NADL a larger polyprotein of 3988 amino acids. With one single ORF pestivirus, protein synthesis almost certainly follows that of other flaviruses in that a large polyprotein precursor is translated and processed by either co-translational or post-translational proteolytic cleavage.

The final protein products of the ORF of pestiviruses are represented diagrammatically in Fig. 1. The functions of most of these proteins have been established for the cytopathic NADL strain by Collett and his colleagues (1988). The first product of the ORF is the non-structural protein p20, which has proteolytic activity (Wiskerchen *et al.*, 1991). There then follow four structural proteins, the
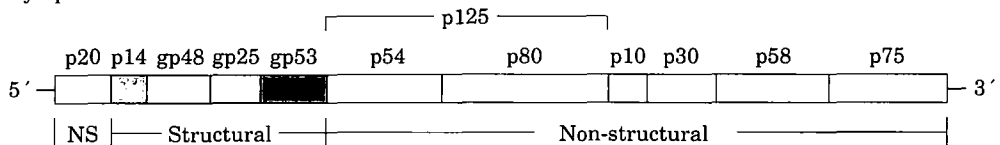
Fig. 1. Diagrammatic representation of final protein products of non-cytopathic (NCP) and cytopathic (CP) pestiviruses. Adapted from Collett *et al.* (1993) and Deng and Brock (1992). The major non-structural protein produced in cells infected by NCP pestiviruses is p125. In cells infected with CP virus this protein is cleaved to release the immunodominant p80 protein, with p125 and p54 proteins also being detectable.

first of which, p14, is the viral nucleocapsid protein. Of the three mature envelope glycoproteins (gp48, gp25, and gp53) two are involved in important host recognition functions. Mouse mabs have shown that gp53 is the immunodominant glycoprotein in neutralization reactions and binding to cell receptors (Donis *et al.*, 1988; Bolin *et al.*, 1988; Paton *et al.*, 1992*a*; Xue & Minocha, 1993). A bovine mab against this protein has also shown neutralizing activity (Onisk *et al.*, 1991). The gp48 of BVDV also contains a neutralizing epitope (Xue *et al.*, 1990) suggesting that it is also exposed on the surface of the virion. There is no such evidence, however, for gp25, which probably represents a transmembrane protein. The remaining 75% of the ORF represents the non-structural coding region. Only the first protein of this region, the p125 (p54/80), will be discussed here. The known and likely functions of the other non-structural proteins are detailed in recent reviews (Collett, 1992; Collett *et al.*, 1993).

Cytopathogenicity

The p125 protein has attracted attention because of its association with cytopathogenicity. In cells infected with NCP pestiviruses only p125 is produced. In cells infected with CP pestiviruses not only p125, but two proteins derived from p125, namely p54 and p80, are also produced. At the present time, expression of p80 is the only apparent molecular marker of cytopathogenicity (Pocock *et al.*, 1987; Dutia *et al.*, 1990; Meyers *et al.*, 1991). The p80 protein is important because it is a highly conserved and immunogenic protein, with all infected animals having high levels of antibody against it. It has been shown to be a proteinase involved in polyprotein processing (Wiskerchen & Collett, 1991). The p54 protein, however, is variable between isolates, and antibodies against it cannot be detected readily in infected animals.

Because the most easily identifiable biochemical difference between CP and NCP isolates is the proteolytic processing of the p125 to the p80 and p54 subunits, interest has focused on the region of the genome encoding these proteins in an attempt to understand the distinction between CP and NCP isolates. Insertion sequences have been found in the NADL and Osloss reference strains of BVDV (Meyers *et al.*, 1990). Both insertions were located in the area of the genome encoding the p54. The insertion sequence in the Osloss strain encoded a complete ubiquitin-like element, however that in NADL is almost identical to a bovine mRNA sequence. A ubiquitin-encoding insertion sequence was subsequently found in another CP BVDV isolate but not in its 'paired' NCP biotype, and it was proposed that recombination between viral and cellular RNA is responsible for the development of the CP virus (Meyers *et al.*, 1991). However, not all CP isolates of BVDV and BDV have insertion sequences in the region encoding the p54 (De Moerlooze *et al.*, 1990). Further studies on four 'pairs' of CP and NCP BVD viruses have revealed that although viral gene duplications with cellular RNA insertions were present in three CP viruses no insertions or duplications were found in the fourth CP virus (Qi *et al.*, 1992). Of additional interest in this study was the observation that there was greater sequence homology between pairs than to the NADL stain, supporting the hypothesis that CP virus originates by mutation from NCP virus, thereby precipitating MD. In two other 'pairs' of CP and NCP BVD viruses, both CP viruses were distinguishable from their NCP partners only by duplication

and rearrangements of two regions of their genomic RNAs (Meyers *et al.*, 1992). Thus there is evidence that the generation of CP BVDV is not restricted to recombination between cellular and viral sequences but can also be achieved by rearrangement of viral sequences.

The mutation of NCP viruses to CP viruses within the host would, therefore, appear to happen in more than one way. An important prerequisite for the generation of p80 is the introduction of a protease cleavage site at the amino terminus of this protein. So far, host RNA insertions, and viral RNA duplications and rearrangements have been associated with cytopathogenicity. It is likely that yet further mechanisms remain to be elucidated since there are two reports in which the majority of CP pestiviruses examined had neither RNA insertions nor gene duplications in the p54 coding region (De Moerlooze *et al.*, 1991; Greiser-Wilke *et al.*, 1993). There still remain the, as yet, unresolved questions as to how p80 kills cells *in vitro* and its role, if any, in precipitating MD *in vivo*.

Serological relationships

The original observation by Darbyshire (1960) that HCV and BVDV were related antigenically came from the recognition of cross-reacting antigens in virus-infected cells using agar gel immunodiffusion (AGID) tests. Similar cross-reactions have been demonstrated among all pestiviruses using AGID, immunofluorescence, and enzyme-linked immunosorbent assay (ELISA). Discrimination among pestiviruses has been possible using polyclonal antisera in cross-neutralization tests and direct staining with mabs. Both methods readily distinguish HCV from the ruminant pestiviruses (Wensvoort *et al.*, 1989; Edwards *et al.*, 1991). The serological relationships among ruminant pestiviruses, however, is not clear-cut and uncertainty surrounds antigenic variation among isolates. It is important for this to be resolved so that vaccines can be developed which protect against all the field pestiviruses likely to be encountered.

Cross-neutralization studies among bovine pestiviruses have led to the view that BVDV isolates belong to a single serologically related group within which there is a spectrum of antigenic cross-reactivity. Viruses fall into groups within the spectrum but none of the groups are sufficiently different to be considered as distinct serotypes (Howard *et al.*, 1987). A comprehensive comparison of viruses from several countries has yet to be reported, however.

