Prevalence of bovine virus diarrhoea virus viraemia in cattle in the UK

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INFECTION of cattle with bovine virus diarrhoea (BVD) virus is common both in the UK (Harkness and others 1978, Stott and others 1980) and elsewhere (Meyling 1984, Bolin and others 1985a). Most infections are clinically inapparent but if they occur in pregnant animals the fetus can become infected. Depending on the stage of gestation, infection may induce a fetal antibody response, produce malformations and death, or result in the birth of persistently viraemic calves that may be stunted and have an increased frequency of neonatal death.

Some persistently viraemic calves survive until maturity and the virus can be passed on to subsequent offspring forming a family of persistently infected cattle (Liess and others 1984, McClurkin and others 1984). It is these calves, persistently infected with non-cytopathogenic strains of BVD virus and immunotolerant, that are at risk from dying with acute clinical mucosal disease later in life (Malmquist 1968, Steck and others 1980, Roeder and Drew 1984), an event triggered by a superinfection with a cytopathogenic strain of the virus (Brownlie and others 1984, Bolin and others 1985b).

Apart from the risk of developing mucosal disease, persistently viraemic cattle are a source of BVD virus infection in a herd which may contribute to an increased incidence of other diseases, eg, respiratory disease (Stott and others 1980, Potgeiter and others 1984, Barber and others 1985).

The purpose of this study was to investigate the frequency with which persistent infection with BVD virus occurred in apparently normal cattle in the UK.

Four groups of cattle were investigated. The first comprised 325 calves aged two to four months held in three groups of about equal size and being reared for beef production on site 1 (Table 1). The second was 341 calves aged two to four months held in six groups that ranged in size from 40 to 97. These were also being reared for beef production but on a second site (site 2, Table 1). The third group of cattle comprised 93 apparently healthy pregnant cows two to three years old selected for export from 15 farms. The fourth group was 165 gnotobiotic calves sampled on the day on which they were derived at this institute between 1980 and 1985.

Serum samples were collected from each animal and stored at −20°C until assayed. Although buffy coat preparations have generally been used for the detection of BVD virus in blood (Bolin and others 1985a), the observations of M. C. Clarke, J. Brownlie and C. J. Howard (unpublished data) and those of Meyling (1984) have shown that serum is satisfactory. A microtitre system was used to assay samples (Howard and others 1986). Briefly, 10 µl of serum was added to duplicate wells in a microtitre tray containing 2×10^4 embryonic bovine lung cells (Rutter and Luther 1984) in 200 µl of Eagles minimum essential medium and 10 per cent heated fetal calf serum. Trays were incubated for six days at 36°C in 5 per cent carbon dioxide in air. The presence of non-cytopathogenic BVD virus in cell cultures was detected by immunoperoxidase staining carried out at room temperature. Cells were fixed by treatment with paraformaldehyde and Nonidet (Laurila and others 1978) and stained with swine anti-BVD virus serum and affinity purified goat anti-pig IgG coupled with horseradish peroxidase (Kirkland Perry Laboratories, USA). The substrate was tetramethylbenzidine used according to the protocol of Miles Laboratories. Cells infected with BVD virus produced bright yellow wells. Positive and negative controls were included in all trays.

TABLE 1: Prevalence of persistent infection with bovine virus diarrhoea (BVD) virus in four groups of cattle

Animal group	Number tested	Number of BVD virus isolations	Number persistently infected
Beef calves: site 1	325	3	1
Beef calves: site 2	341	1	1
Pregnant cows	93	1	1
Gnotobiotic calves	165	2	1
Totals	924	7	4

