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Original Article

Peste des petits ruminants virus antibodies in domestic large ruminants in Bangladesh

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Abstract

Introduction: Peste des petits ruminants (PPR) is an important transboundary animal disease of small ruminants which causes serious damage to the livelihood and food security of millions of small-scale farmers. PPR is endemic in goats in Bangladesh since 1993. The aim of this study was to determine the seroprevalence of PPR in sheep, cattle, and buffaloes in Bangladesh.

Methodology: A total of 434 blood samples from sheep (n = 100), cattle (n = 190) and buffalo (n = 144) were collected aseptically. Sera were separated and antibody titer was determined using a commercially available c-ELISA kit.

Results: The overall seroprevalence was 16% and 3.68% in sheep and cattle, respectively, while buffaloes had a considerably higher seroprevalence of 42.36%. The study suggests that buffaloes are more prone to the PPR virus (PPRV) infection and cattle.

Conclusions: This study provides serological evidence of PPRV infection in cattle and buffaloes. These results may warrant further studies to find out the role of large ruminants in transmitting PPRV infection to small ruminants and vice versa and inclusion of all domestic and wild ruminants for regular surveillance program.

Key words: Seroprevalence; PPRV; c-ELISA; sheep; cattle; buffalo.

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Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease of wild and domestic small ruminants caused by a *Morbillivirus*, the peste des petits ruminants virus (PPRV), under the family Paramyxoviridae [1]. The virus has been reported in many geographic regions and endemic in most of the Saharan and sub-Saharan Africa, Middle East, Indian subcontinent, China, Turkey and recently has been reported in Kazakhstan, Mongolia and Tajikistan [2-3]. Both goats and sheep are susceptible to natural infection, but comparatively goats show increased mortality [4]. The disease is well characterized by high fever consistent with significant lesions affecting respiratory and gastro-intestinal systems leading to pneumonia and severe diarrhea [5-7]. PPRV is primarily spread by aerosol, and close contact between the susceptible and infected animals [8].

PPRV is antigenically related to the rinderpest virus and thus PPRV can also atypically infect cattle and buffalo may create problems in large ruminants [4]. However, no natural outbreaks of PPR in cattle has been recorded so far. Cattle experimentally infected with PPRV showed sub-clinical disease and developed anti-PPRV antibodies [9-10]. In 1995, PPRV was isolated from a rinderpest-like disease outbreak in Indian buffaloes [11]. Furthermore, wild ruminants were affected by PPRV in both natural and experimental settings [12-13]. Pigs and camels experimentally infected with PPRV [14] showed symptoms of subclinical infection [15]. In the past few years, PPR antibodies have been detected in a wide range of domestic and wild animal species from different geographic locations [16-18].

PPR is endemic in Bangladesh and considered as a number one killer disease in goats. So far published work from Bangladesh on PPR have been limited to

goats and mostly on disease investigation, pathology and molecular epidemiology [5-7, 19-22]. Currently, serological surveillance of PPR in sheep and goats is of little value due to current PPR vaccination strategies. On the other hand, Bangladesh has been declared rinderpest free in 2007 and since then there has been no use of rinderpest vaccine in cattle, which could provide cross protection against PPRV. Thus, the inter-species transmission of PPRV from small to large ruminants or vice versa cannot be ignored. In Bangladesh, cattle, buffalo and goats are kept together under mixed farming system, which in turn favors inter-species transmission of pathogens. Therefore, the present study aimed at detecting the seroprevalence of PPR in sheep, cattle and buffaloes from selected areas of Bangladesh.

Methodology

Study area

The survey was conducted in sheep, cattle and buffalo herds in 7 districts (Dhaka, Faridpur, Mymensingh, Netrokona, Pabna, Rajshahi, and Sirajganj) of Bangladesh. The region or locations were selected based on the high density of livestock, the grazing area, and the presence of the PPR endemicity. Animals without prior history of PPR infection or vaccine, as well as no clinical symptoms of PPR or any disease, were selected on a random basis, adult livestock (older than 1 year of age) irrespective of their age or sex.

Sample collection

A total of 434 blood samples were collected (100 from sheep, 190 from cattle, and 144 from buffaloes) over a period of 12 months. The details of sample collection from the selected locations of Bangladesh are stated in Table 1. Blood samples were collected and sera were separated. The collected sera were then stored at -20 °C until use.

Table 1. Total number of samples collected from different areas of Bangladesh.

