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Viral antibodies in bovine fetuses in Argentina

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In order to establish the prevalence of viral infections of the bovine fetus in Argentina, a serological survey for antibodies against viral agents currently affecting cattle in this country was conducted. Antibodies against foot-and-mouth disease virus (FMDV), bovine herpesvirus-1 (BHV-1), bovine leukaemia virus (BLV), bovine rotavirus (BRV), bovine coronavirus (BCV), bovine viral diarrhoea virus (BVDV) and parainfluenza-3 (PI-3) were investigated in a total of 315 fetal serum samples. Conventional techniques were used: indirect immunofluorescence (FMDV, BHV-1, BVDV and BCV), radial immunodiffusion (BLV), ELISA (BRV) and haemagglutination inhibition (PI-3). Antibodies against BHV-1, BVDV and PI-3 were detected in samples from fetuses in the second and third trimester of gestation, with a prevalence of 1.21 per cent (two of 165), 2.03 per cent (four of 197) and 5.08 per cent (nine of 177), respectively. Either antibodies or non-antibody factors able to bind to BRV and BCV antigens were detected with a prevalence of 2.44 per cent (five of 205) and 4.54 per cent (five of 110), respectively. In addition, 14.68 per cent of non-specific inhibitors of PI-3 mediated haemagglutination were found. No seropositives against FMDV and BLV were detected.

The prevalence of viral infections of bovine fetuses under field conditions in Argentina has not been established. The predominant viral pathogens affecting the adult cattle population in this country are foot-and-mouth disease virus (FMDV), bovine herpesvirus-1 (BHV-1), bovine leukaemia virus (BLV), bovine rotavirus (BRV), bovine viral diarrhoea virus (BVDV), parainfluenza-3 (PI-3) and bovine coronavirus (BCV). Infections with bovine enterovirus and adenovirus have also been detected (Zoratti de Verona et al 1979, Lager et al 1981, Schudel et al 1984, Ruiz et al 1989). In this paper, the results of a serological survey of fetuses used for primary tissue culture preparation at the Institute of Virology, INTA-Castelar, are presented. Antibodies against FMDV, PI-3, BHV-1, BVDV, BRV, BLV and BCV were investigated using conventional techniques.

A total of 315 fetuses from different regions of Argentina were obtained at a local abattoir, in Buenos Aires, between August 1990 and April 1991. Blood samples were collected by heart puncture and sera were separated, fractionated and stored at -20°C until assayed. The fetuses were randomly selected, and belonged to several European breeds. They were classified according to sex and age – following the crown-rump length method – as belonging to the first, second or third trimester of gestation (0 to 3, 4 to 6, 7 to 9 months, respectively). Most of the samples (55.9 per cent, 176 sera) belonged to the second trimester; 30.8 per cent (97 sera) to the third, and 13.3 per cent (42 sera), to the first.

Viral antigens were propagated in primary or secondary fetal bovine testis cultures. MA-104, BHK and HRT-18 cell lines were used for BRV assays and preparation of FMDV and BCV antigens, respectively. The viral strains used were: FMDV 01 Campos, BVDV Oregon C-24 V, BHV-1 Los Angeles, BRV serotype 6 Lincoln, a BCV strain obtained from S. McNulty (Veterinary Research Laboratories, Belfast) and a field isolate of PI-3.

Antibodies against BVDV, BHV-1, FMDV and BCV were detected by indirect immunofluorescence (IIF). Bovine rotavirus antibodies were detected by enzyme-linked immunosorbent assay (ELISA) and confirmed by IIF. The authors found the IIF more reliable, since the positive samples had an ELISA reading close to the cut-off value but were clearly positive by IIF. The haemagglutination inhibition (HI) method was employed for detection of anti-PI-3 antibodies. To rule out the presence of non-specific haemagglutination inhibitors the positive samples were treated with neuraminidase and the HI assay was repeated. BLV antibodies were investigated by radial immunodiffusion. Antigen and positive control serum for this assay were kindly provided by the School of Veterinary Medicine, University of La Plata, Buenos Aires.

