Epidemiology of bovine virus diarrhoea virus

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Summary: A better understanding of the epidemiology and pathogenesis of bovine virus diarrhoea virus (BVDV) has emerged in recent years. Fetal infections and in particular those resulting in birth of persistently infected calves are of central importance for the epidemiology of BVDV. A prevalence of persistently infected, viraemic animals of about 1% is found in Denmark and elsewhere by examination of randomly collected blood samples. A recent field study shows that 53% of randomly selected herds in an area in Denmark where BVDV is endemic had one or more persistently infected animals. Persistently infected cows may breed and will always transmit the infection to the calf. Such familial occurrence of persistent infection seems to be a fairly common phenomenon. Persistently infected cattle are important sources of infection to other cattle. Transiently infected cattle following experimental exposure will usually not transmit the infection by contact but this may not always apply to cattle after natural infection. Knowledge of the occurrence and potential for spread of virus from persistently infected bulls is reviewed. Virus is excreted with semen of both persistently and transiently infected bulls and BVDV may be transmitted by use of infected semen for insemination. The potential for spread of the infection through embryo transfer should be avoided by the use of adequate testing and controls.

KEYWORDS: Bovine virus diarrhoea virus - Control - Embryo transfer - Epidemiology - Fetal infection - Infected bulls - Persistent infection - Prevalence - Semen - Transmission.

INTRODUCTION

Bovine virus diarrhoea virus (BVDV), the bovine pestivirus, is a commonly occurring pathogen in cattle. The majority of cattle experience infection during their first years of life. Post-natal infections are usually subclinical although acute clinical disease characterised by diarrhoea, coughing, transient drop in milk production and followed by abortions occasionally occurs. This was the clinical picture given in the first description of the disease (51). Subsequently, fetal infections were demonstrated as being the cause of abortions and malformations (25, 76). Fetal infection causing immune tolerance was suspected and proved as a basis for deficient immune response and viral persistence in calves with chronic infection (43, 44). Field studies showed that persistent infection and failure to respond immunologically to BVDV were a precondition for the other characteristic disease associated with BVDV, the fatal mucosal disease (MD) (35, 60, 69). Field studies also documented the importance of persistently infected animals for the spread of infection between herds (60).

Naturally occurring strains of BVDV may either be cytopathogenic (cp) or non-cytopathogenic (ncp). Fetal and persistent infections are produced by ncp strains,
whereas cp strains occur in association with MD. Experimental work in recent years has shown that MD may be reproduced experimentally if persistently infected animals are superinfected with a cp strain of BVDV that is antigenically closely related to the ncp strain causing the persistent infection (10, 13, 65).

Recent findings and current concepts of the epidemiology, pathogenesis and clinical aspects of BVDV have been dealt with in several reviews (3, 14, 18, 57, 61). An improved knowledge of the subclinical and clinical effects of BVDV infections gained through the works cited above, together with knowledge of the widespread occurrence of BVDV, suggests that the economic losses caused by these infections are substantial. Thorough knowledge of the epidemiology of BVDV is a necessary requirement for the reduction of these losses. A significant number of both quantitative and descriptive epidemiological studies have appeared in recent years. These are reviewed and discussed in relation to results of more recent studies in Denmark.

EVENTS AFTER INTRODUCTION OF BVDV TO A HERD

Typical sequence

BVDV may be introduced to a herd in several ways. Introduction and transmission may occur as a result of direct contact with a persistently infected animal. Such animals shed the virus in all excretions and secretions and are therefore very efficient transmitters of the infection. The virus may also be introduced with a pregnant animal carrying an infected fetus. In connection with the birth of an infected calf, the surroundings immediately become contaminated and the calf will start shedding virus, even though virus multiplication in the animal may be temporarily suppressed by colostral antibodies during the first 4-8 weeks of life. Although generally considered a less likely possibility, virus may also be introduced with an animal that has recently undergone an acute infection. Such animals transiently shed the virus in the early stage of infection and it is possible that transmission to other animals sometimes occurs.

