Expression of non-cytopathogenic bovine viral diarrhoea virus (BVDV) in oocytes and follicles of persistently infected cattle

J. Brownlie, P. J. Booth, D. A. Stevens, M. E. Collins

Veterinary Record (1997) 141, 335-337

EMBRYOS transferred from cattle persistently infected with non-cytopathogenic bovine viral diarrhoea virus (BVDV) are a potential vector for the transmission of this virus. At present, this risk remains equivocal because insufficient evidence has been compiled to conclude that BVDV is unlikely to be transmitted if the recommended International Embryo Transfer Society embryo washing procedures (IETS 1996) are implemented. Following these procedures, the four calves that have been born after transfer of embryos from persistently infected cattle have been diagnosed as non-infected (Wentink and others 1991, Bak and others 1992, Brock and others 1997). It is still unknown however if a proportion of oocytes in infected cattle carry the virus and, if so, whether they are developmentally competent. In order to begin to answer these questions, the presence of both BVDV antigen and ribonucleic acid (RNA) in the ovarian oocytes and follicles of three cows (Aberdeen Angus and Friesian) aged 16 to 19 months that were diagnosed as persistently infected by a blood virus isolation technique (Booth and others 1995) were investigated. BVDV viral antigen was detected in histological sections of cryopreserved ovaries using monoclonal antibodies specific to the BVDV viral envelope glycoprotein g03 and to the non-structural p80 viral protein (Collett and others 1989). The indirect immunofluorescent detection protocol (Booth and others 1995) comprised an initial swine serum blocking step designed to reduce non-specific binding followed by incubation with the monoclonal antibodies. After washing, the sections were incubated in goat anti-mouse immunoglobulin conjugated to fluorescein isothiocyanate. A final washing step proceeded mounting of the sections and their visualisation using a microscope fitted with an ultraviolet lightsource. The specificity of the fluorescence was tested by substitution of the monoclonal antibodies with one of the same
isotype but specific to respiratory syncytial virus, by substitution of either the first or second antibody with phosphate buffered saline, and by similar staining of ovarian sections from a BVDV negative cow.

The detection of RNA encoding BVDV was performed on formalin-fixed, wax-embedded ovarian sections by in situ hybridisation using a digoxigenin-labelled 758 base riboprobe complementary to a highly conserved region of the BVDV genome encoding the non-structural p80 region (Colletti and others 1989). Hybridisation was detected using sheep anti-digoxigenin conjugated to alkaline phosphatase followed by a substrate colour reaction (Desport and others 1992). Specificity of hybridisation was investigated by substituting a riboprobe encoding a neomycin resistance gene and also by performing the technique on ovarian sections from cows not infected with BVDV.

The immunohistochemistry results indicated that over 18 per cent of follicles were infected with BVDV (Table 1) and over 6 per cent of follicles (Table 2) possessed oocytes that were positive for viral antigen (Fig 1). When the infected follicles were classified according to the dual location of BVDV antigen in the oocyte and follicle cell compartments (Table 1), the distribution was found to be approximately equal between those in which both the oocyte and follicle cells were infected, and those in which only the follicle cells were positive. As antigen-positive oocytes are likely to reside within follicles that possess infected follicle cells (Table 1), the small proportion of follicles that exhibited positive oocytes alone in the apparent absence of infected follicle cells probably

<table>
<thead>
<tr>
<th>Animal</th>
<th>Primary follicles</th>
<th>Secondary follicles</th>
<th>Total follicles</th>
<th>BVDDV antigen positive oocytes (%)</th>
<th>BVDDV RNA positive oocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/159 (5-0)</td>
<td>2/2 (100)</td>
<td>10/161 (6-2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>26/219 (11-9)</td>
<td>0/1 (0)</td>
<td>26/220 (11-8)</td>
<td>55/132 (41-7)</td>
<td>23/97 (34-3)</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td>34/378 (9-9)</td>
<td>2/2 (66-7)</td>
<td>36/381 (9-4)</td>
<td>78/199 (39-2)</td>
<td></td>
</tr>
</tbody>
</table>

