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Serological and molecular detection of Bovine Herpes Virus 1 infection in cattle by enzyme linked immunosorbent assay and polymerase chain reaction

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ABSTRACT

The present study was undertaken to assess the seroprevalence of Bovine Herpes Virus 1 (BoHV 1) in cattle in and around Thrissur district employing ELISA and to detect the presence of the virus from suspected cases of abortion in cattle using PCR assay. A total of 182 serum samples were screened using commercially available ELISA kit of which 22 were found positive for BoHV1 antibody with a seropositivity of 12.09 per cent. For the detection of BoHV1 virus in aborted cases, a PCR assay was standardised targeting glycoprotein C gene of BoHV1. Out of a total of 13 aborted foetal tissue samples, six were found to be positive with an amplicon size of 179 bp. Further, the amplicons were confirmed by nucleotide sequencing. Phylogenetic analysis of the sequences showed that our sample from Kerala grouped with isolates from India (Gujarat and UP), Brazil, Switzerland, and USA.

Key words: Bovine Herpes Virus 1, ELISA, PCR, Seroprevalence.

INTRODUCTION

Bovine herpes virus 1 which belongs to the genus *Varicellovirus* under the subfamily *Alphaherpesvirinae* of family *Herpesviridae* is the causative agent of infectious bovine rhinotracheitis (IBR) in cattle. The virus is also known to cause various other disease conditions among cattle such as infectious pastular vulvovaginitis (IPV), infectious balanoposthitis, abortion, infertility, conjunctivitis and encephalitis. They cause significant loss to the dairy industry and livestock farmers in terms of milk production, abortion and infertility (Raizman *et al.*, 2011; Raaperi *et al.*, 2012). Development of latency being a unique feature of the virus makes the carrier animals to remain as silent shedders of the virus and thus acting as a potent source of infection for other animals in the herd (Winkler *et al.*, 2000).

The disease has been recorded from different states like Gujarat, Uttar Pradesh, West Bengal, Bihar, Andaman and Nicobar, Uttaranchal, Karnataka, Tamil Nadu and Andhra Pradesh (Lata *et al.*, 2008; Ganguly *et al.*, 2008; Singh and Sinha, 2006; Sunder *et al.*, 2005 ; Jain *et al.*, 2006; Kiran *et al.*, 2007; Selvaraj *et al.*, 2008). In Kerala, seroprevalence studies have been conducted by Sulochana *et al.* (1982) and Rajesh *et al.* (2003) recording a seropositivity of 49.86 per cent and 14.88 per cent, respectively.

The gold standard test for the laboratory diagnosis of BoHV1 is virus isolation and identification. However, the method is tedious and time consuming. In India, the prevalence of the disease has been studied by means of various serological tests like enzyme linked immunosorbent assay (ELISA). Molecular diagnostic tests like polymerase chain reaction (PCR) have been used for the detection of BoHV 1 DNA in semen, nasal and conjuctival swabs (Van Engelenburg *et al.*, 1993; Vilcek *et al.*, 1994; Deka *et al.*, 2005; Grom *et al.*, 2006; Ranganatha *et al.*, 2013; Ravishankar *et al.*, 2013). However, the studies on BoHV 1 as a significant cause of abortion in cattle employing PCR is scarce (Kaur *et al.*, 2013), since majority of studies on abortion in cattle were concentrated around bacterial agents like *Brucella*, *Leptospira* and *Chlamydia*.

Increasing reports of the occurrence of disease from many parts of the country are of a major concern now. In Kerala, apart from few studies dated back, no leading reports are there regarding the occurrence of the disease. Under these circumstances, the present study was envisaged to analyse the seroprevalence status of BoHV1 in and around Thrissur district, Kerala as well as to standardise a PCR assay to ascertain the potential role of BoHV1 as a cause of abortion in cattle.

MATERIALS AND METHODS

Collection of samples: About two milliliter of blood (without anticoagulant) from 182 apparently healthy animals and aborted fetuses from 13 suspected cases of abortion were collected from cattle reared in different parts of Thrissur and from organised farms under Kerala Veterinary and Animal Science University, Pookot, Kerala during the study period from December, 2017 to February, 2018. Serum was separated by centrifugation at 3000 rpm for five min and

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stored at -20°C until use. The stomach contents were collected aseptically from the aborted fetuses and the specimens were processed for DNA extraction and subsequently stored in -20°C until use.