The losses that follow the spread of virus in a herd are dependent on the immune status of the herd or, more precisely, on the number of seronegative animals in early pregnancy. The acute infection most often passes without any noticeable clinical disease, but coughing, diarrhoea and a temporary drop in milk yield may be seen. Laboratory diagnosticians are rarely involved in outbreaks of acute infection. Some authors doubt that acute post-natal BVDV infections ever cause clinical disease (57). However, this view is contrary to both earlier and more recent accounts of field outbreaks (4, 32, 49, 51) and may be an oversimplification. Acute enteric disease associated with seroconversion to BVDV occurs occasionally but attempts to isolate BVDV from such cases are usually negative (49).

Whether clinical or not, BVDV infection of the seronegative pregnant cow may lead to infection of the fetus. Depending on the age of the fetus at exposure, the outcome may be embryo loss, abortions, stillbirth, congenital abnormalities or persistent infection with growth retardation and death from MD. As it is common practice among cattle owners to plan their calvings to take place over a 2-3 month period, losses may occur in an epidemic form with all the above-mentioned conditions represented.
 Abortions may be the first symptom of BVDV infection and are the result of infections occurring during the first trimester of pregnancy. The time that elapses between virus exposure and abortion varies. Some abortions occur at 3-5 months of gestation and others at 6-7 months. A minimum of three weeks is usually required (74) but it has been reported that abortions occurred as early as nine days after the putative exposure to BVDV in natural infections (32). The virus is not usually isolated from early abortions but these may sometimes be antibody-positive, whereas late abortions may be BVDV-positive (29). The number of abortions associated with spread of the virus in a herd varies from a few (19) to 20-30% of the pregnancies (32, 33). Results of laboratory examination of four herds with multiple abortions are summarised in Table I.

The diagnosis of BVDV-associated abortions is difficult as there is no characteristic pathology and because interpretation of the results of virus isolation tests, on aborted fetuses, is difficult. To arrive at a diagnosis it is often necessary to submit additional material for laboratory examination. This might include paired serum samples from dams that have aborted and from in-contact animals, and precolostral blood samples from newborn calves, for virus isolation and antibody examination.

In seronegative pregnant animals the transplacental infection occurs with very high efficiency, and if the pregnancies are not lost the calf invariably becomes persistently infected when infection of the dam occurs from gestation day 40-90 (17, 28, 43).

Fetal infection between days 90 and 125 (43, 62) may still result in persistent infection, but at this stage of gestation the fetus develops the ability to respond immunologically. Therefore, the virus may be eliminated but the early immune response, until about day 150, may cause severe malformations such as atrophy of both cerebrum and cerebellum and ocular defects (11, 12, 28, 36, 50, 62, 73). The clinical picture is characterised by a wide range of locomotor disturbances, from slight ataxia to severe incoordination, inability to stand, opisthotonus and torticollis (73). Infection of the fetus soon after the onset of immune responsiveness may be associated with post-natal stunting of the calf. The condition is difficult to diagnose but close examination may also reveal locomotor dysfunction caused by damage of the central nervous system. The prognosis of this condition is unknown (62).

Calves with persistent infection resulting from early fetal infection generally appear normal at birth although, as mentioned previously, stunting and trembling caused by hypomyelogenesis may occur in some individuals. But neonatal mortality may be high as a result of secondary infections which cause enteritis, pneumonia and arthritis (42, 43). Reduced weight gain and growth retardation occur to a varying extent and persistently infected calves often appear unthrifty and undersized (17, 46, 57).

The most conspicuous consequence of fetal infection for the persistently infected calf is the fatal MD. The disease usually occurs in 6- to 24-month-old cattle and in its classical form is characterised by profuse diarrhoea, excessive salivation and nasal discharge. Sometimes a series of cases is spaced within a few weeks in 5-25% of the animals in the age group, but generally the morbidity is low. The disease is invariably fatal (56, 57). Even if an outbreak of MD has been preceded by significant losses caused by abortion and neonatal mortality, these events may occur too sporadically to be associated with a single cause. It is therefore quite common that a BVDV infection in a herd is first diagnosed when cases of MD begin to occur.
TABLE I
Occurrence of abortions and results of laboratory examination * on material submitted from four herds (1985-1987) with multiple abortions

