



Efficacy of Indirect Enzyme Immunosorbent Assay and Passive Haemagglutination Test for the Diagnosis of Bovine Herpes Virus -1 (BHV-1) Infection

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ABSTRACT

A total of 255 sera were collected from unvaccinated crossbred (174) and indigenous (81) cattle and buffalo having history of respiratory and reproductive disorders. All the sera sample were subjected to indirect ELISA and passive haemagglutination (PHA) test for the detection of BHV-1 antibodies. Indirect ELISA and PHA test detected significantly ($P < 0.01$) varied prevalence of BHV-1 antibody *i.e.* 60.39 % and 35.69%, respectively. The sensitivity and specificity of PHA test in comparison with indirect ELISA was 55.19% and 94.06%, respectively. Moderate agreement ($kappa = 0.442$) was noticed between ELISA and PHA for the diagnosis of BHV-1 antibodies. Significantly ($p < 0.01$) higher prevalence of BHV-1 antibodies was observed in crossbred (70.11%) as compared to indigenous cattle and buffalo (46.91%). Hence, it is be concluded from present investigation that the indirect ELISA could be reliable and sensitive test than PHA test to screen BHV-1 antibody.

Keywords: Bovine herpes virus-1, ELISA, PHA, sensitivity, specificity

Bovine herpes virus -1 (BHV-1), a member of family *Herpesviridae* is an economically important pathogen of cattle. It causes a wide range of disease manifestations including respiratory disease and abortion, with worldwide distribution (Cowley *et al.* 2011). The economic losses are due to storms of abortion in pregnant animals, loss of milk production, loss of draught power, infertility and immunosuppression.

All ages and breeds of cattle are susceptible to BHV-1 infection (Donkersgoed and Babuik, 1991). The transmission of BHV-1 infection to the non-infected cattle mainly occurs through getting contact with infected animals, aerosol route and insemination with virus-contaminated semen from BHV-1 infected bulls (Ampe *et al.* 2012).

Indirect enzyme linked immunosorbent assay (ELISA) and passive haemagglutination (PHA), both the tests can be used for the assessment of BHV-1 antibodies among the cattle population (Satyanarayana and Suri Babu, 1987; Roshtkhari *et al.* 2012). Both the tests have their own

limitations and advantages. However, there is no previous report on comparison of indirect ELISA and PHA test in the diagnosis of BHV-1 antibodies. Also, vaccination for BHV-1 in cattle and buffalo is generally not practiced in Tamil Nadu, hence regular screening of cattle and buffalo is required for the presence of BHV-1 antibodies. Hence, the present study was designed with two objectives, firstly to evaluate the efficacy and relationship between indirect ELISA and PHA test for the detection of BHV-1 antibodies and secondly to determine the seroprevalence of BHV-1 among unvaccinated crossbred and indigenous cattle and buffalo population.

MATERIALS AND METHODS

Location of study

Present study was conducted in the cattle and buffalo brought at Madras Veterinary College hospital and 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.

Collection of samples

Blood samples were collected from 255 unvaccinated cattle and buffalo of different breeds (Jersey crossbred, Holstein Friesian crossbred, Murrah buffalo, Surti buffalo and nondescript bovines) with history of respiratory and reproductive disorders. Sera was separated by centrifuging at 3000 rpm for 10 min. and inactivated at 56°C for 30 min. in water bath. Serum samples were then preserved in sterile labeled vials by adding 0.1% sodium azide and stored at -20°C till further use.

Reference Sera

The OIE standard IBR-EU1 serum (Strong positive) and IBR-EU3 serum (negative) from bovine origin were received from Central Institute for Animal Disease Control Lelystad, The Netherlands.

Preparation of BHV-1 antigen

The BFA-OCS-W strain of BHV-1 was used as a standard virus for antigen preparation. The BHV-1 was propagated in Madin-Darby Bovine Kidney cell line for four serial passages and a titre of $10^{6.362}$ TCID₅₀ per 50 µl of the BHV-1 was used as reference antigen. TCID₅₀ was calculated as per Reed and Muench (1938) method. Optimum protein concentration of BHV-1 tissue culture antigen (0.6 g/100 ml) was estimated as per the method of Lowry *et al.* (1951) and was used in serological diagnosis.

Indirect Enzyme linked immunosorbent assay (ELISA)

ELISA was performed as per the protocol of Florent and Demarneffe (1986). The optimum dilutions of coating antigen, sera and antibovine 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.

Passive Haemagglutination (PHA) test

Ingredients for PHA were prepared as per the protocol described by Singh *et al.* (2001). 100 µl of each serum

sample was adsorbed by equal volume of glutaraldehyde fixed sheep erythrocytes at 37°C for one hr to avoid non specific reaction. After complete settlement of erythrocytes, the supernatant sera were utilized for the test. Further, PHA was performed as described by Shimizu *et al.* (1972) using “U” bottom microplates (Laxbro, India). The titre was expressed as the reciprocal of the highest dilution of serum giving 100% agglutination. The titre of 1:8 and above was considered as positive.