Serological detection of antibodies against BoHV1: Competitive ELISA was conducted for the detection of antibodies against BoHV1 in cattle sera using infectious bovine rhinotracheitis antibody ELISA kit (EVL, Nether- lands) following the manufacturer's recommendations.

`The absorbance of the wells was read at 450 nm in microplate reader (Bio-Rad® Laboratories, USA) and analysis was done using appropriate software. Calculation of the titre was performed based on the results of absorbance of each sample. The S/P value (ratio of sample OD to mean OD of the positive control) of each sample was calculated and the S/N value corresponding to the test samples were interpreted such that those values more than 0.7 were considered as negative while those less than 0.6 were considered as positive (Table 1 and 2).

Molecular diagnosis of BoHV1: The stomach contents and foetal tissues preserved in PBS (10 per cent) were appropriately processed and DNA was extracted using commercial DNA purification kit (Hi-Media, India) and subjected to PCR.

PCR assay was standardised using specific primers (Table 3) targeting the glycoprotein C gene of BoHV 1 for amplification with initial denaturation at 95ºC for 10 min, followed by 35 cycles of denaturation at 95º C for 4 sec, annealing at 64.5º C for 1 min and extension at 72º C for 1 min and a step of final extension at 72ºC for 10 min. The IBR viral DNA procured from Indian Veterinary Research Institute (IVRI), Barreily, UP was used as positive control. A total of 13 aborted foetal tissue samples were analysed for presence of BoHV1 antigen. The DNA samples were also subjected to PCR assays targeting *Brucella*, *Leptospira* and *Chlamydia*.

The positive amplicons were sequenced using automated sequencer employing Sanger's dideoxy chain termination method at SciGenome Labs Pvt. Ltd., Cochin. The obtained sequences were aligned with other sequences of BoHV1 available in GenBank using BLASTn.

Phylogenetic analysis: Phylogenetic analysis was performed using MEGA 7 software for the partial Glycoprotein C gene sequences as per Kumar *et al*. (2016). The sequences obtained during this study and those downloaded from GenBank were aligned using the Clustal W program of the MEGA 7 software. The Tamura 3-parameter model was determined as the best fit based on BIC scores (Bayesian

Table 1: S/N ratios of test samples for first ELISA plate.

Negative control	0.405439	0.960445	0.690977	0.52286	0.81582	0.70086	0.94808	0.20148	0.68974	0.71940	0.27194
Negative control	0.71199	0.875155	0.859085	0.93572	0.95673	0.71322	0.65760	0.67614	0.67367	0.77503	0.75278
Positive control	0.652658	0.678616	0.68356	0.67119	0.68603	0.70333	0.67861	0.68108	0.69962	0.81829	0.93448
Positive control	0.956737	0.657602	0.688504	0.66378	0.68356	0.67367	0.67367	0.69839	0.69468	0.67614	0.75896
0.69715	0.834363	0.828183	0.707046	0.82571	0.70086	0.70333	0.68726	0.68356	0.70951	0.68479	0.49814
0.69344	0.781211	0.506799	0.351051	0.67985	0.46477	0.97651	0.67490	0.52904	0.79975	0.26823	0.67651
0.97033	0.693449	0.758962	0.379481	0.59579	0.67737	0.39555	0.65883	0.66996	0.28306	0.39678	0.67490
0.69344	0.697157	0.87021	0.399258	0.68974	0.68850	0.68232	0.40914	0.66625	0.67861	0.68974	0.67490

S/N ratio >0.7 is considered as negative.
S/N ratio ≤0.6 is considered as positive.

Table 2: S/N ratios of test samples for second ELISA plate.

Negative control	0.78166	0.6960	0.78649	0.875754	0.851628	0.683957	0.716526	0.920386	0.757539	0.931242	0.79614
Negative control	0.67430	0.73341	0.717732	0.516285	0.517491	0.87696	0.603136	0.721351	0.765983	0.804584	0.794934
Positive control	0.68516	0.91556	0.761158	0.787696	0.669481	0.6731	0.718938	0.917973	0.71339	0.52111	0.791315
Positive control	0.68033	0.80458	0.913148	0.683957	0.787696	0.922799	0.802171	0.778046	0.895054	0.201448	0.680338
0.793727	0.79010	0.70808	0.680338	0.686369	0.792521	0.671894	0.512666	0.932449	0.704463	0.516285	0.765983
0.924005	0.72014	0.77442	0.927624	0.675513	0.809409	0.952955	0.911942	0.705669	0.780458	0.768396	0.956574
0.826297	0.79734	0.78890	0.773221	0.781665	0.806996	0.798552	0.751508	0.6731	0.800965	0.77684	0.851628
0.790109	0.79372	0.80096	3.310811	0.915561	0.913148	0.714113	0.895054	0.800965	0.927624	0.793727	0.791315

S/N ratio >0.7 is considered as negative.