Animals	Location/District	No. of serum sample
Sheep	Dhaka	60
(n = 100)	Mymensingh	20
	Netrokona	20
Cattle	Dhaka	10
(n = 190)	Mymensingh	50
	Faridpur	40
	Sirajgonj	65
	Rajshahi	15
	Pabna	10
Buffalo	Dhaka	100
(n = 144)	Sirajgonj	16
	Rajshahi	4
	Pabna	24
3 species	7 districts	434

Competitive Enzyme linked Immunosorbant assay (c-ELISA)

Sera were analyzed for the anti-PPR antibodies using two different commercial competitive ELISA Kits developed by IDVet Innovative Diagnostics, CIRAD-EMVT, Montpellier, France (ELISA kit 1) and another one developed by Genevieve Libeau, CIRAD, France (ELISA kit 2) [23]. Both ELISA procedures were identical except for a difference in calculation method. The ELISA was performed following manual instructions provided by the kits.

The OD (Optical density) values were read at 450nm and 492nm using the ELISA Reader ELx800 (BioTek Instruments, USA) in case of ELISA kit 1 and 2, respectively. The inhibition of mAb binding in the presence of serum is expressed as competition percentage (CP) and percentage inhibition (PI) and it was calculated from the mean OD value by using the instructions of the kits.

Statistical analysis

Both mean CP and mean PI values along with standard deviation were determined from the obtained CP and PI values of the serum samples. Paired *t*- test was carried out to determine the significance of variation of CP and PI values among the different animals. The samples with CP values $\leq$ 35% were considered as positives in case of ELISA kit 1 and the samples with PI $\geq$ 50 % were considered as positives in case of ELISA kit 2 for PPRV infection.

Results

A total of 434 sera from sheep, cattle, and buffaloes from different areas of Bangladesh were tested by c-ELISA using two different ELISA kits. Table 2 shows the number of positive and negative samples from each district, as well as the mean PPRV antibody percentage values in sheep, cattle, and buffaloes.

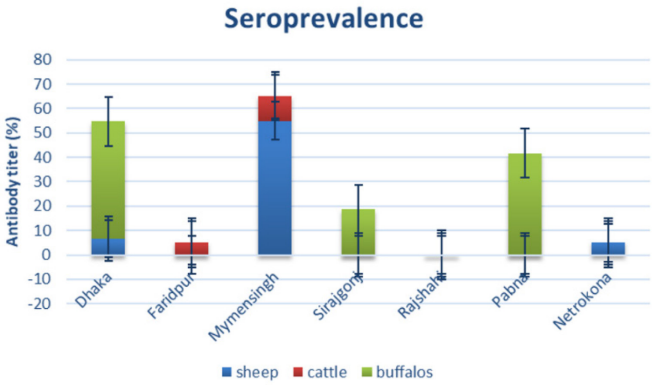
A total of 16 out of 100 sera samples from sheep showed PPRV-specific antibody collected from Dhaka, Mymensingh, and Netrokona districts with seroprevalence of 6.67% (4/60), 55% (11/20), and 5% (1/20), respectively. In comparison, 334 serum samples from large ruminants revealed a PPR seroprevalence of 20.35% (68/334) with a significant disparity between cattle and buffaloes. The mean competition percentage (CP) values obtained in c-ELISA from tested cattle and buffaloes are depicted in Table 2. In cattle, the mean positive and negative antibody titers were 16.77% and

80.49%, respectively, while in buffaloes, the mean positive and negative antibody titers were 17.07% and 83.20%, respectively. PPRV antibodies were detected in 5% (2/40) and 10% (5/50) of cattle in Faridpur and Mymensingh districts, respectively, while no positive sample was found in Dhaka, Sirajganj, Rajshahi, and Pabna districts (Figure 1). Furthermore, a high percentage (48%) of seropositive buffaloes (48/100) were found in Dhaka district, followed by 18.75% (3/16) and 41.66% (10/24) in Sirajgang and Pabna districts, respectively (Figure 1). Therefore, the overall prevalence of PPR was reported 16% (16/100) in sheep, 3.68% (7/190) in cattle and 42.36% (61/144) in buffaloes (Figure 2).

