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The use of polymerase chain reaction assay for the detection of bovine herpes virus-1 infection (BHV-1) in cattle in organized and un-organized farms

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Abstract
This research study was carried out to detect the antigen prevalence of Bovine Herpes Virus-1 (BHV-1) infection among two groups of cattle, which were maintained and reared in organized farms and in un-organized farms. A total number of 107 serum sample were collected randomly from both the farms, comprising of 54 serum samples (n=54) from the organized farms and 53 serum samples (n=53) among un-organized farms, The genomic DNA extraction from the serum samples were carried out for the detection of Bovine Herpes Virus-1 infection by Polymerase Chain Reaction (PCR) using gI gene specific. PCR detected 25 samples from organized farms and 24 samples from un-organized farms, the percentage prevalence of Bovine Herpes Virus-1 infection in organized farms was 47.16 percent (25/53) and 44.44 percent (24/54) in the un-organized farms. The present study emphasizes the diagnosis of BHV-1 infection in Cattle in organized farms and un-organized farms based on Polymerase Chain Reaction (PCR) result.

Keywords: Bovine herpes virus-1, polymerase chain reaction, gI gene, cattle

Introduction
Infectious bovine rhino tracheitis (IBR) caused by BHV-I virus is a highly infectious disease of production animals especially large ruminants. The disease manifests various systemic infections and thereby leading to heavy economic losses to the livestock industry attributable to drop in production. Though all age group of animals are susceptible, young calves following weaning are found to be highly susceptible due to wane out Colostral immunity. Infectious Bovine Rhinotracheitis is categorized under multispecies diseases (OIE, 2010) [8], this disease causes reproductive, respiratory, and nervous problems among Bovine population predominantly in Cattle. (Ganguly *et al.*, 2008) [2]. In India, the disease has been reported since 1976 (Mehrotra *et al.* 1976) [5]. However (Kiran *et al.* 2005) [3] considered this disease as one of the most prevalent respiratory and reproductive viral disease of cattle in India. Sinha *et al.* 2003 [9] and Malmarugan *et al.* 2004 [4] reported the BHV-I prevalence of 2.75 to 81.0% in other bovine species i,e buffaloes in India.

Materials and Methods
A total of 107 whole blood samples were collected randomly among cattle population using anticoagulant blood collection tube, the samples were centrifuged and the serum were later separated and transferred into microfuge tubes. The total number of serum samples subjected to study (n=107) comprising of 54 samples from organized farms and the remaining 53 samples from un-organized farms.

Genomic DNA Extraction
Serum samples were used in the preparation of genomic DNA. DNA was prepared using the MEDOX-Easy ™ ultra pure genomic DNA minipreps kit. The genomic DNA was extracted as per the manufacturer's instructions, the samples collected in 500µl phosphate buffered saline (PBS) was mixed with equal quantity of suspension buffer and pelleted. The pellet was suspended in lysis buffer and incubated at 56 °C for 30 minutes followed by centrifugation at 10,000 rpm for 1 min. The fluid was collected in to a column and spun as above and the flow through was discarded. The column was washed with 500 µl 70% ethanol for twice a time. Further, the DNA was eluded using the elution buffer and the same was stored at -20°C until further use.

Primers

A set of primers for amplification of gI gene by PCR.

Table 1: Polymerase Chain Reaction (PCR)

S. No	Name of the Oligos	Sequences (5' → 3')	Fragment Size
1.	Forward gI	5' CACGGACCTGGTGGACAAGAAG (22) 3'	468bp
2.	Reverse gI	5' CTACCGTCACGTGAGTGGTACG (22) 3'	

Detection of BHV-1 DNA by Polymerase Chain Reaction (PCR)

The genomic DNA extracted was subjected to Polymerised Chain Reaction. The primers, forward gI CACGGACCTGGTGGACAAGAAG (22) and the Reverse gI CTACCGTCACGTGAGTGGTACG (22) were designed as per Vilcek *et al*., (1993) [10]. The PCR was performed in 25 µl reaction volume, which included master mix, nuclease free distilled water, forward and reverse gI gene primers and 3.0 µl DNA template.

Thermal Cyclic Conditions for PCR

PCR was performed in a total reaction volume of 25 µl, in Master Cycler (eppendorf) with following thermal cyclic conditions as per Deka *et al*. (2005) [1] with a little change in temperature and timings. Standardization of PCR was carried out using positive control and negative control DNA.