<table>
<thead>
<tr>
<th>Number of fetuses abortionsexamined</th>
<th>fetuses BVDV+</th>
<th>precolostral blood samples</th>
<th>BVDV+ diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd 1 (40 cows)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov. 85</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec. 85</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr. 86</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>May 86</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Aug. 86</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Oct. 86</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dec. 86</td>
<td>72</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Herd 2 (110 cows)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun. 86</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Jul. 86</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Aug. 86</td>
<td>2</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Sep. 86</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Oct. 86</td>
<td>2</td>
<td>1</td>
<td>1 + 2**</td>
</tr>
<tr>
<td>Nov. 86</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Dec. 86</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Herd 3 (70 cows)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec. 85</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 86</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Jun. 86</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Jul. 86</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Aug. 86</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sep. 87</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Herd 4 (140 cows)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sep. 85</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb. 86</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar. 86</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr. 86</td>
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<tr>
<td>May 86</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun. 86</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul. 86</td>
<td>2</td>
<td>2</td>
<td>1**</td>
</tr>
<tr>
<td>Aug. 86</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Oct. 86</td>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Nov. 86</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Dec. 86</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Feb. 87</td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Jun. 87</td>
<td>64</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

* Carried out as described in ref. 45.
** BVDV antibody in fetus
PERSISTENTLY INFECTED ANIMALS

Occurrence in apparently healthy cattle

The growing awareness of the importance of persistently infected animals for the transmission and maintenance of BVDV infections as well as losses, has prompted surveys in different countries on the prevalence of persistent viraemia in cattle populations. Some of the surveys were random and others were more or less biased (Table II). Recently, a survey on BVDV infections in Danish cattle was conducted in 19 herds as part of an epidemiological study. The herds had previously been selected by the National Institute of Animal Science for research projects in cattle production systems (Houe et al., unpublished). The herd owners were not aware of any particular disease problems that could be ascribed to an infectious disease like BVDV. A total of 2,570 cattle in the 19 herds were examined for BVDV antibody and viraemia. BVDV was isolated from 37 animals in 10 (53%) of the herds. The prevalence of viraemia in these 10 herds was 2.9% and the proportion was 1.4% for all the animals. After 3-4 months a new sample was obtained from 29 of the viraemic animals and virus was reisolated from 28. The prevalence of antibody carriers for all the herds was 64%. For herds with persistently infected animals the prevalence of antibody carriers was 87% and in herds without, it was 43%. The incidence of viraemia found in this study agrees well with results of similar studies elsewhere (Table II) (49), but a prevalence rate of infected herds in an infected area has not been reported previously.

Table II

Prevalence of BVDV viraemia in cattle

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Material examined</th>
<th>Number tested</th>
<th>BVDV isolations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>(45)</td>
<td>adult random</td>
<td>1,332</td>
<td>12 (0.9)</td>
</tr>
<tr>
<td>Germany</td>
<td>(37)</td>
<td>adult random</td>
<td>2,317</td>
<td>22 (0.9)</td>
</tr>
<tr>
<td>Japan</td>
<td>(64)</td>
<td>calves selected</td>
<td>149</td>
<td>12 (8.1)</td>
</tr>
<tr>
<td>Sweden</td>
<td>(1)</td>
<td>adult random</td>
<td>711</td>
<td>12 (1.7)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>(31)</td>
<td>adult and calves random</td>
<td>924</td>
<td>7 (0.8)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>(20)</td>
<td>selected</td>
<td>3,151</td>
<td>56 (1.8)</td>
</tr>
<tr>
<td>USA</td>
<td>(9)</td>
<td>calves and adults selected</td>
<td>3,157</td>
<td>54 (1.7)</td>
</tr>
</tbody>
</table>

The prevalence of persistently infected animals in any cattle population is primarily determined by the incidence of infection of animals in early pregnancy. The incidence of infection can be inferred from data of serological surveys. If the age-specific antibody prevalence of the cattle population is known, it is possible to calculate the incidence of infection during any year of life by subtracting the prevalence at one
age from that of the next. On the basis of results of a serologic survey in the UK, it was estimated that 39% of the cows had been infected during their first three pregnancies. Assuming that the fetuses were vulnerable to BVDV infection for 180 days per year, it could be calculated that one in 16 calves born had been exposed to the harmful effects of the virus (17, 27). Similar calculations have been made to estimate the number of persistently infected calves born. Assuming that birth of a persistently infected calf would be the likely result if the cow is infected between days 40 and 120 of gestation and that each cow on average would have five calves, the proportion of calves persistently infected at birth could be calculated to 1.6% (59). In Denmark it is more realistic to assume that the average number of calvings per cow is three. The proportion will then be 2.4%.

