Bovine herpesvirus type 5 in the semen of a bull not exhibiting clinical signs


BOVINE herpesvirus type 1 (BHV-1) has a major impact on the cattle industry. The virus is the causative agent of infectious bovine rhinotracheitis/infectious postural vulvovaginitis (IBRV), It is also recognised as an important cause of abortion and a number of other syndromes, including, albeit rarely, encephalitis (Gibbs and Rweyemamu 1977, Roels and others 2000). Bovine herpesvirus type 5 (BHV-5), a close relative of BHV-1, is the agent of bovine herpesvirus encephalitis (Roizman and others 1992). In view of the high cross-reactivity of BHV-5 antibodies with those induced by its close relative BHV-1, BHV-5 infections have been distributed as yet unknown. Nevertheless, BHV-5 infections are being identified by an increasing frequency, particularly in Brazil and Argentina, (Carrillo and others 1988, Heinlein and others 1993, Salvador and others 1993). The disease is characterised by an often fatal meningoencephalitis (Carrillo and others 1983, Heinlein and others 1993, Salvador and others 1998). The number of infections without any clinical signs is probably high. It is an important agent of diseases of the central nervous system, and therefore BHV-5 (as well as BHV-1) must be considered in the differential diagnosis of bovine spongiiform encephalopathy. BHV-5 has been isolated from brain tissue, nasal and ocular secretions and from abortions (Heinlein and others 1993). However, to date, BHV-5 has not been associated with genital lesions, which are frequently reported for BHV-1 (van Oirschot 1995). Moreover, BHV-5 isolates have not been identified in male genitalia or semen. In this report, communication reports the isolation and characterisation of BHV from the semen of a naturally infected Aberdeen Angus bull exhibiting no clinical signs.

During surveillance for viruses at an artificial insemination centre (Esmeraldeo 1996), a virus was isolated from the semen of a bull exhibiting no clinical signs. The isolation induced typical herpesvirus cytopathology on Madin-Darby bovine kidney cells (ATCC-CCL-24), characterised by rounding and ballooning of cells with formation of syncytia (Esmeraldeo 1996). The isolate, named V175, was initially identified as a bovine herpesvirus with the aid of an immunoperoxidase monolayer assay (IPX) with a monoclonal antibody (mAb) that reacts with both BHV-1 and BHV-5 (2cG5) (Table 1), as described by Roche and others (1997). The virus then had its IPX reactivity profile determined against a panel of BHV-1 and BHV-5-specific mAbs (Souza and others 2002). The reactivity profile displayed by isolate V175 was identical to that of the BHV-5 strains included for comparison: N569, A663 and EV430 (Table 1). In addition, it was distinct from the profile of BHV-1 strains (Cooper, Oxford and SV265) (Table 1).

To confirm the type of V175, a differential PCR was designed on the basis of the sequence of the BHV-1 glycoprotein C (gC) gene, a region known by its low degree of homology between BHV-1 and BHV-5 (Chowdhury 1995). The primers designed were 5'GCGTGGCTCTTGCG3' (forward), corresponding to nucleotides 187 to 202 along the BHV-1 gC sequence, and 5'CTCGGAGGCGATCCG3' (reverse), corresponding to nucleotides 434 to 419 (Chowdhury 1995). The PCR reaction was carried out in a volume of 15 μl containing 2mM magnesium chloride, 1.5 μl Taq buffer 10x, 1.5 μl dimethyl sulfoxide, 0.2mM of each deoxynucleotide, 1 U Taq polymerase, 15 pmol of each primer and 1 μl of the DNA sample. Amplification was performed in a thermocycler (Techne) with one initial cycle of denaturation (94°C for three minutes) followed by 35 amplification cycles consisting of denaturation (94°C for one minute), annealing (60°C for one minute) and extension (72°C for one minute). One final extension step was carried out for seven minutes at 72°C. The reaction revealed an amplicon of the expected size (247 base pairs [bp]) from BHV-1 strains, whereas a larger amplicon of approximately 330 bp (as determined by gel electrophoresis) was obtained from BHV-5 strains (Fig 1). As shown in Fig 1, the amplicon from V175 was similar to those of BHV-5 (2Cg5, A663 and EV340), but different from those obtained from the BHV-1 strains (Cooper, Oxford and SV265). In addition, viral DNA was submitted to restriction endonuclease analysis with BstEII as previously described (Engels and others 1981, 1993, 1993).

TABLE 1: Antigenic profile of reactivity of the V175 semen isolate with monoclonal antibodies (mAbs) by an immunoperoxidase monolayer assay. Representative strains/isolates of bovine herpesvirus type 1 (BHV-1) and type 5 (BHV-5) are included for comparison

<table>
<thead>
<tr>
<th>Type</th>
<th>Virus Strains/isolates</th>
<th>BHV-1</th>
<th>BHV-5</th>
<th>EV430</th>
<th>N569</th>
<th>A663</th>
<th>2Cg5</th>
<th>11H6</th>
<th>7F12</th>
<th>54S</th>
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<tr>
<td>BHV-5</td>
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</table>

+ Positive reaction, – Negative reaction

Origins of the BHV-5 strains, A663, N569, EV430 and the BHV-1 strains, Cooper, Oxford and SV265 are in Souza and others (2002).
D’Arce and others (2002). The restriction endonuclease profile observed confirmed the similarity of V175 with other BHV-5 strains (Fig 2).

Viruses with antigenic and genomic BHV-5 profiles have previously been isolated from two cases of abortion (Heinlein and others 1993) and from outbreaks of respiratory disease in the absence of neurological signs (P. M. Roche, unpublished observations). However, to the authors’ knowledge this is the first report of the isolation and characterisation of BHV-5 from semen. Like its close relative BHV-1, BHV-5 appears to be capable of disseminating in herds via the genital route. Therefore, sexual contact and semen from BHV-5-infected bulls might become a potential source of infection for cows, and appropriate care must therefore be taken to avoid transmission. Proper testing of bulls to ensure their negativity for BHV-5-specific antibodies must be encouraged. It must be borne in mind that, as no BHV-5-specific antibody assays are yet available, serological testing for bovine herpesviruses is usually performed by serum neutralisation tests. BHV-1 neutralising antibodies are highly cross-reactive with BHV-5 and a proportion of BHV-5 seropositive animals may not be diagnosed when the serum neutralisation test is performed only against BHV-1 (Teixeira and others 1998). Therefore, a negative serological test to BHV-1 does not exclude the possibility of previous contact with BHV-5. In such cases, effective monitoring for BHV-5 antibodies should include serum neutralisation tests against BHV-5. If BHV-5-specific serology is unavailable, proper screening of bulls must include virus isolation from genitalia and/or semen otherwise, despite controlling BHV-1 shedding, BHV-5 might be disseminated.

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