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Natural infection with bovine virus diarrhoea virus in a dairy herd: A spectrum of symptoms including early reproductive failure and retained placenta

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Abstract

The consequences of natural infections by bovine virus diarrhoea virus (BVDV) in a dairy herd comprising approximately 60 cows were studied over a 3 year period. The outcome of 39 pregnancies after artificial insemination or natural service during a 4 month period of risk for contracting BVDV was nine abortions, one mummified foetus, one stillbirth, three calves that died within 1 week of age, 12 calves persistently infected (PI) with BVDV (11 males and one female) and 13 non-PI calves. Retrospective studies showed that only two of the 13 dams (15.4%) of non-PI calves had been inseminated once, which is a significantly \((P<0.001)\) lower rate than for 192 other gestations (66.7%) during the 3 year period.

The gestational duration of multiparous cows (but not of heifers) was longer \((P<0.01)\) for five cows with PI calves (mean \(\pm SD\) 287.6 \(\pm\) 9.5 days) than for 53 other gestations in 35 cows (280.6 \(\pm\) 3.8 days). Five of the 12 dams of the PI calves had not expelled the foetal membranes within 2 days after calving, which is a higher \((P<0.001)\) incidence than the seven cases of retained placenta observed after 198 other calvings. Furthermore, there was a 4.4-fold higher risk \((P<0.01)\) of treatment for enteritis and/or pneumonia in calves and a 6.0-fold higher risk of calf mortality associated with the introduction of BVDV. The mean heart girth of the PI calves was less \((P<0.05)\) than that of non-PI calves, at both 80 and 180 days of age.

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1. Introduction

Bovine virus diarrhoea virus (BVDV) is a serious pathogen of the bovine foetus. The outcome of transplacental infections with this pestivirus includes abortions and foetal anomalies such as cerebellar and thymus hypoplasia and ocular defects (for a review, see Van Oirschot, 1983). When an infection with a non-cytopathic strain of BVDV occurs before 120 days of gestation, the foetus may develop immune tolerance to the virus and thereby a pre- and postnatal lifelong infection (McClurkin et al., 1984). Because of this tolerance, persistently infected (PI) calves have no, or only a low level of, antibodies to BVDV. The PI calves may develop normally and reach breeding age, though they are often small and have a poor growth rate and an unthrifty appearance. PI calves also run the risk of developing the fatal condition called mucosal disease (Roeder and Drew, 1984; Brownlie et al., 1984). The latter is thought to be the consequence of a mutation of the persistent non-cytopathic infecting virus to a cytopathic form (Howard et al., 1987) or a reinfection with a cytopathic strain (Brownlie et al., 1984).

PI calves excrete the virus via their bodily secretions and are therefore a potent source of infection to cattle with which they have contact (Travén et al., 1991). Even though the postnatal infection is often subclinical, it can result in serious disease (Corpai et al., 1989; Rebhun et al., 1989). BVDV is also regarded as an immunosuppressive agent which may exacerbate symptoms of a concurrent infection (Potgieter et al., 1984).

The aim of this study was to elucidate the consequences of BVDV infections on reproductive performance in a dairy herd. This was accomplished by comparing reproductive data for 3 consecutive years, i.e. 1 year before, the year during, and 1 year after BVDV was introduced into the herd. Furthermore, PI calves were compared with non-PI calves in terms of the incidence of disease and heart girth.

2. Materials and methods

The herd comprised approximately 60 milking cows and 100 heifers and both heifer and bull calves in a loose housing cowshed. Most of the animals were of Swedish Red and White breed but some were of Swedish Friesian breed or cross-breeds. The calves were reared either for dairy replacement or as finished beef. The cows and most of the heifers were artificially inseminated by the local AI association, though some heifers were naturally mated with a bull. For reasons of economy and practicality, the animals were served between October and July. Consequently, most of the calvings occurred between July and April.
In early January 1990 there were signs of a mild respiratory disease among most of the animals, which lasted for about 1 week. About 1 week later, an outbreak of diarrhoea occurred among the cows, accompanied by a fall in milk production. On 1 March one cow died after a few days of diarrhoea. An offspring of this cow, calved in late February, developed diarrhoea on 5 March and was slaughtered on 10 March. At necropsy, among other findings, there were erosions of the epithelia of the mucosa in the mouth, oesophagus and abomasum. Attempts to isolate BVDV from sera of the two animals failed. At this time, however, BVDV was isolated from a 1-month-old calf that was slaughtered because of severe pneumonia. On 27 March, milk samples were collected from all lactating cows and blood samples from all non-lactating animals. All but three animals proved antibody-positive to BVDV. The three antibody-negative animals were found to be BVDV-negative.