Discussion

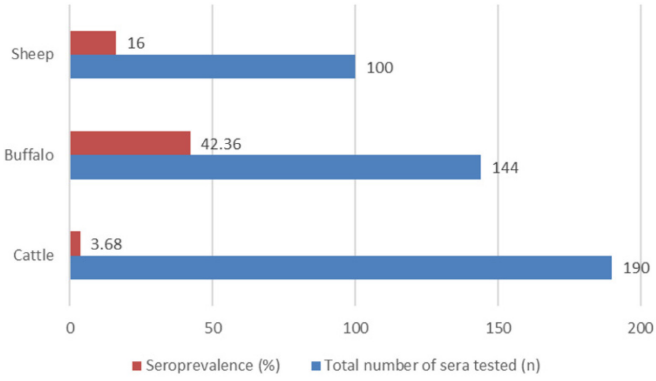
The present study provided data on the serological status of PPR in sheep, cattle and buffaloes from selected areas of Bangladesh. Although a large number of buffaloes were found seropositive, clinically PPR disease was not noticed in buffaloes in Bangladesh. This may indicate that buffaloes were exposed to PPRV and the target animal have not shown any clinical signs. Similar assumption can be made for cattle as well. However, in this study, only healthy animals were considered for sampling, therefore, the abnormal reproductive and production performance of the exposed animals were not reported. PPR virus transmission requires intimate contact between infected and vulnerable hosts [24], hence higher seroprevalence in large ruminants could be related to the coexistence with infected goats or sheep. To meet the requirement of protein, a huge number of livestock industries have been developed around the capital city of Dhaka where a large number of livestock animals are transported daily. This may favor the spread of virus and spill into

Figure 1. Seroprevalence of PPR in sheep, cattle and buffalo in selected areas of Bangladesh.



The bar graph shows the antibody percentage level of PPR in three species in each individual district. The error bar indicates calculated standard error.

Figure 2. Overall seroprevalence to PPR in three studied animals in relations to the total number of sera collected.



According to the bar graph, buffalo had the highest seropositivity against PPRV, followed by sheep and cattle.

Table 2. The number of tested sera and their antibody titers in serum samples from sheep, cattle, and buffalo in selected areas of Bangladesh.

Animals	Location	No. of sample	No. of Positive sample	No. of Negative sample	Mean positive PI/CP values ± SD*	Mean negative PI/CP values ± SD*
Sheep	Dhaka	60	4	56		
(n = 100)	Mymensingh	20	11	9	54.61 ± 4.85	38.01 ± 4.42
	Netrokona	20	1	19		
Cattle	Dhaka	10	0	9		
(n = 190)	Faridpur	40	2	38		
	Mymensingh	50	5	43	16.77 ± 3.43	80.49 ± 12.31
	Sirajgonj	65	0	65		
	Rajshahi	15	0	15		
	Pabna	10	0	10		
Buffalo	Dhaka	100	48	47		
(n = 144)	Sirajgonj	16	3	13	17.07 ± 4.4	83.20 ± 8.86
	Rajshahi	04	0	4		
	Pabna	24	10	14		

* In sheep percent inhibition (PI) and in cattle and buffalo competition percentage (CP) was considered for result interpretation; SD: standard deviation.

the environment. Sirajganj and Pabna districts are considered as livestock paradise for Bangladesh where community based mixed livestock farming industry has been established. However, farm biosecurity is very poor and livestock is densely populated. This may allow close contact between infected and non-infected susceptible species. However, cattle were exposed similarly in the same environment but lower seroprevalence was recorded in cattle than buffaloes. This could mean that the virus adapted faster in buffaloes than in cattle. PPRV adaptation in large ruminants may result in a shift in virulence of the strain in that location, necessitating ongoing surveillance to better understand the molecular epidemiology and clinical state of animals. Higher prevalence in buffaloes may indicate possibility of continuous circulation of PPR in this species. However, spillover from sheep and goat also could not be ruled out as all the animals are grassed and housed together in Bangladesh. Therefore, buffaloes should be included in the national control program. Prevalence of PPRV antibodies in cattle and buffaloes has been reported from different countries [18, 25-26]. This may indicate that PPRV has gradually adapted in large ruminants.

Seroprevalence rate in sheep, cattle and buffalo varied among different locations of Bangladesh. A variety of regional factors have been identified related to high seroprevalence PPR in field samples from different typical (sheep, goat) and atypical (cattle, camel, Buffalo) hosts [16-18, 24-26]. Buffalos are unique among PPR atypical hosts that are geographically dispersed across PPR endemic areas in the world and their numbers are rapidly increasing [24].

Conclusions

In conclusion, the existence of PPRV-specific antibodies in cattle and buffaloes suspected of having a subclinical, inapparent, or non-lethal infection means that cattle and buffaloes are naturally exposed to PPRV, either directly or indirectly. However, the detection of PPRV antibodies in an animal species does not essentially mean a clinical relevance to the infection [16]. It is important to investigate whether the existence of the virus in cattle and buffalo has any epidemiological significance. Since the effects of these positive antibodies in ruminants are unclear at this moment, further research might be needed to fully comprehend their importance. Active surveillance including all domestic and captive wild ruminants is needed to better understand the spread of the disease across a larger geographic area in Bangladesh. Furthermore, research into the molecular characteristics of PPR virus in other hosts is required to know whether the existence of the virus in cattle and buffalo has any epidemiological significance for virus perpetuation and infection to other animal species.

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