

Original Article

## Seroprevalence of Peste des Petits Ruminant Virus specific antibody in goats in different regions of Bangladesh

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### ABSTRACT

**Objective:** The study was undertaken with an objective to determine the seroprevalence of Peste des Petits Ruminants (PPR) in goats of different age groups.

**Materials and methods:** A total of 606 goats (414 vaccinated and 192 unvaccinated) were randomly selected from Rajshahi, Sirajganj and Gazipur districts. The goats were categorized into different age groups; (i) 0-6 months, (ii) 12-24 months, and (iii) >24 months. Blood samples were collected from the goats and sera were prepared. The sera were examined for the presence of antibodies against PPR virus (PPRV) by competitive enzyme linked immunosorbent assay (c-ELISA).

**Results:** In the unvaccinated goats, overall seroprevalence was 8.70% (n=36/414). The highest seroprevalence was recorded in Rajshahi (28.57%; n=18/63) which was followed by Gazipur (16%; n=12/75) and Sirajganj (2.17%; n=6/276). The age-based overall seroprevalence in the unvaccinated samples from 0-6 months age group was 9.43% (n=15/159). Similarly, 12-24 and >24 months age groups of goats revealed the presence of 6% (n=9/150) and 11.43% (n=12/105) seroprevalence against PPRV. Of the vaccinated samples, overall 76.04% (n=146/192) were seropositive against PPRV. Within the age group of 0-6 months, vaccinated samples had the highest seroprevalence (80.25%; n=65/81) as compared to 12-24 (70.83%; n=34/48) and >24 months (74.60%; n=47/63) age groups of goats, respectively.

**Conclusion:** The seroprevalence in the unvaccinated samples indicates that PPRV is circulating in Bangladesh which is inducing to produce natural antibody in goats. This study also states that the field level vaccination against PPRV could give protection to the goats.

### KEYWORDS

c-ELISA, Goats, PPR, Seroprevalence

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## INTRODUCTION

Peste des Petits Ruminants (PPR) is a viral disease of small ruminants. The disease is manifested by fever, ocular and nasal discharges, anorexia, necrotic stomatitis, foul smelled diarrhea, enteritis, and bronchopneumonia followed by either death or recovery ([Lefèvre et al., 1991](#); [Balamurugan et al., 2014](#)). The causative agent of this economically important disease is PPR virus (PPRV) belonging to genus Morbillivirus ([Özkul et al., 2002](#)) under the family Paramyxoviridae ([Murphy et al., 1999](#)). PPRV naturally infects goats and sheep, but goats show more susceptibility and consequentially, disease severity in goat is higher than in sheep. Susceptibility age of goats in endemic areas is >4 months up to 24 months ([Samad, 2008](#)). Mortality due to PPR is mostly associated with severe immunodeficiency that makes the goats more susceptible to secondary infection ([Haeney et al., 2002](#)).

After the first report of PPR from Ivory Coast, the disease has been reported in many countries like Middle East, the Arabian Peninsula, and most parts of Africa ([Abu-Elzein et al., 1990](#); [Shaila et al., 1996](#); [Balamurugan et al., 2014](#)). Frequent outbreaks of PPR have also been recorded in south asian countries like Pakistan, Bhutan, Nepal, Afghanistan, India and Bangladesh ([Banik et al., 2008](#)). The PPRV was first identified by [Sil et al. \(1995\)](#) in Bangladesh during a severe outbreak in 1993. The PPR outbreaks caused 74.13% morbidity and 54.83% mortality in Black Bengal goats in this country ([Islam et al., 2001](#); [Das et al., 2007](#)).