Occurrence in diseased cattle

The occurrence of viraemia in animals with clinical disease appears in Table III. Confirmation of BVDV infection in live animals with enteric disease, wasting and respiratory disease is commonly made by virus isolation from blood samples. Many of the mentioned clinical conditions are MD or various chronic disease syndromes of persistently infected animals. The prognosis is poor when virus isolation is positive in clinically affected animals. A mortality of more than 80% has been found by us and others (22). The isolation rate on autopsy material is also included in Table III. In material from aborted fetuses the isolation rate is 4-6%, which agrees with results reported elsewhere (41).

### TABLE III

<p>| Isolation rates of BVDV in material from Danish cattle submitted for laboratory examination* |</p>
<table>
<thead>
<tr>
<th>Samples</th>
<th>1986</th>
<th>1987</th>
<th>1988</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood samples clinical suspicion</td>
<td>3741/1183 (32)**</td>
<td>3669/1191 (32)</td>
<td>3651/1099 (30)</td>
</tr>
<tr>
<td>Calves or organ material submitted for autopsy</td>
<td>1117/201 (18)</td>
<td>602/259 (43)</td>
<td>580/134 (23)</td>
</tr>
<tr>
<td>Aborted fetuses</td>
<td>178/11 (6)</td>
<td>305/15 (5)</td>
<td>322/13 (4)</td>
</tr>
</tbody>
</table>

* Carried out as described in ref. 45
** total/virus isolation (%)

Persistently infected maternal families

The healthy persistently infected cow may breed. The virus is always transmitted vertically to the calf, which is born with persistent infection. In this way the virus may be perpetuated in an affected herd. The calves of persistently infected dams show the same variation in viability as other persistently infected calves. In one study all calves born of persistently infected dams died shortly after or several weeks after birth (42). The results of other reports show that half the calves reached breeding age (8, 71). It is not known how frequently families with persistent infection occur.
In Australia, approximately half the clinical cases of MD that are confirmed by virus isolation and in which there has been an opportunity to examine the dams, have been the progeny of clinically normal but persistently infected cows (39). As the fecundity of persistently infected cows is below normal, and as they also have higher mortality rates, either from MD or other causes, than normal uninfected cows, the persistently infected families tend to die out. New persistently infected families are generated by exposure of susceptible cows early in pregnancy and this may be a recurring event if the herd size is above a certain level and the management practice allows some animals to be raised sufficiently isolated to avoid exposure to virus before mating. In Denmark, the prevalence of BVDV viraemia in adult cattle is approximately 1% (45). Therefore, the occurrence of vertical transmission of BVDV should be fairly common. Among the previously mentioned ten herds with persistently infected animals, cows that had passed the infection to their calves were found in two herds; 4 calves among 36 viraemic animals had been born from persistently infected dams.

TRANSMISSION AND SOURCES OF INFECTION

Persistently infected animals as sources of infection

Although the persistently infected animals are often characterised by varying degrees of growth retardation and more or less unthrifty appearance, a significant proportion are clinically normal. This is of major epidemiological significance. The persistent infection is a generalised infection and the virus is present in many tissues, especially in epithelial and endothelial cells and in cells of the reticulo-endothelial system. Therefore, the virus is shed continually and may be isolated from virtually any secretion or excretion including nasal discharge, saliva, semen, urine, tears and milk (16, 57, 71). Faeces is usually a poor source of virus (15). The presence of persistently infected animals maintains a continuous source of virus, and the level of immunity in herds with persistently infected animals is therefore high. Usually 85-100% of the animals are antibody carriers (Fig. 1). If the persistently infected animals die or are removed and no animals are introduced, the herd becomes susceptible in the course of a few years (Fig. 2).

