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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)

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Bovine herpesvirus l (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 $TCID_{50}$ was added to each well except cell control wells, and the plate was incubated at 37 °C for 24 hrs in the CO_2 incubator. Then, 100 μl of MDBK cell suspension containing 3 x 10^4 cells were added to each well and the plate was further incubated at 37°C for 2 days in the CO_2 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 $TCID_{50}$ 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-

E-mail: snandi1901@yahoo.com; fax: +91-581-2302188. *Present address: Biological Products Division, IVRI, Izatnagar, Bareilly, U.P. (243122)

Abbreviations: BoHV-1 = bovine herpesvirus 1; VNT = virus neutralization test; IPB = infectious pustular balanoposthitis; c-ELISA = competitive ELISA; PI = percentage of inhibition

system). 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 bulls 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 indicating the absence of antibodies to BoHV-1 and were considered negative. The requirements of the kit that the negative control should have a minimal mean A_{450} value of 0.8 and the PI of positive control should be equal to or lower than 30% of the negative control, were fulfilled in the test. In VNT, 93 (57.4%) positive serum samples showed lower titre (1:2 to 1:8), 45 (27.7%) moderate titre (1:16 to 1:32), and 24 (14.8 %) very high titre (1:64 and above). Clearly, BoHV-1 infection was present in high percentage in the farms and our results were in accordance with the published results, which showed overall 61% positivity to the BoHV-1 in most of organized farms in India employing indirect ELISA (*4*). A study employing monoclonal antibody based ELISA showed that 29.28% of sera of cattle and buffalo bulls from semen collection centers of Gujarat state were positive for the antibody against BoHV-1 (*1*). Possible association of infectious bovine rhinotracheitis and bovine abortion was recorded in 55.4% of the aborted crossbred cows (*8*). Furthermore, BoHV-1 has the tendency to remain latent in the trigeminal or sacral ganglion of the infected animals. Latently infected animals can excrete the virus in semen, vaginal secretion, and respiratory discharges only under naturally or artificially induced stress condition. Therefore, the semen from seropositive bulls should be screened before being used for artificial insemination purposes.

Although a great deal of work has been carried out on the surveillance of BoHV-1 infection in cattle and buffaloes, very limited information has been available about the comparison of VNT and c-ELISA. This study has been carried out to detect the presence of antibodies to BoHV-1 by VNT and c-ELISA and to find out the relative diagnostic sensitivity and specificity and the agreement between the c-ELISA and VNT. In this study, the diagnostic sensitivity and diagnostic specificity of c-ELISA in comparison to VNT was 99.3 % and 90.9 % respectively (Table). 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. On the other hand, 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.

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

Serum samples	c-ELISA	
	Positive	Negative
Total 272	171 (62.8%)	101 (37.2%)
VNT-positive 162 (59.5%)	161	1
VNT-negative 110 (40.5%)	10	100

c-ELISA sensitivity = (161/162) x 100 = 99.4%; c-ELISA specificity = (100/110) x 100 = 90.9%.

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