

## Maternal recognition of foetal infection with bovine virus diarrhoea virus (BVDV)—the bovine pestivirus

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### Abstract

**Background:** Pestiviruses are the veterinary viruses with genome homology to human hepatitis C virus (HCV). This group includes classical swine fever virus (CSFV), border disease virus of sheep (BDV) and bovine virus diarrhoea virus (BVDV). There are some similarities in the pathology of all three virus infections; in utero transmission to the foetus can cause early embryonic losses, severe congenital abnormalities and, particularly with BVDV, lifelong persistent infections. In situ hybridisation studies have demonstrated virus within reproductive tissues and the germinal centres of lymphoid tissue.

**Objectives:** To examine the immune response of cattle throughout their pregnancy following infection with bovine pestivirus (BVDV) during the first trimester (before 110 days).

**Study design:** In two experimental studies, heifers were infected with BVDV before 98 days gestation. Their antibody response was monitored during the remainder of the pregnancy. In another study, the antibody response of pregnant cattle was monitored following a natural infection of BVDV on a farm. Calves of the dams from all these three studies were examined, following birth, for persistent BVDV infection.

**Results:** It was observed that in dams carrying persistently infected foetuses, the immune response was markedly higher ( $13811 \pm 1273$  U ELISA antibody) than in those dams carrying uninfected foetuses ( $2542 \pm 588$  U ELISA antibody). These results were used to establish an antibody threshold (10000 U ELISA antibody) to predict the virus status of unborn calves during a farm outbreak of BVDV infection. The combined results of experimental and farm studies showed that in dams with low antibodies, 5/15 calves were infected whereas in dams with high antibodies, 17/19 calves were infected.

*Abbreviations:* HCV, hepatitis C virus; CSFV, classical swine fever virus; BDV, border disease virus; BVDV, bovine virus diarrhoea virus; CNS, central nervous system; MHC, major histocompatibility complex; ELISA, enzyme-linked immunosorbent assay; PI, persistently infected.

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**Conclusions:** The predictive reliability of the assay appeared valuable but not secure. The ability of BVDV to infect the foetus with consequent maternal recognition, whilst remaining inaccessible to maternal immune exclusion, is a novel finding. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Pestiviruses; Bovine virus diarrhoea virus (BVDV); Maternal recognition; Foetal infection

## 1. Introduction

Bovine virus diarrhoea virus (BVDV) is a pestivirus within the family Flaviviridae. It is of considerable veterinary importance in cattle and has genome homology to hepatitis C virus (HCV). It has a catholic tropism for different tissues of the body; the consequences of which induce the varied clinical signs of disease. Clinically, acute infection with BVDV may be inapparent but this virus is recognised as a major component of respiratory disease, particularly in calves. However, it is the acute infection of reproductive tissues that can have profound delayed effects and there is now a growing realisation of its economic importance.

### 1.1. Variation of virulence of acute pestivirus infections

The first description of BVDV was of a transmissible diarrhoea of adult cattle (Olafson et al., 1946) and, in some of the early studies, severe lesions were reported (Carlson et al., 1957). However, in recent years, it has been accepted that acute infections are widespread and are usually mild or inapparent (Harkness et al., 1978, Baker, 1990). The reasons for the differences have been considered (Brownlie, 1991) although remain without any real explanation.

The major change in our perception of the significance of acute BVDV infections is a direct consequence of the report of fatal disease in veal calves (Rebhun et al., 1989). The virus responsible (BVDV strain CD87) was shown to cause profound thrombocytopenia in experimental studies (Corapi et al., 1989). Further outbreaks of acute fatal disease have subsequently been recorded in many American states and in Canada. The BVDV strains isolated from these outbreaks are genomically and serologically distinguishable from classical BVDV strains (Pellerin et al., 1994) and, initially, have