The spread of the infection may take place at varying speeds depending on the conditions under which the animals are kept (57), but sometimes the spread is slow even when the animals are housed together. Transmission may encompass several calving seasons (4). An indication of the time it has taken for the infection to spread through the herd can be deduced from the difference in age of the persistently infected animals in the herd (disregarding cases where persistently infected cows have passed the infection to their calves). We have studied outbreaks of MD in which there were up to seven months difference in age between the youngest and oldest case, indicating that spread of the virus had taken at least four months to pass through the pregnant animals. As mentioned previously, the persistently infected animal has a key role in the transmission of the infection both within and between herds but the infection may also be introduced with a pregnant cow carrying an affected fetus. Trade of pregnant animals is common in connection with both national and international sales, and passage through salesbarns, markets for live animals and quarantine stables constitutes a special risk. Buyers are in a difficult position because only purchase
Occurrence of 75 serum-neutralising antibody carriers (95%) and 4 viraemic animals (5%) in a herd comprising 79 cattle following diagnosis of MD. All SN antibody negative animals are viraemic (+).

(Diagnostic case. Laboratory examinations carried out as described in ref. 45)

of virus-negative and antibody-negative animals will guarantee that the fetus is not infected. Such animals will have to be brought directly to the buyer's herd — a requirement that will be impossible to fulfil in export situations.

Transiently infected animals as sources of infection

After acute post-natal infections there is transient viraemia, and virus is shed in most secretions from days 4 to 10. In some instances the virus has been recovered until day 19 after infection (15). But the amount of virus is low and therefore often of no consequence. In most experimental infections with BVDV there is no transmission of the infection to fully susceptible in-contact animals. This was found when the virus was transmitted by insemination (47) or when animals were infected by parenteral injection of virus (56). However, these experiments may not give a completely true picture of the circumstances under which natural infections occur. If a transient infection occurs concurrently with a respiratory disease, transmission is likely to be facilitated by way of aerosols. A significant relationship has been established between bovine respiratory disease and infection with BVDV (70) and there is experimental evidence to suggest that some strains are pneumopathogenic (53, 54). If BVDV is an important agent in bovine respiratory disease it is also possible that this syndrome has significance in the dissemination of BVDV.
HEALTHY HERD NO HISTORY OF BVD

![Bar chart showing occurrence of antibody carriers in a closed milking herd](chart.png)

**FIG. 2**

Occurrence of antibody carriers in a closed milking herd with 132 animals, of which 6 (4.5%) were SN antibody positive. Five of 7 animals older than 8 years and 1 animal in the 1-year-group had antibody.

(The herd was examined prior to purchase of animals for experimental use. Laboratory examination was carried out as described in ref. 45)

The rate at which the infections spread may be dependent on the strain of BVDV. High virulence strains of hog cholera virus spread very rapidly whereas low virulence strains spread more slowly, as with most BVDV strains (75). It is possible that such differences also exist among BVDV strains. In some of the early works on BVD there is a discrepancy between the description of the very contagious nature of the acute virus diarrhoea in the field and the indications of how readily some of the viral isolates spread by contact after experimental infection (56). After the transient viraemia and virus excretion, the acutely infected animal produces antibodies, which lead to lifelong immunity. There is only limited evidence for latent infection (68) and excretion in immune animals (48).

Acute infections may be important in the spread of BVDV observed when large numbers of cattle originating from different sources are assembled in quarantine stables before shipment. BVDV infections diagnosed as seroconversions or transient viraemias occur commonly under these conditions in spite of the fact that appropriate laboratory examination has been made to ensure that no persistently infected animal
is brought into the stables. This experience was acquired in recent years in connection with export of live animals (Table IV). Others have reported seroconversions in a closed group of cattle in the absence of persistently infected animals (15).

**TABLE IV**

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Animals</th>
<th>Seroconversions and 4-fold titre rises to BVDV Animals</th>
<th>Viraemia Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Groups</td>
<td></td>
</tr>
<tr>
<td>1984</td>
<td>1024</td>
<td>13</td>
<td>51</td>
</tr>
<tr>
<td>1985</td>
<td>2769</td>
<td>37</td>
<td>96</td>
</tr>
</tbody>
</table>

* ref. 45

The bull and semen as sources of infection

Since 1978 (16, 42), when attention was first drawn to the existence of apparently healthy bulls persistently infected with BVDV, several reports describing the clinical condition and reproductive performance of such cases have appeared (5, 8, 34, 58, 71, 77). The persistently infected bull is of epidemiological importance in several ways. As carrier of the virus, the bull may transmit the infection to susceptible pregnant animals in herds to which it is either sold or hired. If allowed to enter an artificial insemination (AI) centre as semen donor, the persistently infected bull will transmit the infection to other bulls causing transient excretion of the virus in semen; if the semen quality is not severely affected, the persistently infected bull might even be used as a semen donor for AI. The amount of virus excreted in the semen of persistently infected bulls is very high ($10^4$-$10^6$ TCID$_{50}$/ml), and the semen quality is often poor due to such changes as low density, low motility and abnormal sperm morphology. The changes in sperm morphology are seen as deformities of the sperm head such as “collapsed heads” or heads surrounded by swollen cell membrane (58). Some persistently infected bulls are so severely affected that the spermiogenetic epithelium is destroyed and the semen consequently without sperms (8, 71).

