The effects of bovine viral diarrhoea virus on cattle reproduction in relation to disease control

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Abstract

Bovine viral diarrhoea virus (BVDV) is a major reproductive pathogen in cattle. Infection of the bull can lead to a fall in semen quality and the isolation of infectious virus in the ejaculate, while infection in the cow leads to poor conception rates, abortions and congenital defects. BVDV also reduces the animal’s resistance to other respiratory and enteric pathogens. The prevalence of BVDV is primarily due to the efficiency with which the virus crosses the placenta of susceptible females. Calves that survive infection during the first trimester of pregnancy are born with a persistent and lifelong infection. These persistently infected (PI) animals represent between 1.0% and 2.0% of the cattle population and continuously shed infectious virus.

The availability of reliable diagnostic ELISA and PCR techniques, which can test milk or serum samples for virus or antibodies, has simplified BVDV surveillance and improved the prospects for control. Although PI animals are the principal vectors within and between herds, they can be readily identified and removed. By contrast, cows carrying a PI foetus are particularly problematic. These animals have been compared to ‘Trojan Horses’ because they are virus-negative and antibody-positive but they deliver PI calves. In general, acutely infected cattle are much less efficient vectors but infections at the onset of puberty have resulted in a localised and persistent infection within the testes. Under these circumstances, virus shedding into the semen may remain undetected.

Transmission of BVDV can be controlled through vaccination or eradication. BVDV vaccine technology has been developing over the past 30 years, but currently available vaccines are still of the conventional inactivated or attenuated sort. In general, vaccination has not been applied with sufficient rigor to make a significant impact on the level of circulating virus, unlike the national...
and regional eradication programmes established in areas such as Scandinavia, Austria, the Netherlands and Scotland. Eradication confers the added advantage of improved herd health; however, it also creates a susceptible cattle population that needs to be protected by stringent biosecurity.

In this article, we discuss how BVDV influences reproductive function, the potential for viral transmission during breeding and the measures that must be taken to avoid the spread of infection to susceptible cattle populations via semen, embryos, culture fluids and infected cows. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Bovine viral diarrhoea virus (BVDV) is a major reproductive pathogen in cattle populations throughout the world, with an incidence of infection often in excess of 70% (Paton et al., 1998; Houe, 1999). As a consequence, this virus is responsible for considerable animal suffering and economic loss (Duffell et al., 1986; Houe, 1999).

BVDV is a member of the *Pestivirus* genus in the virus family *Flaviviridae*. Along with the other pestiviruses, classical swine fever virus (pigs) and border disease virus (sheep), it has a single-stranded, positive-sense RNA genome of approximately 12.5 kilobases. BVDV is most commonly associated with cattle populations but infection of a variety of domesticated and exotic ungulates has also been reported (Løken, 1995). The virus exists as two biotypes: the non-cytopathogenic (NCP) and the cytopathogenic (CP) biotypes. The NCP biotype is most commonly isolated in the field; it replicates in cultured cells without inducing cell death, and can cross the placenta to establish a persistent and lifelong infection. In contrast, the CP biotype, which arises through mutation of NCP virus (Donis, 1995), induces apoptotic cell death in cultured cells (Zhang et al., 1996) and is unable to establish persistent foetal infections (Brownlie et al., 1989). Both NCP and CP viruses can be sub-divided into two genetic groups: genotypes 1 and 2 (Donis, 1995). Most work has been concentrated on type 1 strains.

Cattle carrying persistent infections are frequently unthrifty (Houe, 1993; Baker, 1995) and if exposed to an immunologically identical CP biotype, will succumb to mucosal disease, which is fatal (Brownlie et al., 1984). In areas where adequate control measures are not implemented between 1.0% and 2% of cattle are estimated to be persistently infected (PI) at any one time (Houe, 1999). These PI animals represent the principal route of transmission within and between herds. However, the virus can also be transmitted from acutely infected cattle and by fomites, bovine sera, rectal examination, fluids used for embryo transfer, infected semen and contaminated vaccines (Bolin et al., 1991; Lang-Ree et al., 1994; Nettleton and Entrican, 1995).

Acute infections are usually mild or inapparent, but severe thrombocytopenia with high morbidity and mortality has accompanied some outbreaks, particularly those involving the type 2 viruses (Baker, 1995). Of particular relevance to this article is foetal infection and the inapparent infection of post-pubertal animals. Unfortunately, transient reproductive problems in the field are frequently missed when other clinical signs are
absent. This leads to the misconception that BVDV is not affecting herd fertility. In the following article, we discuss the affects of BVDV infection on reproductive function and how this may influence disease transmission and control.