When isolates from cattle and sheep are compared by cross-neutralization there is evidence that some sheep isolates similar to the Moredun reference strain belong to a serotype distinct from cattle isolates (Laude & Gelfi, 1979; Nettleton, 1987). Further evidence to support this distinction has come from studies with mabs. When Edwards *et al.* (1988) examined 84 bovine and 16 ovine isolates the results indicated the presence of at least two antigenic groups of pestiviruses one of which predominates in cattle and one in sheep.

Genomic comparisons of ruminant pestiviruses

The use of the polymerase chain reaction (PCR) and/or direct nucleotide sequencing has allowed the comparison of pestiviruses at the genome level. The differentiation of HCV from ruminant pestiviruses has been confirmed and extended by these studies (Katz *et al.*, 1993; Wirz *et al.*, 1993). Although the direct

comparison of cattle and sheep isolates is hampered by a lack of sequence data on
BD viruses evidence is accumulating that most sheep isolates are distinguishable
from cattle pestiviruses (Becher *et al.*, 1994). The data suggest that BD viruses may
be evolutionary 'younger' than BVD viruses, having undergone fewer mutations
(Berry *et al.*, 1993). In a recent comprehensive study of 129 pestiviruses, compris-
ing 33 isolates from pigs, 79 from cattle and 17 from sheep differentiation
between the viruses was achieved by cutting PCR-amplified products with restric-
tion endonucleases. At least three genogroups were identified: group 1 (HCV)
contained 32 of the pig isolates; group II (BVDV) contained all the cattle isolates
tested plus six sheep isolates; and group III (BDV) contained 11 sheep isolates and
one pig isolate (Vilcek *et al.*, 1994).

CLINICAL DISEASE

Ruminant pestiviruses are important causes of congenital and enteric disease with
a wide range of clinical manifestations. Diseases in cattle, sheep, goats and other
species will be considered separately.

Cattle
   Serological studies have shown that most adult cattle throughout the world have
antibody to BVDV. The relative lack of clinical disease attributable to the virus sup-
ports the belief that the great majority of natural infections are subclinical or
cause only mild disease. Nevertheless, the virus is known to be an important cause
of enteric and congenital disease and has been associated with other disease
syndromes.

   Enteric disease.   In the field, two distinct syndromes have been described which
remain named after their first descriptions. Bovine virus diarrhoea is an acute
disease characterized by diarrhoea, pyrexia and mild depression with a high mor-
bidity and low mortality whereas mucosal disease is a low morbidity acute or
chronic disease with diarrhoea, mucosal erosions, profound depression and
death. Not all outbreaks of clinical disease, however, fit neatly into one or other of
these syndromes and differential diagnosis from conditions such as salmonellosis
and necrotizing enteritis of beef suckler calves can be difficult (Penny *et al.*, 1994).
Moreover, there is evidence that the pathogenicity of BVDV isolates can vary and
that enteric disease in a herd is more severe after initial introduction than later in
the course of the disease (Barber *et al.*, 1985). In addition to causing the clinical
signs seen in BVD some isolates in the USA have been associated with thrombocy-
topaenia and petechial and ecchymotic haemorrhages throughout the body. In
such outbreaks of haemorrhagic disease mortality can approach 20%.

   Congenital disease.   Pestivirus infection of pregnant cattle usually results in mild
or inapparent disease in the dam, but transplacental infection of the foetus can
result in foetal resorption, mummification, abortion, stillbirths, or the birth of
small weak calves some of which may have central nervous system (CNS), eye or
coat defects.

   Other disease syndromes.   Mixed infections of BVDV and another co-infecting

microorganisms (e.g. bovine herpesvirus-1, *Pasteurella* and *Salmonella* sp.) have been shown both experimentally and in field outbreaks to cause respiratory or enteric disease syndromes. The severity of the diseases has been greater than that caused normally by either agent alone, and has been attributed to the immuno-suppressive effect of BVDV.

Sheep

Border disease is a congenital disease of sheep and clinical disease is usually manifest at lambing time. Occasionally, however, enteric disease can be associated with pestivirus infection.

Congenital infection. (i) Neonatal disease: the presence of BD in a flock can be indicated by an excessive number of barren ewes, abortion, stillbirths, and the birth of small weak lambs some of which have abnormal body conformation, tremor and/or fleece changes sometimes with excessive pigmentation. The fleece changes result from long hairs rising above the fleece to form a 'halo' effect especially along the neck and back. This effect is most obvious in smooth-coated breeds and is much less obvious in the coarser-fleeced breeds. Affected lambs are commonly termed 'hairy-shaker' or 'fuzzy-lambs'.

(ii) Enteric disease: occasionally, losses at lambing time are low and the first evidence of disease is seen in older lambs, especially around weaning time, when some have died and others are scouring and/or ill-thriven. There may or may not have been a history of having a few 'hairy-shakers' at lambing time. Such enteric disease also occurs among groups of PI sheep held in isolation and has many similarities to MD in cattle (Nettleton *et al.*, 1992*a*).

Primary infection. Aveyron disease: ovine leucopaenic enterocolitis [also called Aveyron disease, Syndrome X or petega ovina (sheep plague)] was first reported in December 1983 in the Aveyron region of France among sheep reared inten-sively for milk production. The epidemic killed 1500 ewes and 24,000 lambs in 1984. The principal symptoms were severe depression, pyrexia and diarrhoea and some recovered ewes later aborted or gave birth to weak shaking lambs with poor viability. The incidence of the disease fell sharply in 1985 and has remained low. Although the cause of the epidemic was never established conclusively there was evidence that an unusually virulent BDV isolate (AV2 strain) had made a signifi-cant contribution to causing the disease (Chappuis *et al.*, 1986).

Goats

Field cases of a clinical disease in goats caused by pestiviruses are rare with only one documented case of a kid showing severe body tremors from birth resulting in locomotor dysfunction (Loken *et al.*, 1982).

Experimental infection of pregnant goats with BVDV produces severe placen-titis and high foetal death rates. Unlike cattle and sheep it would appear to be very difficult to establish persistently infected kids (Depner *et al.*, 1991).