been denoted as Group II BVD viruses (Ridpath et al., 1994) (an alternative taxonomy has been suggested for the four pestivirus groups, Becher et al., 1995). These apparently new viruses are a serious challenge to our understanding of acute BVDV infections. There are still, as yet, no authenticated epidemics of haemorrhagic BVDV disease due to Group II viruses outside of the North American continent and even within this region, the virulence of Group II viruses is far from uniform. The correlation of Group II viruses with increased virulence of BVDV is real but, as a cautionary note, three outbreaks of fatal BVDV disease in adult cattle have been recorded in the UK (Hibberd et al., 1993; David et al., 1994), none attributable to this genotype of virus. Serologically, the viruses responsible for the UK outbreaks are all classical Group I BVD viruses; one virus from one of the outbreaks (Ho 916) has been genotyped and is a typical Group I virus (Collins, Paton and Brownlie, unpublished data). Other examples of severe disease with Group I BVD isolates have been recorded. Thus, although it is clear that Group II viruses have an altered and increased pathogenicity, some Group I viruses also have the capability to cause severe disease. In the search for virulence genes, the clinical and experimental results of severe disease associated with both the Group I and Group II viruses must be noted.

### 1.2. Acute infection of reproductive tissues

The main interest in BVDV infections of reproductive tissues has been the possibility that the virus may cross the placenta and establish itself within the foetus. The outcome of such in utero transmission is dependent, to a great extent, on the age of the foetus. Infection of the early foetus with the non-cytopathogenic biotype can result in persistent viraemia (Casaro et al., 1971) but is often associ-

ated with pathology (Kahrs, 1973). Infection of the mid-term foetus can also result in fetopathology, abortion, or in utero growth retardation. However, later in pregnancy, the bovine foetus is immunocompetent and can develop an effective immune response.

The tropism of BVDV for oviduct and granulosa cells is marked (Booth et al., 1995) and explains the very high levels of virus recovered from ovarian follicular fluids. The presence of virus in both ovarian and oviductal fluids explains the increased reproductive losses in cattle infected with BVDV around the time of insemination (McGowan et al., 1993). An earlier observation by Virakul et al. (1988) showed that natural infection around the time of insemination resulted in a significant reduction in conception rates. More recently, the presence of BVDV within germ-line cells has been confirmed by the demonstration of non-cytopathogenic virus in oocytes and follicles of persistently infected cattle (Brownlie et al., 1997).

Two reports have indicated unexpected long-term implications for acute BVDV infections of reproductive tissues. An ovaritis, with associated viral antigen, was demonstrated over 60 days following intramuscular infection (Sentonga et al., 1980). A similar long-term effect has been noted in testicular tissues (Whitmore et al., 1978) with recovery of virus from semen and abnormal sperm cells 60–80 days following acute infection (Paton et al., 1989).

Thus, it is inescapable that there can be serious implications for the proper functioning of reproductive tissues following acute BVDV infection. These observations also highlight an important potential for effective vaccination strategies and may explain why some studies with live vaccines have demonstrated serious pathological consequences (Roth and Kaeblerle, 1983; Liess et al., 1984).

### 1.3. *In utero and congenital infections*

BVDV rarely infects the foetuses of sero-positive cattle. Maternal antibodies effectively prevent the access of virus through the placenta. It appears that the problem of in utero and congen-

ital infections is restricted either to persistently viraemic dams or to sero-negative dams that are acutely infected in the first two trimesters of gestation.

During acute infection the virus invades the placenta, replicates and may cross to the foetus without producing lesions (Casaro et al., 1971). In sheep, BVDV has been shown to damage the maternal vascular endothelium within 10 days of infection and the resulting cellular debris is ingested by the foetal trophoblast (Barlow, 1972). This could be a mechanism of virus transfer from dam to offspring and may also account for the placentitis that leads to the high level of abortion following BVDV infection. It is well recorded that early embryonic death, infertility and 'repeat breeder' cows are often the sequel to pestivirus infection during pregnancy (Van Oirschot, 1983). In a herd infected with BVDV, the conception rates were reduced from 78.6% in the immune cows to 22.2% in BVDV infected cattle (Virakul et al., 1988).

There is less certainty about the pathway and timing of foetal invasion where the dam is persistently viraemic because all tissues, including the uterus, are continually infected with virus. All foetuses born of viraemic dams are themselves persistently viraemic. Whether infection of these foetuses occurs at the level of the germ cell, or subsequent to the rupture of the zona pellucida upon implantation, is still a contentious issue; Brownlie et al., (1997) demonstrate viral RNA (39%) and antigen (19%) within oocytes and follicles of persistently infected animals, whereas Tsuboi and Imada (1998) reported only two of 17 oocytes and embryos from persistently infected cattle positive. It has been reported that border disease virus (BDV) antigen can be found in the germinal cells of the sheep ovary (Gardiner, 1980).