Even though abnormalities of the semen of persistently infected bulls may cause more or less reduced fertility, their performance need not be so poor as to exclude them from use. Following a screening of Danish AI bulls, a persistently infected bull that had acceptable semen quality and fertility was identified. The semen from this bull was used experimentally to inseminate twelve seronegative heifers, all of which became pregnant. One of the heifers gave birth to a persistently infected calf, and this calf transmitted the infection to seronegative animals in the same cow-house (47). The results of this study illustrate the most serious implication of the use of persistently infected bulls as AI donors.

If persistently infected bulls are used for natural service the cows may conceive when immunity has developed, but this will not result in birth of calves with prenatal infection (4, 42). Local antibody in the uterus may be important in the protection
of the embryo after both insemination and natural service. The zona pellucida effectively prevents infection of the embryo until hatching on days 8-9 after fertilisation and, at that time, local immunity is already active in response to virus introduced with semen.

When BVDV is infused into the uterus at the time of breeding, a significant reduction in conception rate is seen in seronegative animals. The same effect is not seen in seropositive animals or if the animals are inoculated orally and intranasally at the time of breeding (79). One study showed that large amounts of cp BVDV introduced into the uterus seven days after insemination caused degeneration of the embryos (2). Another study showed that BVDV interfered with fertilisation when the virus was introduced at the time of insemination (26). On the basis of these studies it might be concluded that BVDV contributes to "repeat breeding" in the field. But conception failure was not seen in twelve heifers experimentally inseminated with the semen of a persistently infected bull (47).

The semen of transiently infected bulls may either be without changes or exhibit a deterioration in quality (52, 78). In a recent study, transient virus excretion was demonstrated after the viraemic stage, i.e. on days 10, 12, 14 p.i. in experimentally infected bulls. The virus titre in the semen was much lower in transiently infected than in persistently infected bulls (52).

When pooled semen samples are tested for viral contamination, BVDV is frequently demonstrated (63). This contamination could be due to semen from both persistently and transiently infected bulls. Considering the harmful effects of semen contamination by BVDV it is important to ensure that no bull which is persistently infected with BVDV is allowed to act as AI donor. Since 1985, pre-entry testing for BVDV has been prescribed in Danish AI centres and similar regulations are in force in the UK (40). Before that time the AI bulls in all Danish centres had been tested for BVDV antibody and virus using the samples taken for routine monitoring for IBR, leukosis and brucellosis. Of 1,398 bulls tested in 1985, 1,081 (78%) had antibodies to BVDV. Pre-entry testing greatly reduces but does not completely eliminate the risk of semen-borne infection. A significant number of bulls are antibody-negative and this means that occasional transient infections with subsequent excretion of BVDV in semen may take place if BVDV is introduced into a centre. The best way to identify a persistently infected bull is by virological examination of a blood sample (58).

**Embryo transfer**

Embryo transfer (ET) is a potential route of transmission for BVDV. In theory the virus might be transferred either on or within the embryo or in the wash fluids used for the transfer. BVDV does not seem to be taken up by preimplantation embryos, and established washing procedures effectively remove contaminating virus provided the zona pellucida is intact (55, 66, 67). It is not known if the oocytes of persistently infected cows are infected with BVDV, as are the oocytes from lambs persistently infected with Border Disease virus (23). Laboratory examination of donor cows for viraemia would guard against this possibility.

Contamination of wash fluids by the use of BVDV-contaminated fetal serum constitutes a real risk of infecting recipient cows and the fetus with virus. So far, there is no report on the experimental transmission and production of a persistently infected ET calf in this way. In experiments performed by us (Jensen et al., unpublished), embryos washed and transferred in fluid with even small amounts of
ncp BVDV resulted in loss of the embryo. It is possible that this is the most likely outcome of direct BVDV infection of the hatched embryo in the uterus. If the embryo can be infected transplacentally by back transmission from the transiently infected recipients, there is a possibility for production of a persistently infected calf.

