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Research Article

Detection of Bovine Herpesvirus–l (BHV–l) Infection in Cattle by Antigen Detection ELISA and Multiplex PCR

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ABSTRACT

In present cross sectional study, nasal swabs were collected from cattle of organized and unorganized herds (n=333) from seven districts of Uttar Pradesh state, and examined for presence of BHV–1 using antigen detection by ELISA and subsequently confirmed by multiplex–Polymerase chain reaction (multiplex–PCR). Overall percent positivity of BHV–1 antigen in cattle of Uttar Pradesh examined was 11.1% (37/333). The percent positivity of BHV–1 was higher in organized herd (15.38%) than that of unorganized herd (4.4%). All the 37 samples positive by ELISA were processed further for molecular characterization using gB and gC based multiplex PCR. Out of 37 samples, 20 (54.0%) samples were positive with gB and gC based multiplex PCR. Among these, 20 positive samples by PCR, 11(29.7%) samples were positive with both gB and gC gene primers of multiplex PCR, while 6 (16.2%) samples were positive only with gB gene primer and 3 (8.1%) samples were positive only with gC gene primer. From the study, it can be concluded that the BHV–1 virus is circulation in cattle of Uttar Pradesh, India, which causes significant economic impact to dairy industry and export. Although this study was performed using less number of sample from limited geographical area, so a detailed study should be performed using more number of samples from vast geographical area.

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INTRODUCTION

Worldwide, livestock health problems particularly of reproductive system leads to direct and indirect economic losses to dairy industry by abortions, still births, early embryonic mortality, retention of placenta, poor fertility, and loss of production (Poulsen and McGuirk, 2009; Gay and Barnouin, 2009; Raaperi et al., 2012). Among these health problems majority are of infectious origin such as brucellosis, leptospirosis, campylobacteriosis, listeriosis and infectious bovine rhinotrachitis (Kumar et al., 2009; Verma et al., 2014). Infectious bovine rhinotracheitis (IBR) is a major, economically important and emerging disease of cattle, caused by bovine herpesvirus–1 (BHV–1), causing various clinical syndromes viz., respiratory, reproductive (vulvovaginitis or balanoposthitis), conjunctivitis, encephalitis and generalized systemic infections (Gibb and Rweyemamu, 1977; Straub, 1991; Nandi et al., 2009; Jacevicius et al., 2010; Verma et al., 2014).

BHV–1 genome consist of linear, double stranded DNA of about 1,36,000 base pairs, enveloped with glycoproteins spikes on its surface and its structure is typical of herpesviruses of group D (Roizman, 1992). There are 8 known glycoproteins viz., gB, gC, gD, gF, gH, gI, gK and gL. Out of these, gC, gD, gE, gG, gI, UL49h and thymidine kinase genes are involved in viral virulence (Smith, 1991; Smith et al., 1994; van Engelenburg et al., 1994; Kaasheek et al., 1998). Studies show that the BHV–1 glycoproteins gB, gC, gD, gE, gH, gk, gL are required for virus entry (Schroder and Keil 1999). BHV–1 isolates were classified into subtype 1 (BoHV–1.1) and BoHV–1.2 according to distinct restriction enzyme profiles of the genomes. In India, disease was first time reported in Uttar Pradesh state and since then many reports have been published regarding its occurrence in different states of the country (Mehrotra et al., 1976; Renukaradhya, 1996; Rajkhowa et al., 2004; Sunder et al., 2005; Ganguly et al., 2008; Nandi et al., 2010; Verma et al., 2014). For effective control of disease, early and confirmatory diagnosis is very important. Recently, emphasis has been given to reduce the time required for diagnosis of infections. Virus isolation in cell culture is most frequently used for diagnosing BHV–1 but it is laborious, time consuming, and requires samples of good quality. Hence alternative techniques like ELISA and polymerase chain reaction have been tried. The present manuscript describes the epidemiological studies of BHV–1 using antigen based ELISA and multiplex PCR in cattle of Uttar Pradesh, India.