Previous study demonstrated that small ruminants including goats can develop positive level of antibody titer against PPRV under natural situation. The seroprevalence of PPRV specific antibodies has been recorded from Bangladesh to be 49.17% in goats, 19.05% in cattle, and 36.0% in sheep ([Razzaque et al., 2004](#)). To develop disease control strategy against PPR, it is always necessary to have updated baseline seroprevalence data of the PPRV specific antibodies in the small ruminants. Therefore, the present study was undertaken with an objective to determine the seroprevalence of PPRV-specific antibodies in unvaccinated goats in some selected areas of Rajshahi, Sirajganj and Gazipur districts in Bangladesh. Furthermore, it is necessary to have serosurveillance data of PPRV-specific antibodies in the vaccinated goats to investigate whether the existing field level vaccination program undertaken by the government is being effective. Hence, the present study also had an objective to monitor of PPR specific antibody in goats after field level vaccination.

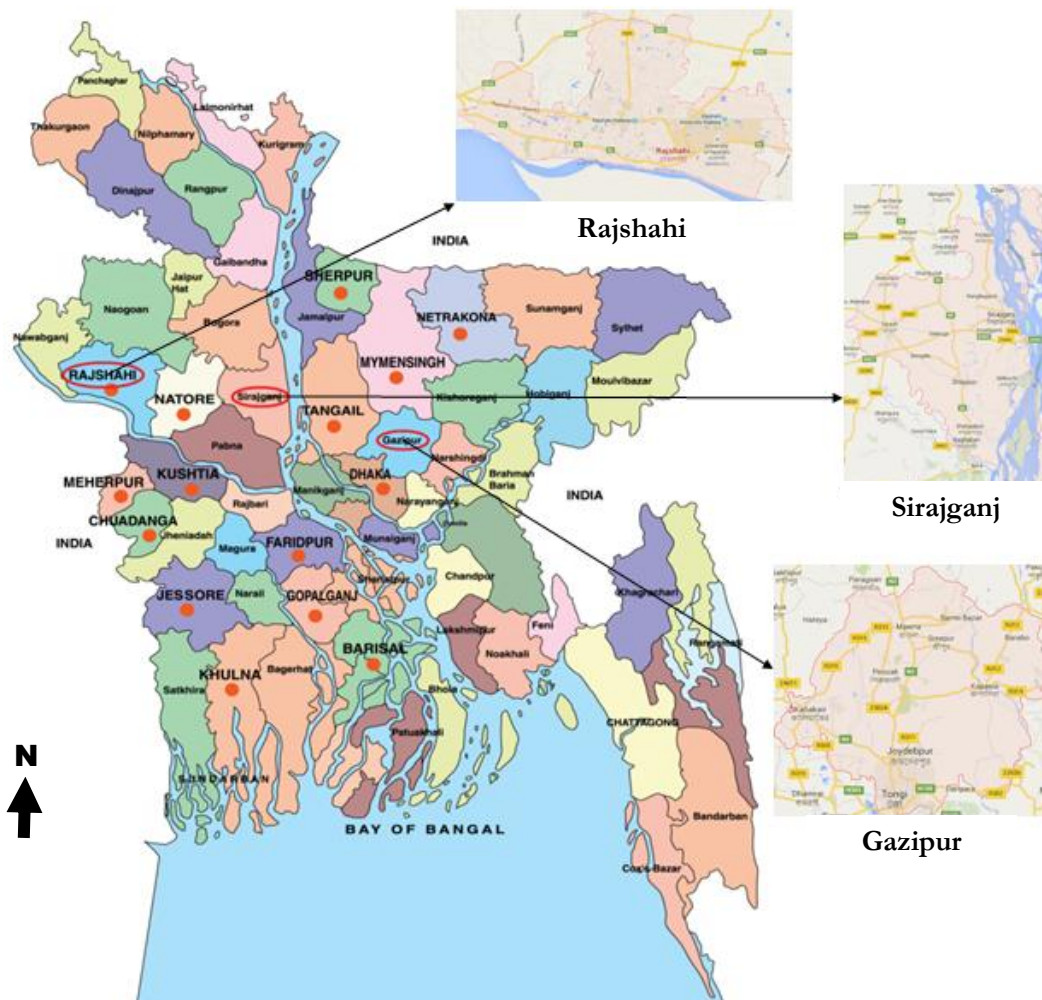
## MATERIALS AND METHODS

The study was conducted during the period of January 2015 to June 2015 at the SAARC Regional Leading Diagnostic Laboratory for PPR (SAARC RLDL-PPR), Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka.