A reproductive problem was evident during the winter and spring of 1990. Cows returned for service several times and abortions occurred. From July to September 1990, 12 calves were born with a persistent BVDV infection.

2.1. Reproductive performance

The reproductive performances during the following periods were compared: October 1988 to July 1989, October 1989 to January 1990 (BVDV risk period, i.e. the time of conception of the 12 dams producing PI calves), February to July 1990, and October 1990 to July 1991. The reproductive data of a cow were ascribed to a certain period according to the time of first insemination. The cattle inseminated during the BVDV risk period were divided into three groups: cows producing (a) PI calves, (b) non-PI offspring, and (c) cows that aborted or produced stillborn calves or calves that died within 2 days of age.

Reproductive data were obtained from the protocol devised by the AI personnel and from the farmer's records. The following parameters were studied.

(1) The mean number of inseminations per pregnancy (Ins_{mean}). Any re-inseminations within 6 days were excluded. A pregnant state was determined by rectal examination, usually 6–8 weeks after insemination.

(2) Possible early abortion, defined as an interval between two inseminations of 60 days or more.

(3) Abortion, defined either as the visible expulsion of a foetus before 250 days of gestation, or as proved non-pregnant by rectal palpation after a confirmed pregnant state.

(4) Duration of gestations producing calves that survived for more than 2 days after birth. For each multiparous cow (at least three previous calvings), the mean gestational duration over the 3 year period was calculated, starting from the fourth pregnancy.

2.2. Disease incidence among calves

The incidence of disease among calves, between 2 days and 4 months of age, was obtained from the protocol devised by the farmer's veterinary surgeons. Any death of a calf was recorded by the farmer.
2.3. Heart girth

A measuring tape was used to estimate the heart girth of nine PI calves (eight males and one female) and 23 non-PI calves (13 males and ten females) when they were 80 and 180 days of age (three PI bull calves were sold for research purposes and were therefore not available). The heart girth at 80 days was estimated by simple linear regression of the results from two measurements, the first between 60 and 80 days and the second between 80 and 100 days. Similarly, the heart girth at 180 days was estimated from two measurements, between 150 and 180 days and between 180 and 210 days, respectively.

2.4. Retained placenta

The foetal membranes were considered to be retained if not expelled within 2 days after calving. Cases of abortion were excluded, since the information on foetal membranes was considered unreliable.

2.5. Samples, virological methods and persistent infection

Blood or milk samples were taken from all animals on 27 March 1990 and blood samples from all calves born from March 1990 to May 1991 once or more when they were 8 weeks or older. An enzyme-linked immunosorbent assay (SVA-NOVA Biotech, Uppsala, Sweden) was used to detect antibodies to BVDV in serum (Juntti et al., 1987) and in milk (Niskanen et al., 1989). Serum and milk samples were deemed negative for BVDV antibodies if the absorbance values ($A_{450}$) were less than 0.21 and 0.05, respectively. The presence of BVDV in sera was tested by inoculation on cultures of embryonic bovine turbinate cells. Briefly, 20 μl of serum and 100 μl of turbinate cells ($10^5$ cells ml$^{-1}$) were added per well in quadruplicate in microtitre plates. After 4 days of incubation at 37°C, the cultures were examined for cytopathic effects and the presence of BVDV was determined by an indirect immunoperoxidase test (Meyling, 1984).

A calf was considered to be PI if the BVDV was isolated from the serum on two occasions 2 months apart. A calf was considered to be non-PI either if it had a moderate (0.40 $< A_{450} < 0.70$) or a high level of BVDV antibodies, or if it had no, or a low level of BVDV antibodies ($A_{450} < 0.40$) and was virus-negative.