MATERIALS AND METHODS

Study Design, Area and Sample Collection

This cross–sectional study was conducted in seven districts (Agra, Bareilly, Etawah, Ghaziabad, Lucknow, Mainpuri and Mathura) of Uttar Pradesh, India (Figure 1). A total of 333 nasal swabs were collected from cattle of 1–4 years of age. Samples were taken from different farms, semen collection centers and gaushalas in Uttar Pradesh. Among these 333 nasal swabs, 243 samples were taken from organized herd and 90 samples were

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taken from unorganized herd (Table 1). The nasal swabs were dipped in Eagle's MEM containing antibiotics, thoroughly shaked and centrifuged at 1000g for 10 min at 4ºC. The supernatants from nasal swabs were taken for ELISA and viral DNA extraction.

Table 1: Distribution of samples collected in the study

S. No.	Organized herd		Unorganized herd	
	Place of sample collection	Number of samples	Place of sample collection	Number of samples
1.	Gaushalas	116	Bareilly Mandal	40
2.	Dairy farms	93	Etawah	30
3.	Semen collection centers	34	Mainpuri	20
	Total	243	Total	90

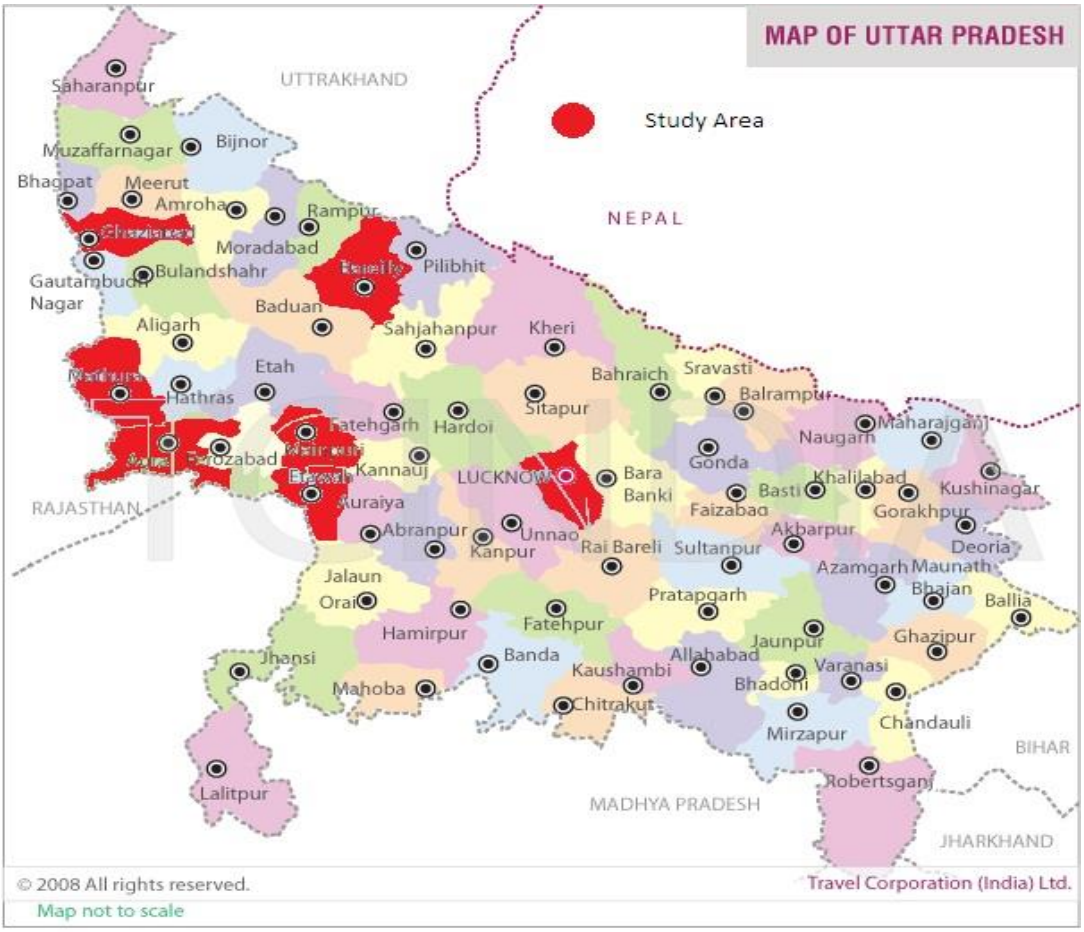

Figure 1: Uttar Pradesh State map showing the districts of study

Laboratory Examination

The laboratory analysis was conducted at Department of Veterinary Epidemiology and Preventive Medicine, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvidhyalaya Evum Go–Anusandhan Sansthan (DUVASU), Mathura, India by antigen detection (using sandwich ELISA) and viral DNA detection (using Multiplex polymerase chain reaction).

Antigen Detection by Sandwich–ELISA

The nasal samples were tested to detect presence of BHV–1 antigen, using a commercially available sandwich enzyme linked immunosorbent assay (ELISA) kit (BIO–X Pulmotest BHV–1 ELISA kit) following manufacturer's recommendations.

Viral DNA detection by Multiplex PCR

The samples, which were positive by antigen detection sandwich ELISA, were processed further for Multiplex PCR using the specific primers (Table 2) For amplification in thermocycler (Techne, Japan) an initial denaturation (5 min at 95°C) was followed by 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 60°C) and extension (1 min at 72°C) and a step of final extension for 10 minutes at 72°C. The amplicons of 173bp and 478bp were visualized under UV illuminator after agarose gel electrophoresis (5 volts/cm) using 1.5% agarose made in 0.5X Tris–borate buffer (TBE) containing ethidium bromide (0.5µg ml^{-1}).