There have been reports of a remarkably high frequency of persistent infections among ET calves (38). The reason for this has not been definitely established, but ET calves are often produced under conditions that would favour a high prevalence of persistent infection, irrespective of the breeding technique used. It is common practice to assemble recipients, often originating from many different farms, into large herds where embryo transfer is performed. It is obvious that such practices increase the risk of BVDV infection of the recipients. It is, therefore, important not only to examine the recipients before ET for viraemia but also to screen all animals before they are introduced into farms housing recipients. Furthermore, it seems advisable to use only seropositive animals as recipients.

Transmission from ruminants other than cattle

Other species besides cattle may be involved in the epidemiology of BVD, and pestivirus infections in ruminants other than cattle will be dealt with elsewhere in the present issue. Suffice it to say that inter-species spread of bovine and ovine pestiviruses seems to take place readily (21, 24). The importance of this factor for transmission of the infection to cattle depends on husbandry practices and, in particular, the extent to which cattle and sheep are allowed to have contact. Wild ruminants are also infected with pestiviruses, and this reservoir may also directly or indirectly have some importance for infection in cattle.

Transmission through vaccines

In countries where live vaccines against BVDV are in use, vaccination of animals in early pregnancy may result in birth of persistently infected animals (36). Transmission is also possible via live vaccines contaminated with the BVDV from fetal serum used for growing cell cultures.

Transmission by untraceable sources

Even if the key role of persistently infected animals in the transmission of BVDV is unquestionable, it should be stated that retrospective inquiries and investigations in connection with severe outbreaks of BVD or MD often fail to identify a persistently infected animal as source of the infection. In a study of ten severe outbreaks of BVDV, it was found that six occurred in closed herds to which no animals had been introduced for a number of years (Houe, unpublished). The widespread occurrence and slow spread of the virus in infected herds often make it difficult, if not impossible, to identify positively the means by which the virus was introduced. The critical events may have occurred months or years previously. A possible scenario would be a contact that results in subclinical infection in one pregnant animal leading to the birth of a persistently infected calf that in turn infects a number of pregnant animals whose offspring will develop clinical MD eighteen months later. Neighbourhood contacts and participation in animal shows are events that may be important in this context, but such events may often be impossible to point out.
In an early account of BVDV it was stated that the disease was spread from farm to farm by veterinarians and livestockmen, presumably through contaminated clothing and footwear or veterinary instruments (56). This view is difficult to reconcile with the present knowledge of how readily BVDV spreads. But the common practice in veterinary medicine of not changing syringes and needles and of not discarding partly used bottles between herds must give reason for concern. This is a significant way of transmitting classical swine fever (72) and it may also be important with BVDV.

**ECONOMIC LOSSES AND CONTROL**

The majority of cattle in most cattle-producing countries experience infection with BVDV. The serious subclinical and clinical effects are, as reviewed above, well documented. These facts make it evident that the economic losses caused by BVDV must be of considerable importance. Good quantitative estimates of the losses, together with their precise identification, are necessary for the selection of sound control strategies both at the national and farm level. With the present knowledge of the epidemiology of BVDV there is little doubt that the greatest importance must be attached to pre-natal infections, which are estimated to cause more than three-quarters of financial losses (28). Analyses of the losses at the farm level have shown that total costs of BVDV infections in a breeding herd presumed to be previously unexposed are likely to be twice the figure calculated from proven cases of post-natal disease alone (19). In the UK, studies on cost-benefit analyses and BVDV-associated losses have recently been published. The results of these studies do not support adoption of control programmes for BVDV on a national scale (6, 28). Apart from the costs which are known or can be calculated, there are several other aspects of BVDV that have to be taken into account, such as possible transmission of the virus from sheep and wild ruminants and transmission by other as yet unknown pathways (28).

The control strategy at the farm level should primarily aim at the prevention of pre-natal infections. How this is achieved must depend on the management practice and purchasing habits of the individual farm and whether licensed vaccines are available or not. Computerised processing of farm data and data on the risk of transmission might be used in advising the farmers (7, 30).