2.6. Statistical analyses

Student's $t$-test, $\chi^2$ analysis and Fisher's exact test were used to evaluate the data. The results are expressed as mean values ± SD, unless otherwise indicated.
3. Results

3.1. Reproductive performance

Of the 39 cows and heifers artificially inseminated or mated with a bull during the BVDV risk period (October 1989 to January 1990) and which became pregnant, nine (23.1%) aborted, one delivered a mummified foetus at full term, three (7.7%) gave birth to a stillborn calf or calf that died within 2 days of birth, while 12 (30.8%) gave birth to PI calves (Table 1, Fig. 1). The remaining 14 animals gave birth to 13 non-PI calves and one calf that died of haemorrhagic gastroenteritis at 1 week of age. This calf had a moderate level of antibodies to BVDV ($A_{450}=0.50$) but attempts to isolate BVDV from the serum failed.

Four of the nine abortions occurred between 2 and 5 months of gestation and three during the last trimester. Two heifers proved pregnant at 2 and 5 months, respectively, but at the expected day of calving, one was found not pregnant and the other was in the third month of gestation. In one case, the abortion occurred 3 months after the dam was known to be seropositive to BVDV.

The highest InSmean was observed during the BVDV risk period, 1.92, when 14 of 35 (40.0%) animals became pregnant after one insemination. As shown in Table 2, this conception rate is significantly ($P<0.01$) lower than for other pregnancies during the 3 year period (116/170, 68.2%). The low rate of pregnancy

Table 1
Numbers of cows and heifers served by bull or artificial insemination (ins) during the periods indicated, and the outcome of gestation. The dams of calves persistently infected (PI) with BVDV conceived during the period October 1989 to January 1990 (BVDV risk period)

|                      | Oct. 1988–July 1989 (10 months) | Oct. 1989–Jan. 1990 (4 months) | Feb.–July 1990 (6 months) | Oct. 1990–July 1991 (10 months) |
|----------------------|---------------------------------|--------------------------------|--------------------------|---------------------------------|
| 1 or 2 ins, not pregnant, culled | 3                               | 0                               | 1                        | 3                               |
| ≥ 3 ins, not pregnant, culled     | 2                               | 2                               | 1                        | 3                               |
| Inseminated, pregnant           | 72                              | 35                              | 31                       | 67                              |
| Mated with bull, pregnant       | 3                               | 4                               | 9                        | 4                               |
| Total number of gestations      | 75                              | 39                              | 40                       | 71                              |

**Outcome of gestation**

|                      |                      |                      |                      |                      |
|----------------------|----------------------|----------------------|----------------------|----------------------|
| Cows culled          | 0                    | 0                    | 0                    | 2                    |
| Abortions            | 0                    | 103                  | 1                    | 0                    |
| Stillbirth or calves dead | 4                  | 3                    | 2                    | 1                    |
| within 2 days        |                      |                      |                      |                      |
| Dystocia, calf dead  | 1                    | 0                    | 0                    | 1                    |
| Live calves          | 70                   | 264                  | 37                   | 67                   |

1Includes two dams of PI calves.
2Six other heifers were also mated with bull and conceived but were later sold.
3Includes a mummified foetus.
4Includes 12 PI calves and one calf that died at 1 week of age.
Fig. 1. Relation between parity and the outcome of pregnancies in cows and heifers artificially inseminated or naturally served by a bull during the 4 month period when dams of persistently BVDV-infected (PI) calves had conceived. Stillbirth includes calves that died within 1 week of age.