Other species

Pestiviruses have been isolated from at least 12 other ruminant species, and out-breaks of disease with similarities to bovine mucosal disease have been described

Table I
Effects of pestiviruses on the immune system

Species	Observations	Reference
Cattle	**Acute infection**	
	Leukopaenia and neutropaenia in the first 7 days of infection	Ellis *et al.* (1988)
	Production of soluble factors by foetal lung cells infected *in vitro* which impair peripheral blood leucocyte proliferation to a T cell mitogen	Markham and Ramnaraine (1985)
	Production of an interleukin-1 inhibitor by monocytes infected *in vitro*	Jensen and Schultz (1991)
	Persistent infection	
	Virus protein in B cells, T cells (including γd T cells) and monocytes in peripheral blood	Bielefeldt Ohman *et al.* (1987) Sopp *et al.* (1994)
	More rapid antibody response to challenge with cytopathic bovine virus diarrhoea in persistently infected cattle compared with non-infected controls	Westenbrink *et al.* (1989)
	Decreased antibody response to infection with bovine leukosis virus	Roberts *et al.* (1989)
	Decreased proliferation of peripheral blood leukocytes to T cell mitogens, and impaired neutrophil function	Brown *et al.* (1991)
	No difference in humoral or cell-mediated immune responses between persistently infected and control calves	Houe and Heron (1993)
Sheep	B cell hyperplasia in peripheral blood	Burrells *et al.* (1989)
	T cell (CD8) hyperplasia in peripheral blood	Woldehiwet and Sharma (1990)
	Abnormal expression of the T19 cell surface marker (associated with γd T cells) in efferent lymph	Entrican *et al.* (1992)
	Virus RNA and virus protein in efferent lymphocytes	Entrican *et al.* (1991)

in captive ruminants in zoos. Nevertheless, in captive or free-living ruminants no clinical syndromes have yet been shown conclusively to result from pestivirus infection (Nettleton, 1990).

PATHOGENESIS

The wide variety of clinical signs seen in pestivirus-infected ruminants, and the variable results following intrauterine infection indicate the fascinating and unique complexity of ruminant pestivirus pathogenesis. The mechanisms involved in acute, congenital and persistent infections will be considered separately.

Acute infection of non-pregnant ruminants
    Experimental infection of normal healthy cattle and sheep with virtually all isolates of pestiviruses is short-lived and mostly subclinical. Mild pyrexia, transient

diarrhoea and increased nasal and ocular discharges may be observed for up to 2 weeks. Leucopaenia due to a decrease in both T and B lymphocytes is common and usually coincides with detectable viraemia 3–14 days post-infection. Recovery coincides with the first detection of serum antibody at 2–3 weeks after infection, and peak antibody levels occur 8–10 weeks later. It is generally accepted that animals with high serum antibody titres following acute infection are immune to reinfection, but antibody titres do decline indicating that immunity following natural infection may not be lifelong (Howard, 1990). In addition to antibody there is a cellular component of the response to acute infection. It is known that peripheral blood leukocytes (PBL) recovered from acutely-infected cattle can be stimulated to proliferate in response to live BVDV (Larrson & Fossum, 1992). The importance of the cellular response to BVDV has also been demonstrated by the in vivo depletion of T lymphocyte subsets in acutely infected gnotobiotic calves. The removal of CD4-positive cells but not CD8-positive cells resulted in prolonged viraemia and increased virus titres in the blood (Howard et al., 1992). Other effects of pestiviruses on the immune system following acute infection are shown in Table I.

Some pathogenic pestiviruses have been described which produce significant disease in acutely infected animals. A recent isolate of BVDV designated CD-87 caused severe disease and deaths of 20 adults in a dairy herd of 100 animals in New York State, USA. Experimental infection of 1–8 week-old calves with this isolate caused severe thrombocytopaenia, haemorrhagic disease and the death of two of seven susceptible calves, with virus being detectable in the blood of calves for up to 40 days post-infection (Corapi et al., 1989). Fatal pestivirus infection of apparently normal immunocompetent adult cattle manifested as severe enteritis has also been described recently in the UK with death rates up to 10% reported (David et al., 1994).

Two pestivirus isolates have a high pathogenicity for sheep: the AV-2 strain isolated from a case of ovine leucopaenic enterocolitis in France has been shown to produce profound leucopaenia and death in 50% of 3–5-month-old lambs (Chappuis et al., 1986); another isolate, recovered in the Netherlands, was a pathogenic ruminant pestivirus contaminant of a live HCV vaccine. The contaminant was probably of sheep origin since its most likely source was the secondary lamb kidney cells used to prepare the vaccine. Lambs given the contaminated vaccine developed fever, prolonged leucopaenia, anorexia, conjunctivitis, nasal discharge, pale conjunctivae, dyspnoea, diarrhoea and four of eight lambs died (Wensvoort & Terpstra, 1988).

Infection of pregnant ruminants

The most serious consequences of pestivirus infection occur when the virus infects susceptible dams during pregnancy. Infected animals commonly show no clinical signs but virus spreads rapidly to the placenta and crosses to the foetus. In sheep, the virus can cross to the foetus within 1 week of experimental intranasal infection of the dam, and in cattle field evidence suggests that foetal infection may occur as rapidly. The dam's immune response clears virus from maternal tissues but it has no effect in the foetus where virus can persist.

Foetal infection. The outcome of transplacental foetal infection with pestiviruses

is very variable but is more serious the earlier in gestation it occurs. In this respect the pestiviruses have similarities with rubella virus infection of humans and comparisons have been drawn in a review by Van Oirschot (1983).

Most information has come from experimental studies in sheep that have shown that the outcome depends on the strain and dose of virus, the breed of the foetus and its ability to repair damage and, most importantly, the stage of foetal development at which infection occurs (for review see Barlow & Patterson, 1982).

The age at which the foetus gains immunological competence is critical in determining the distribution and persistence of virus which in turn influences the extent of foetal damage. The ovine foetus can first respond to an antigenic stimulus between approximately 60 and 80 days gestation. Before this time virus replication is uncontrolled and approximately 50% of all foetuses will be killed. Death may occur rapidly leading to resorption or the unnoticed abortion of small foetuses or may be delayed until months after infection when the subsequent abortion or stillbirths are obvious. In lambs that survive infection in early gestation, virus is widespread in virtually all organs, and there is no evidence of any inflammatory reaction. The principal pathological findings are myelin deficiency in the CNS which accounts for the tremor and an increase in the number of primary hair follicles causing 'hairiness'. The low pathogenicity of some virus strains, however, means that some lambs can be born persistently infected with virus without showing any clinical signs and with only minimal pathological lesions (Bonniwell et al., 1987). Pre-colostral blood samples from 'hairy-shaker' and other PI lambs contain readily detectable amounts of infectious virus. Such lambs are tolerant to the virus and have a persistent infection which continues long into post-natal life (Terpstra, 1985; Nettleton et al., 1992a).