**Experimental animals and study locations:** In this study, serosurveillance was conducted for the antibodies of PPRV in randomly selected goats with no clinical signs of PPRV at different age groups i.e. (0-6) months, (12-24) months and >24 months age from three different districts of Bangladesh, namely Rajshahi, Sirajganj and Gazipur (**Figure 1**). For this purpose, a grand total of 606 goats were selected for sampling, wherein 63, 276 and 75 unvaccinated samples were from Rajshahi, Sirajganj and Gazipur, respectively, and 192 vaccinated samples were from Rajshahi.

**Blood collection and sera preparation:** Blood (5 mL) was aseptically collected from each goat by puncturing jugular vein using disposable plastic syringes in a gentle manner after swabbing with 70% alcohol or iodine. The loaded syringe was kept in inverted condition for 30 min for blood clotting. After clotting of blood, it was retracted and then transferred in incubator for release of serum. Clear serum was transferred to eppendorf tube, labelled and stored at -20°C.

**Competitive Enzyme Linked Immuno-sorbent Assay (C-ELISA):** PPR c-ELISA kit (ID Screen® PPR Competition Kit) was obtained from IDvet, France. Each serum sample was subjected to test for PPRV antibodies as per the protocol described by the manufacturer. Briefly, 25 µL of dilution buffer were added to each well of the ELISA micro titration plate. Then, 25 µL of the positive control (PC) was added to the wells A1 and B1 and 25 µL of the negative control (NC) was added to the wells C1 and D1. 25 µL of the each sample was added to the remaining wells. Then, the plate was incubated at 37°C for 45 min and washed 3 times with approximately 300 µL of the washing solution. After washing, conjugate 1X was added to each well. Again the plate was incubated at 21°C for 30 min followed by washing 3 times with approximately 300 µL of the washing solution. Then, 100 µL of the substrate solution was added to each well. The plate was then incubated at 21°C in the dark place for 15 min. 100 µL of the stop solution was added to each well in order to stop the reaction. Finally, the micro titration plate was read for OD values with multichannel spectrophotometric ELISA plate reader with interference filters of 450 nm and the reading data was placed into



**Figure 1:** Study areas (indicated by red circles)

data sheet of Microsoft® Excel program and saved in the computer hard disc with specific identification name. The test was considered validated if  $OD_{NC} > 0.700$  and  $OD_{PC} / OD_{NC} < 0.3$ .

For each sample, the competition percentage was calculated using the following formula-

$$S/N\% = \frac{OD_{sample}}{OD_{NC}} \times 100$$

The value of S/N% less than or equal to 50% was considered positive, while greater than 50% and less than or equal to 60% was considered doubtful, and greater than 60% was considered negative.

**Statistical analysis:** All the statistical data were analyzed by Graphpad Prism 5.0 (Graphpad software).

## RESULTS AND DISCUSSION

Peste des Petits Ruminants (PPR) is now considered as endemic and a great threat towards the development of

goat farming in Bangladesh (Islam et al., 2001; Dhar et al., 2002; Das et al., 2007). Several studies had been reported on different aspects of PPRV in goats in Bangladesh; for example, pathological investigation (Khan et al., 2005), evaluation of antibiotic combined hyperimmune serum therapy (Islam et al., 2003), and seroprevalence (Razzaque et al., 2004). The present study investigated the seroprevalence of PPRV antibodies under natural condition in unvaccinated goats which may be helpful in developing disease control strategy for encountering PPR. It is crucial that, effective implementation of control strategies for PPR requires regular vaccination with effective vaccine and sero-monitoring of immunity against PPRV. Furthermore, a question is often raised by farmers and field veterinarians about the potency of the vaccines after field level vaccination. Thus, the present study also adopted a sero-monitoring of PPR specific antibody in the vaccinated goats after field level vaccination in a selected area of Bangladesh. For seroprevalence, a total of a 606 serum from vaccinated and unvaccinated goats were collected and tested by c-ELISA.

