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Detection of Respiratory Viral Antigens in Nasal Swabs of Bovine by Sandwich ELISA

Rakhi Gangil[1], Gurpreet Kaur, P.N. Dwivedi

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ABSTRACT

Bovine Respiratory diseases are responsible for economic losses in livestock industry worldwide. The present study aiming to investigate the occurrence of respiratory viral antigens of Bovine herpesvirus-1 (BoHV-1), Bovine respiratory syncytial virus (BRSV), Bovine viral diarrhoea virus (BVDV) and Bovine parainfluenza 3 virus (BPI3V) in bovines and describe the distribution of respiratory viruses in unvaccinated animals in Punjab. A total of 55 nasal swab samples were collected from the cattle and buffaloes exhibiting the clinical signs of respiratory disease. All samples were tested for the detection of all four viral antigens by commercially available sandwich ELISA kit. BoHV-1 and BPI3V antigen were detected in 1 and 3 samples with the percentage of 1.8 and 5.4, respectively, whereas none of the sample was found positive for BRSV and BVDV antigen. The present study concluded the existence of BoHV-1 and BPI3V infection in respiratory tract and plays the important role in the respiratory viral infections of bovines. This study suggests the prevention measures should be taken to control the economic losses due to respiratory diseases in cattle and buffaloes.

Key words: BoHV-1, BPI3V, BRSV, BVDV, Respiratory disease, Sandwich ELISA.

INTRODUCTION

Livestock sector has remarkable contribution in the national economy and emerged as a tool for socio economic development of the farming community. Dairy farming is now not only for household consumption but also as a source of supplementary income to the small and marginal farmers. India is a largest milk producer in the world and it can continue to hold the first position if some of the diseases affecting the productivity of the animals is taken care. Respiratory and reproductive disorders in dairy animals due to various etiological agents had led to severe economic losses to farmer in terms of decrease milk production as well as loss of feedlot animals.

Bovine respiratory diseases (BRD) occur all over the world and considered to be the major cause of morbidity and mortality within feedlot and dairy cattle (Iglseder *et al.,* 2011). It is a multifactorial disease, such as stress due to weaning, transportation, pooling of cattle from multiple sources, dusty conditions, parasitism, concurrent diseases and weather extremes and environmental factors ultimately resulting in bronchopneumonia (Klima *et al.,* 2014). Respiratory viruses *viz*. bovine herpes virus-1 (BoHV-1), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV) and Bovine Para influenza 3 virus (BPI3V) are the most important viral agents that cause respiratory infection in bovine worldwide.

Bovine Herpesvirus-1 (BoHV-1) belongs to family *Herpesviridae*, subfamily *Alpha herpesvirinae*. It is an economically important disease characterized by severe inflammation of upper respiratory tract together with dyspnoea, depression, nasal flow and deconditioning in cattle and buffaloes (Yavru *et al.,* 2005). Besides this, infection may cause acute gastroenteritis, conjunctivitis, encephalitis, abortion, mastitis and repeat breeding in cattle.

Department of Veterinary Microbiology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana-141 004, Punjab, India. [1]Department of Veterinary Microbiology, College of Veterinary Science and AH Mhow-453 446, Madhya Pradesh, India.

Corresponding Author: Rakhi Gangil, Department of Veterinary Microbiology, College of Veterinary Science and AH Mhow-453 446, Madhya Pradesh, India. Email: rgangil@gmail.com

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Bovine viral diarrhea virus (BVDV) is a *Pestivirus* in the *Flaviviridae* family and it causes disease in bovines and characterized with respiratory symptoms, abortions, congenital anomalies, still birth and birth of persistently infected carrier animals and can lead to fatal mucosal disease (Bedekovic *et al.,* 2013).