Whether, following acute or persistent infection, the virus infects the foetus by either direct cell to cell transmission or systemic spread is not clear. The time taken for the passage of virus from dam to foetus is variable but it has been recorded that abortions due to BVDV can occur within 10–18 days after intramuscular injection (Virakul et al., 1988). Our own experience has shown that abortions can take place several months after foetal infection.

The outcome of foetal infection is dependent on two main variables; the age of the foetus at the time of infection and the biotype of the infecting virus. There is uncertainty about the pathogenesis of infection during the first 30 days of pregnancy. There is good evidence that BVDV will reduce the conception rate during this period (Virakul et al., 1988; McGowan et al., 1993) and that the virus will replicate freely in the maternal placenta (Parsonson et al., 1979). However, there is also the view that only limited transplacental infection occurs during this early stage (Whitmore et al., 1981) because the contact between maternal epithelium and foetal trophoblast is not sufficiently intimate for vertical transmission until the 'bridge' formation at around 30 days (Barlow, 1972; Kendrick, 1971). This has implications for the use of infected semen or even during embryo transfer (Meyling and Jensen, 1988).

There is little doubt that foetal infection will occur after this 30-day period and following an acute infection, the outcome is influenced by the stage of pregnancy during which challenge occurs, first trimester (up to about 110–120 days), the second (to about 180–200 days), or third trimester (to full-term, about 280 days). Only infection during the first trimester can produce calves that remain persistently viraemic for life whereas abortions (see below) can result from infection during trimesters one or two (Gillespie et al., 1967). Calves infected during the last trimester are able to mount an active immune response (Brown et al., 1979).

#### 1.4. Abortions

The outcome of infection with the non-cytopathogenic biotype during the first and second trimesters is frequently death, abortion or mummification of the foetus (Huck, 1957; Casaro et al., 1971; Kendrick 1971, 1973). Foetal death can follow directly from viral invasion but damage of the maternal placenta may contribute by disrupting its vascular supply of nutrients. Experimental infections during this period have shown that more than 30% of foetuses are aborted (Brownlie et al., 1986) but recovery of virus from aborted tissues is poor. However, experimental infection

of cattle during the first trimester of pregnancy with the cytopathogenic biotype does not give abortions and there is some doubt whether this biotype can even establish in the early foetus (Brownlie et al., 1989).

#### 1.5. Teratogenesis

Viruses that establish in the early foetus during organogenesis can cause bizarre malformations in the developing foetus. BVDV has a well documented teratogenic effect, in common with other pestiviruses (Van Oirschot, 1983). When the lesions induced by BVDV infection are particularly severe, the foetus will die and be aborted. However, it is evident that the non-cytopathogenic biotype can replicate in the early foetus, often causing damage to selected tissues but not sufficient to cause death. Such calves are born with a variety of clinical signs that range from apparently normal to the weakly, unthrifty calf or occasionally brain damaged.

The pathogenesis of this wide range of lesions is unlikely to be due to a single defect and reflects the wide-ranging cellular tropism of the virus. BVDV has a preference for mitotically active cells, particularly those of the central nervous system (CNS) and lymphoid tissues (Brown et al., 1973; Done et al., 1980; Bielefeldt Ohmann, 1988; Fernandez et al., 1989). Whether the pathogenic event is an inhibition of normal cell division and differentiation or due to a direct action of the virus is difficult to determine. Certainly, BVDV causes significant intrauterine growth retardation in many tissues of the foetus, particularly in the CNS and the thymus (Done et al., 1980) and a direct cytolytic effect has been suggested for the hypoplasia in the germinal layer of the cerebellum (Brown et al., 1973) and other tissues (Casaro et al., 1971). Hypomyelination of the CNS, which is often associated with thymic hypoplasia, has also been observed (Anderson et al., 1987). A further consistent finding within the pestiviruses is the localisation of the virus in the vascular endothelium and from the resulting vasculitis, there can be inflammation, oedema, hypoxia and cellular degeneration (Van Oirschot, 1983).

