

Prevalence of Bovine Herpesvirus-1 in cattle and buffaloes in Punjab

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Received: 08-10-2012, Accepted: 07-11-2012, Published online: 16-03-2013

How to cite this article:

Kaur G, Dwivedi PN, Verma R and Sharma NS (2013) Prevalence of Bovine Herpesvirus-1 (BHV-1) in cattle and buffaloes in Punjab, *Vet. World* 6(6): 343-345, doi: 10.5455/vetworld.2013.343-345

Abstract

Aim: The aim of the present study was to identify the prevalence of Bovine Herpesvirus-1 (BHV-1) in cattle and buffaloes in the Punjab using PCR as diagnostic tool.

Materials and Methods: A total of 63 samples (Semen- 57, placental cotyledons-1, vaginal secretions-1, foetal stomach contents-1 and tracheal swabs-3) from cattle and buffaloes were processed for identification of BHV-1 using PCR

Results: From January 2007 to December 2010 (Semen- 57, placental cotyledons-1, vaginal secretions-1, foetal stomach contents-1 and tracheal swabs-3) from cattle and buffaloes were collected. The DNA was extracted from a total of 63 samples and subjected to PCR revealed that none of the sample positive for the BHV-1 infection.

Conclusion: From the study it was concluded that the farms screened were free from BHV-1 infection.

Keywords: Bovine Herpesvirus-1, buffaloes, cattle, PCR, prevalence.

Introduction

Bovine herpesvirus type-1 (BHV-1) is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*. It is an important pathogen of cattle worldwide [1] and is classified as BHV-1.1 (respiratory form), BHV-1.2 (genital form) and BHV-1.3 (encephalitic form) [2]. It is responsible for great economic losses to livestock industry in terms of loss of milk production and abortion. The virus causes numerous clinical manifestations like rhinotracheitis, vulvovaginitis, balanoposthitis, abortion and encephalitis. Besides this infection, it can cause acute gastroenteritis, conjunctivitis, mastitis and repeat breeding in cattle [3]. BHV-1 generally infects cattle greater than 6 months of age once maternal immunity has waned [4]. The virus is excreted through nasal, ocular secretion, placenta of aborted animals and through semen. In the bovine semen, BHV-1 is the most common viral pathogen and it poses a great threat to cattle industry as the virus can spread by artificial insemination causing variety of genital disorders [5]. Following an acute infection, the latent virus is established mainly in the sensory ganglia, for the rest of the life of the animal [6]. This latent virus can be reactivated and consequently excreted, with the risk of infecting herd mates which are free from BHV-1. Thus, vaccination can control the clinical disease but latent infection cannot be prevented, therefore, once cattle that have been infected must be regarded as lifelong potential shedders of BHV-1 [7]. Bulls infected with BHV-1 are lifelong carriers and potentially shed virus intermittently in their semen.

Laboratory examinations are required for the diagnosis of BHV-1. Isolation of virus is the most frequent

method for the detection of BHV-1 in the suspected clinical samples. However, this method is time consuming and laborious. The other conventional methods for detection of BHV-1, such as the fluorescent antibody test, ELISA and serological testing, lack sensitivity, particularly when applied to semen donor bulls [8]. Molecular technique such as PCR has been standardized and is commonly used to detect the presence of BHV-1 in various samples effectively because of its enhanced sensitivity, specificity and rapidity [9]. Thus, in the present study clinical samples were examined with the help of PCR for the detection of BHV-1 in these animals.

Materials and Methods

Samples: A total of 63 samples including semen (57), placental cotyledons (1), vaginal secretions (1), foetal stomach contents (1) and tracheal swabs (3) from various farms, submitted in the Department of Veterinary Microbiology, College of Veterinary Science, GADVASU from January 2007 to December 2010 were processed for the BHV-1.

Sample Processing: The frozen semen straws were thawed at 37°C and diluted to 1:10 in sterile normal saline solution and was clarified by centrifugation at 8000 rpm for 10 minutes in a refrigerated centrifuge. The supernatant was collected and kept at -20°C till further use.

The placental cotyledons and vaginal secretions were collected in phosphate buffer glycerol (PBG) and were then triturated in mortar and pestle using sterilized sand and normal saline solution. It was then clarified by centrifugation at 8000 rpm for 10 minutes in refrigerated centrifuge. The supernatant was also collected and stored at -20°C till further use.

The foetal stomach content was diluted 1:10 in phosphate buffer saline and then clarified by centrifugation at 8000 rpm for 10 minutes in refrigerated centrifuge. The

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Table-1. Total number of samples tested via PCR for the detection of BHV-1

| Sr. No. | Sample | PCR (Positive/Total) |
|---------|--|----------------------|
| 1. | Semen sample (33 Cattle; 24 Buffaloes) | 0/57 |
| 2. | Placental Cotyledons (Buffalo) | 0/1 |
| 3. | Vaginal Secretion (Cow) | 0/1 |
| 4. | Foetal Stomach Contents (Buffalo) | 0/1 |
| 5. | Tracheal Swab (Buffalo) | 0/3 |

supernatant was collected and stored at -20°C till further use.

The tracheal swab was collected in a test-tube having 2ml of sterilized tryptose phosphate broth. It was centrifuged at 8000 rpm for 10 minutes. The supernatant was collected and stored at -20°C till further use.