The results are shown in Table 1. Three of the 325 sera assayed from site 1 were found to contain BVD virus (Table 1). None of these infected sera contained antibody detectable by enzyme-linked immunosorbent assay (Howard and others 1985). However, sera taken six weeks later from two of these calves contained antibody indicating that seroconversion had occurred as a result of infection. The third animal remained seronegative when tested six and 12 weeks after the original sampling indicating it was an immunotolerant and persistently viraemic calf. BVD virus was isolated from one of 341 sera tested from site 2. A second sample collected about six months later also yielded virus indicating the animal was persistently viraemic. Antibody was not detected in this second serum sample. BVD virus was isolated from one of the 93 sera taken from pregnant cows and a second serum sample taken about two months later also contained the virus, but did not contain antibody, indicating that this animal was persistently infected. Furthermore, during this time the cow had produced a calf that was also viraemic and antibody negative. Sera from two out of 165 gnotobiotic calves contained virus. In one case a second sample was not available but in the other a second sample taken about two months after birth contained BVD virus, indicating the animal was persistently viraemic. As expected antibody was not detected.

Three of the isolations were from sera stored for at least three years at −20°C indicating storage in this manner is adequate for retaining viral infectivity scored as positive or negative. Persistently viraemic cattle contain 10^4 to 10^6 TCD50/ml serum (M. C. Clarke and others, unpublished data) and a moderate fall in titre with storage should not prevent the detection of viraemic cattle. In this context it can be noted that batches of fetal calf serum used for cell culture clearly remain contaminated with BVD virus for long periods when stored at −20°C (Nuttall and others 1977).

In summary, seven animals out of 924 were found to have BVD virus in their sera, a prevalence of 0·8 per cent. Of these one could not be studied further and two seroconverted so that four animals (0·4 per cent) were identified as being persistently infected and immunotolerant. A survey of 1332 sera taken from cattle at slaughter in Denmark indicated 0·9 per cent were viraemic (Meyling 1984). Some of these may have been transiently infected and the disease status is unknown but the percentage obtained is similar to the present value of 0·8 per cent. A survey of 3157 cattle by Bolin and others (1985a) indicated 1·7 per cent were persistently infected but this was not a random survey of cattle as several of the herds had been selected for a previous history of BVD virus infection.

Fetal infection that results in the production of viraemic cattle is likely to continue as long as BVD virus circulates in the cattle population and infects susceptible pregnant cattle. Screening of animals by a microtitre system similar to the one used here could provide an inexpensive way of detecting vir-

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aemic animals and remove a potential source of infection. Finally, the survey provides a figure (0·4 per cent) for the number of persistently infected cattle likely to be present in apparently normal herds in the UK.

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Rapid dectection of toxigenic *Pasteurella multocida* by an agar overlay method

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THE clinical signs of atrophic rhinitis of pigs include atrophy of the nasal turbinate bones and twisting and shortening of the snout (Switzer and Farrington 1975). The disease was first associated with *Pasteurella multocida* by Gwatkin and others, (1953) but experimental reproduction of severe, progressive and irreversible turbinate lesions in pigs was achieved only when toxigenic type D strains of *P multocida* were selected for infections (Pedersen and Barfod 1981, Rutter and Rojas 1982, Rutter 1983, Pedersen and Elling 1984).

Toxigenic isolates were differentiated from non-toxigenic strains because cell-free extracts were lethal for mice and caused skin lesions in guinea pigs (de Jong and others 1980) or were cytotoxic for embryonic bovine lung cells (Rutter and Luther 1984). The crucial role of the toxin in the pathogenesis of atrophic rhinitis was shown by the reproduction of turbinate atrophy after intranasal (Il'ina and Zusukhin 1975) or intraperitoneal injection (Rutter and Mackenzie 1984) of crude extracts. This was confirmed when intraperitoneal injection (360 ng protein/kg) of germ-free pigs with a toxic polypeptide (MW 155,000) purified from crude extracts caused 50 per cent turbinate atrophy within 14 days (Chanter and others 1986).

Until now, the most rapid method for the detection of toxigenic *P multocida* has relied on inoculation of samples onto selective culture medium (Rutter and others 1984) and into mice, selection and identification of *P multocida*, passage in broth culture and titration of sterile culture supernatants, along with those of controls, in mice, guinea pigs or embryonic bovine lung cells (Rutter and others 1984). This process can take seven to 10 days and the number of samples to be pro-