### 1.6. Persistent viraemia

Another outcome of foetal infection during the first trimester is the establishment of a viraemia that persists for life (Kahrs, 1973). Before 110–120 days, the foetal immune system has not developed sufficient immunocompetence to recognise the infecting virus (BVDV) as 'foreign'. Soon after this 110–120 day period, 'self' antigens are recognised and the virus is accepted as a 'self' tissue and there is established specific immunotolerance to the persisting virus. It is this immunotolerance, reflected by the lack of specific antibody to the persisting virus, that allows the virus to persist in the blood and tissues for the lifetime of the animal. It is worthy of mention that in all the recorded field and experimental data there is no evidence for persistence with the cytopathogenic biotypes (Brownlie et al., 1989).

There is considerable variation in the signs and pathology described for these persistently viraemic cattle. Their identity is based on the isolation of non-cytopathogenic virus in high titre on successive occasions and the lack of antibody to the persisting virus. Their clinical appearance can range from normal to the grossly abnormal. Why some are more damaged than others can, at present, only be a speculation about the age, size, and timing of viral challenge for the early foetus. The pathology of the grossly abnormal calf reflects the viral tropism for the CNS, lymphoid and epithelial cells. Within the CNS, the predilection sites for viral persistence are the cerebral cortex and the hippocampus (Fernandez et al., 1989). Lesions in such tissues are often more severe when the foetus is infected during the second trimester (Scott et al., 1973; Binkhorst et al., 1983) and account for the depression and inco-ordination seen in some new-born calves. Frequently these calves fail to survive and grossly abnormal brain lesions, such as cerebellar hypoplasia (Done et al., 1980) can be seen at post-mortem.

Lesions within the lymphoid tissues, apart from the reduced size of organs such as the thymus (Done et al., 1980), are not so evident. The gross changes, seen in the Peyer's patches of the small intestine during mucosal disease, are not observed

(Brownlie et al., 1984). However, there are cellular changes that are said to account for the immunosuppression seen in persistently viraemic animals (Ellis et al., 1988). There is a reduction in the recirculating B-cells (Muscoplat et al., 1973) and also in T-cells (Reggiardo and Kaerberle, 1981). There are preliminary data to show that the recirculating gamma/delta T-cells are also depressed (Howard, Clarke and Brownlie, unpublished data). It has been estimated that 4.4% of blood leucocytes, 5.4% of T-cells and 2.1% of B-cells are infected with virus (Bolin et al., 1987). By use of a technique for increasing permeability of cells with increased penetration of immunolabel, greater infectivity for lymphoid cells has been demonstrated (Sopp et al., 1994). Interestingly, in sheep persistently infected with BDV, it was demonstrated that B-cell numbers were significantly increased whereas the T-cells and lymphocytes, expressing class I major histocompatibility complex (MHC) antigen were decreased (Burrells et al., 1989).

Several epithelial tissues sustain BVDV replication. BVDV antigen can be demonstrated within the keratinocytes of the tongue, skin and labia (Bielefeldt Ohmann, 1983) and this may account for the erosive oral lesions which characterise clinical disease.

### 1.7. Maternal recognition of persistent foetal infection with BVDV

The persistently infected animal remains the main reservoir of virus for infecting other cattle; the most serious consequence, as explained above, is for the early pregnant dam. Animals that are persistently infected are readily diagnosed from blood or tissue biopsy samples. However, a dilemma for veterinary clinicians and diagnostic virologists alike has been to identify those dams that are carrying a persistently infected foetus. Unless the dams are themselves persistently infected, in which case there is a 100% vertical transmission of virus to the foetus, acutely infected dams make an antibody response and rapidly clear the virus. These dams may carry a BVDV-infected foetus for prolonged periods (e.g. 180–250 days) until parturition and it was an investigation into the dams immune response during this period that is presently reported.



