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Experimental infection of cattle in early pregnancy with a cytopathic strain of bovine virus diarrhoea virus

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Nine pregnant heifers, in early gestation (63 to 107 days), were infected intranasally or in utero with cytopathic bovine virus diarrhoea virus (BVDV) and each dam seroconverted. All nine calves developed to full term; four were stillborn, of which one had seroconverted but virus was not recovered from their tissues. One of the five liveborn calves appeared to have seroconverted in utero to an adventitious BVDV infection in late pregnancy but the remaining four were not viraemic and showed a normal secondary antibody response to BVDV infection at about six months old. Thus, in contrast to results with non-cytopathic virus there was no evidence that infection in utero with cytopathic virus could result in a persistent viraemia or immunotolerance. It is suggested that cells able to support a persistent viraemia with cytopathic virus may not be developed in the young fetus.

INFECTION of the bovine fetus with bovine virus diarrhoea virus (BVDV) during the early stages of pregnancy can have a severe outcome. Under farm and experimental conditions there may be abortions, stillbirths or the birth of calves either congenitally damaged or persistently viraemic and immunotolerant (Malmquist 1968, Kendrick 1971, Brownlie et al 1986a). The viraemic calves may fail to thrive and later succumb to mucosal disease (Steck et al 1980, Brownlie et al 1984).

The observation that all viraemic animals are infected with the non-cytopathic form (biotype) of BVDV has been a constant finding (Brownlie et al 1984, Barber et al 1985, Bolin et al 1985, Brownlie et al 1987, Clarke et al 1987). In utero infections with a cytopathic isolate (NADL) have been reported (Casaro et al 1971, Kendrick et al 1971, Braun et al 1973) and when fetuses were infected beteen 100 and 256 days of gestation many seroconverted. Virus was recovered from some samples of fetal tissue examined between six and 56 days after infection but abortions did not occur. On the other hand abortions were observed when infection was between 51 and 99 days but virus was not recovered. McClurkin et al (1984) also used the NADL strain for in utero infection between 86 and 114 days, there was one abortion and the remainder seroconverted. In contrast when a different cytopathic isolate was used by Braun et al (1973) evidence of fetal infection before 95 days was not obtained. Done et al (1980) observed abortion and seroconversion with a pool of 10 isolates but reisolated only a non-cytopathic biotype. However, there appears to be no documented case of persistence and immunotolerance, as a result of fetal infection, with the cytopathic biotype. This anomaly does not appear to have been investigated but there are several possible explanations: (i) the cytopathic virus cannot cross the maternofetal barrier at the placentome; (ii) cytopathic virus is so pathogenic that the fetus, infected early in its development, does not survive to full term; (iii) the cytopathic biotype may infect the early fetus but becomes non-cytopathic; (iv) cytopathic virus may be more susceptible than the non-cytopathic form to inactivation by fetal cells or fluid; and (v) cytopathic BVDV may require a 'target-cell' that does not mature until after the fetus becomes immunocompetent. This paper describes attempts to resolve some of these possibilities by infection of seronegative cattle, during early pregnancy, with cytopathic virus.

Materials and methods

Animals

BVDV is endemic in the Compton herd but nine Friesian heifers were selected that had no detectable antibodies to the virus, were not viraemic and were between 63 and 107 days pregnant. They were housed in an isolation unit in two groups. The heifers were infected with BVDV, intranasally or in utero, and were examined daily for behavioural signs of oestrus, as an indication of termination of pregnancy and abortion. After eight weeks in isolation the animals were returned to the farm.

At parturition the calves remained with their dams and were allowed to suck maternal colostrum for 24 hours but were then reared separately.