Table 2
Reproductive data of cows inseminated and pregnant, in relation to parity and period. The dams of calves persistently infected (PI) with BVDV conceived during the period October 1989 to January 1990

| Period             | Heifers | Uni- and bi-parous cows | Multiparous cows | Total |
|--------------------|---------|-------------------------|------------------|-------|
|                    | N Ins₁ | InS₁ mean               | N Ins₁ | InS₁ mean | N Ins₁ | InS₁ mean | N Ins₁ | InS₁ mean |
| Oct. 1988–July 1989 | 22 16  | 1.36                    | 26 18$^{a}$ | 1.35 | 24 18 | 1.38 | 72 | 52$^{a}$ | 1.36 |
| Oct. 1989–Jan. 1990 | 10 7   | 1.40                    | 11 1$^{b}$ | 2.27 | 14 6 | 2.00 | 35 | 14$^{bc}$ | 1.92 |
| Feb. 1990–July 1990 | 3 2    | 1.33                    | 18 10$^{a}$ | 1.67 | 10 8 | 1.30 | 31 | 20$^{ae}$ | 1.51 |
| Oct. 1990–July 1991 | 20 14  | 1.33                    | 27 19$^{a}$ | 1.44 | 20 11 | 1.55 | 67 | 44$^{a}$ | 1.44 |

InS₁, number of animals conceiving after first insemination; InS₁ mean, mean number of inseminations per gestation. Within columns, the absence of a common superscript indicates a significant ($P<0.05$) difference in frequency.

After first insemination was attributed mainly to the poor performance of the cows and not of the heifers.

Among the animals inseminated during the BVDV risk period, the 13 dams of non-PI calves had the highest InS₁ mean value (2.38). Only two of the 13 animals (15.4%) produced calves after just one insemination. One of these two cows proved seronegative to BVDV after the risk period (27 March) and the other was inseminated late in the risk period. Seven of the 13 dams of non-PI progeny had an interval of 60 days or more between two inseminations. These reproductive data of the dams of non-PI calves differ from those of the dams producing PI calves and of dams inseminated during the other three observation periods (for details see Table 3).
Table 3
Reproductive performance of heifers and cows artificially inseminated and proven pregnant. The number of calves persistently infected (PI) with BVDV conceived during the period October 1989 to January 1990

| Dams (N)   | No. of inseminations per animal | Possible early abortion | Retained placenta |
|------------|---------------------------------|-------------------------|-------------------|
|            | 1 | 2 | ≥ 3 | Mean | N (%) of total | N (%) of total |
| Oct. 1988—July 1989 | | | | | | |
| All dams   | 72 | 52a | 15 | 5 | 1.36 | 5a (6.9) | 2a (2.8) |
| Oct. 1989—Jan 1990 | | | | | | |
| Dams that produced PI calves | 10 | 6ab | 3 | 1 | 1.50 | 0ab | 5b (50.0) |
| Dams that aborted or produced calves stillborn or dead within 2 days1 | 12 | 6ac | 3 | 3 | 1.75 | 2abc (16.7) | 1a (8.3) |
| Dams that produced non-PI calves | 13 | 2cd | 5 | 6 | 2.38 | 7c (53.9) | 0ac |
| All dams   | 35 | 14bed | 11 | 10 | 1.92 | 9bc (25.7) | 6c (17.1) |
| Feb.—July 1990 | | | | | | |
| All dams   | 31 | 20ab | 7 | 4 | 1.51 | 1a (3.2) | 1ac (3.2) |
| Oct. 1990—July 1991 | | | | | | |
| All dams   | 67 | 44a | 18 | 5 | 1.44 | 5a (7.5) | 3a (4.5) |

1 Includes a dam of a calf that died at 1 week of age. Within columns, figures the absence of a common superscript indicates a significant (P<0.05) difference in frequency.

3.2. Dams of PI calves

Six of the dams of the PI calves were heifers, one was a uniparous cow and five were multiparous cows (at least three previous calvings). All but two heifers had been served by AI. The Ins_mean for these animals was similar to that for dams inseminated during the non-BVDV risk periods (Table 3).

The mean duration of gestation of the multiparous cows was significantly (P<0.01) longer for the five cows of PI calves (287.6±9.5 days, range 280–304 days) than for the other 53 gestations in 35 cows (280.6±3.8 days, range 269–292 days). The gestational duration of the four heifer dams of PI calves (278.3±4.2 days) was not significantly different from that of 43 other heifers served by AI (279.7±7.2 days).