Bovine respiratory syncytial virus (BRSV) is a member of family *Paramyxoviridae*, genus *Pneomovirus*. BRSV infection is characterized with dyspnea, coughing, rhinitis, nasal discharge, fever, bronchiolitis, edema emphysema and in some cases disease progressing to severe pronchopneumonia may end with death (Ellis *et al.,* 2001). Bovine Parainfluenza-3 virus (BPI3V) is also associated with respiratory infections, characterized by rhinitis, acute and silent fever nasal secretions (Solis-Calderon *et al.,* 2003). It is a member of family *Paramyxoviridae* and subfamily *Paramyxovirinae*. Parainfluenza virus causes generally clinical and subclinical infections and this infection predispose the host to secondary bacterial infections and other viral infections may lead to death.

Diagnosis of these viral pathogens have been made by various methods such as Enzyme Linked Immunosorbant Assay (ELISA), Serum Neutralization Test (SNT), Fluorescent Antibody test (FAT), Polymerase Chain Reaction (PCR) and Reverse Transcriptase PCR (RT-PCR). It has been opined that virus isolation is a gold standard and more sensitive for antigen detection in diagnosis, but it is time consuming, expensive and cumbersome procedure than ELISA and requires skilled personnel and established specified laboratory for cell culture (Fulton and Confer 2012). However ELISA has been test of choice for detection of antigen and antibody due to advantage to perform this test within a short time frame in the laboratory and is more efficient in the differential diagnosis as combined infection can also be detected simultaneously for different associated viral agents (Avci *et al.,* 2014). The present study planned to detect the various respiratory viral antigens in nasal excretions of cattle and buffaloes and determine the BRD occurrence by sandwich ELISA.

MATERIALS AND METHODS

Sample collection

A total of 55 nasal swab samples were collected in PBS from cattle and buffaloes exhibiting clinical signs of respiratory infections *viz*., coughing, depression, fever, nasal and ocular discharge, and anorexia during November 2016 to March 2018 from different Gaushalas in and around Ludhiana and Teaching Veterinary Clinical Complex, College of Veterinary Science, Guru Angad Dev Veterinary Science University Ludhiana, Punjab. Two ml of each sample were centrifuged at 825 x g for 15 minutes. Supernatant were collected and stored at -20°C for further use. All the applications were performed under strict sterile conditions.

Sandwich ELISA

Nasal swab samples were analyzed for detecting mentioned viral antigens by a commercially available sandwich ELISA (Biox Diagnostics, Pulmotest Respiratory Tetra ELISA kit, Belgique) where detection of these agents can be assayed simultaneously from a single sample. The test was performed in Virology laboratory of Department of Microbiology, College of veterinary Science, Guru Angad Dev Veterinary Science University Ludhiana, Punjab.

The sandwich ELISA was performed as per manufacturer instructions. The plates were read on an ELISA reader (Thermo, USA) at 450nm. The optical density (OD) in well coated with viral antibody was corrected by subtracting the OD value of corresponding negative control. Percent positivity values of sample were calculated as given below:

$$\frac{S}{P}\ \% = \frac{OD_{\text{corrected value of sample}}}{OD_{\text{corrected value of positive control}}} \times 100$$

Samples value was then compared with positive reference supplied with manufacturing kit.

RESULTS AND DISCUSSION

Bovine respiratory disease (BRD) is a major problem of cattle and buffaloes affecting all ages of animals worldwide and causes economic loss for producers (Houe, 2003). In present study, specific BoHV-1 antigen was detected in one (1.8%) sample and 3 (5.4%) samples were found positive for BPI3V antigen among all tested samples (Fig 1). However none of the sample was positive for BRSV and BVDV antigen. Not a solitary sample tested showed positive reaction for presence of two antigens concomitantly.