The results are shown in Table 1. Twenty-four sera...
were positive for antibodies against BVDV, BHV-1, BRV, BCV or PI-3. One of the samples had antibodies against both BHV-1 and BVDV. No positives for FMDV or BLV antibodies were detected. Non-specific inhibitors of the haemagglutination were also detected. As expected, positive samples were from fetuses in the second and third trimester which are fully able to synthesise immunoglobulins (Schultz 1973).

The prevalence of BVDV antibodies was 2.03 per cent (four of 197); this is equivalent to the prevalence found in the USA in 1973, when a study involving 147 fetuses showed three to be seropositives (Hubbert et al 1973). However, a recent report described that 1.21 per cent of 1608 pools of fetal sera from that country had BVDV antibodies. Each pool included sera from two to three fetuses, and the percentage of seropositive fetuses would therefore be at least 4.6 per cent (Bolin et al 1991). The different values may reflect a current greater activity of the virus in the field, or be a result of the application of modified live vaccines against BVDV. In Argentina, vaccination is limited and carried out only with inactivated virus, and fetal antibodies are elicited by the fixation procedures in these assays, a sero-neutralisation test against 1000 TCID50 of bovine rotavirus serotype 6 was conducted (not shown). Partial neutralisation occurred, demonstrating binding of the sera to the native proteins in the virion. The possibility exists, and should be investigated, of the antibodies being against a different serotype of rotavirus, as shown by Brüssow et al (1991), or that sera contain non-anti-

### TABLE 1: Fetal antibodies response according to age

| Gestation (months) | BHV-1 | BVDV | FMDV | BRV | PI-3 | BLV | BCV |
|--------------------|-------|------|------|-----|------|-----|-----|
| 0-3                | 0/22  | 0/27 | 0/11 | 0/27 | 0/23 | 0/27 | 0/14 |
| 4-6                | 0/92  | 2/104| 0/59 | 2/109| 1/82 | 0/109| 3/67 |
| 7-9                | 2/51  | 2/66 | 0/58 | 3/89 | 8/62 | 0/69 | 2/26 |
| Total              | 2/165 | 4/197| 0/128| 5/205| 9/177| 0/205| 5/110f |
| Per cent           | 1.21  | 2.03 | 0.00 | 2.44 | 0.00 | 0.00 | 0.54 |

* Non-specific inhibitors of PI-3 mediated haemagglutination: 26/177 = 14.68 per cent
† One female fetus, eight months old; one male fetus, seven months old
‡ Two female fetuses, four months old; one female fetus, eight months old (same as in 1), one male fetus, seven months old
§ Two male fetuses, six months old; two male fetuses seven months old; one female fetus, eight months old
** One male fetus, six months old; two male fetuses seven months old; six female fetuses, seven months old
*** Four male fetuses four, six, seven and eight months old; one female fetus, four months old

The present results allow for an estimation of the probability of reproductive losses caused by BVDV and BHV-1, which are highly abortogenic (Kendrick 1971, Wyler et al 1989). In addition, BVDV may establish persistent infections in the fetus during the first 120 days of gestation (Brownlie 1990). Assuming that the rate of fetal infection with either virus during the first trimester of pregnancy equals that of the second and third trimesters (given by the prevalence of antibodies, Table 1), the chances of abortion due to BHV-1 would be, at least, 1 per cent and the chances of abortion or births of calves persistently infected with BVDV would be around 2 per cent.

Reported figures analysing BHV-1 and BVDV as causes of bovine abortions differ; 2.9 per cent and 0.5 per cent of more than 3800 bovine abortions were caused by BHV-1 and BVDV, respectively, in the USA (Hubbert et al 1973); in Britain, Lucas et al (1986) detected BVDV in 2.4 per cent of the 330 aborted fetuses analysed, with 4 per cent of BVDV antibodies in fetal fluids, and no BHV-1 antibodies or antigen. In Australia, BVDV was detected in 2 per cent of 265 aborted fetuses (Jerrett et al 1984). In Argentina, abortions attributable to both agents occur (Ruiz et al 1989, Campero et al 1991), but no studies of a large number of cases have been made. Since the present samples were taken at random, and originated in different herds from different regions, the gross estimation of fetal losses induced by BHV-1 or BVDV might be quite accurate.

