



Detection of bovine herpes virus (BoHV-1) infection in respiratory tract of bovines

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Livestock is an important part of the Indian economy and it plays a significant role in welfare of rural population of India. Bovine herpesvirus-1 (BoHV-1) infection is a major causal agent of respiratory diseases apart from causing reproductive disorders. It is enveloped virus which belongs to the subfamily alpha herpesvirinae in the family of Herpesviridae. The viral genome is a linear double stranded DNA molecule of 137 kb that encodes several glycoproteins and which are responsible for infection. Infectious Bovine Rhinotracheitis (IBR) is a severe respiratory form of BoHV-1 infection in high producing cattle and buffaloes. The disease was reported first time in Uttar Pradesh state and since then many reports have been published regarding its occurrence in different states in country (Nandi *et al.* 2009, Verma *et al.* 2014).

Naturally occurring BoHV-1 in respiratory form usually go unnoticed and do not cause high mortality amongst affected animals, but persistence of the virus causes the economic losses due to reduced production, impaired work ability, abortion etc. (Ravishankar *et al.* 2012). However, several reports are available about prevalence of virus in semen and in other reproductive disorders (Majumdar *et al.* 2015). But the report of viral detection in pneumonic cases of bovine is scarce in India except few solitary reports of isolation in nasal secretion (Ranganatha *et al.* 2013, Patil *et al.* 2016). For effective control of disease, early and confirmatory diagnosis is very important. Diagnosis of IBR virus is usually based on ELISA, PCR and virus isolation tests. Recent emphasis has been given to reduce the time required for diagnosis of infections. Virus isolation is laborious, time consuming and requires skilled person along with good quality samples.

Nasal swabs (100) and tracheal lavages (6) were collected from all ages of cattle and buffaloes exhibiting clinical signs of respiratory infections from clinics of

College of Veterinary Science, Guru Angad Dev Veterinary Science University Ludhiana, Punjab. Four lung tissues samples were procured from Department of Pathology, COVS, GADVASU, Ludhiana. Virus inoculum was prepared in PBS by centrifugation 825 × g for 15 minutes. Supernatant was collected and stored at –20°C for further use. All the applications were performed under strict sterile conditions.

All nasal swab samples were analyzed for detecting IBR virus (BoHV-1) antigen by a commercially available sandwich ELISA kit (Biox Diagnostics, Belgique). The sandwich ELISA was performed as per manufacturer instructions. The plates were read on an ELISA reader (Thermo, USA) at 450 nm. The optical density (OD) in well coated with viral antibody was corrected by subtracting the OD value of corresponding negative control. Samples were considered positive as per value provided by manufacturer.

Template DNA was prepared from all samples by using Sambrook and Russell (2001) for molecular detection of IBR virus. The DNA was also extracted from reference virus procured from the School of Animal Biotechnology, GADVASU, Ludhiana used as a positive control. PCR amplification was carried out for highly conserved region of glycoprotein I (gI) gene with specific published primers (Vilcek *et al.* 1994) for diagnosis of IBR virus. The nucleotide sequences of the forward and reverse primers were gI F (624-CACGGACCTGGTGGACAAGAAG-645) and gI R (1070-CTACCGTCACGTGAGTGGTACG-1091) for amplification of 468 bp product of gI gene. The PCR reaction was optimized by adding 2.5 µl of 10× PCR buffer (with 15 mM MgCl₂), 1.0 µl of forward and reverse primer (20 pm/µl) each, 1.0 µl of dNTPs mix (10 mM each), 0.2 µl Taq DNA polymerase (5 units/µl), 10 µl of extracted DNA (150–400 ng) and the reaction was made up to 25 µl using nuclease free water. The optimized thermal cycler conditions was initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 51.8°C for 1 min, extension at 75°C for 1 min and final extension at 75°C for 10 min. The negative control consisted of sterile water instead of DNA template while positive control

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consisted of DNA extracted from reference virus. The amplified product was electrophoresed in 1.5% agarose gel and visualized as a single compact band of expected size under in gel documentation system (Syngene, USA). A clear compact band of 468 bp was regarded as a positive for BoHV-1.

Specific BoHV-1 antigen was detected only in one nasal swab sample (1%) in antigen detection sandwich ELISA. Out of hundred nasal swab samples, 3 nasal swab samples were found positive by PCR yielding a product size of 468 bp. The remaining 97 nasal samples, 6 tracheal lavages and 4 lung tissue samples failed to produce the targeted amplification.

Thus in the present study, the overall incidence of BoHV-1 was found to be 3.0% in nasal swab samples in bovines by using PCR and 1% in antigen detection ELISA. BoHV-1 infection was reported only in 4% female crossbred cattle out of 75 cattle in PCR whereas none of the buffaloes was found positive. The animals older than 3 year of age showed the higher incidence (4.2%) than animal up to 3 years of age which had lower incidence (2.8%).