Infection of animals

Virus. A cytopathic isolate of BVDV, Pe515c Cl,

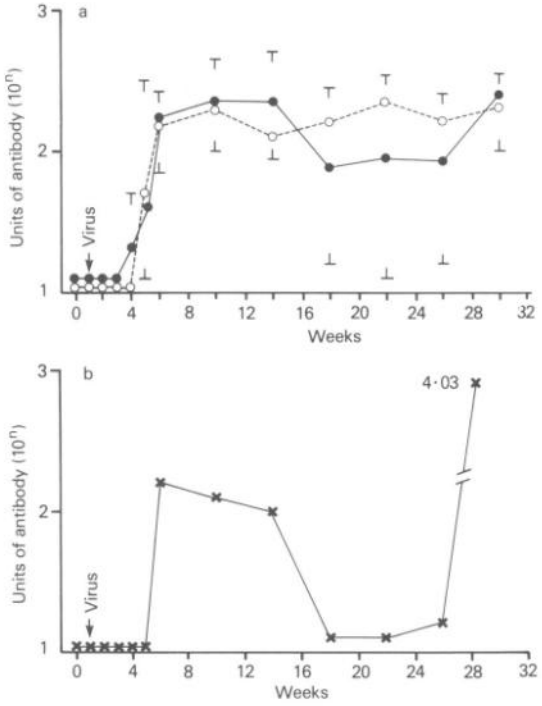

FIG 1: (a) Antibody response (mean ± SEM) measured by ELISA in pregnant cattle challenged with cytopathic BVDV. Five animals were infected intranasally (— ● —) and four in utero (— —○— —). (b) Antibody response measured by ELISA in cow V139 challenged with cytopathic BVDV

from a field case of mucosal disease (Brownlie et al 1984) was used. The virus was grown in cultures of calf testis cells and was cloned by three cycles of plaque purification.

Intranasal inoculation. Five heifers were inoculated intranasally with 5×10^7 TCD50 of virus in 5 ml cell culture medium divided between both nostrils.

In utero inoculation. Four heifers were presented for surgery to allow direct infection of the fetus, thus bypassing the maternofetal barrier. The surgical approach was in the left sublumbar fossa. Local anaesthetic was infiltrated along a 20 cm vertical line in the centre of the fossa. The skin and first muscle sheet were incised by scalpel, the two remaining muscle layers were parted by blunt dissection to reveal the peritoneum and this, in turn, was lifted and incised vertically with scissors. The pregnant uterine horn was brought forward to the incision and either the fetus or fetal membranes were injected with 5 ml of cell culture fluid containing 10^6 TCD50 of virus. Finally, the incision was closed with nylon sutures with rubber sleeves on the skin to prevent pressure necrosis. Twenty millilitres of longacting terramycin was given intramuscularly.

Rechallenge of calves. Calves that were born live were returned to the herd at about three months old and their normal development assessed for a further three months. They were then returned to an isolation unit, rechallenged with 5×10^7 TCD50 of cytopathic virus by intranasal aerosol and examined daily for evidence of anorexia, salivation or diarrhoea.

Assay for antibodies to BVDV

Samples of serum were collected at weekly intervals from the heifers for six weeks after infection and then at monthly intervals until they calved.

Calves were bled at birth (after colostrum) and at weekly intervals for 10 weeks and for five weeks after rechallenge. Sera were assayed for antibodies to BVDV by ELISA (Howard et al 1985).

Assay of samples for BVDV

Suspensions were prepared from various tissues and seeded to cultures of calf testis cells and observed for cytopathic effect. Coverslip preparations were tested for the presence of non-cytopathic BVDV using a fluorescent antibody method. Negative cultures were subjected to a second passage in calf testis cells. The tissues examined included the following: tonsil, thymus, lung, liver, spleen, small intestine (Peyer's patch and non-Peyer's patch), mesenteric and prescapular lymph nodes, brain, testis and blood.