The authors can also estimate the probability of births of calves with central nervous system lesions induced by BVDV, because infected fetuses 90 to 150 days old are prone to develop such lesions (Roeder et al 1986). The prevalence of BVDV antibodies in two fetuses four months old (two of 197, Table 1) indicates that roughly 1 per cent of the calves may be born with these lesions.

Five of 205 fetuses (2.44 per cent) were positive for BRV antibodies, and five of 110 (4.54 per cent) were positive for BCV antibodies. These are enteric viruses, for which no viraemia has been described; for this reason the finding of antibodies was unexpected. However, there is at least one report describing the detection of antibodies against these agents in fetal and precolostral serum samples in Japan, using the HI and neutralisation tests (Sato et al 1980). For BRV antibodies the present authors employed an ELISA and the IIF test for confirmation. As it was possible that the sera contained some non-specific factor(s) able to bind to BRV antigens denatured by the fixation procedures in these assays, a sero-neutralisation test against 1000 TCID50 of bovine rotavirus serotype 6 was conducted (not shown). Partial neutralisation occurred, demonstrating binding of the sera to the native proteins in the virion. The possibility exists, and should be investigated, of the antibodies being against a different serotype of rotavirus, as shown by Brüssow et al (1991), or that sera contain non-anti-
body factors which partially neutralise the virus (Tokuhsa et al. 1981).

For the detection of the BRV and BCV antibody the percentage of seropositives found was considerably lower than that detected in Japan. Tokuhsa et al (1981) detected non-specific virus-binding factors able to neutralise these viruses. The differences in prevalence observed may be due to an authentic lesser proportion of infected fetuses, to the absence of those factors in the samples used, or to a greater specificity of the IIF assay employed.

PI-3 antibodies were detected in nine of 177 samples (5.0-8 per cent). Although most authors were not able to detect anti-PI-3 antibodies in fetal sera (Horner et al 1973, Hubbert et al 1973), this was the highest prevalence in the present survey. By treatment of the samples with neuraminidase the authors detected non-specific inhibitors of haemagglutination in 14.68 per cent of the samples, as did Horner et al (1973). The inhibitors were found exclusively in the oldest sera, and the authors assume that they were derived from the denaturing of sera during storage. Although in Argentina most of the adult bovine population are seropositive against PI-3, and the dams are usually immune (not allowing the virus to reach the fetus), the detected seroprevalence is comparatively high. The fetopathic properties of PI-3 under field conditions are not clear, but the virus can be recovered from aborted fetuses (Lucas et al 1986) and HI antibodies have been detected in fetal fluids and sera from aborted fetuses (Schultz 1973, Jerrett et al 1984). No conclusive data are available, and whether fetuses infected with PI-3, especially during the first trimester, will be aborted, remains an open question.

The present authors did not detect FMDV or BLV antibodies. Since FMDV is endemic in most of Argentina, the negative results in the search for FMDV antibodies and FMDV isolation attempts in fetal organs (not shown) supported the hypothesis that the virus was unable to pass the placental barrier (Schudel and Sadir 1986).

In Argentina, BLV antibodies can be detected both in adult cattle and in precolostral sera by the immunodiffusion assay employed. The negative results can be explained by the low number of samples studied. The percentage of seropositive samples in infected herds is relatively low (3 to 6 per cent of the infected dams) (Toma et al 1990) and it is probable that the analysis of a greater number of sera would give some positive results.

Since only fetuses older than four months are able to produce an antibody response, and some viral agents induce abortions, viral infections of fetuses in the first trimester of gestation are easily missed, unless viral isolations are attempted. The analysis of the seroprevalence is only a first approach to the study of fetal infections, but the results emphasise the need for prophylactic measures and routine controls of diagnostic and pharmaceutical reagents derived from fetal bovine tissues.

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