**Table 1:** Serological status of PPRV antibodies of **(A)** unvaccinated and **(B)** vaccinated goats.**(A)**

Location	Age (months)	No. of samples	Positive samples	Seropositivity prevalence (%)	Seropositive competition (S/N)% means±S.E.M.
Gazipur	0-6	15	0	0	NA
	12-24	39	9	23.08	8.77±3.16
	>24	21	3	14.29	36.65±2.57
	<b>Total</b>	<b>75</b>	<b>12</b>	<b>16.0</b>	<b>15.74±7.32</b>
Rajshahi	0-6	27	12	44.44	11.63±0.80
	12-24	18	0	0	NA
	>24	18	6	33.33	8.44±0.87
	<b>Total</b>	<b>63</b>	<b>18</b>	<b>28.57</b>	<b>10.56±0.87</b>
Sirajganj	0-6	117	3	2.56	10.42±4.62
	12-24	93	0	0	NA
	>24	66	3	4.54	46.12±4.36
	<b>Total</b>	<b>276</b>	<b>6</b>	<b>2.17</b>	<b>28.27±17.85</b>
<b>Grand total (Unvaccinated)</b>		<b>414</b>	<b>36</b>	<b>8.70</b>	<b>15.24±3.67</b>

NA=Not applicable

**(B)**

Location	Age (months)	No. of samples	Positive samples	Seropositivity prevalence (%)	Seropositive competition (S/N)% means±S.E.M.
Rajshahi	0-6	81	65	80.25	21.66±3.13
	12-24	48	34	70.83	13.77±0.77
	>24	63	47	74.60	15.06±2.34
<b>Total (Vaccinated)</b>		<b>192</b>	<b>146</b>	<b>76.04</b>	<b>16.07±1.35</b>

**Seroprevalence of PPRV antibody in the unvaccinated goats:** Among a total of 414 unvaccinated sera samples, overall 36 (8.70%) were seropositive (**Table 1**). Previously, in India, overall seroprevalence of PPRV antibody in goats was observed as 35% ([Balamurugan et al., 2014](#)), whereas in Tanzania and Sudan it was 49.5% ([Swai et al. 2009](#)) and 59.15% ([Osman et al. 2009](#)), respectively. Thus, the difference in the seropositivity of PPRV antibody among different countries might be attributed to the difference in the agroclimatic condition of different countries.

In our study, in the seropositive samples, the mean competition was 15.24±3.67, (**Table 1**). In the midst of the unvaccinated samples, 75 were from Gazipur with seropositivity 16.0%, while 63 and 276 samples were from Rajshahi and Sirajganj with seropositivity 28.57% and 2.17%, respectively (**Table 1**). So, the samples from Rajshahi and Sirajganj showed the highest and lowest seropositivity, respectively. The results of seroprevalence of this study extended the previous findings of [Razzaque et al. \(2004\)](#) who reported sero-prevalence of PPRV antibodies in 49.17% goats in Mymensingh district, and also with [Banik et al. \(2008\)](#) who reported seroprevalence of PPRV antibodies in 25% goats. In a study in Sudan, [Salih et al. \(2014\)](#) detected 45.6% seroprevalence of PPRV in sheep and goats using c-ELISA, which is much higher than our findings. This variation might be due to

difference in geography. However, the findings of this study indicated that a certain percentage of goats in different areas of Bangladesh persistently possess antibody against PPRV which might counteract subsequent natural PPR challenge ([Bidjeh et al., 1999](#); [Islam et al., 2003](#)). Additionally, these data are clear indication that the percentage of the seropositive antibody titer is not same at different areas of Bangladesh, and thus indicates the presumable differential exposure of the goats towards PPRV.