Table 1. Prevalence of BHV-1 antibodies by indirect ELISA

Name of the Place	No. of sera tested	Total positive	Average OD value	Prevalence rate (%)
Madras Veterinary College Hospital	154	96	0.237	62.34*
Organized farms in Tamil Nadu	101	58	0.31	57.43*
Total	255	154	0.274	60.39

*No significant difference in BHV-1 antibodies prevalence between cases from Madras Veterinary College Hospital and organized farms in Tamil Nadu ($p \geq 0.05$)

OD value of known negative serum: 0.080

OD value of known positive serum: 0.160

Statistical analysis

The seroprevalence of BHV-1, sensitivity and specificity of ELISA and PHA and agreement between PHA and ELISA tests were assessed statistically using chi-square test as per the procedure of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Out of 255 sera sample, 154 (60.39%) samples were found positive for BHV-1 antibodies by ELISA (Table 1) whereas only 35.69% samples were detected positive by PHA test (Table 2) as ELISA could find even low level of BHV-1 antibodies in test sera (Shirvani *et al.* 2011; Trangadia *et al.* 2012). The present study is comparable with the earlier reports on BHV-1 antibody prevalence (56%) in Iranian non-vaccinated cattle population (Sadri, 2012) and 64.72% prevalence in Andhra Pradesh, India (Suri Babu *et al.* 1984). In contrast, Trangadia *et al.* (2012)

Table 2. Prevalence of BHV –1 antibody titres by PHA

Name of the place	No. of sera tested	PHA titre									Total positive	Prevalence rate (%)
		< 8	8	16	32	64	128	256	512	1024		
Madras Veterinary College Hospital	154	101	7	19	6	10	5	3	2	1	53	34.42*
Organized farms in Tamil Nadu	101	63	4	6	6	9	10	1	2	—	38	37.62*
Total	255	164	11	25	12	19	15	4	4	1	91	35.69

*No significant difference in BHV-1 antibodies prevalence between cases from Madras Veterinary College Hospital and organized farms in Tamil Nadu ($p \geq 0.05$)

reported low prevalence of BHV – 1 infection (23.94%) in Gujarat.

Table 3. Breed wise prevalence of BHV-1 antibodies in cattle and buffalo

Breed	No. of sera tested	Total positive	% positives	Overall % positive
Crossbred	Jersey			
	Crossbred	127	90	70.87
	Holstein Friesian			
Indigenous	Crossbred	47	32	68.09
	Murrah			
	Buffalo	34	17	50.00
	Surti Buffalo	20	11	55.00
	Non-descript	27	10	37.04
				46.91*

*The prevalence rates are significantly higher in crossbred animals than indigenous breeds ($p < 0.01$).

The breed wise seroprevalence of BHV-1 antibodies using ELISA were 70.11% and 46.91% in crossbred (Jersey and Holstein Friesian Crossbred cows) and indigenous (Murrah buffalo, Surti buffalo and non-descript) cattle and buffalo, respectively. The prevalence was significantly higher in crossbred cattle than indigenous one ($p < 0.01$) as illustrated in Table 3. In present investigation, crossbred cattle showed higher prevalence of BHV-1 antibodies than indigenous cattle which is in agreement with findings of Satyanarayana and Suri Babu (1987). Less susceptibility of indigenous cattle and buffalo might be due their inherent resistance to BHV-1 infection.

The sensitivity and specificity of PHA in comparison with standard ELISA was 55.19% and 94.06%, respectively. Likewise, Cowley *et al.* (2011) reported 100% sensitivity and 97–100% specificity of indirect ELISA. In congruence with present observation, Edwards *et al.* (1986) also reported PHA as less reliable and sensitive than indirect ELISA since PHA failed to detect BHV-1 antibodies in some low titre sera (Solsona *et al.* 1980). The moderate

Table 4. Comparison of PHA with ELISA for BHV-1 antibody detection

No. of sera tested	No. of positive sera by PHA	ELISA positive and PHA positive	ELISA positive and PHA negative	ELISA Negative and PHA positive	ELISA Negative and PHA negative	Sensitivity (%) of PHA	Specificity (%) of PHA	Agreement (kappa statistic)
255	91*	85*	69*	6	95	55.19	94.06	0.442

*Significant difference was found between PHA and ELISA for detecting BHV-1 antibodies ($p < 0.01$).

agreement ($kappa = 0.442$) was estimated between ELISA and PHA for detecting the BHV-1 antibodies using Kappa statistics (Table 4). On contrary, Beccaria *et al.* (1982) observed good agreement between ELISA and serum neutralization test for the detection of BHV-1 antibodies.