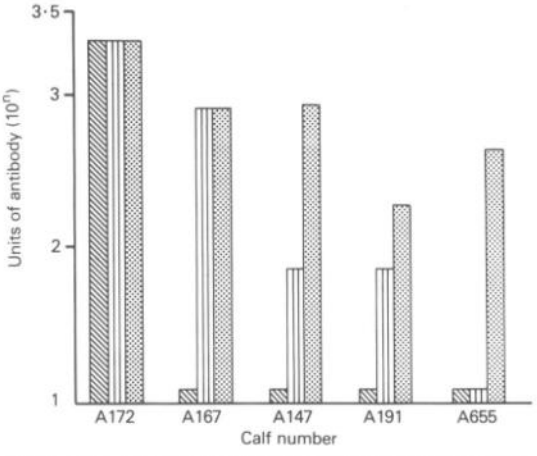

FIG 2: Antibody to BVDV assayed by ELISA in sera of calves at birth (▨), six months old when rechallenged with cytopathic virus (▥) and five weeks after challenge (▩)

TABLE 1: Infection of pregnant heifers with BVDV

Heifer number	Route of inoculation	Length of pregnancy (days)	Age of fetus when infected (days)	Outcome of pregnancy	Calf number
T487	In utero	274	89	Stillbirth	–
T668	In utero	278	79	Stillbirth	–
V139	In utero	279	85	Live calf	A172
V605	In utero	285	90	Live calf	A167
T433	Intranasal	280	63	Stillbirth	–
T470	Intranasal	280	107	Live calf	A147
T676	Intranasal	281	80	Stillbirth	–
T689	Intranasal	280	65	Live calf	A191
T699	Intranasal	281	102	Live calf	A655

Results

Antibody response of pregnant heifers to BVDV infection

All the heifers seroconverted following BVDV infection. Those that had received an intranasal inoculation started to respond at four weeks and reached a maximum titre by about 10 weeks (Fig 1a). The heifers whose fetuses had been inoculated also seroconverted and showed similar dynamics of response (Fig 1a). One heifer (V139) had a weak primary response and antibody had become undetectable by about week 17. However, during the month preceding parturition, there was a considerable secondar response (Fig 1b). This was reflected in the antibody titre of the serum of the calf, A172, from this cow (Fig 2).

Outcome of pregnancy

All nine animals continued their pregnancies to full term but four calves were stillborn (Table 1). Calculations showed that these fetuses were infected between 63 and 107 days of development.

Assay of samples from stillborn calves for BVDV

Samples of tissues from four stillborn calves (Table 1) were tested for the presence of BVDV but neither cytopathic nor non-cytopathic virus was detected. However, serum from one of these (calf from dam T668) had antibody to the virus.

Assay of blood from liveborn calves for BVDV

Samples of whole blood collected from calves at birth (after colostrum) and when they were about two months old were assayed but BVDV was not detected.

Antibody levels in liveborn calves

Sera from each of the liveborn calves were assayed for antibody to BVDV. Titres of samples from four calves showed a decline over several weeks with a mean half-life of 22 days and consistent with maternal antibody. The remaining calf, A172 (dam V139, Table 1), had no decline in antibody titre and thereby showed evidence of an active immune response.

Antibody response in rechallenged calves

The five liveborn calves developed normally and when rechallenged at six months old showed no clinical signs of disease.

The titres of antibody to BVDV in these calves at birth, at six months old when rechallenged and five weeks later are shown in Fig 2. Calf A172, that had seroconverted in utero, showed no changes and A167 appeared to have seroconverted before challenge. The other three animals showed a typical serological response.

Discussion

Each of the nine heifers inoculated with BVDV developed an immune response and this indicated that an infection with cytopathic virus was established. The increase in antibody titre was similar to that of gnotobiotic calves infected intranasally (unpublished results). There were no apparent differences in the dynamics of the response when the two routes of infection, intranasal or in utero, were compared. This provides support for the observations of McClurkin et al (1984) that cytopathic virus is able to cross the maternofetal barrier although the possibility of infection from trace amounts of inoculum left in the needle track, following fetal infection, cannot be eliminated.