If foetal infection occurs when the immune system is first developing (60–80 days) the outcome is less predictable. Foetal deaths are fewer. Some lambs are born PI, that is virus-positive and antibody-negative, while others will be born virus-negative and antibody-positive. Infection at this stage can also result in widespread inflammatory lesions particularly in the CNS leading to cerebral cavitation and cerebellar dysplasia—the alternative pathology of BD (Barlow & Patterson, 1982). Lambs thus affected can have severe nervous symptoms and major locomotor disturbances, and frequently have a high concentration of serum antibody to BDV (Roeder et al., 1987).

Foetal infection after 80 days gestation is met by an immune system capable of eliminating the virus. Foetal death is uncommon and virtually all lambs will be born apparently normal with no viraemia but with demonstrable antibody. There is evidence, however, that virus antigen persists in such lambs for at least a year in areas of nodular arteritis affecting medium to small arterioles principally in the CNS but also occurring mildly in a range of non-neural tissues. The lesion has been considered to be caused by a cell-mediated immune response (Barlow & Patterson, 1982).

The consequences of foetal infection in cattle are similar to those seen in sheep although bovine foetuses do not succumb easily to pestivirus infection. Foetal survival following early intrauterine infection is common and can be as high as 70%. Obvious clinical signs of tremor or coat defects have been reported in cattle but are rare. In many cases calves appear normal at birth but some may be smaller

than expected. Again, the age of the foetus when infection occurs is a major determinant of the outcome.

Before 100 days gestation foetal infection results in the development of virus persistence in virtually all organs and the birth of a calf which remains infected for life. Some infections result in foetal death with the calves being resorbed, mummified or aborted. Abortions can occur at any time from 30 days after infection up to the time of normal parturition. Stillborn calves with or without growth retardation can also result from early intrauterine infection.

Between approximately 100 and 150 days, foetuses gain the capability of mounting an immune response and infection during this period is associated with evidence of inflammation. Since this is also a time of final organogenesis of the nervous system and eyes, viral damage to developing cells can result in cerebellar hypoplasia and dysplasia, cavitation of the cerebrum and retinal lesions. At birth, affected calves can show a wide variety of nervous signs and eye defects. They can be PI but are more usually free of infectious virus and have high levels of circulating anti-pestivirus antibody.

Infection of the foetus after approximately 150 days gestation results in a fully competent immune response and elimination of the virus. Although foetuses may suffer growth retardation, they rarely die and at birth are virus-free and antibody-positive.

Persistent infection

The only time that persistent infection can be established is in approximately the first half of foetal life. The most likely explanation is that the virus infects the foetus before its immune system is sufficiently developed to mount a response. As the foetal immune system subsequently develops it cannot distinguish the virus from self. This apparent immunotolerance is highly specific since subsequent infection with other pestivirus isolates in post-natal life can elicit an immune response (Steck *et al.*, 1980) which is directed only against epitopes which are different from those on the persisting virus (Bolin, 1988). Evidence to support this comes from the fact that the persisting virus is not eliminated.

The possibility that pestiviruses can somehow adversely affect immune function in PI animals has been a focus of attention for several years. There is a prevailing assumption that PI animals tend to succumb to intercurrent infections. However, the number of potential contributory factors makes this a difficult phenomenon to prove. Several studies have been conducted in an attempt to elucidate what effects, if any, pestiviruses have on the immune system of the host. Some of the more recent observations are summarized in Table I.

Leucocytes from PI cattle and PI sheep have been shown to be infected with pestiviruses. The effect that this has on immune function is unclear because there is conflicting evidence on antibody responses in PI cattle and on alterations in lymphocyte subsets in PI sheep. If pestiviruses do affect immune function, they may do so at several levels. There are many components in operation during an effective immune response to a particular pathogen which include antigen processing and presentation, antigen recognition, cell proliferation and cytokine production. Pestiviruses have been reported to affect cell proliferation and exper-

imentally infected cells appear capable of producing soluble factors which affect immune function.

The main reasons that the question of immunocompetence of PI animals has not been fully resolved are four-fold. First, it is more difficult to identify potential differences in parameters such as cell proliferation between PI and control animals since the range in responses to mitogens or nominal antigens in outbred animals is highly variable. Second, the reagents to study immune mechanisms in detail in ruminants have become available only recently. Third, different pestivirus isolates probably affect animals in different ways; and finally there is the variation between individual PI animals, since every PI animal is unique in the degree of damage suffered by its vital organs during its intrauterine development.

One major consequence of the immunological studies has been that the recognition of the presence of virus in PBL has allowed the development of a number of rapid techniques to diagnose pestivirus infection using blood as a readily available clinical specimen. These techniques include ELISAs (see below) and flow cytometric analysis of PBL which harbour virus antigen (Qvist *et al.*, 1991).

EPIDEMIOLOGY

Ruminant pestiviruses are among the most widespread and successful animal viruses in the world. Virtually all cattle populations are infected, with antibody prevalence rates among adult animals often in excess of 60%. Among sheep, antibody prevalence is much more variable (0–50% of adults) and can have a geographical distribution reflecting different husbandry practices (Harkness & Roeder, 1988). Studies on virus stability outside the host (Depner *et al.*, 1992) suggest that the virus is too unstable for it to survive for long periods in the environment, and its successful transmission is almost certainly due to spread by PI animals.

Host range

Pestiviruses appear to infect naturally only the even-toed ungulates belonging to the order Artiodactyla, within which there are 11 species of pig and 173 species of ruminant. Despite attempts to adapt pestiviruses to grow in a variety of other hosts only rabbits appear to support virus replication (Fernelius *et al.*, 1969). There have been reports of antibody to pestiviruses in human sera, pestivirus antigen in human stools and the isolation of pestiviruses from human blood (Potts *et al.*, 1987; Yolken *et al.*, 1989; Giangaspero *et al.*, 1993). Such reports require rigorous substantiation. In this laboratory, sera from staff handling pestiviruses *in vitro* and closely exposed to PI sheep have never contained detectable antibodies to BDV (Moredun) or BVDV (NADL and Oregon C24V) when tested by ELISA, serum neutralization test (SNT) or indirect immunofluoresence tests (IIFT).

Among domestic animals, ruminant pestiviruses infect pigs, cattle, sheep, goats, red deer (*Cervus elaphus*), alpaca (*Lama pacos*), llamas (*Lama glama*) and camels. In captive and free-living ruminants infection has been demonstrated serologically in more than 40 species, and outbreaks of disease similar to those seen in domestic ruminants have been described with pestiviruses being isolated from some such

cases. It is reasonable to suppose that many other species of ruminants harbour pestiviruses (Nettleton, 1990).