Table 1  
BVDV antibody titres recorded in pregnant cattle challenged with live BVDV during the first 100 days of pregnancy

Animal number	Days of gestation at challenge	Antibody (U/ml) at 180 days post challenge	Viraemic calf (Y/N)	Active immunity in calf (Y/N)
D10	98	2689	N	Y
D12	95	2147	N	Y
D14	82	11 840	Y	n/a
D558	95	4136	N	Y
D559	66	14 700	Y	n/a
D571	81	4260	N	Y
D577	90	964	N	Y
D581	66	18 650	Y	n/a
0110	68	1054	N	N
0128	72	14 690	Y	n/a
0144	31	14 910	Y	n/a
0170	68	14 220	Y	n/a
0251	30	7665	Y	n/a

Active immunity is classified as rising titres in the neonate (indicative of seroconversion) rather than falling titres (indicative of declining maternal antibody). n/a, not applicable.

## 2. Method and materials

### 2.1. Cattle in experimental studies

In a series of two experimental studies, heifers that were seronegative to BVDV, were collected and held in strict isolation within a large animal cattle unit to avoid adventitious infection. They were randomly allocated to groups for either vaccination with an inactivated BVDV vaccine (Brownlie et al., 1995) or as non-vaccinated controls. All heifers were synchronised to permit artificial insemination, thereby allowing all pregnant animals to be challenged as a group with BVDV at the end of the first trimester of pregnancy (before 100 days of pregnancy). The antibody response of all animals was monitored throughout the remaining months of pregnancy.

Details of the virus challenge, virus re-isolation and BVDV antibody assays used in this study have been reported elsewhere (Brownlie et al., 1995).

All calves or, where appropriate, aborted fetuses born to the BVDV-challenged control dams were checked for persistent BVDV infection.

### 2.2. Cattle in field outbreak of BVDV infection

A severe BVDV infection occurred in adult cattle during November and December, 1992 (Hibberd et al., 1993); subsequently, the surviving pregnant cattle, many of whom were in early pregnancy, were monitored throughout the remaining pregnancy for their antibody status. All calves born to these animals were screened for persistent BVDV infection.

## 3. Results

### 3.1. Antibody kinetics during pregnancy of cattle in experimental studies

At the time of challenge with BVDV, the antibody status of all heifers in the control group was negative (i.e. below 100 U/ml enzyme-linked immunosorbent assay (ELISA) antibody). The heifers were challenged with BVDV between 30–98 days gestation and their sera examined for BVDV antibodies at intervals during the remainder of their pregnancy. The complete data for sera at 180 days post-challenge is given (Table 1). The antibody titres of sero-positive cattle (6) that were carrying uninfected calves and bled at 180 days

gave a mean value of  $2542 \pm 588$  U (ELISA antibody), whereas the antibody titre in those heifers (7) with persistently infected foetuses gave equivalent values of  $13811 \pm 1273$  U. It is interesting to note only one of the dams that produced a viraemic calf (D577) had an antibody titre below 10000 U.

### 3.2. Antibody kinetics during pregnancy of cattle following field infection with BVDV

All cattle were bled throughout their pregnancy and on the basis of their BVDV antibody titre, at about 180 days of pregnancy, classified into a low antibody group (below 10000 U ELISA antibody) and into a high antibody group (above 10000 U ELISA antibody). Although the predictive value of this grouping may appear arbitrary, it was based on the results from the experimental studies shown in Table 1 and Fig. 1. This proposed distinction, between the low and high antibody dams, was then assessed in a natural BVDV outbreak against the live/dead and virus status of the new-born calf (Table 2). In an analysis of the data (Fig. 2), the number of persistently infected calves in the low antibody group is 4/13 whereas in the high antibody group it rises markedly to 11/13.

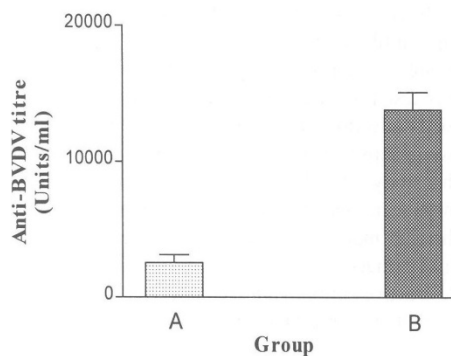


Fig. 1. BVDV antibody titres recorded after 180 days of pregnancy in groups A (dams carrying normal calves) and group B (dams carrying persistently viraemic calves). Data are means  $\pm$  S.E.

