



Comparison of Three Immunological Assays to Detect Infectious Bovine Rhinotracheitis (IBR) Antibodies in Buffaloes

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ABSTRACT

The present study involved in analysis of 81 sera samples collected from Murrah (34), Surti (20) and non-descript buffaloes (27) with history of respiratory and reproductive disorders for IBR antibodies by indirect Enzyme linked immunosorbent assay (ELISA), Virus neutralization test (VNT) and Passive haemagglutination assay (PHA). The immunological assays viz., indirect ELISA, VNT and PHA revealed that 50.62%, 43.21 % and 27.16 % of IBR positivity respectively. The overall positivity irrespective of serological test was found to be 54.32 %. The sensitivity, specificity of PHA and VNT in comparison with ELISA were 46.34 %, 70.73 % and 85.00 %, 92.50 % respectively. Moderate agreement between ELISA and VNT ($kappa = 0.56$) and fair agreement between ELISA and PHA ($kappa = 0.38$) were found for the detection of IBR antibodies. Significant difference ($p < 0.01$) was found between indirect ELISA and PHA in the detection of BHV-1 antibodies. VNT and PHA were found to be useful as the quantitative tests to estimate antibody level in buffalo whereas ELISA could be performed for herd screening programme.

Keywords: IBR, PHA, VNT, indirect ELISA, sensitivity, specificity

Infectious bovine rhinotracheitis (IBR) is one of the most economically important emerging contagious disease of cattle and buffalo caused by Bovine herpes virus 1 (BHV-1), a member of the *Herpesviridae* family and clinically characterized by mucopurulent nasal discharge, fever, depression, inappetence, conjunctivitis, abortion, reduced milk yield and encephalitis (Rola *et al.*, 2005). All ages and breeds of cattle and buffaloes are susceptible to IBR infection. Transmission of IBR infection to the non-infected cattle and buffaloes occurs through direct contact, aerosol and insemination with virus-contaminated semen (Ampe *et al.*, 2012).

Virus neutralisation tests (Bitsch, 1978) and various ELISAs (Kramps *et al.*, 1993) are used for detecting antibodies against BHV-1 in serum. Because of latency, identification of serologically positive animals provides a useful and reliable indicator of infection status.

Various reports revealed that there was highly significant correlation between ELISA, VNT and PHA for detection of IBR antibodies in cattle and buffaloes (Kramps *et al.*, 1996). Hence, the present study was designed to find out congruity among these diagnostic tests for detection of IBR antibodies in buffaloes and also to evaluate their efficacy.

MATERIALS AND METHODS

Location of study

Present study was conducted in buffaloes brought to Madras Veterinary College hospital and in organized farms of Tamil Nadu, India. The cell culture and antigen preparation work were carried out at Department of Animal Biotechnology and the diagnostic tests were performed at



Department of Veterinary Preventive Medicine, Madras Veterinary College, Chennai.

Test and Reference Sera

A total of 81 blood samples with history of respiratory and reproductive disorders were collected from Murrah (n=34), Surti (n=20) and non-descript buffaloes (n=27). Then, the sera were separated and preserved by adding 0.1 per cent sodium azide and stored at -20°C . The OIE standard sera for IBR i.e. IBR-EU1 and IBR-EU3 were utilized as known positive and negative reference sera, respectively.

BHV-1 antigen preparation

BFA-OCS-W strain of BHV-1 was propagated in Madin-Darby Bovine Kidney (MDBK) cell line and the final titer of $10^{6.362}$ TCID₅₀ per 50 ml of BHV-1 obtained after four serial passages in MDBK cell line was used to perform the serological tests (Reed and Muench, 1938). The protein concentration of BHV-1 tissue culture antigen was estimated as 0.6 g/100 ml as per the method of Lowry *et al.* (1951) and was utilized to perform serological tests.

Serological tests

Indirect Enzyme linked immunosorbent assay (ELISA)

Indirect ELISA was performed as per the protocol of Florent and Demarneffe (1986). The optimum dilutions of coating antigen, sera and anti-bovine IgG – HRP conjugate were standardized by the checker board titration as 1:50, 1:10 and 1:50, respectively. The mean optical density (OD) value twice and above the negative OD value was taken as positive.

Virus neutralization test (VNT)

VNT was performed as described by Dubuisson *et al.* (1992). Two fold dilution of 50ml test sera were made in 50ml of maintenance medium from 1/2 to 1/256 in 96 well tissue culture Falcon plates. Then 50ml of 100 TCID₅₀ BHV-1 was added to each well and the mixtures were kept in an incubator at 37°C for one hour. Then the serum virus mixture was transferred into 96 well tissue

culture plate (Tarson) containing confluent monolayer of MDBK cell line and 100ml of maintenance medium. The plates were incubated for 6 days at 37°C with 5 percent CO₂ for BHV-1 specific CPE. Negative, positive sera and cell control were included in the test. The neutralization titer was calculated as the reciprocal of the highest dilution resulting in complete inhibition of BHV-1 specific CPE.