Intraspecies transmission

By far the most important source of pestivirus infection is the apparently healthy PI virus carrier. Virtually all body excretions and secretions contain infectious virus with nasal discharges and saliva being the most potent sources. Thus, under natural farming conditions any husbandry which increases nose-to-nose contact will hasten the spread of virus from PI to susceptible animals.

Among cattle, intensive housing with trough feeding will ensure rapid virus spread in groups containing PI animals, with susceptible cattle seroconverting 3–6 weeks after mixing. There is evidence, however, that virus may spread slowly through a herd. Following the introduction of BVDV into a susceptible Scottish dairy herd of approximately 200 milking animals the virus took 2 years to spread to all the adult cows. In contrast, the younger stock among which PI calves were widespread all seroconverted rapidly (Barber et al., 1985). Studies of 19 Danish dairy herds have also demonstrated high antibody prevalence among groups of calves containing PI animals confirming rapid virus spread among such groups (Houe & Meyling, 1991). There is little available information on the spread of BVDV in beef suckler herds, but virus spread appears to be efficient. Investigations by our laboratory into such herds experiencing BVDV-related disease have revealed from 50–100% seropositivity among adults with 10 PI calves detected in one herd of 182 cattle (Nettleton, 1986).

Persistently infected heifers reaching breeding age may have reduced fertility but those that do calve will always produce PI offspring and this has been shown to be an important way of maintaining high levels of infectious virus in some herds (Straver et al., 1983).

Much of what has been said for cattle also applied to sheep in which species the first observation of PI mothers producing PI offspring was made (Westbury et al., 1979). When housed, PI sheep readily transmit virus to susceptible in-contacts (Nettleton et al., 1992a) whereas at grass the likelihood of transmission is low (Bonniwell et al., 1987). There is little information on the transmission of pestivirus following acute post-natal infections and there is a need to establish whether such infections are capable of maintaining the virus in the absence of PI animals. Available knowledge suggests that PI animals are essential for virus survival but that virus may continue to circulate in a herd for up to 2 years after the removal of PI cattle (Barber & Nettleton, 1993).

Interspecies transmission

Compared with within-species transmission, pestivirus spread across species barriers is a rare event under current European farming conditions where species tend to be segregated. When contact between and among ruminants and pigs occurs pestiviruses will cross between species and can cause disease. It has been known for more than 20 years that a low percentage of pigs in countries free of virulent HC were seropositive to pestiviruses, the conclusion being that pigs were being infected by pestiviruses from cattle. Recently, disease in pigs resembling hog cholera on a farm in England was shown to be due to a bovine pestivirus (Paton et

al., 1992b). An earlier outbreak of HC-like disease in pigs also in England was shown to have been caused by an ovine pestivirus (Roehe *et al.*, 1992).

Pestivirus exchange between sheep and cattle occurs readily. Given the higher prevalence of PI cattle compared with sheep it is not surprising that outbreaks of BD have been caused by transmission of virus from cattle to sheep (Carllson, 1991). No report of disease in cattle due to spread of virus from sheep has been documented, but such field infection could occur given the ease with which virus can be transmitted experimentally (Barlow & Patterson, 1982). There must also be the unlikely possibility of transmission to susceptible domestic ruminants from free-living species of artiodactyls since virus has been isolated from three species of deer and from four other free-living species (Nettleton, 1990).

Other sources of pestivirus infection

Vaccines.   Pestiviruses are important contaminants of modified live virus (MLV) vaccines administered to ruminants and pigs (Nettleton *et al.*, 1992*b*). 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. Contaminating ruminant pestiviruses are known to have caused disease in pigs following the administration of certain batches of HCV vaccine (Wensvoort & Terpstra, 1988) and Aujesky disease virus vaccine (Vannier *et al.*, 1988) as well as disease in goats following the use of an orf virus vaccine (Loken *et al.*, 1991). In addition, both sheep pox and orf virus vaccines administered to sheep have been incriminated as vectors of pestivirus infection (Spais *et al.*, 1975; Liess *et al.*, 1982). In cattle a pestivirus-contaminated MLV rota-coronavirus vaccine given to pregnant cattle resulted in serious losses of calves (Lohr *et al.*, 1983). A BVDV-contaminated vaccine against bovine respiratory disease has also been reported (Kreeft *et al.*, 1990), while contaminated rinderpest vaccines have been shown to result in seroconversion to BVDV and have been implicated as the cause of BVD-like disease (Rweyemamu *et al.*, 1991).

Artificial breeding programmes.   Artificial breeding programmes aimed at synchronizing the reproductive cycles of a large number of susceptible cattle or sheep have the potential for resulting in major outbreaks of pestivirus-induced disease. Persistently infected bulls and rams continuously excrete large amounts of infectious virus in semen and must be rigorously excluded from all breeding programmes. By contrast acute infections are much less important although it has been demonstrated that acutely infected bulls excrete low levels of infectious virus in semen for 4–6 days between 7–14 days after infection (Kirkland *et al.*, 1991).

Embryo transfer (ET) has also been associated with pestivirus-induced disease in cattle (Kirkland *et al.*, 1990) emphasizing the need to ensure that donors, recipients and all fluids used for the collection and manipulation of embryos are free of pestiviruses. Similar vigilance is also needed during the exploitation of *in vitro* fertilization.

Transmission by blood and nasal secretions.   Given levels of up to one million infectious virus particles per millilitre of blood in PI cattle and sheep the potential exists for infection to be carried from such animals to others by hypodermic needle or other contaminated veterinary instruments. It has been shown, for

example, that contaminated ear punches can transmit infection. Virus levels in nasal secretions are also high and bull nose holders have been shown to be capable of spreading infection (Gunn, 1993).

Under experimental conditions virus has been isolated from three species of blood-feeding flies, allowed to bite a PI bullock. One species, *Haematopota pluvialis* transmitted virus to susceptible sheep 15 min after their infective meal (Tarry *et al.*, 1991).

DIAGNOSIS

Clinical diagnosis

Responsibility for the diagnosis of pestivirus-induced disease in a herd or flock lies with the veterinarian attending the sick animals. Even the most experienced clinician, however, has difficulty reaching an unequivocal diagnosis of pestivirus infection based purely on presenting signs. Confirmatory laboratory tests are virtually always required and the correct samples to be collected for laboratory examination are detailed below.

