

# Seroprevalence of Peste des Petits Ruminants in Small and Large Ruminants in Selected Bordered Areas of Bangladesh

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## ABSTRACT

**Background and Objective:** Peste des petits ruminants (PPR) is endemic in Bangladesh and bordered areas of the country play a key role in virus dissemination as it is a transboundary disease. Therefore, the present study was planned and conducted for the seromonitoring of PPR in goats and cattle in bordered areas such as the Sylhet and Jhenaidah Districts of Bangladesh. **Materials and Methods:** A total of 200 serum samples from 80 goats and 120 cattle randomly were collected from Sylhet (cattle serum from 03 Upazillas and goat serum from 02 Upazillas) and Jhenaidah District (cattle serum from 03 Upazillas and goat serum from 02 Upazillas) of Bangladesh. Blood samples were collected and then serum was separated and used for PPR viral antibody detection by cELISA. **Results:** The overall seroprevalence was found 25% in Cattle and 32.5% in goats in study areas. The district-wise seroprevalence was found in the case of cattle 30 and 20% and in the case of goats 35 and 30% in Sylhet and Jhenaidah, respectively. Among Upazilla's highest percentages were recorded at Jaintapur Upazilla (40%) of Sylhet and Harinakundha Upazilla (40%) of Jhenaidah District in the case of Cattle and Jaintapur Upazilla (40%) of Sylhet in case of goats. **Conclusion:** It is concluded that the seropositivity against the PPR viral antibody of the study animals is much higher, indicating the natural circulation of the PPR virus in the bordered areas. Seropositive cattle also indicate that the PPR virus might have extended its host range.

## KEYWORDS

Antibody, PPR virus, ruminant animals, bordered areas, seromonitoring, disease eradication

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## INTRODUCTION

The PPR is an infectious, viral (genus Morbillivirus) and transboundary disease of sheep and goats with high morbidity and mortality. The virus has an antigenic relationship to Rinderpest, Measles and Canine distemper virus<sup>1,2</sup>.



USD 2.1 billion in financial harm annually is caused by PPR globally. About 300 million families are facing different difficulties in regard to their sustenance, safer food and occupation opportunities due to PPR<sup>3</sup>. In 1942, PPR was first reported in Côte d'Ivoire and now spread to more than 70 countries excluding many more without OIE-PPR status. The OIE-PPR status Asian countries are India, Nepal, Bangladesh, Pakistan, Afghanistan and Iran. The PPR has been endemic in Bangladesh since 1993 and bordered areas are considered a red alert zone as animal trespass (both legal and illegal) can play an important role in the dissemination of transboundary diseases like PPR. Bangladesh shares around 4,096 km<sup>2</sup> of the area as an international border with the neighbor country India and around 300 km<sup>2</sup> with Myanmar. Both Sylhet and Jhenaidah districts of the country accommodate a large part of the bordered areas and illegal animal trespass and disobeying of animal quarantine act are the common scenario. That's why a study on PPR serosurveillance was essential in these districts of Bangladesh.

Usually, the severity of natural infection is more in goats than in sheep. Still, now, no natural outbreak has been reported in cattle in Bangladesh, though cattle may be infected sub-clinically. Clinical signs may be similar to rinderpest in infected cattle. The disease has been reproduced in calves experimentally followed by the death of calves. Buffaloes may also be infected. Govindarajan *et al.*<sup>4</sup> isolated PPR Virus from a PPR-like outbreak that occurred in Indian buffaloes in 1995. Different tools and techniques may use for the diagnosis of PPR. Monoclonal antibody-based ELISAs, Anti-haemagglutinin (H) monoclonal antibodies based ELISA like blocking ELISA or competitive ELISA<sup>5</sup>. Virus neutralization tests (VNT) etc., are effective techniques. Epidemiological data on PPR in goats, sheep, cattle and buffaloes in Bangladesh is scanty. The Rinderpest virus has been eradicated and no vaccination is going on. From Bangladesh's perspective, it is essential to conduct serosurveillance on PPR viral antibodies, to find out whether the PPR virus is extending its host range or not. After considering the aforementioned points, this study was carried out with the aim: "Study on seroprevalence of peste des petits ruminants (PPR) in goats and cattle in selected bordered areas of Bangladesh".

## MATERIALS AND METHODS

**Study duration:** The study was carried out from July, 2021 to June, 2022.

**Selection of study area:** Bangladesh is a South Asian, unelevated, overcrowded and marshy country having a coastline of 580 km of the Bay of Bengal. The country shares a border on three sides (east, west and north) with India, on the south by the Bay of Bengal and on the southeast with Myanmar. Bangladesh has a tropical monsoon because of crossing the tropic cancer line at the midline of the country, therefore, the climate can be described as heavy seasonal rainfall, high temperatures and high humidity. Both Sylhet and Jhenaidah are two districts that lie in the bordered area of Bangladesh and bear importance due to huge animal trespass (legal and illegal) and livestock-populated areas. Therefore, these two-bordered districts were selected for this study based on zoogeography and PPR outbreaks.

**Selection of Farmers:** A survey was conducted using a pre-structured questionnaire to collect data concerning farmers' mass awareness about PPR vaccination and PPR disease to facilitate easy identification of unvaccinated and natural infection-free animals.

Farmers who belong to remote areas, far away from the Local Veterinary Hospital and unaware of PPR vaccination and disease were selected for this study under the direct supervision of respective Upazilla livestock officers, based on information regarding natural PPR outbreaks and PPR vaccination program conducted by Upazilla livestock office.

