LETTER TO THE EDITOR

Comparison of ELISA and virus neutralization test in assaying serum antibodies to bovine herpesvirus 1

S. NANDI, M. KUMAR*

Virology Laboratory, Centre for Animal Disease Research and Diagnosis (CADRAD), Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, U.P. (243122)

Received October 21, 2010; accepted March 17, 2011

Keywords: bovine herpesvirus-1; bulls; seroprevalence; c-ELISA; virus neutralization test

Bovine herpesvirus 1 (BoHV-1) causes a variety of diseases including respiratory, nervous, and reproductive disorders in domestic as well as in wild bovines and occurs throughout the world including India. Infectious pustular balanoposthitis (IPB), a reproductive form of the disease in bulls and buffalo bulls, is prevalent in India (1, 3, 4, 7). This exotic disease has been disseminated in India as a consequence of cross-breeding program of the indigenous cattle conducted for the germplasm improvement. Preliminary diagnosis of the disease can be made by its clinical manifestations, but for confirmatory diagnosis the virus isolation in cell culture, fluorescent antibody technique, ELISA, virus neutralization test (VNT), and PCR are commonly used (5, 6). In this study, a comparison has been made between VNT and competitive ELISA (c-ELISA) for the detection of antibodies to BoHV-1 in serum samples of the cattle of three different farms in India.

In the present study, a total of 272 serum samples of adult cattle were collected from three different organized farms at Meerut, Bhopal, and Rohtak in north-central subtropical part of India. Most of the male animals were apparently healthy except for a few animals showing erythematous and necrotic lesions of 4–5 mm in diameter all over the mucosa of penis and prepuce. The semen samples of a few bulls were light yellow in color and low in volume. The cows were apparently healthy except for a few animals affected by abortion. Up till now, a vaccination against BoHV-1 has not been practiced in India.

Serum samples were transported in cold boxes to the Virology Laboratory, Centre for Animal Disease Research and Diagnosis (CADRAD), Indian Veterinary Research Institute (IVRI), Izatnagar within 2 days after collection. The serum samples were inactivated at 56°C for 30 mins in the water bath. MDBK cell line and BoHV-1 virus isolate (216 IBR II) maintained at the laboratory of CADRAD were used in this study. For VNT, two-fold serial dilutions of the serum samples including positive and negative control serum were diluted from 1:2 up to 1:64 in Dulbecco’s minimum essential medium (DMEM) containing 2% FCS. 50 μl of each dilution was added to the triplicate wells of 96-well tissue culture microtitre plate and 50 μl of BoHV-1 containing 100 TCID₅₀ was added to each well except cell control wells, and the plate was incubated at 37°C for 24 hrs in the CO₂ incubator. Then, 100 μl of MDBK cell suspension containing 3 x 10⁴ cells were added to each well and the plate was further incubated at 37°C for 2 days in the CO₂ incubator and evaluated for the presence or absence of cytopathic effect. The end point titre was determined on the basis of highest dilution of serum neutralizing 100 TCID₅₀ of the virus in 50% of wells (6).

The c-ELISA was performed according to the method provided by manufacturer (Institut Pourquier, France) (2, 9). The plate was read at 450 nm in ELISA reader (Thermo Lab-
Sera with a percentage of inhibition (PI) equal or greater than 55% were considered as negative for the presence of specific antibodies to the gB protein of BoHV-1. Sera with PI equal or lower than 50% were considered as positive for specific antibodies to the gB protein. Sera with PI between 50–55% were considered as doubtful. The diagnostic sensitivity and diagnostic specificity of the c-ELISA was calculated by the comparison with results of VNT, a gold standard and Office International des Epizooties (OIE) recommended test. The agreement between the tests was evaluated by applying Kappa statistic (10).

In this study, 272 serum samples of cattle and buffaloes were tested by VNT and c-ELISA. A total of 171 (62.8%) and 162 (59.5%) serum samples were found positive by c-ELISA and VNT, respectively, whereas 101 (37.2%), and 110 (40.5%) were found negative in c-ELISA and VNT, respectively. 161 and 100 serum samples were found positive and negative, respectively, in both tests. The results indirectly indicated that the positive animals might have been exposed to the BoHV-1 and the lesions present in male animals were suggestive of IPB. A total of 10 serum samples showed positivity in c-ELISA, but negativity in VNT, whereas 1 sample was negative in c-ELISA, but found positive in VNT. Most of the positive serum samples (127) having PI values below 25 indicated the presence of high antibody levels. On the other hand, 101 serum samples had the PI values above 56 greater than 55% were considered as negative for the presence of antibodies to BoHV-1. As the disease is endemic in India, a regular monitoring and surveillance program and zoosanitary measures for the successful control of the disease.

Table Relative diagnostic sensitivity and specificity of c-ELISA and VNT

<table>
<thead>
<tr>
<th>Serum samples</th>
<th>c-ELISA</th>
<th>VNT</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Total 272</td>
<td>171 (62.8%)</td>
<td>101 (37.2%)</td>
</tr>
<tr>
<td>VNT-positive 162 (59.5%)</td>
<td>161</td>
<td>1</td>
</tr>
<tr>
<td>VNT-negative 110 (40.5%)</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

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c\text{-ELISA sensitivity} = \frac{(161/162) \times 100}{100} = 99.4\%; \ c\text{-ELISA specificity} = \frac{(100/110) \times 100}{100} = 90.9\%.
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The agreement between the tests was evaluated by applying the Kappa statistic and Kappa value was found to be 0.91, which indicate almost a perfect agreement. VNT is considered as a gold standard and an OIE recommended test (6). However, there is a need of the cell culture facility, handling the live virus and the test itself lasts 3 days. In India, most of the small veterinary diagnostic laboratories do not have the cell culture facility as it needs skilled staff and expertise to maintain the cell culture in ideal condition. Therefore, ELISA and particularly c-ELISA is a very sensitive test for the examination of large number of serum samples for the presence of antibodies to BoHV-1. As the disease is endemic in India, a regular monitoring and surveillance of the herds for BoHV-1 infection is warranted in order to take decision on the implementation of immunization program and zoosanitary measures for the successful control of the disease.

Acknowledgement. The authors thank the Director of the Indian Veterinary Research Institute for providing facilities to carry out this study.

References