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Sero-positivity of Bovine Herpes Virus Type 1, Parainfluenza Type 3 Virus and Respiratory Syncytial Virus in Healthy and Pneumonic Cattle

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Abstract

Seropositivity of bovine herpes virus type 1, bovine respiratory syncytial virus and bovine parainfluenza type 3 viruses was determined in 41 cattle presented to large animal hospital, GADVASU with respiratory diseases and 25 cattle were kept as control animals. The seropositivity of BoHV-1, BRSV and BPIV-3 in control and diseased group was 16.0 Vs 41.5, 28.0 Vs 51.2 and 36.0 Vs 65.8 per cent, respectively. There was statistically significant difference ($p<0.005$) in the proportion and degree of seropositivity' s between diseased and control animals against BoHV-1 and BPIV-3. Exact role of viruses in the disease process could not be established due to non-availability of paired sera from the animals and investigation of the animals at the initial stage of the setting up of disease. Nevertheless, the present study confirms the presence of these respiratory viruses in cattle population of Punjab, India.

Keywords: Bovine Respiratory Viruses, Cattle, ELISA, Pneumonia, Punjab

Introduction

Respiratory diseases are important both from an economic and an animal welfare standpoint. Outbreaks of respiratory diseases have been responsible for direct (increased mortality and treatment costs) and indirect losses (impact of the diseases on the work load, the growth rate of animals, the age at first calving, premature culling and the milk production). Respiratory disease in cattle is a multi-factorial disease that involves an interaction of stressors and infectious agents. A number of studies have demonstrated a synergistic role of viruses in bovine respiratory disease in increasing the pathogenicity of both viral and bacterial concomitant infections (Shahriar *et al*., 2002). Bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (BPIV-3), bovine herpesvirus type 1 (BoHV-1), bovine viral diarrhea virus (BVDV) and bovine coronavirus (BCV) are the main viruses causing respiratory diseases in cattle. These viruses suppress the immune system of the host and increase the risk of secondary bacterial infections (Valarcher and Hägglund, 2006). Clinical disease may not occur until other pathogens are present or when adverse environmental conditions precipitate the clinical disease (Radostits *et al*., 2000).

Currently, the available data on seroprevalence of respiratory viruses in cattle are usually from large dairy farms. Many previous studies have reported the seroprevalence of respiratory viruses, but published reports are lacking regarding the association of respiratory viruses with the disease occurrence. Thus, the main objective of the present study was to document the exposure of cattle to BoHV-1, BRSV and BPIV-3 viruses associated with clinical respiratory disease in cattle.

Materials and Methods

The study included two groups. Diseased group comprised of 41 cross bred cattle with clinical signs (fever, dyspnea, open mouth breathing, coughing) and radiographic examination suggesting pulmonary diseases. Confirmatory diagnosis was made on the basis of tracheo-bronchial aspirate cytology. Control group included 25 cows without any respiratory signs. No vaccination was done for any of the viruses tested in both the groups.

Ten ml of blood was collected from each animal aseptically from the jugular vein in a wide mouth test tube. The test tube was kept in slanting position at room temperature till clotting. The serum was collected from clotted blood by centrifuging at 3000 rpm for 10 minutes. The separated serum was collected in a screw capped plastic vial and stored at -20^{0}C till used. The serum samples were investigated for antibodies to bovine herpes virus, respiratory syncytial virus and parainfluenza virus-3.

Serodiagnosis of BoHV-1, BRSV and BPIV-3

Commercial indirect ELISA kits developed by Bio-X Diagnostics®, Belgium, were used. The ELISA kit for BoHV-1 used microtitration plate sensitized by purified BoHV-1 virus. The ELISA kit for BRSV used microtitration plate sensitized by monoclonal antibodies specific to F protein of BRSV virus, which were used to trap recombinant F protein, whereas the one for BPIV-3 used microtitration plate sensitized by monoclonal antibodies specific to one of the antigenic determinants of BPIV-3 virus.