After a case of MD has been diagnosed in a herd, identification and removal of all persistently infected animals should always be implemented because mortality is usually very high in the remaining persistently infected animals. Future epidemiological studies and cost-benefit analyses in herds living "in peace" with the infection will reveal whether similar intervention can be justified in such herds.

To avoid reintroduction of the infection, all cattle entering the herd must be isolated and tested. In the absence of vaccination this strategy is risky and may lead to heavy losses if BVDV is reintroduced. Selective use of efficacious and safe vaccines, therefore, should be part of a control strategy for BVDV.
ÉPIDÉMIOLOGIE DU VIRUS DE LA DIARRHÉE VIRALE BOVINE. — A. Meyling, H. Houe et A.M. Jensen.

Résumé: On comprend mieux, depuis quelques années, l'épidémiologie et la pathogénie du virus de la diarrhée virale bovine (virus BVD). Les infections fœtales, en particulier celles qui aboutissent à la naissance de veaux infectés de manière persistante, sont d'une importance centrale pour l'épidémiologie du virus BVD. Au Danemark et ailleurs, l'examen de prélèvements sanguins recueillis au hasard montre que le taux de prévalence des animaux infectés persistants et virémiques est d'environ 1 %. Une étude récente réalisée sur le terrain a révélé que 53 % des troupeaux choisis au hasard, dans une zone du Danemark où le virus BVD est endémique, comprenaient un ou plusieurs animaux infectés persistants. Une vache infectée persistante peut reproduire ; dans tous les cas, elle transmettra l'infection à son veau. Ce caractère familial de l'infection persistante paraît être un phénomène assez fréquent. Les bovins infectés de manière persistante sont une source importante d'infection pour les autres bovins. En général, un bovin infecté de manière transitoire, après contamination expérimentale, ne transmettra pas l'infection par contact, mais cela n'est pas forcément vrai à la suite d'une contamination naturelle. Les auteurs passent en revue les connaissances acquises sur la fréquence et les risques de diffusion du virus par des taureaux infectés persistants. Les taureaux infectés, que ce soit de manière transitoire ou persistante, excréteront le virus avec leur sperme, et le virus BVD peut être transmis par de la semence infectée utilisée pour l'insémination artificielle. On doit éviter les risques de diffusion de l'infection par le transfert d'embryons en pratiquant les examens et les contrôles adéquats.


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EPIDEMIOLOGÍA DEL VIRUS DE LA DIARRÉA VIRAL BOVINA. — A. Meyling, H. Houe y A.M. Jensen.

Resumen: Se comprende mejor, desde hace algunos años, la epidemiología y la patogenia del virus de la diarrea viral bovina (virus BVD). Las infecciones fetales, en particular aquéllas que provocan al nacimiento de terneros infectados de manera persistente, son de una importancia central respecto de la epidemiología del virus BVD. En Dinamarca y otros países, el examen de tomas de sangre efectuadas al azar muestra que la tasa de prevalencia de los animales infectados persistentes y virémicos es de aproximadamente 1%. Un estudio de campo reciente reveló que 53% de los rebaños elegidos al azar en una zona de Dinamarca en que el virus BVD es endémico contaban con uno o varios animales infectados de manera persistente. Una vaca infectada persistentemente puede reproducir; en todos los casos transmitirá la infección a su ternero. Este carácter familiar de la infección persistente parece ser un fenómeno bastante frecuente. Los bovinos infectados de manera persistente son una fuente de infección importante para los demás bovinos. En general, un bovino infectado transitoriamente mediante contaminación experimental no transmitirá la infección por contacto, pero esto no es forzosamente así cuando se trata de una contaminación natural. Los autores pasan revista a los conocimientos con
que se cuenta respecto de la frecuencia y riesgo de difusión del virus por toros infectados de manera persistente. Los toros infectados transitoria o persistentemente excretan el virus con su esperma y el virus BVD puede ser transmitido por el semen infectado utilizado para la inseminación artificial. Deben evitarse los riesgos de difusión de la infección por la transferencia de embrones practicando exámenes y controles adecuados.

PALABRAS CLAVE: Epidemiología - Infección fetal - Infección persistente - Prevalencia - Profilaxis - Semen - Toros infectados - Transferencia de embrones - Transmisión - Virus de la diarrea viral bovina.

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