2. BVDV infection and male fertility

Transmission of BVDV can occur following the use of raw, extended or cryo-pre- 
served semen (Meyling and Jensen, 1988; Schlafer et al., 1990). The impact of 
distributing BVDV contaminated semen could be considerable, so particular care must 
be taken to ensure all bulls standing at semen collection centres are virus-free. Most cell 
types within the bull’s reproductive tract are permissive to BVDV but infection of 
spermatozoa has not been demonstrated conclusively. High viral titres (1 × 10^4.7–7.6 
TCID_{50}/g) can be isolated from the accessory glands, testes and semen of infected 
bulls, sometimes in association with testicular lesions (Barlow et al., 1986). Despite 
these high viral titres, PI bulls can produce semen of an acceptable quality (Kirkland et 
al., 1991; Bielanski and Loewen, 1994), although they are often associated with poor 
fertility. This poor fertility is not restricted to breeding with sero-negative cows 
(Meyling and Jensen, 1988; Paton et al., 1990; Kirkland et al., 1994) and may be more 
closely related to the frequency of spermatozomal abnormalities and low motility 
(Revell et al., 1988). Nevertheless, PI bulls can sire clinically normal offspring. Those 
calves that are born PI (Meyling and Jensen, 1988) may arise following recirculation of 
the virus in susceptible dams or through viral persistence within the urogenital tract. 

Acute infections are also accompanied by BVDV-contaminated ejaculates that may or 
may not be associated with a transient dip in semen quality (Paton et al., 1989; Kirkland 
et al., 1991, 1997). The level of virus shed in semen is low and the period of shedding is 
usually quite short, although it may continue slightly beyond the last day of detectable 
viraemia (Paton et al., 1989). However, Voges et al. (1998) have reported a bull that was 
strongly sero-positive and non-viraemic, and yet shed virus persistently in semen. This 
situation may arise if bulls become infected at puberty, i.e. during the formation of the 
immunological ‘blood–testes’ barrier, thus allowing the virus to replicate within the 
testes and evade immune surveillance. Experimentally induced infections add support to 
this hypothesis (Bruscheck, C.J.M., 1999, personal communication).

The use of hire bulls represents a serious disease risk to what may otherwise be 
closed herds. As a minimum requirement, the bulls should be certified as not PI and 
quarantined before use to reduce the risk of virus transmission in case they are 
undergoing an acute infection. AI centres with inadequate screening facilities also risk 
distributing contaminated semen. Within EU countries, all bulls entering export-ap- 
proved studs are subjected to a single virus isolation test before entry (EU directive 
88/407). Similar standards are frequently adopted before semen is collected for 
domestic use. Although this legislation reduces the risk of distributing semen collected 
from PI bulls, it is not sufficiently rigorous to detect BVDV, which has evaded immune 
surveillance within the ‘blood–testes’ barrier (Voges et al., 1998). Therefore, it may be 
necessary to review testing arrangements and to introduce additional screening of 
ejaculates before the semen is distributed. This should be conducted on diluted semen
because of the cytotoxicity of the raw ejaculate (Revell et al., 1988). However, RT-PCR may well be a more convenient and reliable method of screening semen (Da Silva et al., 1995).

3. Ovarian function following BVDV infection

All the major organs within the female reproductive tract are permissive to BVDV and the distribution of virus is similar in animals carrying acute or persistent infections. Virus can be recovered from cells within the oviduct, endometrium, myometrium, as well as placental membranes (Fredriksen et al., 1991; Booth et al., 1995). Within the ovary the virus has been located in interstitial, luteal, granulosa and thecal cells, as well as follicular fluid (Booth et al., 1995; Grooms et al., 1996; Fray et al., 1998). In addition, both RNA and viral antigens have been detected in oocytes recovered from PI cows (Brownlie et al., 1997; Fray et al., 1998) although this has not been reported to date following an acute infection.