Table 3: Primer sequences used in PCR for the amplification of BoHV 1 glycoprotein C gene.

Forward and Reverse primers	Primer sequences (5′-3′ × direction)	Product size
IBR(F)	CTGCTGTTGGTAGCCCACAACG	179 bp
IBR(R)	TGTGACTTGGTCCCCATGTCGC	

Table 4: Nucleotide sequence of BoHV1.

BoHV1 Kerala	TTTGACTTGGGGCCCATGGCGCGG CGCCAAGGGGACACCCCCTCGGTAG ATGCGCCGTCAGAAGCCACGGTCAG GGGCAAGTTGCGGGGGTCGGCGGGC GAAGGGAAATATAGTTGTCGCCCAG CTCCGGCTACGGTACGCGATCGAGC CGTTGTGGGCTACAAACAGCAGA

Information Criterion) and the evolutionary history was inferred by Maximum likelihood method.

RESULTS AND DISCUSSION

Enzyme linked Immunosorbent Assay: In the present study, out of 182 bovine serum samples tested, 22 (12.09%) were found to be positive. Previous reports (Sulochana *et al.*, 1982; Rajesh *et al.*, 2003) stated a seropositivity of 49.86 per cent and 14.88 per cent, respectively in Kerala. The samples had been collected from apparently healthy animals from organised government farms as well as private farms. The animals were not vaccinated against BoHV1. Hence, the present study suggests that IBR is still a major concern in India.

According to Bolton *et al.* (1981), the assay is a superior choice for the diagnosis of BoHV1 infection because of its ability to detect non- neutralizing antibodies and its high sensitivity and low cost as compared to virus isolation. Cho and Bohac (1985) also pointed out the increased specificity and sensitivity of ELISA for detecting BoHV1 antibodies over other serological detection methods. The commercially available ELISA kits for the detection of antibodies against BoHV1 were used by many researchers (Ravishankar *et al.,* 2012; Verma *et al.*, 2014) and were found to be effective, rapid, and reliable in nature in comparison with the conventional assays which were time consuming and expensive.

Polymerase Chain Reaction: The potential role of BoHV 1 as a major cause of abortion in cattle in the state is not studied so far. The BoHV1 infection inflicted abortion in cattle is mainly diagnosed using immunohistochemistry, presence of compatible histological lesions and by PCR. In comparison with other methods, PCR assay was found to be easier, faster and more sensitive to detect the virus from tissue samples (Sasani *et al.,* 2013). In the present study, a total of 13 aborted foetal tissue samples were subjected to PCR assay and six of them were found positive, revealing an amplicon size of 179 bp corresponding to the *gC* glycoprotein gene of BoHV1 (Fig 1). Samples such as stomach content and fetal tissues from suspected cases of abortion were collected and used for PCR.

PCR has become an important molecular diagnostic tool for the detection of an array of viral diseases affecting animals. In addition to the speed, sensitivity and specificity, it offers an additional advantage that, requirements of a good quality diagnostic sample is eliminated, because only a short sequence of target nucleic acid is necessary for detection in PCR.

The assay was employed by many research workers (Van engelenberg *et al.*, 1995, Sreenivasa *et al.,* 1996, Deka *et al.,* 2005) to detect BoHV1 from suspected cases of IBR and IPV and reported that PCR assay was more sensitive than virus isolation. A similar PCR assay was also done by Fuchs *et al.* (1999) on blood samples collected from naturally infected cattle to amplify gB, gC, gE glycoprotein genes of BoHV1.

