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Molecular detection of bovine herpes virus -1 (BHV-1) infection in cattle in organised and unorganised farm in Bidar, Karnataka

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Abstract
The present study was carried out to detect the prevalence of Bovine herpes virus-1 among cattle in Bidar. A total of 321 samples, comprising of serum, nasal & ocular samples were collected from a total of 53 and 54 cattle maintained in organized and un-organized farms during the period of January 2016 to January 2017 in Bidar, 159 samples were collected from the organized farm, which included serum (n=53), nasal (n=53) and ocular (n=53) samples. The remaining 162 out of 321 samples were collected from the un-organized farm, which included serum (n=54), nasal (n=54) and ocular (n=54) samples. The gI gene specific Polymerase Chain Reaction (PCR) detected 47.16 percent (25/53), 49.05 percent (26/53) and 35.84 percent (19/53) as positive in serum, nasal and ocular samples respectively in the organized farm and in the un-organized farm the positivity was 44.44 percent (24/54), 14.81 percent (8/54), 11.11 percent (6/54) in serum, nasal and ocular samples respectively. The overall percent positivity as based on the cattle samples tested in the organized farm was 40.70 percent and in the un-organized farm it was 23.22 percent.

Keywords: gI gene, Cattle, IBR-1, organised and unorganised farms

Introduction
Bovine Herpes Virus -1 (BHV-1) is an agent responsible for the development of a severe respiratory form of infection known as Infectious Bovine Rhinotracheitis (IBR) in high producing cattle and Infectious Pustular Vulvo-Vaginitis (IPV) and Infectious Pustular Balanoposthitis (IPB) in cows and bulls respectively [1]. Bovine herpes virus -1 infection has a worldwide distribution and depicting significant variation in the incidence and prevalence at the regional level.

The disease poses various clinical and pathological problems in affected cattle and causes a considerable economic loss to the livestock industry attributable to the decreased milk production, reduced feed efficiency and reproductive abnormalities [2]. All age groups of cattle are susceptible to the infection although young calves following weaning are found to be highly susceptible that may be associated with a reduction in cholesterol immunity [3]. In India, IBR was first reported by Mehrotra *et al.* [3, 1]. Kiran *et al.* [4] described this disease as one of the most prevalent respiratory and reproductive viral disease of cattle in India [5, 6] reported the IBR prevalence of 2.75 percent and 81.0 percent in buffaloes respectively in India. Renukaradhya [7] reported the sero-prevalence of 50.9 percent and 52.5 percent in cattle and buffaloes respectively.

Infectious bovine rhinotracheitis (IBR), is a well described clinical syndrome, caused by bovine herpesvirus-1 (BHV-1). It is a potential viral pathogen of cattle responsible for remarkable economic losses to the livestock industries around the world. Infectious Bovine Rhinotracheitis is described under multispecies diseases (OIE, 2010), characterized by the development of an acute, contagious, severe respiratory, reproductive and nervous problems in cattle [8, 9]. The disease is of worldwide in distribution and in India, the endemic prevalence of IBR has been extensively reviewed by Nandi *et al.* [1]. In addition to IBR, the virus also causes infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IPB) syndromes in cattle. Bronchopneumonia and mortality are the important complications caused by secondary bacterial infections [10].

Currently, PCR is becoming an inevitable molecular technique used in the diagnosis of various diseases because as it is more sensitive and more rapid than virus isolation technique [11]. Despite the presence of colostral immunity, the virus maintains latency in the trigeminal ganglion of the affected cattle and as and when the cattle are stressed out due to various reasons they shed the virus in the environment and become the source for infecting the other susceptible cattle. It could be due to immune evasion mechanisms and reactivation of virus following stress. Various intrinsic and extrinsic factors also influence the prevalence of infection among cattle population [12]. The present study focused on the diagnosis of BHV-1 infection in Cattle in organized and un-organized farm based on PCR