Vilcek *et al.* (1994) standardized the gI gene based PCR and reported that PCR is specific and sensitive assay for detection of IBR virus from nasal swab samples from infected animals. However, ELISA was also used by various researchers for detection of BoHV-1 infection in respiratory route. Iscan and Duman (2011) detected the viral antigen only in 0.8% of nasal swab samples of infected animals by ELISA while seropositivity was higher (21.2%) in same sampled animals. Singh *et al.* (2013) also described the 11.1% prevalence of BoHV-1 infection in nasal swab samples of cattle of Uttar Pradesh by using PCR and ELISA technique. In West Bengal, Saha *et al.* (2010) had detected the BoHV-1 virus in only one sample (1.5%) out of 65 nasal swab samples tested by PCR. Nisavic *et al.* (2018) examined 110 bovine nasal swab samples from Serbia and only four (3.6%) samples were found positive in PCR. These findings were in line with present study in which 3.0% incidence were recorded to detect infections through respiratory route in bovines. However, Patil *et al.* (2016) reported 15% (10/65) nasal samples positive in virus isolation and confirmed by PCR. In their study all the samples were collected from suspected animals showing clinical sign of IBR. Shedding of virus is more in clinical phase of disease, which increases the sensitivity of virus isolation and PCR (Sobhy *et al.* 2014).

In present study, all crossbred female cattle were found positive for BoHV-1 infection and none of the buffalo was positive. Similarly, Ranganatha *et al.* (2013) examined nasal and conjunctival swab samples from bovines for detection of bovine herpes virus-1. Out of total 40 samples, 3 (7.5%) samples were found positive in PCR in which 2 samples were from cattle and 1 was from buffaloes. Ei-Kholy (2005) examined 148 bovines for BoHV-1 infection in different specimens of bovines and also recorded higher incidence in cattle 16% (15/93) than buffalo 7.2% (4/55). More susceptibility of crossbred cattle was due to high production

stress and less adaptation to the Indian climatic conditions whereas buffaloes are generally reared from ancient time for household consumption, less production stress and well suited to Indian climate.

Sex wise evaluation revealed that all crossbred cattle were female. It may be because of production stress in female animals. Moreover, it is known that artificial insemination is the main transmission route for BoHV-1 virus because semen is the source of infection. In agreement to current study, Deka *et al.* (2005) also recorded the higher prevalence in crossbred than the indigenous cattle and females were more prone to infection than male.

Present results described the higher incidence (4.2%) in adult cattle (> 3 year age) than young animal (2.8%) (up to 3 year age). The risk of infection is more in animals that had a parity of two or more and in crossbred of Holstein Friesian. The increase in the incidence of IBR infection with age could be due to the fact that as animals grow older, they are more likely to be exposed to the virus since they are more likely to come up in to contact with other animals which have recovered from the disease but remains carrier.

There are so many seropositive reports available from different states of India, which show the higher prevalence of infection in animals and mainly cattle were affected (Nandi *et al.* 2009, Samrath *et al.* 2016). But lower rate of virus detection may be because of the latency of virus in ganglions and the animal remains seropositive life long, and it can be reactivated by stress and such animals become a potential threat to spread the virus around the environment periodically (OIE, 2008). The detection of BoHV-1 is feasible only during pyrexia phase, which lasts for few days only. The lesser number of positive samples from nasal swabs in the present study was probably due to the lower concentration of virus excreted from the respiratory route. The number of positive samples could be increased if repeated swabs would have been taken from the suspected animals or if swabs would have been taken during stress period of animal. Since maximal virus replication and shedding occurs between the third and sixth days which is early acute phase of the disease, swabs for virus isolation should be taken early in the course of disease when the discharge is serous rather than mucopurulent (OIE, 2008).

The present study revealed the existence of BoHV-1 virus among the cattle populations of Punjab and also shedding the virus through respiratory route. The virus can be detected from the upper respiratory tract from BoHV-1 infected animals. This study was performed using less number of samples from limited geographical area, so a detailed study is required using more number of samples from vast geographical area.

SUMMARY

Infectious bovine rhinotracheitis (IBR) is considered commonest form of BoHV-1 respiratory tract infection. The current work was designed to study the incidence of IBR using molecular technique and antigen detection sandwich ELISA. In the present study, nasal swab samples (100) and tracheal

lavages (6) were collected from bovines, which were showing respiratory symptoms from clinics of College of Veterinary Science, GADVASU, Ludhiana. Four lung tissues were also collected from dead animals. All nasal samples were tested for IBR virus antigen by using commercial sandwich ELISA kit. The antigenic incidence of BoHV-1 was reported to be 1% in tested animals. For molecular detection of BoHV-1 in respiratory tract, the DNA was extracted from all samples (nasal swabs, tracheal lavages and lung tissues) and subjected to gI gene specific PCR by using published primers. The overall incidence of BoHV-1 in nasal swabs of animals with the history of respiratory symptoms was found to be 3% and rest of the samples were found negative for IBR virus in PCR. Species wise evaluation of BoHV-1 infection revealed that out of 75 cattle, 3 (4.0%) cattle were found positive in PCR where as none of the buffaloes were found positive in present study. The animals older than 3 year of age showed the higher incidence (4.2%) than animal up to 3 years of age (2.8%). In this study, all positive cattle were crossbred females. According to sex wise status, it was observed that females are more prone to infection, which may be because of production stress in female animals.

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