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Brief Communication

An Unusual Congenital Malformation in a Calf with Serological Evidence of Foetal Bovine Viral Diarrhoea Virus Infection

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Bovine viral diarrhoea (BVD) virus is maintained in the environment by persistently infected animals (*Derget & Loewen,* 1995). The BVD virus in an immunocompetent pregnant animal is capable of crossing the placental barrier and invading the foetus (*Kahrs* 1973, *Done et al.* 1980). The principal determinant of foetal response to infection is the age of the foetus at the time of infection (*Baker* 1987), and the differing ability of different strains of BVD virus to produce congenital defects (*Hafez et al.* 1976, *Sanders et al.* 1983). Breed variation and immune status of the host may also be important factors in determining the foetal effect.

The possible outcomes of foetal infection include foetal resorption, abortion, mummification, congenital malformations, birth of weak and undersized calves, birth of calves persistently infected with BVD virus, and birth of normal calves. Foetopathology caused by BVD virus infection during the first trimester has been well documented (*Kahrs et al.* 1970, *Casaro et al.* 1971, *Scott et al.* 1973, *Brown et al.* 1974, 1975, *Done et al.* 1980, *Van Oirschot* 1983, *Binkhorst et al.* 1983, *Wilson et al.* 1983, *Ohmann* 1984, *Roeder et al.* 1986). The following congenital defects have been described: cerebellar hypoplasia, hydrocephalus, hydranencephaly, with or without cranial deformation, dysmyelination of the spinal cord, lenticular cataracts, microphthalmos, chorioretinopathy, alopecia, brachygnathia, intrauterine growth retardation and thymus hypoplasia.

This report describes an unusual congenital malformation in a calf, where there was serological evidence of foetal BVD virus infection. The male calf was born to a 3.5-year-old dairy cow after a prolonged gestation (294 days), and 15 min after the calving the animal died. The first female calf born to this cow, one year previously, was normal. The well managed dairy cattle herd (Israeli-Holstein breed), comprising 40 lactating cows, was kept under a zero-grazing management system in open barns, all the year round, with a rolling herd milk production average of 9,000 kg. The herd had not been routinely vaccinated against BVD infection. This unusual malformation was one-off occurrence, and there were no other indications of BVD virus – associated in this herd. Serological survey by ELISA test showed a prevalence of 89% for BVD virus in this particular herd.

Pre-colostral serum from heart blood of the newborn calf and a blood sample from the dam

were collected for detection of neutralizing antibodies and for virus isolation.

Cell culture: Kidneys and lungs from bovine foetuses, obtained from a local abattoir, formed the source for the cell cultures. Preparation of the cell suspension was performed according to standard procedures (*Mahy & Kangro* 1996).

Screening for adventitious BVD virus: Five millilitres of the final cell suspension were cultured separately, passaged three times at weekly intervals, and each passage was tested for the presence of BVD virus by an indirect immunofluorescence (IF) assay (Hyclone Laboratories, Inc., UT, USA). Briefly, at each passaging, a drop of the cell suspension (approximately 10 000 cells/drop) was dried on a glass slide, fixed in 100% acetone for 10 min and allowed to dry. The spotted sample was incubated with diluted bovine anti-BVD virus antiserum in a humid chamber for 30 min at 37 °C, washed 3 times with carbonate/bicarbonate buffer, then incubated with diluted goat anti-bovine IgG/FITC in a dark humid chamber for 30 min at 37 °C. After 3 additional washes with carbonate/bicarbonate buffer, a mounting buffer of 50% glycerol was applied and the slide was observed under epifluorescent lighting (Nikon Optiphot, Osram XBO 100 W OFR mercury lamp, FITC filter). Positive and negative controls were included in each test.

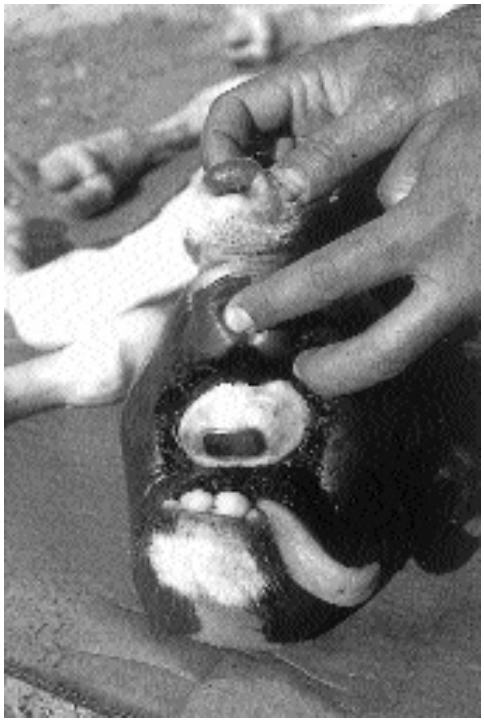

Figure 1. A single large eye in the middle of the face.