Five of the 12 (41.7%) dams of PI calves had a retained placenta, which is a higher (P<0.001) incidence than the seven cases of retained placenta observed after 198 other calvings (3.5%) over the 3 year period. During the post-partum period, four dams of PI calves, of which three had retained placenta, had vaginal discharge and did not come on heat normally and were therefore treated with hormones once or twice. The four animals subsequently came on heat and two conceived after the first insemination, whereas the other two did not become
pregnant in spite of three and four inseminations, respectively. One dam was culled for reasons other than reproductive failure. The Ins_{mean} for the nine dams that had given birth to PI calves and then became pregnant was 1.22.

3.3. Calf health

Calf health was affected when BVDV was introduced. Ten out of 61 calves (16.4%) born between October 1989 and September 1990 were treated by a veterinarian for respiratory disease and/or enteritis and eight calves (13.1%) died before 4 months of age. These incidences are higher (P<0.01) than for the 134 calves (3.7% and 2.2%, respectively) born the year before and the year after BVDV was introduced (Table 4).

Table 4
The number of calves treated for disease by a veterinarian and calves that died between 2 days and 4 months of age

| Period when calves were born | No. of calves | Symptoms | Arthritis or peri-arthritis | Others | Mortality rate |
|-----------------------------|--------------|----------|-----------------------------|--------|---------------|
|                             |              | Diarrhoea or respiratory symptoms |                     |        | N (% of total) | N (% of total) | N (% of total) | N (% of total) |
|                             |              |         |                             |        | N (% of total) | N (% of total) | N (% of total) |                  |
| Oct. 1988-Sept. 1989        | 71           | 2^{a} (2.8) | 2 (2.8) | 2 (2.8) | 2^{a} (2.8) |
| Oct. 1989-Sept. 1990^1      | 61           | 10^{bc} (16.4) | 3^{3} (4.9) | 2 (3.3) | 8^{b} (13.1) |
| Oct. 1990-Sept. 1991^4      | 63           | 3^{ab} (4.8) | 0 | 0 | 1^{a} (1.6) |

1Period when BVDV was introduced into the herd.
2Includes two of the persistently BVDV-infected calves.
3Includes one of the persistently BVDV-infected calves.
4Calves persistently infected with BVDV present in the herd for most of the period.

Within columns, the absence of a common superscript indicates a significant (P<0.05) difference in frequency.

Fig. 2. The heart girth of eight male calves persistently infected (PI) with BVDV (black dots) and of 13 male non-PI calves (lines indicate mean ± 1.96 × SD) at the age of 80 and 180 days.
3.4. The PI calves

Eleven of the 12 calves were males, which is a significantly higher ($P > 0.001$) proportion than among other calves (99/195). Two of the PI calves (16.7%) were treated for diarrhoea and/or respiratory disease and one was treated for pericarpitis before 4 months of age. The heart girth of the female PI calf was less at both 80 and 180 days of age (87 cm and 103 cm, respectively) than the lower 95% confidence limit for ten female non-PI calves (93.5 cm and 116.9 cm, respectively). As shown in Fig. 2 the mean heart girth of eight male PI calves ($96.3 \pm 4.7$ cm) was less ($P < 0.05$) than for 13 male non-PI calves ($100.5 \pm 2.3$ cm) at 80 days of age and also less at 180 days ($123.3 \pm 8.8$ cm vs. $130.2 \pm 2.0$ cm, $P < 0.05$). The female and the four smallest male PI calves were slaughtered at an age of 9–10 months. Three of the remaining four PI calves developed mucosal disease within 1 week at an age of 10–12 months and died or were emergency slaughtered. The fourth PI calf was then also slaughtered.