Materials and Methods

serum, nasal & ocular samples were collected by using polypropylene tube containing sterile cotton swabs(PW003, Hi media Lab, Pvt. Ltd. Mumbai, India). A total of 321 samples, comprising of serum, nasal & ocular samples were collected from a total of 53 and 54 cattle maintained in organized and un-organized farms respectively in Bidar during the period of January 2016 to January 2017**.** 159 samples were collected from the organized farm, which included serum (n=53), nasal (n=53) and ocular (n=53) samples. The remaining 162 out of 321 samples were collected from the un-organized farm, which included serum (n=54), nasal (n=54) and ocular (n=54) samples

Extraction of genomic DNA

All serum, nasal and ocular samples from organized and unorganized farms were subjected to gI gene based PCR to detect *T. gondii*. DNA extraction was carried out from pooled samples of nasal, ocular, and serum using the DNeasy Kit as per the manufacturer's instructions (Bio-Basic Inc. Canada). The extracted DNA was stored in aliquots at -20 °C.

Polymerase chain reaction (PCR)

PCR assay targeted to GI gene (468 bp) was used for diagnosis of infectious bovine rhinotrachetitis as per Vilcek *et al* [13]. The nucleotide sequences of the forward and reverse primers are as follows for gI gene (468 bp size) [14].

Forward: 5' CACGGACCTGGTGGACAAGAAG 3' and

Reverse: 5' CTACCGTCACGTGAGTGGTACG 3'

PCR amplification of gI gene fragment was setup in 25 µl reactions. The reaction mixture consisted of 4.0 µl (58 ng) of template DNA, 12.5 µL of 2x master mix which consist of 10x PCR buffer, 10 mM dNTP mix, and Taq DNA polymerase and 1.0 µl each (10 pmol) of the forward and the reverse primer. The volume was made up to 25 µl by nuclease free water (6.5 µl). The cycling conditions were as follows; the first series of thermal cycling (pre-PCR) consisted of initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation 94 °C for 45 s annealing at 60°C for 1 min and extension at 72 °C for 2 min. Final extension was performed at 72 °C for 10 min. The PCR products (5 µl) were loaded into the respective wells. Molecular weight marker (100 bp) and positive and negative controls were also run. The electrophoresis was carried out at 100 V for 45 min or until the tracking red dye migrated more than two third of the length of the gel tray. The gel was placed under UV trans-illuminator and the results were documented in a gel documentation system (Bio rad) to analyze and document the results.

Statistical analysis

Statistical calculation revealed the significant difference ($P<0.05$) in the prevalence of BHV-1, between the samples tested in the organized farm and highly significant difference ($P<0.01$) in the un-organized farm. Similarly, the calculation also revealed the highly significant difference ($P<0.01$) in the prevalence between the samples tested in both the farms.

Results

The gI gene specific Polymerase Chain Reaction (PCR) detected 47.16 percent (25/53), 49.05 percent (26/53) and 35.84 percent (19/53) as positive in serum, nasal and ocular samples, respectively in the organized farm in un-organized farm the positivity was 44.44 percent (24/54), 14.81 percent (8/54), 11.11 percent (6/54) in serum, nasal and ocular samples respectively. The overall percent positivity as based on the cattle samples tested in the organized farm was 40.70 percent and in the un-organized farm it was 23.22 percent.