In the present cross–sectional study, a total of 333 nasal samples (243 samples from organized herd and 90 samples from unorganized herd) of cattle from seven districts of Uttar Pradesh state, India were analyzed for presence of BHV–1 antigen. Overall positive of BHV–1 antigen in cattle of Uttar Pradesh examined was 11.1% (37/333). In samples from organized herd screened, 15.38% (33/243) exhibited positive

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reaction, while 4.4% (4/90) nasal swab samples from unorganized herds exhibited positive reaction (Table 3).

With variability in the percentage presence of IBR antigen (virus) in various secretions of the animals having the clinical history of the disease. In the present study, higher percent positivity of BHV–1 was observed in organized herd in comparison to that of unorganized herd. This might be due to spread of infection from one animal to other either by close contact between the animals or during natural service with infected bulls as well as poor hygiene practices like improper disposal of aborted fetuses, foetal membranes, uterine and vaginal discharges.

Table 2: Detail of Primers for multiplex PCR

Name of Gene	Sequences	Primer length	Primer Location	Product length (bp)	References
gB1(F)	5'–TACGACTCGTTCGCGCTCTC–3'	20	883–902	478	Fuchs *et al.*–1999
gB2 (R)	5'–GGTACGTCTCCAAGCTGCCC–3'	20	1341–1360		
gC1 (F)	5'–CTGCTGTTCGTAGCCCACAACG–3'	22	763–785	173	Van Engelenburg *et al.* (1993)
gC2 (R)	5'–TGTGACTTGGTGCCCATGTCGC–3'	22	913–935		

Table 3: Distribution of BHV–1 antigen positive samples in cattle of Uttar Pradesh, India

Sr. No.	Place of Sample Collection	Number of Samples Tested	Number of Samples Positive
Organized herd			
1.	Gaushalas	116	18 (15.5%)
2.	Dairy farms	93	15 (16.12%)
3.	Semen collection centers	34	0 (0%)
	Total	243	33 (13.58%)
Unorganized herd			
1.	Bareilli mandal	40	1 (2.5%)
2.	Etawah	30	2 (6.6%)
3.	Mainpuri	20	1 (5%)
	Total	90	4 (4.4%)
	Overall total	333	37 (11.1%)