Ovarian hypoplasia has been reported in PI cows (Grooms et al., 1996) and may be a factor in the poor response of PI cows to super-ovulatory treatments (Brock et al., 1997). Acute NCP infections also alter ovarian function. Viraemia during the pre-ovulatory period reduces the rate of follicle growth in the mono-ovulatory cow (Grooms et al., 1998a; Fray et al., 1999) but not the super-ovulated cow (Kafi et al., 1997). However, the number of ovulations and the quality of embryos recovered following super-ovulation is dramatically reduced (Kafi et al., 1997). BVDV infection of the ovary may be prolonged. Experimental infections have been followed by the recovery of infectious virus and viral antigen (Ssentongo et al., 1980; Grooms et al., 1998b,c) in ovarian tissue up to 60 days post-challenge. Consistent with the slow clearance of the viral antigens, Ssentongo et al. (1980) reported a diffuse interstitial ovaritis extending to 61 days post infection, while Grooms et al. (1998a) noted that pre-ovulatory follicle growth was retarded over two consecutive cycles, post-challenge. How BVDV compromises ovarian function is uncertain but three possible mechanisms suggest themselves. Infected pituitary gonadotrophs may be unable to provide adequate gonadotrophic support (Anderson et al., 1987); the suppressed plasma oestradiol levels seen during infection may be sufficient to silence oestrus and prevent ovulation (Fray et al., 1999); while the leucopenia that accompanies acute infections (Bolin et al., 1985) may be reflected by a deficient ovarian leucocyte population, these cells being vital for normal follicular dynamics (Adashi, 1990).

4. Embryo manipulation and the risk of virus transmission

Since the advent of commercial embryo transfer, questions have been asked concerning the risk of concurrent transfer of infectious agents, particularly BVDV. Calves born to PI dams are themselves PI. Whether vertical transmission occurs through the germ line, or is simply a consequence of development in an infectious environment is uncertain. Both routes are possible: BVDV can be detected within the oocyte (Brownlie et al., 1997; Fray et al., 1998) and high viral titres can be recovered from uterine
flushings (Brock et al., 1990). However, 7-day-old in vivo derived embryos can be washed free of virus after exposure to BVDV in vitro (Singh et al., 1982). In addition, embryos recovered from PI donor cows, washed, and then transferred into BVDV naive recipients have failed to transfer the virus to either the recipient or the calf (Wentink et al., 1991; Bak et al., 1992; Brock et al., 1997), suggesting that BVDV fails to infect the pre-implantation embryo. Nevertheless, Archbald et al. (1979) reported the presence of virus-like particles in the zona pellucida following an experimental intra uterine infection. In addition, using PCR techniques BVDV RNA has been detected in a small number (2/17) of zona pellucida intact embryos recovered from PI donors (Tsuboi and Imada, 1998). These data demonstrate that BVDV can associate with the pre-implantation embryo but it is unclear whether these observations reflect infection or the recovery of adherent, non-infectious material. In fact, doubt has been cast over whether the pre-implantation embryo is permissive to BVDV. Bielanski and Hare (1988) showed that in vivo-derived embryos with damaged zona pellucidae could resist a 48-h incubation with CP virus and Potter et al. (1984) failed to recover infectious virus from blastocysts stripped of their zona pellucidae after an in vitro inoculation. However, this may reflect the age and the origin of the embryo. Brock and Stringfellow (1993) have shown that the development of in vivo-derived, hatched embryos is reduced in the presence of CP virus, while Vanroose et al. (1998) demonstrated that zona pellucidae-free, in vitro-produced embryos are permissive to both CP and NCP virus, with increasing susceptibility as they get older. These observations are consistent with the view that free virus does not cross the zona pellucida following ovulation and zona hardening. Therefore, if in vivo-derived embryos are free from adherent cells and washed in accordance with the International Embryo Transfer Societies (IETS) recommendations (Stringfellow, 1998) before transfer, the risk of BVDV transmission is probably minimal. However, a note of caution must be raised here because the IETS washing procedure failed to remove all infectious virus attached to a blastocyst and a degenerating oocyte recovered 18 days post-infection (Fray et al., 1997).

In vitro-produced embryos appear to present a particular problem for biosecurity. The majority of reports show that NCP and CP viruses do not disrupt fertilisation or development of the zona pellucida intact embryo (Zurovac et al., 1994; Tsuboi and Imada, 1996; Stringfellow et al., 1997) although hatched embryos are vulnerable to CP virus (Brock and Stringfellow, 1993). In contrast, Guerin et al. (1992) demonstrated that BVDV-infected semen reduces blastocyst formation, an effect that is apparently reversed by the addition of anti-BVDV sera (Allietta et al., 1995). Booth et al. (1998) reported a reduced cleavage rate when oocytes were cultured on infected feeder cells but the overall rate of blastocyst formation was unchanged. Collectively, these data imply that adventitious infections could remain undetected unless the IVF cultures were screened for BVDV. This is particularly relevant to embryo hygiene because it is now apparent that infectious NCP and CP BVDV can be recovered from washed IVF embryos (Bielanski et al., 1998; Trachte et al., 1998; Booth et al., 1999). The ease with which virus is recovered from in vitro-, but not in vivo-, produced embryos probably reflects structural and biochemical differences in the zona pellucida.

