# EVALUATION OF THREE IMMUNOLOGICAL ASSAYS IN DETECTION OF BOVINE HERPES VIRUS-1 (BoHV-1) ANTIBODIES IN BUFFALOES

**M. Saravanajayam[1,*], K. Kumanan[2] and A. Balasubramaniam[1]**

## ABSTRACT

A total of 81 sera samples from Murrah (34), Surti (20) and non-descript buffaloes (27) with the history of respiratory and reproductive disorders were collected from northern part of Tamil Nadu, India to detect bovine herpes virus-1 (BoHV-1) antibodies. All samples were subjected to indirect Enzyme linked immunosorbent assay (ELISA), Virus neutralization test (VNT) and Passive haemagglutination test (PHA) to detect BoHV-1 antibodies. Among the serological tests, PHA, VNT and ELISA revealed 27.16%, 43.21% and 50.62% BoHV-1 positivity respectively. The overall positivity irrespective of tests 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 statistic, kappa = 0.56) and fair agreement between ELISA and PHA (kappa = 0.38) were found for the detection of BoHV-1 antibodies. Significant difference was found between indirect ELISA and PHA. VNT and PHA were found to be useful as the quantitative tests to estimate antibody level in buffalo where as ELISA could be performed at field level for large scale screening programme as it is rapid, efficient, reliable and highly sensitive.

**Keywords**: *Bubabus bubalis*, buffalo, BoHV-1, PHA, VNT, indirect ELISA, sensitivity, specificity

## INTRODUCTION

Bovine herpes virus-1 (BoHV-1) is a member of the Herpesviridae family and is one of the most economically important emerging contagious virus of cattle and buffalo causing Infectious bovine rhinotracheitis / Infectious pustular vulvovaginitis / Balanoposthitis and clinically characterized by mucopurulent nasal discharge, fever, depression, inappetance, conjunctivitis, abortion, reduced milk yield and encephalitis (Rola *et al*., 2005). All ages and breeds of cattle and buffalo are susceptible to BoHV-1 infection. Transmission of BoHV-1 infection to the non-infected cattle and buffalo occurs through getting contact with infected animals, aerosol route and insemination with virus-contaminated semen from infected bulls (Ampe *et al*., 2012). Virus neutralisation tests (Bitsch, 1978) and various ELISAs (Kramps *et al*., 1993) are usually used for detecting antibodies against BoHV-1 in serum. Because of latency, identification

[1]Veterinary University Training and Research Centre, Tamil Nadu, India, *E-mail: saravet78@gmail.com

[2]Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

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 in the detection of BoHV-1 antibodies in cattle and buffalo (Kramps *et al*., 1996). Hence, the present study was designed to find out congruity among these diagnostic tests in detection of BoHV-1 antibodies in buffalo and also to evaluate their efficacy.

## MATERIALS AND METHODS

**Sera samples**

A total of 81 blood samples with history of respiratory and reproductive disorders from Murrah buffalo (34), Surti buffalo (20) and Nondescript buffalo (27) were collected from northern part of Tamil Nadu. Then, the sera were separated and preserved by adding 0.1% sodium azide and stored at -20°C. The OIE standard sera for IBR that include IBR-EU1 serum (strong positive) and IBR-EU3 serum (negative) were utilized as known positive and negative.

**Virus and Cell culture**

BFA-OCS-W strain of BoHV-1 was propagated in Madin-Darby Bovine Kidney (MDBK) cell line and was used as antigen for tests.

**Serological tests**

Indirect ELISA was performed as per the protocol of Florent and Demarneffe (1986). The protein concentration of BoHV-1 tissue culture antigen was estimated as per the method of Lowry *et al*. (1951).

VNT was preformed as described by Dubuisson *et al*. (1992). The neutralization titre was calculated as the reciprocal of the highest dilution resulting in complete inhibition of BoHV-1 specific cytopathic effect. PHA was done as per the protocol described by Singh *et al*. (2001) with a modification of using sheep erythrocytes for antigen labelling instead of chicken erythrocytes.

The efficacy of PHA, VNT and ELISA tests in detecting BoHV-1 antibodies in buffaloes was assessed statistically as per Snedecor and Cochran (1980).

## RESULTS AND DISCUSSION

The final titre of $10^{6.362}$ $TCID_{50}$ per 50 µl of BoHV-1 obtained after four serial passages in MDBK cell line was used as antigen. Protein concentration of BoHV-1 antigen was found to be 0.6 g/100 ml. Indirect ELISA detected 41 (50.62%) positives against BoHV-1 antibodies. The mean optical density (OD) value of known negative serum (IBR-IU3) was 0.080, twice and above of which was taken as positive. As per the earlier reports, ELISA was found to be more sensitive and specific than PHA and VNT (Riegel *et al*., 1987). ELISA was considered as standard test to detect the BoHV-1 antibodies in buffaloes, hence, it was taken for comparison. Virus neutralization test revealed 35 (43.21%) samples of positives with the titres ranging from 4 to 64. In the present study, the sensitivity and specificity of VNT compared with ELISA were 70.73% and 92.50% respectively. By this observation, VNT was found 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 22 (27.16%) samples of positives with the titres of 1:8 and above which

was considered as positive. Edwards *et al*. (1986) reported that PHA and one h neutralization test were failed to detect BoHV-1 antibodies in low titre 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 agreed with report of Edwards *et al*. (1986) and fair agreement (Kappa Statistic, kappa = 0.38) was noticed between PHA and ELISA (Table 1). 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 ($P<0.01$) (Table 2).

Among the 81 sera tested, 44 sera sample were positive for BoHV-1 antibodies irrespective of tests and the breedwise BoHV-1 prevalence was 22.22%, 14.81% and 17.28% in Murrah, Surti and non-descript buffaloes 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 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 in northern Tamil Nadu as reported by Miller (1991).

The present study revealed that indirect ELISA detected maximum number of BoHV-1 positive reactors (50.62%) as it could find even low level of BoHV-1 antibodies in test sera and similar findings were reported earlier (Shirvani *et al*., 2011). Many studies revealed that the ELISA and 24 h VNT was 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% to 100% and comparatively less expensive (Roshtkhari *et al*., 2012).

It could be concluded that VNT and PHA are useful as a quantitative test to estimate antibody level of BoHV-1 in buffalo where as ELISA could be performed for large scale screening programme

Table 1. Comparison of PHA, VNT with ELISA for detection of BoHV-1 antibodies in buffaloes.

| Test | Passive heamagglutination test | Virus neutralization test |
|---|---|---|
| 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 dpecificity | 85.00 | 92.50 |
| Agreement (Kappa statistic) | 0.38 | 0.56 |

Table 2. Results of various diagnostic tests in the detection of BoHV-1 antibodies in buffaloes.

| Sl. No. | Test | No. of Sera tested | No. of positives | Percentage of positives |
|---|---|---|---|---|
| 1 | Passive haemagglutination test (PHA) | 81 | 22 | 27.16* |
| 2 | Virus neutralization test (VNT) | 81 | 35 | 43.21 |
| 3 | Enzyme linked immunosorbent assay (ELISA) | 81 | 41 | 50.62* |

*Significant difference noticed between PHA and ELISA for detecting BoHV-1 antibodies (P<0.01).

as it is rapid, efficient, reliable and highly sensitive.

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