Acute infections. Acute infections of susceptible ruminants with pestiviruses occur normally through the oronasal route and are predominantly subclinical. In cattle, however, three manifestations of acute disease have been recognized namely BVD, haemorrhagic syndrome, and clinically severe mixed infections which may present as respiratory or enteric disease. Diagnosis is best achieved by serological testing of paired sera collected from at least five animals at the time of disease and 3 weeks later. The recovery of virus from blood can also be attempted from recently sick animals although low virus titres frequently make this approach unrewarding. From freshly dead animals, a wide variety of tissues should be collected for virus detection/isolation.

Congenital infections. The outcome of foetal infection is variable but is more severe the earlier in gestation it occurs. Infection before mid-gestation commonly results in foetal death leading to resorption, mummification or abortion which can pass unnoticed. Animals, therefore, present as repeat breeders the cause of which is notoriously difficult to establish. Paired serology on at least 10 animals can be helpful. Absence of pestivirus antibody conclusively excludes this agent as the cause, but presence of antibody requires careful interpretation. Evidence of seroconversion in several animals would be needed to confirm recent infection, whereas high antibody titres in virtually all sera would be suggestive of infection contributing to the disease under investigation. Foetal death rates due to pestivirus infection in early gestation vary among ruminant species. Goats appear most susceptible with deaths approaching 100% (Depner *et al.*, 1991). Sheep foetuses have approximately a 50% mortality rate (Nettleton *et al.*, 1992a), while approximately 30% of infected cattle foetuses die before birth (Brownlie, 1991). The demonstration of pestiviruses from aborted or stillborn foetuses is difficult but can be attempted from thyroid, thymus, brain, spleen and kidney. Serum from heart blood or pleural, pericardial or peritoneal fluid should be tested for the presence of pestivirus antibody since its detection will confirm intrauterine infection later

in gestation. Maternal serology can be helpful although the value of paired sera can be limited by the fact that seroconversion usually precedes the abortion.

Foetuses that survive early intrauterine infection with pestiviruses will be born virus-positive and antibody-negative. Those infected in later gestation will be antibody-positive and virus-negative. A heparinized or clotted blood sample collected at birth will be sufficient to confirm either of these conditions. However, once an animal has sucked colostrum from its antibody-positive dam it will itself be seropositive. Colostral antibody can mask the presence of virus in the blood of young PI animals although well-washed leucocytes from heparinized blood are a good source of infectious virus or pestivirus-specific antigen. PI animals more than 4 months old have usually lost all colostral antibody and test virus-positive, antibody-negative.

Persistent infection. Persistently infected ruminants have a constant viraemia and excrete virus continuously especially in nasal secretions and saliva. Any animal suspected of being PI should be blood sampled and tested for the presence of pestivirus and pestivirus antibody. Virtually all PI animals are virus-positive and antibody-negative. A repeat virus-positive sample 3 weeks later will confirm persistent infection and distinguish it from an acute infection. In PI animals dying of MD, virus is readily demonstrable in blood and, from animals that die, thyroid, spleen and lymph nodes should be collected for virus detection/isolation.

Cattle dying of MD are often the first indication of pestivirus infection in a herd. They frequently represent only the 'tip of the iceberg' of infection and follow-up blood samples from in-contacts are advised in order to determine the extent of infection on the farm and identify further PI animals.

Laboratory diagnosis

The laboratory diagnosis of pestivirus infection relies on the detection of the virus or viral components and/or the demonstration of a serological response to the virus. Pestiviruses are notorious contaminants of laboratory cell cultures and cell culture medium. It is essential that laboratories undertaking virus diagnosis of animals have a guaranteed supply of pestivirus-free susceptible cells and foetal bovine or equivalent serum which contains no anti-pestivirus activity and no contaminating virus. It is not sufficient to rely on suppliers' quality assurance data. Experience in our laboratory has shown that approximately 15 batches of commercially available foetal bovine serum have to be screened to identify one batch suitable for pestivirus diagnosis.

Virus detection. Given the importance of viraemic PI cattle and sheep in the spread of infection, techniques which identify such animals from a single blood sample are of prime importance. Virus isolation from serum, plasma or blood clots are all effective provided fully susceptible cells are used. Cells from the same species as those being investigated are most sensitive. We use primary or secondary foetal lamb kidney (FLK) cells for sheep virus isolations and bovine embryonic kidney (BEK) cells for isolating viruses from cattle. The cells are derived from local abattoir foetuses, and contaminating NCP pestiviruses are detected in 2% of FLK and 13% of BEK cells. There is considerable batch-to-batch variation in the susceptibility of both cell types to pestivirus growth. Weekly monitoring of all cells

for latent pestivirus and sensitivity to infection with stock viruses of known infectivity titre are essential quality control procedures in laboratories diagnosing pestivirus infections.

The most sensitive way of confirming pestivirus viraemia is to wash leucocytes three times in culture medium before cocultivating them with susceptible cells. After 5–7 days the cells are stained for the presence of pestivirus using an immunofluorescence (IF) or immunoperoxidase (IPX) test. Although this is the 'gold-standard' against which other methods should be judged it is impractical for testing large numbers of bloods. Herd screening based on the isolation of virus from sera in microtitre plate cell cultures stained by an IPX method as first described by Meyling (1984) has gained wide acceptance.

Anti-pestivirus mabs have provided a wealth of information on the virus proteins important for neutralization and have also identified those proteins containing antigenic epitopes that are highly conserved between isolates. The latter has led to the development of a new generation of highly sensitive diagnostic tests for identifying ruminants that are persistently infected or those that have been previously infected and have seroconverted.

A panel of mabs to the p125/p80 non-structural protein has been generated from mice immunized with lysates of BDV-infected cells as opposed to whole virus (Dutia et al., 1990). None of the 10 mabs were neutralizing and all reacted with both CP and NCP biotypes.

These mabs had very broad cross-reactivity against a range of isolates and four had apparent pan-pestivirus reactivity, indicating the highly conserved nature of the p125/p80 among pestivirus isolates. Mabs have been produced to the p125/p80 of BVDV which also have broad pestivirus reactivity (Deregt et al., 1990; Lecomte et al., 1990; Paton et al., 1991b). Such pan-pestivirus specific mabs have led to the development of a whole range of tests for diagnosing pestivirus antigen and antibody in the blood of ruminants.