* No secondary oocytes were observed in these histological sections

† Immunohistochemistry and in situ hybridisation performed in contralateral ovaries

NO No data

FIG 1: Detection of BVDV antigen (light green staining) in ovaries of persistently infected cows using an indirect immunofluorescent technique. (a) Immunofluorescent and (b) light microscopic (after subsequent staining with haematoxylin) photograph of the same ovarian cortical section. A non-infected small primary follicle (F1) with one layer of follicle cells (C) is shown beside a more developed primary follicle (F2) that has between one and two layers of follicle cells and expresses antigen in both the oocyte (O) and the majority of the follicle cells. Note that BVDV antigen is not expressed in the nucleus (Gy Germinal vesicle) which is characteristic for this RNA type of virus. (c) Immunofluorescent and (d) phase contrast microscopie photograph of the same ovarian section, showing a secondary follicle containing an infected oocyte (O) and scattered infected follicle cells present within the cumulus oophorae (CO) and zona granulosa (ZG). The zona pellucida (ZP), follicle basement membrane (BM), theca (T) and the follicular antrum (FA) are clearly defined.
by the recommended IETS embryo washing and trypsin treatment protocol, the threat of transmission of this virus by embryo transfer is minimal. However, until this is established, these results suggest that in vitro fertilisation laboratories and embryo transfer practitioners should consider the health status of their donor cattle or abattoir derived ovaries, in order to prevent the potential transmission of BVDV.

References

Caries and odontoclastic resorptive lesions in a chinchilla (Chinchilla lanigera)

D. A. Crossley, R. R. Dubielzig, K. G. Benson

Veterinary Record (1997) 141, 337-339

IT IS well recognised that domesticated chinchillas (Chinchilla lanigera) suffer from a range of dental problems (Kennedy 1970, Emily and Pennman 1990, 1994, Webb 1991, Strake and others 1996), the most frequent of which is the result of reduced and uneven coronal wear of the premolar and molar (cheek) teeth with resultant left elongation and secondary malocclusion (Crossley 1995, 1997). Although rarely detected clinically, destruction of dental hard tissue can often be recognised at postmortem examination and in prepared skulls (Figs 1 and 2). This short communication reports the histological confirmation of caries and odontoclastic resorptive lesions in a chinchilla.

A postmortem examination was performed on a debilitated pet chinchilla which had been euthanased following five days of anorexia. On physical examination halitosis and malocclusion were evident. The dependent left mandible had an undulating profile. Gross postmortem examination of the dentition confirmed coronal overgrowth, particularly of the mandibular premolar teeth, and root elongation with distortion of the cortical bone plates over the apices of all the cheek tooth roots. Interproximal periodontal pocketing and food impaction was evident. Areas of brown staining of occlusal and interproximal tooth surfaces suggested the

D. A. Crossley, Animal Medical Centre, 511 Wilbringham Road, Chorlton, Manchester M21 0CB
R. R. Dubielzig, Department of Veterinary Pathobiology, K. G. Benson, Department of Medical Sciences, University of Wisconsin, 2015 Linden Drive West, Madison, Wisconsin 53796, USA

represented an underestimate. This reflects the fact that each follicle was analysed in only one histological section and that in some follicles only a minority of follicle cells were deemed infected. A comparison of BVDV antigen and RNA expression in infected oocytes (Table 2) suggested that in situ hybridisation was a much more sensitive technique for the detection of virus since a four-fold greater percentage of infected oocytes was recorded especially in animal 2 where the comparison was between contralateral ovaries. An alternative explanation for this difference may be the absent co-expression of antigen in virus infected tissues. The data also established that viral antigen was present in both primary and secondary follicles (Table 2) indicating that infected follicles had some developmental competence (Figs 1 and 2). Antigen was observed in oocytes residing in follicles possessing from one to more than 10 layers of follicle cells (Fig 1) and, although only a small number of secondary follicles were observed, such follicles possessed on average a seven-fold higher proportion of infected oocytes than primary follicles (Table 2).

If the proportion of oocytes that are infected with non-cytopathogenic BVDV are eventually proven to be non-viable, and embryos which are not infected can be externally disinfectable.