In each region, unvaccinated samples were further divided into three groups on the basis of their age. Within the age group of 0-6 months, unvaccinated seropositive samples had an overall prevalence of 9.43% and mean competition of 11.39±0.66 (**Figure 2A, B**).

Region-based prevalence and mean competition for the group of 0-6 month's unvaccinated seropositive samples revealed that, samples from Rajshahi had the highest prevalence rate (44.44%) with a mean competition of 11.63±0.80, while samples from Gazipur did not show any seropositivity (**Table 1**). While considering the age group of 12-24 months, unvaccinated seropositive samples showed an overall prevalence of 15.79% and mean competition of 8.77±3.16 (**Figure 2A, B**). Within this age group, only samples from Gazipur showed the seropositivity with a prevalence of 23.08% and mean

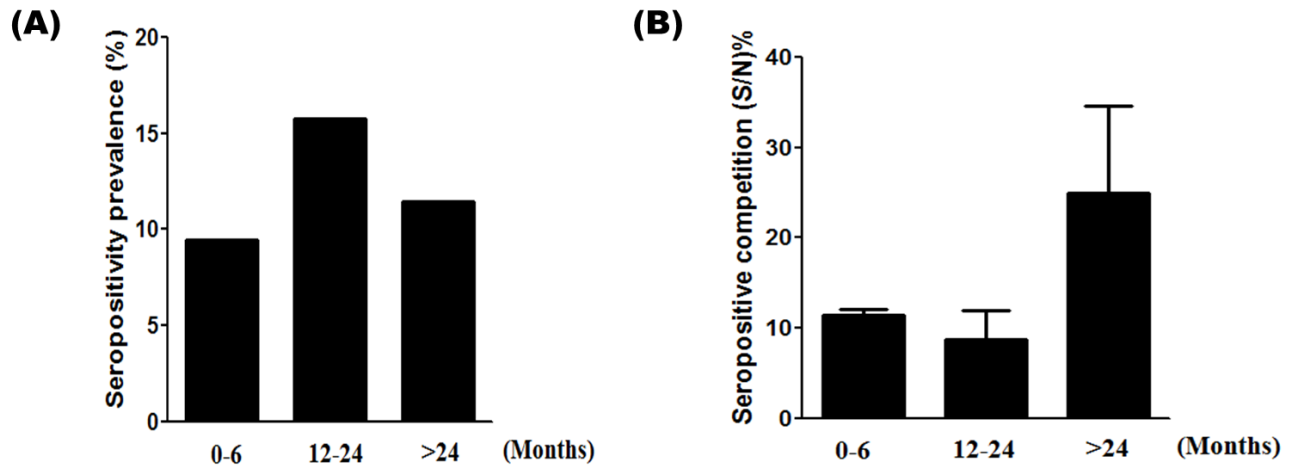


Figure 2: Age-based (A) seropositivity prevalence and (B) seropositive competition (S/N)% in the unvaccinated goats of three districts.