The serologic and virologic evidence of BoHV-1 infection caused by respiratory viruses has been demonstrated previously (Majumdar *et al.,* 2015; Patil *et al.,* 2017). The present study revealed 1.8% occurrence of BoHV-1 antigen in sandwich ELISA. Similarly, Iscan and Duman (2011) reported 0.8% (2/250) prevalence of BoHV-1 antigen in dairy cattle from nasal swab samples. While in the serological part of their study of same sampled animals revealed 21.2% prevalence of BoHV-1 specific antibody. The lower rate of detection of BoHV-1 viral antigen in present study by ELISA may be because of latency of virus at a time of collection of nasal swab. BoHV-1 has tendency to become latent in ganglion of infected animals and shedding of virus in nasal secretions/ viraemia occurs only after reactivation of virus in immunocompromised host or fresh infection (OIE, 2008). However, seroconversion invariably occurs at the time of

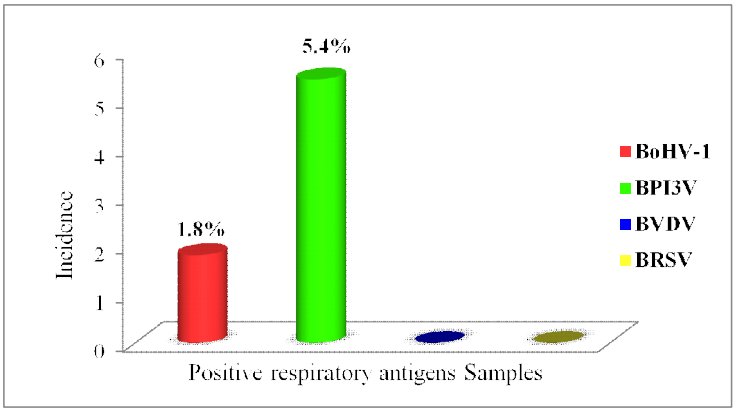

Fig 1: Incidence of respiratory virus antigens detected by in S-ELISA.

initial infection and antibody titre persists for a longer duration, hence many study recorded higher seroprevalence. However, Singh *et al.* (2013) described the 11.1% prevalence of BoHV-1 infection in nasal swab samples of cattle of organized and unorganized herds from seven districts of Uttar Pradesh by using ELISA technique. In accordance of present study Ranganatha *et al.* (2013) also reported lower incidence of BoHV-1 from nasal swabs in their study and suggested it may be due to lower concentration of virus excreted from respiratory route. The number of antigen positive samples could have been increased if repeated swabs would have been taken from the suspected animals because the maximal virus shedding occurs between third and sixth days in acute phase of disease (OIE, 2008).

Edwards *et al.* (1985) experimentally inoculated the calves with BoHV-1 and noticed that by antigen detection ELISA is feasible only during pyrexic phase, which lasts for few days only. Collins *et al.* (1988) evaluated antigen capture ELISA for detection of BoHV-1 antigen in nasal swab specimens and it was judged that ELISA was found to be useful for diagnosis of BoHV-1 antigen when nasal swab specimens were collected during first five days of the infection, when clinical signs were the most apparent. Moreover, sampling in this study was done in all seasons. BoHV-1 infection prevalence was reported to be usually lower in summer than in the other seasons (Woodbine *et al.,* 2009).

Bovine parainfluenza 3 virus (BPI3V) is one of the viruses known to cause respiratory infections. Widespread prevalence of BPI3V antibodies and detection of this virus in lungs were reported worldwide. Parainfluenza infection appears to predispose the host to secondary bacterial infections and it has also been shown to play a role in shipping fever. In the present study, specific parainfluenza antigen was detected in 5.4% of nasal samples by using sandwich ELISA, this finding in accordance with Goswami (2016), who reported 4.83% of parainfluenza infection in lung tissues of bovines. Sex wise incidence also recorded in current study. In present study higher percentage was observed in male (9.1%) then female (4.5%) in bovines among overall male and female tested respectively (Table 1). However, Noori *et al.* (2014) recorded higher BPI3V infection 20% (20/100) in pneumonic lung tissue by sandwich ELISA technique in Sudan. In their study, 19.1% positive for male while 21.9% positive for female. Parainfluenza infection is hard to diagnose due to milder clinical signs. Further death supervenes due to predominant secondary infections (Hagglund *et al.,* 2006). Saeed *et al.* (2016) detected the parainfluenza virus 3 infection in lung tissues of cattle, sheep and goat in Sudan by using Ag-ELISA and reported 12.8%, 9.8% and 47.8% prevalence, respectively and also concluded that parainfluenza infection was causing economic losses due to its associated infection. According to present study, nasal swab can be good sample for diagnosis of disease in live animals while parainfluenza infection mainly reported in lung tissues of dead animals.