The initial expectation that some of the dams would abort their conceptus (Casaro et al 1971, Kendrick 1971, Done et al 1980) was not observed and bypassing the maternofetal barrier by direct infection of the fetus did not result in abortions or congenitally

damaged calves. All the pregnancies developed to full term unlike infection with non-cytopathic virus when a third of fetuses may be aborted (Brownlie et al 1986a). Persistent viraemias were not established and this is also in contrast to infection of cattle, before 110 days of gestation, with non-cytopathic BVDV. Brownlie et al (1986a) reported that 17 of 21 calves, born live, were viraemic. The authors' findings provide an implication that cytopathic and non-cytopathic viruses have different requirements or affinities for cell receptors.

The four liveborn calves that did not seroconvert in utero showed a normal serological response (Fig 2). Therefore, in addition to a lack of viraemia they showed no evidence of immunotolerance with the authors' strain of cytopathic virus. Calf A172 had an active immunity. This appeared to be the result of fetal infection, from non-cytopathic virus present in the Compton herd during the last month of pregnancy when its dam (V139) showed a marked secondary response (Fig 1b). It is also possible, but unlikely, that cytopathic virus had infected the fetus where it had persisted (for about 160 days) to the last month of pregnancy before seroconversion occurred and at the same time reinfected the dam. Another calf that was stillborn (calf from dam T668) also seroconverted in utero. This fetus was infected at the 79th day of pregnancy (Table 1). In two previous studies, fetuses that seroconverted after infection with the cytopathic strain NADL (Casaro et al 1971, Kendrick 1971) were mainly at a later stage of pregnancy than in the present study. The heifers infected intravenously by McClurkin et al (1984) also tended to be at a later stage and it is evident (Casaro et al 1971, Braun et al 1973) that the virus may persist, in which time younger fetuses could attain immunocompetence.

Although the nine calves went to full term there were four stillbirths. Two of these were alive at the start of parturition and required veterinary assistance but did not survive. Another calf was found born dead and the fourth was diagnosed dead at the commencement of parturition. This unexplained finding, 44 per cent stillbirths, must be treated with caution as the numbers are small but the possible influence of infection with cytopathic virus should not be overlooked. For comparison an analysis was made of 29 pregnant heifers, uninfected, of similar age and calving at the same time, in the Compton herd. Six produced dead calves (21 per cent) born between 274 and 287 days of pregnancy and classed as stillborn. The reported figures for stillbirths in heifers is high, 10 to 12 per cent for Dutch Friesians and 15 to 20 per cent for Dutch Red and White heifers (Smidt and Huth 1979). In the experiments reported by Done et al (1980) a number of fetuses died but these could have been the result of the non-cytopathic viruses isolated. Tests for this biotype do not appear to have been included in other studies (Casaro et al 1971, Kendrick 1971) so that its causal role in the abortions observed cannot be ascertained.

Non-cytopathic virus has been recovered from cattle inoculated with a pool of cytopathic BVDV isolates (Done et al 1980) and from sheep inoculated with a brain pool infected with cytopathic Border disease virus (Gardiner et al 1983) and it has, therefore, been suggested that these agents may change as a result of animal inoculation. No evidence was obtained that this occurred in these studies. Unless the viruses are cloned and each form free of the other it may be difficult to ascribe their individual pathogenesis (Brownlie et al 1986b). Also, no evidence was obtained for inactivation/inhibition of virus by amniotic fluid. Both forms of virus were titrated in cell cultures in the presence of 10 per cent amniotic fluid but there appeared to be no effect on the titres obtained (unpublished).

The initial premise of (i) a failure of cytopathic virus to cross the maternofetal barrier; (ii) fatal pathogenesis; (iii) change of biotype; or (iv) inactivation by fetal fluid were not confirmed by the results. An alternative possibility remains: the predilection cell for cytopathic virus in the bovine fetus is not present or has not differentiated before 107 days of gestation. This is supported by the authors' observation (unpublished) that cytopathic virus could not be recovered from tissues of a fetus (of about 85 days) six days after infection. Other studies have shown that cytopathic virus appears to have a qualitative preference for gut lymphoid tissue (Clarke et al 1987) and this tissue, for example, may not be sufficiently developed for cytopathic virus to establish a persistent viraemia in the young fetus.

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