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 10x PCR buffer (with 15 mM MgCl₂), 1.0 µl of forward and reverse primer (20 pmol/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
Infectious bovine rhinotracheitis (IBR) is considered the 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 swab samples of cattle were positive. 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 the 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 or 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 pyrexic 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 the 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 swab samples of cattle were positive. 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 the 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.

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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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REFERENCES