Passive haemagglutination test detected BHV-1 antibody titre ranging from 8 to 1024. Likewise, a titre of 1:8 and above was reported by Singh *et al.* (2001). In present study, six sera sample negative to ELISA were detected positive by PHA test which is supported with report of Gonzalez *et al.* (1985) as PHA test can detect different classes of antibodies.

Based on findings of present study, it is inferred that indirect ELISA could be considered as standard test to detect the BHV-1 antibodies.

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REFERENCES

- Ampe, B., Duchateau, L., Speybroeck, N., Berkvens, D., Dupont, A. and Kerkhofs, P. 2012. Assessment of the long-term effect of vaccination on transmission of infectious bovine rhinotracheitis virus in cattle herds hyperimmunized with glycoprotein E-deleted marker vaccine. *Am. J. Vet. Res.*, **73**: 1787-1793.
- Beccaria, E., Ferrari, A., Nachtmann, C., Bonniolo, A., Bovis, M., Zannino, M. and Petreizelli, E. 1982. Rapid detection of antibodies to infectious bovine rhinotracheitis by macro and micro ELISA. *Dev. Biol. Stand.*, **52**: 141-146.
- Cowley, D.J.B., Clegg, T.A., Doherty, M.L. and More, S.J. 2011. Aspects of bovine herpesvirus-1 infection in dairy and beef herds in Republic of Ireland. *Acta Vet. Scand.*, **53**: 40.
- Donkersgoed, J.C. and Babuik, L.A. 1991. Diagnosing and managing the respiratory form of infectious bovine rhinotracheitis. *Vet. Med.*, **1**: 86-98.
- Edwards, S., Woods, S.B., Westcott, D.G., Emmerson, M., Jones, P.C. and Philips, A.J. 1986. An evaluation of five serological tests for the detection of antibody to bovine herpesvirus 1 in vaccinated and experimentally infected cattle. *Res. Vet. Sci.*, **41**: 378-382.
- Florent, G. and Demarneffe, C.D. 1986. Enzyme linked immunosorbent assay used to monitor serum antibodies to Bovine respiratory disease viruses. *Vet. Microbiol.*, **11**: 309 - 317.
- Gonzalez, E.T., Olira, G., Etchererrigarry, M.E., Schudel, A.A. and Noretb, E. 1985. Application of PHA and IFA tests in the diagnosis of IBR. *Argentina*, **66**(1): 26-30.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275
- Reed, L.V. and Muench, H. 1938. A simple method of evaluating fifty per cent end points. *Am. J. Hyg.*, **27**: 493.
- Roshtkhari, F., Mohammadi, G. and Mayameei, A. 2012. Serological evaluation of relationship between viral pathogens (BHV-1, BVDV, BRSV, PI-3V, and Adeno3) and dairy calf pneumonia by indirect ELISA. *Trop. Anim. Health Prod.*, **44**(5): 1105-1110.
- Sadri, R. 2012. A new way of occurrence and serodiagnosis for Infectious Bovine Rhinotracheitis in Iranian cattle herds. *Iranian J. Vet. Med.*, **6**(2): 99-103.
- Satyanarayana, K. and Suri Babu, T. 1987. Serological survey of bovine herpes virus - 1 (BHV-1), infection in bovines in Andhra Pradesh. *Indian J. Anim. Sci.*, **57**(6): 499-502.
- Shimizu, Y., Isayama, Y., Kawakami, Y., Murase, N. and Kawana, T. 1972. Micro indirect haemagglutination test for detecting antibody against infectious bovine rhinotracheitis virus. *Nat. Anim. Hlth.*, **12**(1): 1-7.
- Shirvani, E., Lotfi, M., Kamalzadeh, M., Bahriari, M. and Abdoshah, M. 2011. Dot-blot Enzyme Immunoassay for the detection of Bovine Herpes Virus-1 (BHV-1) Antibodies. *World App. Sci. J.*, **15**(6): 781-784.
- Singh, R.K., Narayana Bhat, M. and Sastry, K.N.V. 2001. Studies on the prevalence of antibodies to BHV-1 in sheep. *Indian Vet. J.*, **78**: 467-470.
- Snedecor, G.M. and Cochran, W.G. 1967. *Statistical Methods*, 5th ed. Iowa State Univ. Press, Iowa, USA.
- Solsona, M.M., Perrin, B., Perrin, M. and Moussa, A. 1980. Detection of antibodies to Bovine infectious rhinotracheitis virus by the ELISA technique. *Bulletin de l'Academie Veterinaire de France*, **53**(2): 215-255.
- Suri Babu, T., Maclick, B.B. and Das, S.K. 1984. Prevalence of infectious bovine rhinotracheitis virus (BHV-1) antibodies in bovines. *Indian Vet. J.*, **61**: 195-200.
- Trangadia, B.J., Rana, S.K., Nagmani, K. and Srinivasan, V.A. 2012. Serological investigation of Bovine Brucellosis, Johne's disease and Infectious Bovine Rhinotracheitis in two states of India. *J. Adv. Vet. Res.*, **2**: 38-41.