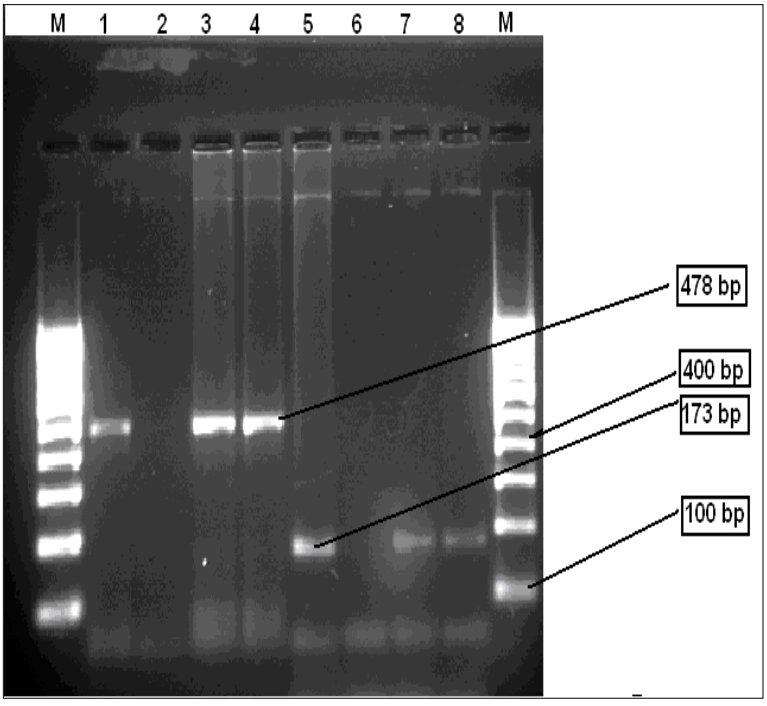

Figure 2: Multiplex PCR assay for detection of BHV–1; Lane M: 100bp Molecular weight marker; Lane 1–4: Field sample with gB gene primer; Lane 5–8: Field sample with gC gene primer

Table 4: Prevalence of BHV–1 using gB and gC gene based multiplex PCR

Sr. No.	Sample From	Total number of samples Processed	Sample Positive by PCR With gB & gC gene	With gB gene	With gC gene	Total
1	Organized Herd	33	10 (30.3%)	5 (15.1%)	3((9.1%)	18(54.5%)
2	Unorganized Herd	4	1 (25%)	1 (25%)	0 (0%)	2 (50%)
3	Grand Total	37	11 (29.7)	6 (16. 2%)	3 (8.1%)	20 (54.0%)

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All the 37 samples, positive by ELISA, were processed further for molecular characterization using gB and gC based multiplex PCR. Out of 37 samples, 20 (54.0%) samples were positive with gB and gC based multiplex PCR. Among these, 20 positive samples by PCR, 11(29.7%) samples were positive with both gB and gC gene primers of multiplex PCR, while 6 (16.2%) samples were positive only with gB gene primer and 3 (8.1%) samples were positive only with gC gene primer (Table 4; Figure 2).

Similarly, various researchers used PCR for molecular detection of BHV–1 in nasal swabs with results range from 25% to 66.6% (Vilcek et al., 1995; Gee et al., 1996; Rola et al., 2005). However in the present study, a multiplex PCR was developed for detection of gB and gC genes. The already known primers of these genes were optimized in the multiplex reactions to perform the multiplex PCR. Results showed percent positivity of gB and gC positive samples was 29.7%, while percent positivity of only gB and only gC gene was 16.2% and 8.1%, respectively. Very few multiplex PCR were developed incorporating these two genes based primers. However the gC gene based primer can detect the virus in latency (Winkler et al., 2000).

CONCLUSION

From the study, it can be concluded that the BHV–1 virus is circulation in cattle of Uttar Pradesh, India, which causes significant economic impact to dairy industry and export. Although this study was performed using less number of sample from limited geographical area, so a detailed study should be performed using more number of samples from vast geographical area.

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