The increasing use of embryo manipulation techniques in cattle production and the ease with which IVF systems become contaminated with BVDV (Avery et al., 1993;
Bielanski et al., 1993 means that there is a need for constant vigilance within the IVF laboratory. Of particular relevance to embryo hygiene is the observation by Booth et al. (1999) that as little as 5-min exposure can result in viral contamination of the embryo. Clearly, the risk of contamination can be reduced by screening the culture reagents, oocyte/semen donors and by using non-permissive co-culture cells such as vero cells or synthetic oviductal fluid in the culture system. Foetal calf serum (FCS) is a particular concern because of the high incidence of BVDV contamination (Bolin et al., 1991). It is therefore advisable to irradiate FCS before use. This will eliminate the low levels of viral contamination that are difficult to detect by conventional screening methods (Sandvik et al., 1997).

5. Conception failure

Herd fertility frequently falls following inapparent or overt BVDV infections. For example, conception rates may fall by up to 44% following experimental infections 9 days before or, 4 days after insemination (McGowan et al., 1993a). Similar results have been reported following infections in the field (Roeder and Drew, 1984; Virakul et al., 1988; McGowan et al., 1993b; Larsson et al., 1994), despite the occasional report to the contrary (Meyling and Jensen, 1988; Kirkland et al., 1997).

Investigations into conception failure have failed to establish how NCP BVDV prevents implantation but neither inadequate luteal function nor altered endometrial sensitivity to oxytocin appear to be responsible (Grooms et al., 1998a; Fray et al., 1999). Although there is evidence to suggest that CP BVDV can adversely affect the integrity of the uterus or the embryo (Archbald et al., 1979; Whitmore et al., 1981; Grahn et al., 1984), the data for NCP virus is equivocal (McGowan et al., 1993a; Kirkland et al., 1997). Nevertheless, NCP virus may have a protracted effect on embryo development because Bielanski and Dubuc (1995) reported a reduced rate of in vitro blastocyst formation when using oocytes recovered 8 days after an acute NCP infection.

6. Infection during pregnancy and foetal pathology

The foetus is vulnerable to infection when susceptible animals come into contact with BVDV. In broad terms, transplacental infections are particularly harmful during the first 180 days of pregnancy and may result in foetal death, congenital deformity, or the birth of PI calves. However, only careful visual and histological examination will reveal the full extent of the lesion profile (Bielefeldt Ohmann, 1995).

BVDV infection of the dam during the pre-implantation period may result in a high incidence of embryo or foetal mortality (Virakul et al., 1988; McGowan et al., 1993a; Larsson et al., 1994), while those infections which occur between implantation and the end of the fourth month of pregnancy (approximately day 40 to 125) are characterised by foetal death, abortion, mummification, the birth of PI calves and a limited degree of teratogenesis (Moening and Leiss, 1995). Abortion rates as high as 40% have been reported following experimental infection on the 100th day of gestation (Done et al.,
1980), although lower rates prevail under field conditions (Duffell et al., 1986). Calves born PI are often weak, undersized and possess a range of macroscopic and microscopic defects. Although some PI animals appear clinically normal, they frequently leave the herd prematurely (Houe, 1993; Baker 1995).

Estimates of the number of PI cattle at large are in the range of 1.0–2.0% (Houe, 1995) but foetal infection may in fact be more common. Levings and Wessman (1991) tested 2224 batches of FCS collected from not more than two different animals and detected live virus or BVDV antibodies in 853 (38%) lots, which concurs with the data of Bolin et al. (1991).

Pre-natal diagnosis of foetal infection is not practised although there is growing evidence that cows carrying PI foetuses have exceptionally high antibody titres (Brownlie et al., 1998; Lindberg et al., 1999). While RT-PCR analysis of foetal fluids can be used to verify a suspected foetal infection, post-natal blood samples need to be interpreted with care because colostrum-derived BVDV antibodies may mask the presence of virus (Palfi et al., 1993).

Foetal infections during mid-gestation (125–180 days) are characterised by a high incidence of congenital abnormality, which may approach 100% following experimental infections. These abnormalities present as alopecia, pulmonary hypoplasia, retarded growth, thymic aplasia, ataxia, cerebellar hypoplasia and other CNS defects and a range of ocular lesions (Baker, 1995).

Transplacental infections during late gestation are not associated with a significant level of congenital deformity. During this period most infections are followed by the birth of a clinically normal calf with high levels of pre-colostral antibodies. However, some late stage abortions and abnormalities have been reported (Moening and Leiss, 1995) and field data indicate that infections late in gestation can compromise the calves’ immune system (Alenius, S., 1999, unpublished observation).