Passive Haemagglutination Assay (PHA)

Ingredients for PHA were prepared as per the protocol described by Singh *et al.* (2001). 100 ml of each serum sample was adsorbed by equal volume of glutaraldehyde fixed sheep erythrocytes at 37°C for one hour to avoid non-specific reaction. After complete settlement of erythrocytes, the supernatant sera were utilized for the test. The test was performed as described by Shimizu *et al.* (1972) and the titer was expressed as the reciprocal of the highest dilution of serum giving 100% agglutination. The titer of 1:8 and above was considered as positive.

Statistical Analysis

The efficacy of PHA, VNT and ELISA tests in detection of IBR antibodies in buffaloes was assessed statistically as per the procedure of Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Indirect ELISA detected 50.62% of buffaloes positive for IBR antibodies among the total 81 sera samples analyzed. ELISA was found to be more sensitive and specific than PHA and VNT (Riegel *et al.*, 1987). Hence, ELISA was considered as standard test to detect the IBR antibodies in buffaloes and it was taken for comparison.

Virus neutralization test revealed that 43.21% of samples positives with the titers ranging from 4 to 64 and found that the sensitivity and specificity of VNT compared with ELISA were 70.73% and 92.50% respectively. VNT was found to be less sensitive than ELISA as reported by Nakajima *et al.* (1989). VNT had moderate agreement (Kappa Statistic, $\kappa=0.56$) with ELISA (Table 1). On the contrary, good agreement between VNT and ELISA was reported by Beccaria *et al.* (1982).

PHA test detected that 27.16% samples were IBR positives with the titers of 1:8 and above which was considered as

positive. Edwards *et al.* (1986) reported that PHA and one hour neutralization test were failed to detect IBR antibodies in low titer sera. In the present study, the sensitivity and specificity of PHA test in comparison with ELISA were 46.34% and 85.00% respectively. This observation was in line with the report of Edwards *et al.* (1986) and fair agreement (Kappa Statistic, $kappa=0.38$) was observed between PHA and ELISA (Table 1) as reported by Saravanajayam *et al.* (2016) that moderate agreement was found between PHA and ELISA for the detection of BHV-1 antibodies. PHA positivity of 3 sera which were negative in indirect ELISA could be attributed to the fact that PHA test detects all classes of immunoglobulins (Ig). On contrary, indirect ELISA was designed to detect only IgG. Similar observation was made by Gonzalez *et al.* (1985). However, there was significant difference noticed between indirect ELISA and PHA ($p<0.01$) in the detection of IBR antibodies (Table 2).

Table 1: Comparison of PHA, VNT with ELISA for detection of IBR antibodies in Buffaloes

Test	PHA	VNT
No. of Sera tested	81	81
No. of Positive	22	35
ELISA positive Test positive	19	29
ELISA positive Test negative	22	12
ELISA negative Test positive	3	6
ELISA negative Test negative	37	34
Percentage of Sensitivity	46.34	70.73
Percentage of Specificity	85.00	92.50
Agreement (<i>Kappa</i> statistic)	0.38	0.56

Table 2: Results of PHA, VNT and indirect ELISA in the detection of IBR antibodies in Buffaloes

Test	No. of sera tested	No. of positives	Percentage of positives
PHA	81	22	27.16*
VNT	81	35	43.21
ELISA	81	41	50.62*

*Significant difference noticed between PHA and ELISA for detecting IBR antibodies ($p<0.01$).

Among the 81 sera tested, 54.32% sera samples were positive for IBR antibodies irrespective of tests. The

breedwise IBR prevalence in Murrah, Surti and non-descript buffaloes was noticed as 22.22%, 14.81% and 17.28% respectively. Analysis of data revealed that buffaloes having respiratory and reproductive disorders among the positive reactors were 53.57% and 56.00% respectively. The prevalence in reproductive disorder is slightly higher probably due to natural service by infected bulls and artificial insemination with infected semen which corroborated with the findings of Loretu *et al.* (1974). The respiratory form of prevalence is due to frequent introduction of buffalo from various parts of country and intensive management practices of buffalo practiced as reported by Miller (1991).

The present study revealed that indirect ELISA detected maximum number of IBR positive reactors (50.62%) as it could find even low level of IBR antibodies in test sera and similar findings were reported earlier by Shirvani *et al.* (2011). Many studies revealed that the ELISA and 24 hour VNT were rapid, reliable and more sensitive than PHA (Edwards *et al.*, 1986) and indirect ELISA using undiluted test serum showed a sensitivity of 100% and specificity of 97 – 100% and comparatively less expensive (Roshtkhari *et al.*, 2012).

Based on the findings of this study, it can be concluded that ELISA could be considered as an immunological tool for large scale screening as it is rapid, efficient, reliable and highly sensitive whereas VNT and PHA are useful as a quantitative test to estimate level of IBR antibody in buffaloes.

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