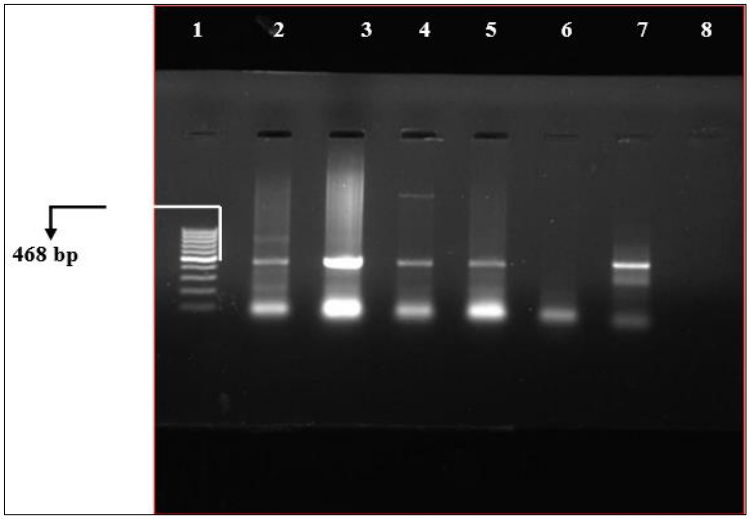
Table 2: Thermal Cyclic Conditions for PCR

Primers	Cyclic Conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final Extension
gI Forward	94 °C	95 °C	57 °C	72 °C	72 °C
gI Reverse	5 min	1 min	1 min	1 min	6 min

Results and Discussion

The samples after subjecting to Polymerase Chain Reaction (PCR) using gI gene specific detected a positive number of 25 samples from 53 cattle (25/53) in the organized farms and a positive number of 24 samples from 54 cattle (24/54) from un-organized farm, the positive samples depicted in fig.1 given below, the prevalence of Bovine Herpes Virus-1 infection in organized farms was 47.16 percent (25/53) and 44.44 percent (24/54) in the un-organized farms. The overall percent positivity tested in the organized farms as well as from the un-organized farms was 45.79%.

Deka *et al.* (2005) [1] found 58.33 per cent samples as positive by gI gene PCR. Moakhar *et al.* (2003) [6] also reported that gI gene has highly conserved sequences that were used as a valuable target for detection of BHV-1 in samples, Deka *et al.* (2005) [1] suggested Polymerase Chain Reaction (PCR) could be the best method and can be used for the frequent detection of BHV-1 infection because of its high sensitivity and specificity. The Chi square test in this study indicated sample wise detection in the organized sector influenced the prevalence of infection by PCR at a significant level whereas the un-organized farm highly influenced the prevalence of BHV-1 infection. Conclusively, sample wise detection by PCR had a highly significant role at both farm levels. It can be assumed that the detection of samples by PCR is highly sensitive (Moore *et al*. 2000) [7] and also more useful for the identification of latently infected cattle population in the farms.



Lane number 1: DNA marker
Lane number 2-6: Test samples
Lane number 2-5: Test samples positive by gI gene PCR
Lane number 6: Test samples negative by gI gene PCR
Lane number 7: Positive control
Lane number 8: Negative control

Fig 1: Polymerase chain reaction agarose gel electrophoresis showing pcr amplified products with bhv-1 gi gene forward and reverse primer

Conclusion

The percent prevalence of Bovine Herpes Virus-1 infection in organized farms was found to be higher as compared to the un-organized farms, this finding may reflect that, the management of Cattle plays an important role in determining the prevalence of Bovine Herpes Virus-1 infection in Cattle, the close proximity of animals maintained in the organized farms whereby the transmission can occur through contaminated oculo-nasal discharges, genital secretions, semen, fetal fluids and droplet infection. The lapse in biosecurity measures or procedures in the organized farms like failing to follow proper quarantine rules or introduction of new stock may cause outbreak in a herd, other factors like production stress and confinement or isolated stress may all attribute to higher prevalence of Bovine Herpes Virus-1 infection in organized farms than to the cattle maintained in Un-organized farms.

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Competing interests

The authors declare that they have no competing interests

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