The development of latency is a unique feature showed by BoHV1 and is significant in the transmission of the disease even after recovery from primary infection. Therefore, it is difficult to establish the occurrence of recent infection in suspected clinical samples. In the present study, positive cases of BoHV1 associated abortion could be detected from cattle and hence could be used as a valuable tool for the rapid detection of BoHV1 associated abortion, which would help in developing appropriate control strategies to combat the infection. The presence of other important agents associated with abortion in cattle (*Brucella*, *Leptospira* and *Chlamydia*) was ruled out by different PCR assays. The nucleotide sequences obtained from the present study showed similarity with reference sequence available in Gen Bank. The sequenced reads were merged using

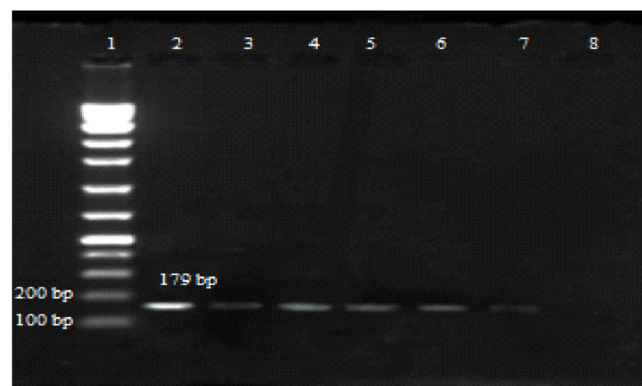

Fig 1: Agarose gel electrophoresis of PCR amplified products of BoHV 1.

Lane 1- 100 bp ladder

Lane 2 - positive control

Lane 3 to Lane 7- positive samples

Lane 8-negative sample

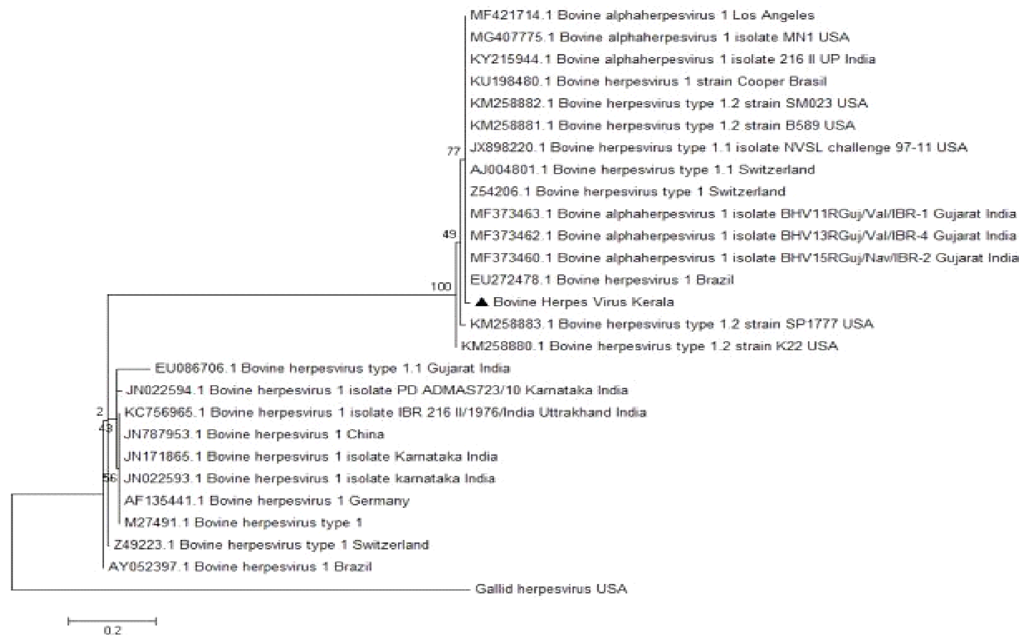

Fig 2: Phylogenetic tree constructed by Maximum Likelihood method based on the basis of the partial sequences of glycoprotein C gene.

EMBOSS merger to obtain the sequences of BoHV1 and further analysed. The sequences were queried in the nucleotide database and blasted to confirm that the BLAST hits were of BoHV1 sequence. The sequence is given in the Table 4.

Phylogenetic analysis: The phylogenetic tree of the partial region of glycoprotein C gene was constructed by Maximum Likelihood Method. The Bovine Herpes virus isolate from Kerala was grouped along with other Indian isolates from Gujarat and UP and also with isolates from Brazil, Switzerland, and USA (Fig 2). All the Bovine herpes virus isolate showed a divergence with the sequence of Gallid Herpes virus forming an out group.

CONCLUSION

Seroprevalence of BoHV1 among cattle population of Thrissur district of Kerala was found to be 12.09 per cent as detected by a competitive ELISA. Polymerase chain reaction assay standardised in the study could detect BoHV1 as a significant cause of abortion among cattle population and the assay was found to be rapid and sensitive so that timely measures can be taken to prevent spread of the infection in the herd.

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