Microtiter plates coated with the respective viral antigens were used as per the manufacturer's guidelines. The optical density of samples and controls (Positive and negative controls provided with commercial kit) were measured at 450 nm within 10 minutes using ELISA reader (Multiskan, Lab systems, USA). The proportions of the animals serologically positive for viruses were calculated. The degree of positivity of serum samples for each virus was recorded using the following formula:

$$Value\ (s) = \frac{Delta\ OD\ sample * 100}{Delta\ OD\ positive}$$

The degree of positivity of serum samples for each virus was scored as instructed by the manufacturer:

BoHV-1										
0		1+		2+		3+		4+		5+
Val<=	30%	<Val=	67%	<Val=	104%	<Val=	141%	<Val=	178%	<Val
BRSV										
0		1+		2+		3+		4+		5+
Val<=	20%	<Val=	40%	<Val=	60%	<Val=	80%	<Val=	100%	<Val
BPIV-3										
0		1+		2+		3+		4+		5+
Val<=	30%	<Val=	67%	<Val=	104%	<Val=	141%	<Val=	178%	<Val

Statistical Analysis

The relative frequency of each virus was expressed as a percentage of the total number of isolates. Chi-square ($\chi 2$) tests were used to determine the relationship between the viruses isolated from samples and the health condition of cattle. The level of significance of the tests was set at 5 per cent.

Results

Seropositivity for BoHV-1, BRSV and BPIV-3 in control group was recorded as 16 per cent, 28 per cent and 36 per cent, respectively. Antibodies to BoHV-1, BRSV and BPIV-3 were found in 41.5 per cent, 51.2 per cent and 65.8 per cent of diseased cattle, respectively. The seropositivity of BoHV-1 and BPIV-3 was significantly higher ($p<0.05$) in diseased group as compared with control animals (Table 1). The difference was non-significant for BRSV.

Table 1: Seropositivity of BoHV-1, BRSV and BPIV-3 in control and diseased groups

Virus	Control group (n=25)	Diseased group (n=41)	Chi square value
BoHV-1	4 (16 %)	17 (41.5 %)	4.64*
BRSV	7 (28 %)	21 (51.2 %)	3.43
BPIV-3	9 (36 %)	27 (65.9 %)	5.58*

**at 5 % level of significance*

Single as well as multiple seropositivity of the viral antigen were observed in the study from both groups (Table 2). In diseased group, 35 (85.4%) out of 41 diseased cows showed positivity for antibody against any of the viral antigen and five cows (14.6%) were negative for any of the three tested viruses. Concurrent seropositivities of three viruses were observed in 21.9 per cent of diseased cattle and none in control group. Antibodies to BRSV were observed concurrently with antibodies to other viruses (either BoHV-1 or BPIV-3 or both) in 18 animals (43.9 per cent). Likewise, antibodies to BPVI-3 were found concurrently with either BoHV-1 or BRSV or both in 18 animals, whereas BoHV-1 was observed in mixed seropositivity in 15 cows. Three diseased cows each were seropositive for BoHV-1 + BPVI-3 and BoHV-1 + BRSV while six were positive for BPVI-3 + BRSV. These proportions were lesser for control group (Table 2).

Table 2: Distribution of seropositivity for BoHV-1, BRSV and BPIV-3 in control and diseased groups

	Control group (n=25)	Diseased group (n=41)
BoHV-1	2 (8 %)	2 (4.87 %)
BRSV	1 (4 %)	3 (7.32 %)
BPIV-3	5 (20 %)	9 (21.9 %)
BoHV-1+BPIV-3 +BRSV	0 (0 %)	9 (21.9 %)
BoHV-1+BPIV-3	0 (0 %)	3 (7.32 %)
BoHV-1+BRSV	2 (8 %)	3 (7.32 %)
BPIV-3 +BRSV	3 (16 %)	6 (14.6 %)

There was significant difference ($p<0.05$) between control and diseased group in terms of degree of positivity of antibodies against BoHV-1 and BPIV-3 from animals. However, no significant difference was observed among the three groups for level of antibodies against BRSV virus (Table 3).

Table 3: Degree of seropositivity (sum of scores) for BoHV-1, BRSV and BPIV-3 in control and diseased groups

	Control group (n=25)	Diseased group (n=41)	Chi square value
BoHV-1	27.32	37.26	**6.13***
BRSV	29.22	36.1	**2.5**
BPIV-3	25.5	38.37	**7.85****

**at 5 % level of significance; **at 1 % level of significance*

Discussion

Serological findings revealed BoHV-1, BPIV-3 and BRSV as common pathogens in cows with respiratory problems. Most of the previous studies have recorded the seroprevalences for the study viruses in apparently healthy animals but few have recorded the seropositivity of respiratory viruses in diseased cows (Nandi *et al*., 2010, Mahajan *et al.*, 2015) as in present study. The presence of antibodies indicated that exposure to these agents is common in the area. Since the animals were not vaccinated for any of the mentioned diseases, the results are indicative that these viruses are circulating within the animals and may be responsible for clinical respiratory problems.