Antigen ELISAs. A highly specific and sensitive ELISA has been developed by coating plates with a pan-pestivirus specific mab which then captures the p125/p80 non-structural protein from lysates of PBL from pestivirus-infected sheep (Fenton et al., 1990). The captured antigen was then detected using a standard polyclonal anti-BDV ovine serum. A similar assay has been published using the same capture mab but a bovine anti-BVDV serum for the detection of infected cattle (Fenton et al., 1991a). Both ELISAs correlated well with the more protracted, labour intensive and expensive technique of conventional virus isolation. However, the risk associated with using a single mab to capture virus antigen is that if the epitope that is recognized by that mab is not present in any particular isolate then it will not be detected by the ELISA. Therefore, a modified ELISA has been developed that incorporates two pan-pestivirus mabs in the capture phase. Both mabs recognize different epitopes on the p125/p80, as determined by competitive binding studies. In addition, the polyclonal sera in the detection phase have been replaced with two directly conjugated mabs which recognize different epitopes on the p125/p80 from the capture mabs. This further standardizes the test by eliminating the use of polyclonal sera which are limited reagents and also has the advantage that the same test can be used for both cattle and sheep (Entrican et al., 1995).

An alternative ELISA for detecting BVDV-infected cattle has been published which incorporates two mabs directed against different proteins in the capture phase (Mignon *et al.*, 1991). Another technique describes the use of polyclonal sera to capture the antigen from the blood of cattle and mabs for detection (Shannon *et al.*, 1991). The ultimate aim of these assays is to have a diagnostic test which is rapid, sensitive and reliable. The advantage of mabs over polyclonal sera is the specificity which they confer and their unlimited availability. However, loss of the epitope recognized by the mab will result in failure to detect the virus. Incorporation of more than one mab greatly reduces this possibility. One potential drawback of these ELISAs and of the previous virus isolation technique comes in the identification of young PI animals. The presence of colostral antibody to pestiviruses has long been known to be a problem in conventional virus isolation techniques. However, this may also be a problem for detection of antigen by ELISA. Shannon *et al.* (1992) have reported that PI calves which tested negative for BVDV antigen by ELISA in the first few weeks of life, subsequently tested positive when retested at 8–15 weeks of age. It is not yet clear if this is due to the antibody inhibiting virus replication *in vivo* thereby resulting in low virus antigen yields during extraction or due to residual serum antibody directly masking antigen in the ELISA.

Antibody ELISAs. ELISAs to measure serum antibodies to pestiviruses offer a rapid and economical alternative to the conventional SNT. There are several reports of ELISAs to measure serum antibody binding to tissue culture-grown virus which is coated onto the plates (for recent examples see Durham & Hassard, 1990; Cho *et al.*, 1991). However, the antigen used to coat the plates must be stringently prepared to avoid the problem of high background staining. A novel approach was adopted by Lecomte *et al.* (1990) who coated plates with recombinant p80 of BVDV Osloss. However, high background was again a problem with sera apparently reacting with the β-galactosidase fusion protein. This could be resolved by using test sera to compete for binding to the recombinant protein with mabs specific for the p80. A competitive binding assay has also been described in which test sera and mabs to the p80 compete for tissue culture grown antigen (Paton *et al.*, 1991*a*).

A more direct method for measuring antibody to the p125/p80 in test sera has been described by Fenton *et al.* (1991*b*). Instead of coating plates with antigen, plates were coated with a pan-pestivirus mab which then specifically captured the p125/p80 from detergent extracts of tissue culture-gown infected cells. Antibodies in test sera then reacted specifically with the capture p125/p80, thereby eliminating high background staining. This ELISA gave a poor quantitative correlation with SNT but there was a good qualitative agreement between the two tests. This is most probably due to the fact that the ELISA measures antibodies to non-structural proteins whereas the SNT measures neutralizing antibodies to structural virus components. This raises the question of reliability of any particular test. The p125/p80 would appear to be the protein of choice for developing a diagnostic pestivirus antibody ELISA, due to its highly conserved nature. The choice of isolate used to prepare the test antigen is also important. Another critical factor is the length of time which antibody can be detected in serum following infection.

Antibody titres to the p80 and also to neutralizing epitopes have been found to persist in some calves held in controlled conditions for up to 3 years following experimental infection (Paton *et al.*, 1991*a*).

Molecular techniques for the diagnosis of pestiviruses

Recent advances in molecular biology have opened up new opportunities for diagnosing pestivirus infections. These novel approaches include dot blot hybridization, *in situ* hybridization and PCR analysis.

Dot blot and in situ *hybridization.* Dot blot hybridization has been used to detect BVDV in serum samples from infected cattle (Brock & Potgieter, 1990). However, correlation with existing virus isolation techniques can prove difficult, as in cases of acute infection where serum antibody can mask the presence of virus. Dot blot hybridization has also been used to detect pestivirus RNA in infected cells. Its potential advantages as a diagnostic tool are that many samples can be run simultaneously and that RNA does not need to be purified from the cells. Critical factors for success are the selection of the correct probes and conditions for hybridization. Lewis *et al.* (1991) showed that under certain conditions a single base mismatch for any of six 20-base probes examined would prevent hybridization. Less stringent conditions resulted in hybridization but this observation illustrates the potential problem of failure to detect certain isolates due to sequence variation in the genome. A cocktail of probes hybridizing to different areas of the genome may overcome this. A dot blot comparison of four probes hybridizing to different areas of the genome of BVDV was performed by Kwang *et al.* (1991). They found that a probe hybridizing to the p80 region of the genome was the most successful at detecting a number of CP and NCP isolates, highlighting the need for probes to highly conserved sequences to ensure success. More recently, probes from the 5'-untranslated region have been shown to be useful for the rapid detection of ruminant pestiviruses from cell culture samples by dot blot hybridization (Qi *et al.*, 1993). *In situ* hybridization has been performed on cells from infected sheep using a probe to the p80 region, but this technique is laborious and while it has great potential as a research tool it has limited use for diagnosis (Entrican *et al.*, 1991).

PCR. The relative sensitivities of dot blot hybridization and PCR for detecting BVDV in serum have been compared. Using a hybridization probe which corresponded to the PCR amplified region, Brock (1991) has shown that PCR was 10–50 times more sensitive than dot blot hybridization.

The detection of pestiviruses by PCR requires the selection of appropriate primers. There are high mutation frequencies among pestivirus isolates (Ridpath & Bolin, 1991) which make it difficult to predict primers which will amplify all virus strains. Computer comparison of the five pestiviruses whose genome sequences are now known identify conserved regions notably in the 5' and 3' non-coding regions and the region coding for the p125/p80 non-structural protein. The accumulated data suggest that the 5' non-coding part of the pestivirus genome, which probably contains sequence elements important for replication, is the most useful region from which to select PCR primers that detect the maximum number of isolates. Ridpath *et al.* (1993) used primers from this region

which amplified all bovine isolates tested but only a minority of porcine and ovine
isolates. Wirz et al. (1993) also selected primers from the 5' region which amplified
all 57 pestivirus isolates tested but their primers gave a product of only 72–74 bp
which is relatively hard to detect. More recently, primers have been identified in
this region which amplified a 288 bp product from all 129 pestiviruses tested
(Vilcek et al., 1994). If such apparent 'universal' primers are shown to detect all
pestiviruses they could play an important role in the development of future diag-
nostic tests.