Bovine respiratory syncytial virus (BRSV) is also important virus affecting respiratory tract in cattle, especially in young calves. The serologic evidences of BRSV infections in India (Goswami *et al.,* 2016 and Hazari *et al.,* 2002) and other countries (Urban-Chmiel *et al.,* 2013 and Saber *et al.,* 1996) have been demonstrated. There are many studies conducted on lung tissues in comparison to nasal swab samples from live animals.

In present study, none of the samples were found positive for BRSV antigen by sandwich ELISA. This finding is in agreement with the observation recorded by Abdallah (2005), who tested 88 samples (37 nasal swabs and 51 lung tissue) by antigen detection ELISA and none of the nasal swab samples were found positive by ELISA while only one lung tissue found positive. However, Masot *et al.* (1993) considered ELISA as a diagnostic tool and most suitable technique for identifying BRSV antigen in the lung tissue. The detection of viral antigen from pneumonic lung tissue also tried by Avci *et al.* (2014) by antigen capture direct ELISA and 16.6% positivity recorded for BRSV antigen whereas could not find presence of BoHV-1, BPI3V and BVDV.

Our results were in accordance with Percivalle *et al.* (1989), who mentioned that ELISA lacks sensitivity in detection of BRSV antigen. Similarly, Quinting *et al.* (2007) compared different antigen detection tests for diagnosis of BRSV antigen, and suggested that ELISA is less sensitive then RT-PCR for BRSV antigen detection in lung tissues. Absence of BRSV antigen can also be explained by sample size, seasonal variation compared to previous findings (Van der Poel *et al.,* 1994) that shows the incidence of BRSV in late autumn and winter.

In the current study, all nasal swab samples were detected as negative for BVDV antigen. Similarly, Goswami (2016) and Avci *et al.* (2014) were also not able to detect BVDV antigen in lung tissues of cattle by antigen detection ELISA. However, Ibrahim *et al.* (2008) examined 33 nasal swab samples of cattle for BVDV antigen detection by capture ELISA. In their study 54% (18/33) prevalence of BVDV antigen were reported. BVDV infection was also reported in other samples such as peripheral blood mononuclear cells (PBMCs) of Indian cattle (Gupta *et al.*, 2014) and milk samples of Korean dairy farms (Park *et al.* 2016) by Ag-Elisa. The serological evidence of BVDV was earlier reported in bovines from India (Puneet *et al.,* 2012 and Selvaraj *et al.,* 2007).

Table 1: Sex wise prevalence of bovine parainfluenza 3 virus antigen (BPI3V-Ag) in nasal swab samples by using sandwich ELISA.

Total tested	Males			Females			Total positive
	Tested	Positive	%	Tested	Positive	%	
55	11	1	9.10	44	2	4.54	3 (5.45 %)

The difference between our results and others may be due to time of sample collection that may be not at the time of acute infection or due to absence of antigen in samples. In the present study all the nasal swab samples were detected as negative for BRSV and BVDV, which may be either due to the animals showing clinical symptoms, might not be having presence/ infection of BRSV and BVDV or during the course neutralizing antibodies might have been produced and neutralized the virus from nasal tract. Since in present study, serological detection of antibodies was not aimed hence nothing can clearly be stated.

CONCLUSION

The present study showed the evidence of the presence of BoHV-1 and BPI3V infection among bovines in Punjab and also shedding the virus through respiratory route. The viral antigen can be detected from the upper respiratory tract of live animal. Further serological and virological examination should be done to investigate the viruses and preventive and control measure must be taken.

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