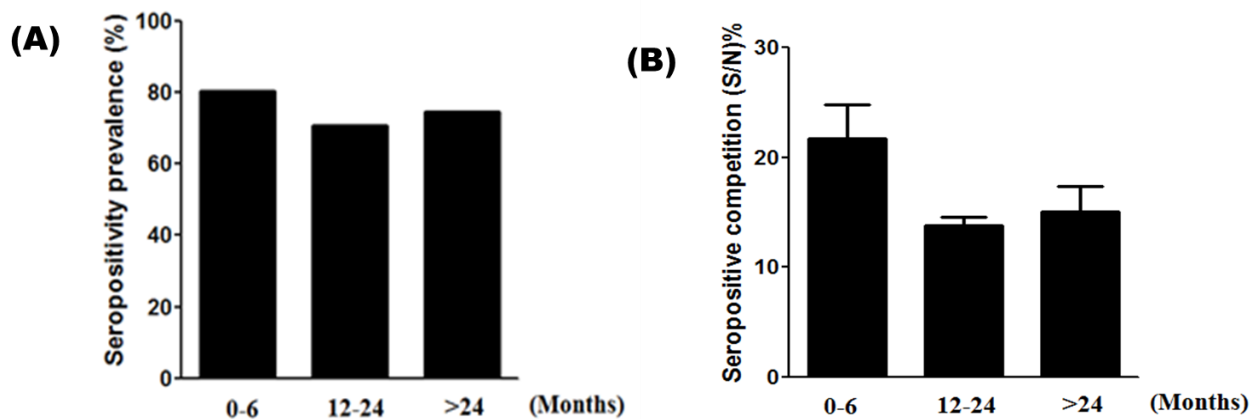


Figure 3: Age-based (A) seropositivity prevalence and (B) seropositive competition (S/N)% in the vaccinated goats of Rajshahi district.

competition of  $8.77 \pm 3.16$  (Table 1). Unvaccinated seropositive samples within the age group of >24 months had an overall prevalence of 11.43% and mean competition of  $24.91 \pm 9.71$  (Figure 2A, B). In this age group region-based prevalence and mean competition of unvaccinated seropositive samples revealed that, samples from Rajshahi had the highest prevalence rate (33.33%) with a mean competition of  $8.44 \pm 0.87$ , whereas samples from Sirajganj showed the least prevalence rate (4.54%) with a mean competition of  $28.27 \pm 17.85$  (Table 1). Thus, the age-based overall seroprevalence showed an upward tendency from 0-6 months age group (9.43%) to 12-24 months age group (15.79%) and at the age group >24 months, it showed a downward trend (Figure 2A). However, in this study, region-based seroprevalence in different age group did not show any unique trend.

**Seroprevalence of PPRV antibody in the vaccinated goats:** A total of 192 vaccinated goat serum samples were collected from Rajshahi and tested by C-ELISA. As shown in Table 1, of the vaccinated samples, 146

(76.04%) were seropositive. In the vaccinated seropositive samples, the mean competition (S/N) % was  $16.07 \pm 1.35$  (Table 1). Vaccinated samples were further divided into three groups on the basis of their age. Within the age group of 0-6 months, vaccinated seropositive samples had an overall prevalence of 80.25 with mean competition of  $21.66 \pm 3.13$  (Figure 3A, B). While considering the age group of 12-24 vaccinated seropositive samples had an overall prevalence of 70.83% with mean competition of  $13.77 \pm 0.77$  (Figure 3A, B). The seroprevalence was 74.60% at the age group of >24 months with mean competition of  $15.06 \pm 2.34$  (Figure 3A, B). Thus, it is evident that the highest seroprevalence was observed in the goats of age of 0-6 months. Previously, Islam et al. (2007) detected the maternally derived antibodies in the kids at 10 to 30 days of age collected from vaccinated dams. This antibody titre gradually declined with the increase of age and could give protection upto 4 months of age. Awa et al. (2002) similarly observed that protective level of maternal derived antibody titre persist upto 3 months of age. So, in

our study, the highest seroprevalence at the age group 0-6 months might be due to the maternally derived antibody. Thus, altogether these data suggest that the field level vaccination is being effective and is able to give protection of the goat population for more than 24 months.

## CONCLUSION

From this study it can be stated that, the PPRV is circulating in Bangladesh and is raising natural positive level of antibody titre in the goat population in Bangladesh by natural transmission. This study also states that the field level vaccination could give protection to the goats as the antibody against PPRV was in protective level. It is noteworthy that, interference of the efficacy of vaccines due to the antibodies developed as a result of natural exposure to PPR infection might be a considerable challenge. So, this fact should be kept under consideration during the field level vaccination. Additionally, in further study, it is also required to investigate whether the positive level of antibody titer possesses viral neutralization capability.

## CONFLICT OF INTEREST

The authors do not declare any competing interest.

## ACKNOWLEDGEMENT

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