Research Article

Detection of Bovine Herpesvirus-1 (BHV-1) 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.


INTRODUCTION

Worldwide, livestock health problems particularly of reproductive system leads to direct and indirect economic problems 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; Raaper 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 rhinotrachitis (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 136,000 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, gE, gH, gL and gK. Out of these, gC, gD, gE, gG, gL, 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 (RoHV-1) and BoHV-1 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; Renukaradhyya, 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 gushalas in Uttar Pradesh. Among these 333 nasal swabs, 243 samples were taken from organized herd and 90 samples were
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 4°C. The supernatants from nasal swabs were taken for ELISA and viral DNA extraction.

### Table 1: Distribution of samples collected in the study

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Place of sample collection</th>
<th>Number of samples</th>
<th>Place of sample collection</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Gaushalas</td>
<td>116</td>
<td>Bareilly Mandal</td>
<td>40</td>
</tr>
<tr>
<td>2.</td>
<td>Dairy farms</td>
<td>93</td>
<td>Etawah</td>
<td>30</td>
</tr>
<tr>
<td>3.</td>
<td>Semen collection centers</td>
<td>34</td>
<td>Mainpuri</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>243</td>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>

**Laboratory Examination**

The laboratory analysis was conducted at Department of Veterinary Epidemiology and Preventive Medicine, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvvidyalya 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 (Techno, 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

<table>
<thead>
<tr>
<th>Name of Gene</th>
<th>Sequences</th>
<th>Primer length</th>
<th>Primer Location (bp)</th>
<th>Product length (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>gB1 (F)</td>
<td>5’-TACGACTCGTTGCCGCTTTC-3’</td>
<td>20</td>
<td>883-902</td>
<td>478</td>
<td>Fuchs et al–1999</td>
</tr>
<tr>
<td>gB2 (R)</td>
<td>5’-GGTAGCTCTCAGTCGGGTC-3’</td>
<td>20</td>
<td>1243-1360</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gC1 (F)</td>
<td>5’-CTGCATGTCGTAACACACAG-3’</td>
<td>22</td>
<td>763-783</td>
<td>173</td>
<td>Van Engelenburg et al. (1993)</td>
</tr>
<tr>
<td>gC2 (R)</td>
<td>5’TGTGACTTGGTGCCCATGTCGC-3’</td>
<td>22</td>
<td>913-935</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample From</th>
<th>Total number of samples Processed</th>
<th>Number of Samples Tested</th>
<th>Number of Samples Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organized Herd</td>
<td>116</td>
<td>18 (15.5%)</td>
<td>25 (54.5%)</td>
</tr>
<tr>
<td>2</td>
<td>Unorganized Herd</td>
<td>40</td>
<td>1 (2.5%)</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>3</td>
<td>Grand Total</td>
<td>333</td>
<td>37 (11.1%)</td>
<td>20 (54.0%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample From</th>
<th>Total number of samples Processed</th>
<th>Sample Positive by PCR With gB &amp; gC gene</th>
<th>With gB gene</th>
<th>With gC gene</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organized Herd</td>
<td>33</td>
<td>10 (30.3%)</td>
<td>5 (15.1%)</td>
<td>3 (9.1%)</td>
<td>18 (54.3%)</td>
</tr>
<tr>
<td>2</td>
<td>Unorganized Herd</td>
<td>4</td>
<td>1 (25%)</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>3</td>
<td>Grand Total</td>
<td>37</td>
<td>11 (29.7%)</td>
<td>6 (16.2%)</td>
<td>3 (8.1%)</td>
<td>20 (54.0%)</td>
</tr>
</tbody>
</table>
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 ranging from 23% to 66.6% (Vilcek et al., 1995; Gee et al., 1996; Rola et al., 2003). 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.

ACKNOWLEDGEMENT

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REFERENCES