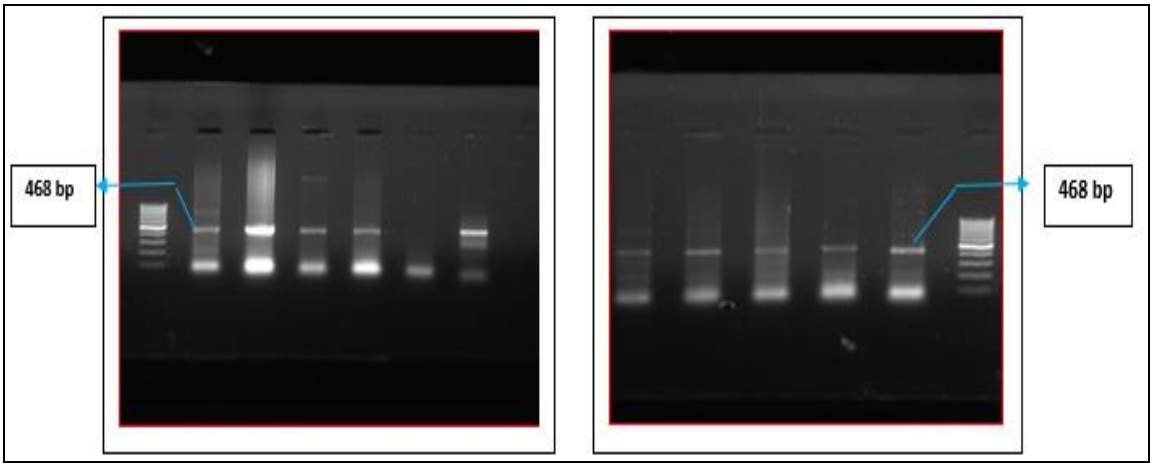
Discussion

In the organized farm, 47.16 percent, 37.50 percent, 49.05 percent, 35.84 percent and 33.96 percent of serum, milk, nasal, ocular and vaginal samples respectively were found as positive, while in the un-organized farm the percent positivity was 44.44 percent, 26.08 percent, 14.81 percent, 11.11 percent and 19.56 percent in serum, milk, nasal, ocular and vaginal samples respectively [15]. identified 44.00 percent of nasal swab samples as positive by PCR and 80.00 percent by nested PCR. Because of the high sensitivity and specificity of PCR, [14, 1] suggested PCR could be the best method and can be used for the frequent detection of BHV-1. In the organized farm, out of 40 cattle samples tested, serum and nasal samples percent positivity in Primiparous was higher (63.63 percent) compared to 18.18 percent/each that was recorded for ocular and vaginal samples but zero percent was recorded for milk samples. Vilcek *et al.* [13, 1] described in detail about the manipulation of swabs samples for performing PCR in order to circumvent the problems that could arise during amplification due to inhibitory substances that might be present in the samples and in addition, they detected 91.66 percent of nasal / ocular swabs samples as positive by gI gene PCR.

In the un-organized farm, the percent positivity in serum and vaginal samples was 42.85 percent/each, and 14.28 percent in nasal and ocular and zero percent in milk samples was recorded in Primiparous cattle whereas in Pluriparous, the percent positivity was 46.15 percent in serum followed by milk, vaginal, nasal, and ocular samples 30.76 percent, 15.38 percent, 12.82 percent 10.25 percent respectively. Gee *et al.* [16] detected BHV-1 in 23.00 percent of nasal swabs samples by PCR however, they able to detect only less number of semen samples as positive by PCR.

The Chi square test in this study indicated sample wise detection in the organized farm 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 level [15]. Reported 82.8 percent of cattle population in Rio Grande do Sul in Brazil was found to be positive for BHV-1 and was also found to be latently infected with the virus. It can be assumed that the detection of samples by PCR is highly sensitive and also more useful for the identification of latently infected cattle population in the farm.

Polymerase Chain Reaction Agarose Gel Electrophoresis Showing PCR Amplified Products with BHV-1 gI Gene Forward and Reverse Primer



Detection of DNA on the Prevalence of Bhv-1 by gI Gene Polymerase Chain Reaction (Pcr)

Conclusions

Cattle in the organized farm showed the overall BHV-1 prevalence of 40.80 percent and in the un-organized farm and Unorganised 23. 20 percent by gI gene PCR at Bidar. Serum and nasal was identified as good source of samples could be used to detect the prevalence BHV-1 and gI gene PCR. The finding assumes significance as far as farm management is concerned because BHV-1 infection in farm is deleterious genome by PCR, Detection of and if established cannot be eradicated easily since virus Bovine herpesvirus 1 from an outbreak of Infectious Bovine hides in the ganglions of the affected animals and persists for the lifetime in the host

Competing interests

The authors declare that they have no competing interests

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