4. Discussion

The precise date when BVDV was introduced into the herd is not known. No animal had been brought to the farm during the 5 year period preceding the reproductive failure, but pregnant heifers were in contact with a neighbour’s heifers during the summer of 1989 while at pasture. It might be that this contact then resulted in the birth of a PI calf that spread the virus during the winter of 1989–1990. No such PI calf was ever identified in March 1990 when all animals were tested, but possibly it was one of those calves that died early in 1990. The first known PI calf was born on 1 July 1990 and according to the established concept of the pathogenesis of persistent infection (McClurkin et al., 1984; Brownlie et al., 1986), this calf was most likely infected as a foetus in December 1989 or January 1990. The spread of BVDV was not very rapid, as three animals were still seronegative to BVDV on 27 March. It is possible that BVDV infection was involved in the respiratory disease, or the outbreak of diarrhoea among the adult cattle, resembling winter dysentery, in January 1990. However, it is not thought unlikely that the diarrhoea was caused by BVDV alone but, more probably, was the result of an infection with bovine coronavirus (for a review see Saif, 1990), with or without a concurrent BVDV infection (Alenius et al., 1991).

The introduction of BVDV into the herd was associated with typical disorders described elsewhere, i.e. abortions at various times during gestation (Roeder et al., 1986; Sprecher et al., 1991; Table 1), increased incidence of diarrhoea/respiratory disease and death among calves (Barber et al., 1985; Table 4), and the birth of PI calves of which some subsequently developed mucosal disease (Roeder and Drew, 1984; Nagele, 1984). This study also confirms the clinical observation by us and others that some (though not all) PI calves are small in relation to their age (Barber et al., 1983; Fig. 2). The increased proportion of males among
the PI calves (11/12) in this study is interesting, but does not accord with our observations of the sex distribution among PI calves in other dairy herds. There are few published reports concerning early embryonic or foetal losses resulting from BVDV exposure (Grahn et al., 1984; Perdrizet et al., 1987). Whitmore et al. (1981) found that BVDV does not inhibit conception when cattle are orally and intranasally infected at the time of breeding. In the present study, only two of 13 cows and heifers inseminated during the BVDV risk period and which subsequently gave birth to non-PI calves had been inseminated once only (Table 3). One of these two cows proved seronegative in March 1990 and was therefore not likely to have been exposed to BVDV during the risk period. Furthermore, seven of 13 dams of non-PI calves had an interval between two inseminations of 60 days or more, strongly indicating early abortions or resorptions. This finding accords closely with the results from experimental and natural infection of heifers 30 days after insemination, in which foetal death occurred in every case (Carlsson et al., 1989). In contrast, the dams of the PI calves had a normal Ins\textsubscript{mean} during the BVDV risk period (Table 3).

This study also adduces new data to the BVDV syndrome—increased gestational length and retained placenta in cows producing PI offspring. However, only multiparous cows (but not heifers) producing PI calves had a longer mean gestation than controls, which is why the implications of these results must be carefully weighed. A long gestation has little or no effect on the incidence of retained placenta, whereas a short gestation increases the risk of retained placenta (Joosten et al., 1991). Moreover, other factors such as abortions, twins, dystocia, calving season, breed, zero-grazing, sire, advancing age of dam, and retention of placenta at a previous calving all increase the risk of retained placenta (Dyrendahl et al., 1977; Bendixen et al., 1987; Joosten et al., 1991). In this study, such risk factors for retained placenta were not present to any greater extent in gestations resulting in PI calves, than for other gestations (data not shown). Therefore, known risk factors cannot account for the high incidence of retained foetal membranes among the dams producing PI calves (41, 7%), which is a considerably higher rate than either the 3.5% observed after 198 other calvings in the herd or the 5.9% reported from an epidemiological study including more than 100 000 cows of Swedish Red and White breed (Bendixen et al., 1987). Thus, carrying a PI foetus can be a risk factor for retained placenta.

In conclusion, this study shows that an ongoing BVDV infection in a dairy herd severely affects the reproductive performance of heifers and cows at risk and also has an adverse effect on calf health. Overall losses caused by BVDV in a herd can be estimated only if adequate diagnostic methods are used in combination with careful recording of reproduction and morbidity data over a lengthy time span. This is why all the adverse effects of ongoing BVDV infections in the past have in most cases been overlooked. After the culling of PI calves, the herd described in this paper became free of BVDV infection, judging from a serosurvey performed in April 1992 on sera from 6–12 month old calves. The owner has decided to keep the herd free of BVDV which, according to our experience, can be achieved by avoiding contact with BVDV-infected cattle.
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