While PCR has revolutionized molecular biology and is an excellent research
tool for providing information on genetic composition, the practicability of using
it for routine diagnosis has yet to be established. PCR assays require rigorous qual-
ity control preferably utilizing four separate air spaces and separate personnel
(Onions & Lees, 1991) and experience with other viruses has shown that PCR can
lead to false-positive diagnoses (Bootman & Kitchin, 1992).

CONTROL

Any control of ruminant pestivirus infections has two essential requirements: (i)
the identification of PI carrier animals; (ii) and the prevention of infection of sus-
ceptible pregnant dams especially during the first half of gestation.

Preventing the introduction of infection

All breeding stock about to be purchased must be blood tested and proved not
to be PI virus carriers. Some countries importing such stock already require certifi-
cation to this effect and it has now been adopted as a recommendation for cattle
by the Office International des Epizooties. A similar requirement at the national
and local level is becoming increasingly feasible as testing becomes easier. Manda-
tory testing before bulls can enter artificial insemination centres is now normal
and sellers of high quality expensive breeding stock are being urged to have their
animals tested before sale. The introduction of some form of certification of
breeding cattle to the effect that they are free of BVDV viraemia is inevitable, and
in areas of high BD virus prevalence a similar scheme for sheep would not be out
of the question.

The best way to prevent the introduction of pestiviruses to a herd or flock is to
use home-bred replacement females and blood test all males before purchase or
in quarantine after arrival on the farm. When female breeding cattle have to be
bought-in they must be blood-tested similarly. The feasibility of blood-testing
replacement ewes should also be weighed against the consequences of a BD out-
break. In the absence of blood testing, and as a matter of course to help 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 cross-
infection between species, it is very important that pregnant ewes are kept separ-
ate from all cattle and that pregnant cattle are not mixed with sheep.

Control in infected herds and flocks

The requirement to control pestivirus-induced disease varies markedly from

farm to farm depending on the type of animal husbandry practices being used. Cattle farmers with a high throughput of bought animals must expect trouble from pestiviruses but can reduce losses by never introducing new stock to pregnant animals and by mixing young stock thoroughly before they reach breeding age to try to ensure natural exposure to a PI virus excretor which will confer immunity. Among closed cattle herds, many have a high rate of BVDV seroprevalence without experiencing any apparent associated disease. Very often the first reported evidence of disease due to pestivirus is a case or cases of mucosal disease. Cases of MD can occur at any age but occur only in cattle that have been PI since early intrauterine infection. The source of the infection can often be traced back to a change of husbandry, usually with the introduction of PI cattle, around that time. Control measures include blood testing to identify PI cattle in the same group as that suffering from MD. Identified PI cattle can then be used as natural vaccinators, housed separately from other stock or sold for slaughter. Antibody testing of other age groups on the farm will identify immune and susceptible groups.

Similar control schemes can be employed with sheep. In a flock which has recently had a sporadic outbreak of BD, the entire lamb crop and the sheep suspected of, or shown to have introduced infection must be removed before the start of the next breeding season. Slaughter is the only way of preventing further spread of disease. In endemically infected flocks, the identification and disposal of PI sheep may not be a practicable way of controlling disease. In such a flock, deliberate exposure of all breeding stock to known PI lambs outwith the breeding season can be used to raise the level of flock immunity. The rate of virus spread will be increased by close herding indoors for at least 3 weeks.

Vaccination

The control of ruminant pestivirus infections would greatly benefit from a safe, effective vaccine that could be given to animals before they reach breeding age. Modified live virus, temperature sensitive mutant virus and killed virus vaccines have all been developed for the control of pestivirus infections in cattle. Several are licensed for use in the United States and most countries in Europe but none has yet been licensed for use in the United Kingdom. The MLV vaccines raise concern over their safety because they may be immunosuppressive, can induce post-vaccinal disease resembling MD, and cross the placenta to cause foetal disease. Although killed BVDV vaccines are safe, they fail to stimulate sufficient immunity to prevent transplacental infection (Bolin, 1990). There have, however, been reports of foetal protection in sheep using killed pestivirus vaccines. Vantsis *et al.* (1980) achieved approximately 50% protection of foetuses born to ewes receiving two doses of formalin inactivated, concentrated BD virus in Freund's adjuvant, while Carllson *et al.* (1991) achieved 86% foetal protection when ewes were vaccinated with an affinity-purified BVDV-ISCOM preparation. In both these trials ewes were infected parenterally with challenge virus. More realistic challenge systems using intranasal delivery or exposure to PI carriers have been suggested. Another important consideration in vaccine development is whether or not single strains of pestivirus are capable of inducing sufficient immunity against the antigenic spectrum of wild-type pestiviruses. Available evidence suggests that effective cattle vac-

cines could be produced from a single strain whereas sheep vaccines for the control of BD will have to contain at least two strains of pestivirus; one antigenically similar to the majority of BDV isolates and the other similar to the antigenically distinguishable viruses that have been recovered from sheep but that are more closely related to BVDV isolates from cattle.

There remains an urgent need for an effective safe pestivirus vaccine for the immunization of cattle and sheep. The development of such a vaccine would be of great help in formulating control strategies. Vaccines with the added advantage of distinguishing vaccinated animals from those infected with wild-type virus by a simple serological assay would be of further benefit.

NOTES ADDED IN PROOF

Outbreaks of severe, acute haemorrhagic disease due to BVDV infection caused mortality rates of 25% among veal calves in Canada in 1993. The virulent strains associated with this syndrome (Group II viruses) were genomically and serologically distinguishable from classical BVDV strains (Group I viruses) Pellerin *et al.,* 1994, *Virology,* **203,** 260–268. In a separate North American study of 140 BVDV isolates two similar groups with antigenic and pathogenic differences were identified. Group I comprised classical BVDVs commonly used in vaccine production, diagnostic tests and research, whereas Group II viruses were isolated predominantly from P1 calves born to dams vaccinated against BVDV, cattle that had died from acute haemorrhagic BVD, or foetal bovine serum. Group II viruses were closely related to two BD viruses included in the study for comparative purposes Ridpath *et al.,* 1994, *Virology,* **205,** 66–74. The emergence of the pathogenic Group II BVDVs capable of causing severe, acute disease represents an important new challenge to our understanding of pestivirus infections.

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