Sera were heated at 56 °C for 30 min and examined in a neutralization assay in microtitre plates, using a 1-h incubation at 37 °C with cytopathogenic BVD virus isolate (100 TCID50 per well) and serial twofold serum dilutions. If inhibition of the cytopathic effect was observed at any dilution, the serum was considered to be negative for BVD virus-neutralizing antibodies.

The following congenital malformations were observed: The 2 orbits had merged and formed a single cavity containing one eye (Figs 1 & 2); generalized alopecia was present, except for the eye, mouth, ears and tail end (Figs 1 & 2); distorted upper jaw and nose, palate cleft or almost totally absent. There was a long median cutaneous protuberance (8 cm long) (Fig. 2) above the single eye. The cerebral hemispheres were fused, with hydrocephalus in the lateral ventricles. The optic nerves were also fused. The pituitary gland was absent, apparently causing an oversize foetus with a prolonged gestation. Serum obtained from the calf had a virus neutralization titre of 1:8192 and that from the dam, 1:512. Blood from the dam was negative on virus isolation.

Malformation may arise when virus infection occurs during organogenesis and thus interferes with growth, differentiation and maturation of foetal tissue, whereas lesions may be the result

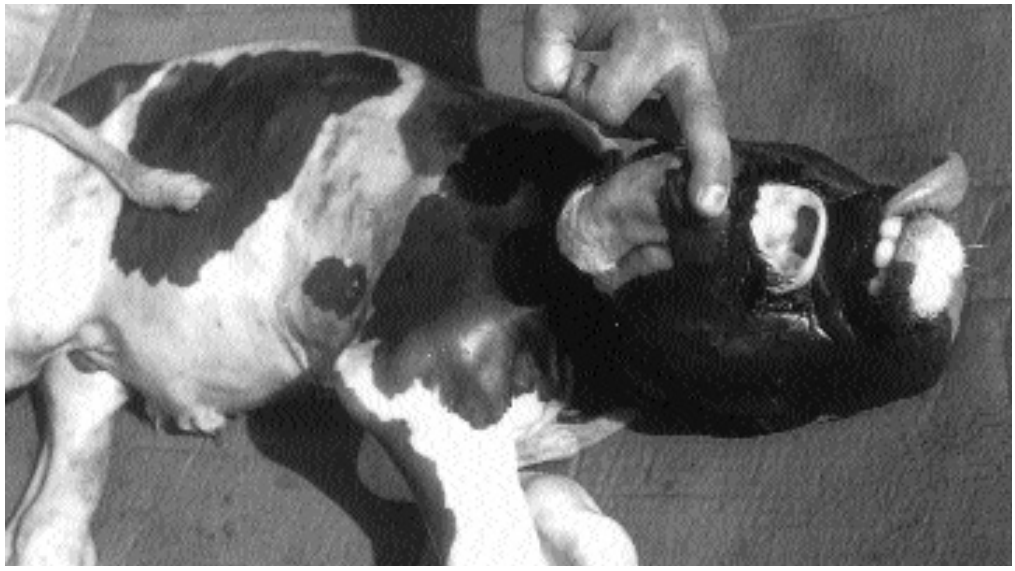

Figure 2. Generalized alopecia, except for the eye, mouth and ears. A long median cutaneous protuberance exists above the eye.

of virus infections of already matured tissue (*Van Oirschot* 1983). Most reports of congenital anomalies of BVD infection have described one or 2 anomalies rather than multiple anomalies (*Binkhorst et al.* 1983, *Wilson et al.* 1983, *Ohmann* 1984).

In the present case, BVD virus replicated apparently in a wide range of foetal tissues. The outcome depends upon the extent of the damage to actively dividing cells, the stage of foetal organogenesis, the development of foetal immune competence, and the ability of the foetus to mount an inflammatory response (*Duffell & Harkness* 1985). Alopecia has been related to maternal infection with BVD virus (*Kendrick* 1971). It seems that, in the present case, inflammatory or necrotizing lesions were severe enough to destroy the germinal epithelium or hair follicles during foetal development, which resulted in some degree of congenital abnormalities (*Casaro et al.* 1971).

The high level of circulating anti-BVD antibodies was the result of an active immune response of the foetus to an intrauterine infection induced by a BVD virus. A similar observation was reported by *Kendrick* (1971) and by *Nettleton & Entrican* (1995), and it indicates once again that the bovine foetuses are immunologically competent early in gestation. No foetal disease has been recognized to result from infection occuring after acquisition of full immune competence - around day 180 of gestation (*Brown et al.* 1979). The time of infection of the foetus in the present case cannot be determined exactly, but the severe and multiple congenital anomalies observed in this newborn calf must have resulted from infection earlier in pregnancy – from about 42 to 125 days of gestation (*Brownlie* 1990).

The presence of high pre-colostrum antibody titres in the serum of the anomalous calf is convincing evidence that a prenatal foetal BVD in-

fection occurred. Although there is serological evidence that the calf had been infected with BVD virus, it cannot be ascertained whether the abnormality was due to the BVD virus infection.

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