Difference ($p<0.05$) in proportion and degree of positivity of antibodies against BoHV-1 and BPIV-3 between control and diseased group suggests role of these viruses in disease process. However, exact role of viruses in the disease process could not be established due to non-availability of paired sera from the animals and investigation of the animals at the initial stage of the setting up of disease.

The seropositivities were more than as recorded by Mahajan *et al*. (2015) as BoHV-1 (13.3 per cent), BRSV (13.3 per cent) and BPIV-3 (20.0 per cent) in cattle with respiratory signs. They also reported multiple seropositivity which corroborates with the present study. Seropositivity for respiratory disease-causing viruses may indicate a latent phase (in BOHV-1 infection) or persistent infection (BPIV-3) and may serve as a risk of infection to non-immunized healthy animals (Aly *et al.,* 2003). The high seroprevalence of BPIV-3 may be due to its ubiquitous nature and world-wide distribution (Bryson, 1990). BPIV-3 has been associated with both acute and chronic pneumonia in cattle. The seroprevalence of BoHV-1 has been reported from 12.0 to 61.2 per cent (Durham and Hassard, 1990, Yavru *et al.,* 2005, Okur-Gumusova *et al.,* 2007, Sakhaee *et al*., 2009, Tresamol *et al.,* 2019). Likewise, seroprevalence of BRSV and BPIV-3 ranged from 29.9 to 100 percent and 36.2 to 100 per cent, respectively (Yavru *et al.,* 2005, Sakhaee *et al*., 2009). Multiple seropositivity were observed in the present study which is in accordance with many studies (Yavru *et al*., 2005; Yousef *et al.,* 2013; Sakhaee *et al*., 2009, Mahajan *et al*., 2015).

Durham and Hassard (1990) reported 78.5 per cent seropositivity of BRSV and 93.9 per cent of BPIV-3 in cattle in Saskatchewan and Alberta which is relatively more as compared to present study. However, seroprevalances of BRSV and BPIV-3 have been reported as high as 93.6 per cent and 100 per cent, respectively (Valarcher and Hagglund, 2006). Contrarily, Yousef and his co-workers reported 17.4 per cent seropositivity rate for BoHV-1, 75.6 per cent BRSV and 69.1 per cent BPIV-3 in apparently healthy cattle in Saudi Arabia (Yousef *et al*., 2013). They also concluded that co-infections with more than one virus were considerably common among non-vaccinated dairy cattle. Erol *et al. (*2007) found specific antibodies for BPIV-3 in 38.2 per cent cattle. Serological study in dairy herds in Kerman province, Iran has been reported with 77.9, 30.4, 100.0, 100.0 and 100.0 % of BVDV, BHV-1, BRSV, BPI-3V and BAV, respectively (Sakhaee *et al*., 2009). Kale *et al.* (2013) has reported the seropositivity of antibodies against BVDV, BRSV, BPI-3V and BAV-3 at rates of 7.2 per cent, 50.0 per cent, 94.6 per cent and 82.1 per cent, respectively, in 56 dairy cattle from Burdur province.

In India, the seropositivity of BoHV-1 from diseased animals was reported as 54.0 per cent (Nandi *et al*., 2010), which neared the findings of present study. Many other studies in India have recorded seroprevalence of BoHV-1 infections from different states of India (Dhand *et al*., 2002; Singh and Sinha, 2006; Ganguly *et al*., 2008; Verma *et al*., 2014), but there is less published data for BRSV and BPVI-3. Seroprevalence of BRSV infection in cattle from Orissa was 46.1 per cent by indirect ELISA and 65.3 per cent by sandwich ELISA (Hazari *et al.,* 2002). These findings are somewhat similar to the findings in our study. The differences observed in seropositivity for tested viruses in the present study may be either due to different rates of exposure of the animal to the virus, different environmental conditions or management practices. Further studies involving large number of animals are required to provide better information regarding the prevalence of these viral diseases in Punjab. Appropriate management

practices and routine vaccination programs may reduce the incidence of the bovine respiratory diseases.

Conflict of Interests

There is no conflict of interest.

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