Isolation of DNA: The total DNA was extracted from 600µl of each of the above processed sample. In brief, twenty microlitres of proteinase K (20mg/ml) and of 10% SDS was added, mixed by vortex for 15 seconds and incubated at 56°C for 2 hours. Subsequently, equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the above solution. It was vortexed for 15 seconds and then centrifuged at 13000 rpm for 15 minutes. To the supernatant equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed gently and centrifuged at 13000 rpm for 15 minutes. To the supernatant, 1/10 volume of 3M sodium acetate solution (pH 5.2) and equal volume of isopropanol was added and stored at -20°C overnight to allow precipitation of DNA. DNA was pelleted by centrifugation at 12000 rpm for 10 minutes at 4°C. The pellet was then washed with 70% ethanol twice, air dried and resuspended in 50µl of Tris-EDTA buffer (pH 8.0) and stored at -20°C till further use.

Polymerase chain reaction (PCR): For the PCR, Forward primer 5'CACGGACCTGGTGGACAAGAAG3' and Reverse primer 5'CTACCGTCACGTGAGTGGTACG3' as reported by Vilcek [10] was used in the present study. The reaction mixture for the PCR included 10µl of template DNA, 1.0µl of forward and reverse primers (10pm/µl) each, 5.0µl of PCR buffer (10x), 2.0µl of MgCl₂ (25mM), 1.0µl of dNTPs (10mM each), 0.2µl of Taq Polymerase (5units/µl) and 29.8µl of nuclease free water to make the final reaction volume to 50µl.

The PCR conditions included an initial denaturation at 95°C for 3 minutes and then 35 cycles of denaturation at 95°C for 60 seconds, annealing at 55°C for 60 seconds and extension at 75°C for 60 seconds and then a final extension at 72°C for 10 minutes. The PCR products were analyzed using 1% agarose gel with 0.05µg/µl ethidium bromide. It was electrophoresed at 5-10 volts/cm for 1 hour. Gene Ruler ladder plus 100 bp (MBI, Fermentas) was run for calculating the relative molecular weight.

In the PCR BHV-1 isolate (IBR 216 II batch No. 9/98), obtained from IVRI, Izatnagar available in the Department of Veterinary Microbiology, Ludhiana was used as positive control in the study.

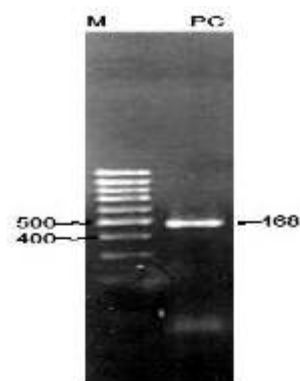


Figure-1. PCR exhibiting a product in a known positive sample (M: Marker; PC: Positive Control)

Results and Discussion

Infectious bovine rhinotracheitis (IBR) is a highly contagious disease caused by BHV-1 affecting cattle and buffaloes [11] and it is present worldwide. BHV-1 infects respiratory and genital tracts of cattle and buffaloes causing various diseases. PCR is an invaluable tool for detection of BHV-1 particularly from clinical samples [12,13,14]. The greatest problem with the infections caused by herpes virus is the induction of carrier state in the animals, due to which, the presence of antibodies in an animal does not indicate the true picture of active infection. Considering this major drawback in serum based tests, detection of virus becomes mandatory to designate an animal as positive for BHV-1 [15]. Out of a total of 63 samples tested in the present study, none of the samples was positive when tested with PCR (Table-1). The reference DNA used in the study showed positive amplification of 468bp in PCR suggests that the assay used was specific and sensitive for BHV-1 and could be used to detect BHV-1 in different samples from infected animals (Fig. 1). In an earlier study conducted in 1998 in the Lusaka province of Zambia, the prevalence of BHV-1 antibodies in bovine sera was reported to be in between 0% to 80% [16]. In another study, two polymerase chain reaction assays specific for glycoprotein B (gB) and glycoprotein C (gC) gene detection were used for the detection of bovine herpesvirus-1 in naturally infected cattle and reported that out of 12 bovine tissue samples tested for gB and gC only one sample was found to be positive for presence of viral genome, producing an amplified PCR product of size 173 bp [17].

PCR was used in another study for the detection of BHV-1 directly from semen samples and it was reported that PCR failed to amplify in some of the semen samples which was attributed due to the inhibitory effect of the bovine semen [18]. However, it was also reported that by using a purification protocol that eliminated the interfering components, the sensitivity of PCR increased markedly [19].

Out of a total of 523 semen samples collected and screened by isolation of virus for BHV-1 in bovine turbinate (BT) and Mardin Darby Bovine Kidney (MDBK) cell lines from the four southern states of India revealed that, only four semen samples showed cytopathic changes indicating that the prevalence of BHV-1 is scarce [20] similar to our findings in which we too failed in detecting BHV-1. Similarly in another study, the prevalence of BHV-1 was found to be 0.8% (2/250) [21]. However, in a study on 574 semen samples from seropositive cattle and buffaloes tested by real time PCR indicated that 1.97% cattle and 3.36% buffalo semen

batches were positive for BHV-1 indicating that real time PCR in adjunct could be used in diagnostic laboratories for identifying BHV-1[22].

Conclusion

It could be concluded that none of the animals screened using PCR, yielded positive results indicating that the farms screened were free from BHV-1 infection. The finding assumes significance as far as farm management is concerned because BHV-1 infection in farm is deleterious and if established cannot be eradicated easily since virus hides in the ganglions of the affected animals and persists for the lifetime in the host.

Authors' contribution

GK designed the study. GK, RV and PND conducted the experiment and analyzed the data. GK and NSS drafted and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors are thankful to Director of Research, GADVAS University and Head, Department of Veterinary Microbiology, for providing the facilities for the smooth conduct of the study. The authors are also thankful to Mudit Chandra for his help in the manuscript revision.

Competing interests

Authors declare that they have no competing interests.

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