### 4. Conclusions

Although hepatitis is not a recorded feature of pestivirus infection, the virus does cause a wide range of pathology in other organ systems; most notably the reproductive, the lymphoid and the central nervous systems. In the early foetus, all these tissues are highly permissive to infection and the severe consequences of this have been reviewed above. A corollary to foetal infection is the production of neonatal calves that remain persistently infected for their lifetime. They become the main reservoir for the virus and are responsible for the initiation of new outbreaks. The early detection of these calves is a central tenet for BVDV control. To detect them before birth would have real advantage for any herd, or even National control scheme.

This paper shows that there is significant immunological recognition by the dam of a persistent infection within its unborn calf. The antibody level in these dams rises throughout pregnancy but rapidly declines following calving and abortion (data not shown). The level of antibody in these dams is extremely high by 180 days of pregnancy (Fig. 1) with a mean value of  $13811 \pm 1273$  U (ELISA antibody) and not typical of levels seen in convalescent sera after acute infection (mean values of  $2542 \pm 588$  U ELISA antibody) or following vaccination with an adjuvanted inactivated BVDV vaccine (range between 1000–2000 U ELISA antibody). The early identification of dams carrying infected calves would be of considerable diagnostic value; such dams could be isolated until the birth of their calf and, at parturition, the calves could be immediately screened. This paper has outlined that pregnant cattle with antibodies of over 10000 ELISA U are highly likely to be carrying persistently infected calves in utero. Further studies are warranted to refine the diagnostic potential of this observation and validate its use in clinical diagnostic virology.

When this test is used in the field as a predictive marker for those dams carrying a persistently infected (PI) foetus, the results (Fig. 2) of the maternal antibody assay (at about 180 days of pregnancy) show marked differences between the

Table 2

Fate of calves born to/aborted from dams which had previously been naturally exposed in early pregnancy to a field isolate of BVDV

Animal number	BVDV antibody grouping(low or high)	Live calvesViraemic (Y/N)	Dead calvesViraemic (Y/N)
901	Low	Y	n/a
717a	Low	N	n/a
411	Low	N	n/a
208a	Low	N	n/a
832	Low	N	n/a
174	Low	N	n/a
651	Low	N	n/a
956	Low	N	n/a
220a	Low	Y	n/a
764	Low	Y	n/a
904	Low	n/a	Y
110a	Low	N	n/a
203	Low	N	n/a
114	High	Y	n/a
222a	High	Y	n/a
305	High	Y	n/a
311	High	Y	n/a
504	High	n/a	Y
608a	High	Y	n/a
614a	High	Y	n/a
706a	High	N	n/a
711	High	n/a	Y
753	High	Y	n/a
773	High	Y	n/a
857	High	N	n/a
371	High	Y	n/a

Dams were classified into low or high anti-BVDV antibody groups based on titre at 180 days gestation. Low antibody = 10 000 U/ml, High antibody > 10 000 U/ml. n/a, not applicable.

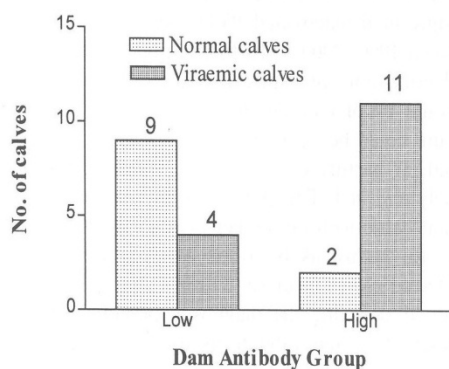


Fig. 2. Fate of calves born to/aborted from dams which had previously been naturally exposed to a field isolate of BVD virus in early pregnancy. Dams were classified into low or high anti-BVDV antibody groups based on titre at 180 days gestation. Low antibody is = 10000 U/ml, High antibody is > 10000 U/ml.

low and high antibody groupings; in the low antibody group 4 of 13 had PI calves whereas in the high antibody group 11 of 13 had PI calves. Although these results may indicate the potential of this test for diagnostic value in the detection of foetal infection, there is some overlap between the two groups which remains to be explained and further study is clearly required to define the thresholds more accurately. However, the mechanism whereby there is maternal recognition of foetally-derived viral antigen but without profound and possible destructive antibody/antigen interaction at the maternal/foetal interface, remains unanswered.

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