A monoclonal antibody-based ELISA allows discrimination between responses induced by bovine herpesvirus subtypes 1 (BoHV-1.1) and 2 (BoHV-1.2)

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Abstract

Bovine herpesvirus type 1 (BoHV-1) has distinct subtypes according to genomic characterization. Immune responses induced by BoHV-1 subtype 1 (BoHV-1.1) are not distinguishable from those induced by BoHV-1 subtype 2 (BoHV-1.2) through conventional serological methods. In the present report, an enzyme linked immunosorbent assay is described that allows discrimination between immune responses in cattle immunized with either subtype, based on a monoclonal antibody that recognizes specifically the amino-terminal region of glycoprotein C (gC) on BoHV-1.1 strains, thus not reacting with BoHV-1.2a. The test displayed a sensitivity of 92%, specificity of 90% and a good correlation with serum neutralization tests on samples from BoHV-1.1-immunized calves (κ = 0.799). The test may be useful to provide new insights into the roles played by each of these two subtypes in the epidemiology of BoHV-1 infections.

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Bovine herpesvirus type 1 (BoHV-1), a member of Herpesviridae family, subfamily Alphaherpesvirinae a major cause of economic losses for cattle industry (Siebert et al., 1999). Clinical manifestations of BoHV-1 infections, although generally referred to as infectious bovine rhinotracheitis (IBR) or infectious bovine pustular vulvovaginitis (IPV), include upper and lower respiratory tract infections, vulvovaginitis, balanoposthitis, abortions and drops in milk yields (Van Oirschot, 1995). Two major BoHV-1 subtypes (subtype 1, BoHV-1.1; subtype 2, BoHV-1.2) have been recognized (Miller et al., 1991; D’Arce et al., 2002). Isolates with tropism for the genitalia were shown later to belong to subtype 2. The occurrence of infections with subtype 1 in Europe in the 1960s led to the first reported cases of infectious bovine rhinotracheitis (IBR) in that continent. That fact led to the association of different subtypes with distinct tropisms on the host’s organism (Edwards et al., 1991). Such association is not, however, absolute, since on occasions both BoHV-1.1 and BoHV-1.2 may be associated with respiratory tract disease (D’Arce et al., 2002) and some strains of BoHV-1.2 may cause severe respiratory illness under experimental conditions (Spilki et al., 2004). However, to date, the epidemiological importance of each of these two subtypes for cattle health is still unknown. In the present study, a monoclonal antibody-based blocking enzyme immunoassay (BoHV-1-subtype-ELISA) that allows discrimination between cattle immunized with either BoHV-1.1 or BoHV-1.2 was tested.

Briefly, for the preparation of BoHV-1 antigen, Madin Darby bovine kidney cells (MDBK) free of bovine herpesviruses and bovine viral diarrhea virus (BVDV) were cultured in Eagle’s minimal essential medium (E-MEM) supplemented with 0% fetal calf serum and enrofloxacin (10 mg/L).
For virus multiplication, the BoHV-1.1 strain EVI 123/98 (D’Arce et al., 2002) was inoculated onto nearly confluent monolayers of MDBK cells at a multiplicity of infection between 0.1 and 1, following standard procedures (Teixeira et al., 2001). When cytopathic effect (CPE) was evident in about 90% of the monolayers, cells and supernatants were frozen at −70 °C, thawed, clarified by low speed centrifugation and used as virus stocks. Titres obtained were typically around 10^7.5 50% tissue culture infectious doses per 50 μL (TCID50). Stocks were used for virus-neutralization assays as well as for the preparation of ELISA antigens.

Cell culture flasks (125 cm^2) were infected the EVI 123/98 virus as above. When CPE was evident in about 60% of the monolayers, the medium was removed and cells overlayed with 0.2% OGP (0.03% H2O2) were added. After 5 min of incubation at 37 °C, the reaction was stopped by the addition of 2 M H2SO4. The optical density (OD) was determined at 492 nm (D’Arce et al., 2002) was inoculated onto nearly confluent 192 F. R. Spilki et al. / Journal of Virological Methods 129 (2005) 191–193.

After 1 h incubation at 37 °C, the plates were washed three times with PBS, filled with another 100 μL of PBST-20 and left to stand for 1 h at room temperature. After adsorption of the antigen, plates were washed once with 100 μL of PBST-20 and left to stand for 1 h at room temperature. The sera under test were diluted 1:2 in PBST-20 and added to duplicate wells. After 1 h incubation at 37 °C, the plates were washed three times with PBST-20 and incubated with a monoclonal antibody (MAb) capable of discriminating between BoHV-1.1 and BoHV-1.2. Such MAb (MAB 71) recognizes an epitope of glycoprotein C (gC) on BoHV-1.1 only, and not on BoHV-1.2 (Risewijk et al., 1999). After 1 h of incubation at 37 °C with MAB 71, three other PBST-20 washings were made. At this moment, anti-mouse IgG peroxidase conjugate (Sigma) was added as secondary antibody and plates incubated for another hour at 37 °C. After three washings with PBST-20, 100 μL of the substrate ortho-phenylenediamine (OPD: Sigma, USA) with 0.03% H2O2 were added. After 5 min of incubation at 37 °C, the reaction was stopped by the addition of 2 M H2SO4. The optical density (OD) was determined at 492 nm in a Multiskan (Titertek) ELISA reader. Levels of inhibition of optical density (IF) were calculated as described previously (IF = optical density MAb − optical density tested serum) / optical density MAb) x 100% (Teixeira et al., 2001). The cut-off point was determined as the mean IF obtained with seronegative control sera; this value was added to 1.76 standard deviations, in order to give statistically significant values (p < 0.05). Values for sensitivity and specificity, as well as Kappa (κ) correlation index were also determined (Jekel et al., 1996).

<table>
<thead>
<tr>
<th>ELISA</th>
<th>BoHV-1.1 immunized</th>
<th>BoHV-1.2 immunized</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA + 23 (a)</td>
<td>2 (b)</td>
<td>25 (a + b)</td>
<td></td>
</tr>
<tr>
<td>ELISA − 18 (c)</td>
<td>20 (c + d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 25 (a + c)</td>
<td>45 (a + b + c + d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Validity analysis: sensitivity (sensitivity = 100 × (c + d)/(a + c) × 100 = 92%; specificity (sensitivity = 100 × (a + b)/(a + b) × 100 = 90%)

Table 1: Analysis between immunization status (BoHV-1.1 or BoHV-1.2) and the responses obtained on MAB 71 discriminative ELISA.

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