7. Control of BVDV: protection of herd fertility

The conclusion from the preceding discussion on fertility and foetal pathology must be that all susceptible cattle need to be protected from adventitious infection throughout the breeding period. The frequency of BVDV transmission and the subsequent risk of conception failure, loss of pregnancy or congenital deformity will depend on the level of immunity in a particular herd. The total impact could be considerable, particularly in naive herds (Duffell et al., 1986; Virakul et al., 1988; Larsson et al., 1994). This notion is supported by the conclusion of Houe (1999) that the infection rate for naive cattle within infected herds is approximately 34% per annum, while Done et al. (1980) estimated that 1 in 16 calves born to cows sero-negative at conception are at risk of death in utero or foetal abnormality.

Clearly the argument for controlling BVDV is very strong, but a decision on the best approach to adopt may not be straightforward. In some countries, regional and national eradication schemes have been established (e.g. Lindberg and Alenius, 1999; Synge et al., 1999). The success of these programmes relies heavily on the identification and
protection of uninfected herds, the detection and elimination of PI cattle from infected herds, adherence to strict biosecurity rules and regular herd screening.

Bulk milk or serum samples can be taken to screen herds for BVDV (Niskanen et al., 1991; Houe et al., 1995; Paton et al., 1998). Where an active infection is suspected, serology should be conducted on homebred stock of 8 months and over, i.e. after the clearance of maternal antibodies. This will establish whether recent sero-conversion has occurred, which would be an indication for blood testing the entire herd in order to detect PI animals. RT-PCR tests on bulk tank milk can be used to reduce the need for individual blood testing of milking cows (Drew et al., 1999). Follow-up blood tests are required to ensure that new born calves are not PI and to confirm that sero-conversions are not still occurring. Once freedom from infection has been established, great care must be taken to avoid its reintroduction. All cattle introduced into the herd must be quarantined and screened for infectious virus and antibodies (Roeder and Drew, 1984). It is particularly important to test all calves born to the imported cattle, especially those born to dams with high titres of BVDV antibodies. Although these cows will be virus negative they often carry PI foetuses (Brownlie et al., 1998; Lindberg et al., 1999) and have been likened to ‘Trojan horses’. It is also essential to establish adequate surveillance procedures so that any breakdown in biosecurity will be detected in its early stages. For a more detailed discussion on the principles of BVDV eradication the reader is referred to Lindberg and Alenius (1999).

In countries without control schemes, the decision on what preventive action to take is more difficult. Because of the likelihood of reinfection, there is little point in attempting to eliminate the virus from a herd if biosecurity cannot be given a high priority. It is uncertain whether or not herds in high-density cattle regions can remain virus negative if their neighbours take no preventative action. If biosecurity cannot be assured, then the main options are to do nothing, or to try to improve herd immunity through vaccination or the deliberate retention of PI animals. Retention of PI animals amongst non-breeding cattle is an effective means of boosting herd immunity but it is difficult to prevent infection spreading to the breeding stock. In addition, herd health will be compromised and the PI animal may succumb to mucosal disease (Edwards, 1997).

A more reliable way of boosting herd immunity is vaccination. Both live attenuated vaccines and killed vaccines are available (Brownlie et al., 1995; Cortese et al., 1998). The primary aim of vaccination is to prevent congenital infection but only a few vaccines have been shown to offer this level of protection (van Oirschot et al., 1999). When used inappropriately, live attenuated vaccines have been associated with ovarian lesions (Grooms et al., 1998b), immune suppression and mucosal disease (Bolin, 1995). It has also been argued that inappropriate vaccination regimes have promoted the spread of virus, with the more pathogenic BVDV (type 2 genotype) strains appearing predominantly in those countries using live virus vaccines. Although killed virus vaccines do not pose these problems, they induce a more transient immune response. The major drawback with vaccination control strategies is the need for consistent and repeated vaccinations, and this is very often not done (Carruthers and Petrie, 1996). For a detailed discussion on the principles of BVDV vaccination policies, the reader is referred to Bolin (1995) and van Oirschot et al. (1999).
8. Concluding remarks

This article has tried to highlight the effects of BVDV infections on reproduction in cattle in relation to disease control. It is obvious that BVDV can be transmitted to naive herds by infected animals, sperm, embryos and contaminated biological products. It is, however, our hope that this article will lead to a greater awareness of BVDV and so help to reduce the spread of this reproductive pathogen through greater surveillance and the use of appropriate diagnosis and hygiene. There is no doubt that BVDV can have a profound effect on herd health and fertility, but even in heavily infected cattle populations, this infection can be controlled.

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