**Selection of experimental animals for blood collection and serum separation:** Experimental animals were handled and used following ARRIVE guidelines<sup>6</sup>. Unvaccinated animals (both cattle and goats) that have no history of getting natural PPR/ PPR-like infection throughout life were selected and used for the collection of blood and serum separation. A total of 200 serum samples from 80 goats and 120 cattle

randomly) were collected from Sylhet (cattle serum from 03 Upazillas such as Jaintapur, Sylhet Sadar and South Surma and Goat serum from 02 Upazillas as Jaintapur and Sylhet Sadar) and Jhenaidah District (cattle serum from 03 Upazillas such as Jhenaidah Sadar, Kaliganj and Harinakunda and goat serum from 02 Upazillas as Jhenaidah Sadar and Harinakunda) of Bangladesh. The blood samples were collected by puncturing the jugular vein. Then serum was separated and preserved in a -2°C deep freezer.

**cELISA test for PPR viral antibody determination:** As cELISA kit (ID screen PPR competition-ID. Vet. Innovative Diagnostics) was used for the determination of PPR viral antibody. While 96-Microwell flat bottom ELISA plate (Thermo Scientific™ Nunc™) was used and the rows of microwell plates were marked with the letter A to H (A1 ... A<sub>12</sub>, similarly, H<sub>1</sub>...H<sub>12</sub>). All the wells of the microwell plate were filled up with 40 µL of dilution buffer-13 at first and then 10µL samples, except A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub> and D<sub>1</sub>. Positive control and negative control were poured into A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub> and D<sub>1</sub> wells, respectively. The microwell plate was then placed into an incubator (ESCO life science). After incubation for 45 minutes at 37°C the plate was removed and washed thrice manually using a wash solution. The wash solution was prepared before starting the procedure as per kit instruction, diluting the wash concentrate (20 X) with double distilled water. The single-strength conjugate was also prepared earlier following kit instructions. About 100 µL conjugate was added to each well and incubated for 30 min. The plate was washed again three times using around 300 µL of wash solution. Then 100 µL substrate solutions were added to each well. The plate was incubated for 15 min in a dark room. Stop solution (100 µL) was added to cease the reaction. Optical density (OD) values were measured at 450 nm using an ELISA plate reader (96 microplate absorbency reader, Benchmark Scientific, United States America).

The neutralization percentage (S/N) was calculated using the following formula on the basis of OD values. Serum Neutralization Percentage (S/N) is  $OD\ sample / ODNC \times 100$  where, NC is negative control and PC is positive control.

Test serum samples showing an S/N value less than or equal to 50% were considered positive, greater than 50% and less than 60% the sample is doubtful and greater than 60% were considered negative. The test was validated if the mean value of the negative control OD was greater than 0.7 (1.0050) and OD Pos/OD Neg is less than 0.3 (0.026866).

**Statistical analysis:** Descriptive statistics were used for this study.

Table 1: Seroprevalence of PPR viral antibody in Cattle of Sylhet and Jhenaidah District

Name of the District and Upazilla		Total	Number of	Number of	Sero-positive serum (%)	Mean/Avg	Overall (%)
District	Upazilla	Number of serum	seropositive serum	sero-negative serum			
Sylhet	Jaintiapur	-	08	12	40	-	-
	South surma	-	06	14	30	30	-
	Sylhet sadar	20	04	16	20	-	25
Jhenidah	Jhenaidah sadar	-	02	18	10	20	-
	Kaliganj	-	02	18	10	-	-
	Harinakunda	-	08	12	40	-	-

Table 2: Seroprevalence of PPR viral antibody in Goats of Sylhet and Jhenaidah District

Name of the District and Upazilla		Total	Number of	Number of	Sero-positive serum (%)	Mean/Avg	Overall (%)
District	Upazilla	Number of serum	seropositive serum	sero-negative serum			
Sylhet	Jaintiapur	-	12	08	40	35	-
	Sylhet Sadar	20	06	14	30	-	32.5
Jhenidah	Jhenaidah sadar	-	06	14	30	30	-
	Harinakunda	-	06	14	30	-	-

## RESULTS AND DISCUSSION

The overall seroprevalence was found in 25% of cattle and 32.5% of goats in this study. The district-wise seroprevalence was found in the case of cattle 30 and 20% in Sylhet and Jhenaidah Districts and in the case of goats 35 and 30% in Sylhet and Jhenaidah, respectively (Tables 1 and 2). In the case of Cattle, the Upazilla-wise seroprevalence of Sylhet District was 40, 30 and 20% in Jaintiapur, South Surma and Sylhet Sadar Upazilla, whereas 10, 10 and 40% in Jhenaidah Sadar, Kaliganj and Harinakundaa Upazillas of Jhenaidah District, respectively (Table 1). In the case of goats, the Upazilla wise seroprevalence was 40 and 30% in Jaintiapur and Sylhet Sadar Upazilla of Sylhet District, whereas, 30% in both Jhenaidah Sadar and Harinakunda Upazillas of Jhenaidah District, respectively (Table 2).

In spite of no natural PPR outbreak or PPR vaccination history in study populations and areas, an overall seroprevalence was found at 25% in cattle and 32.5% in goats in this study which is almost in accordance with the findings of the other study. Chowdhury *et al.*<sup>7</sup> reported a total of 21% of goats are seropositive to PPR in Bangladesh, which varies from district to district. The highest prevalence (49.4%) was found in Jessore and the lowest (6.3%) in Chittagong. Seroprevalence was found at 26.7, 20, 12.5 and 10.5% in Rajshahi, Sylhet, Mymensingh and Dhaka, respectively. Abraham and Berhan<sup>8</sup> found seroprevalence of 49.2% in goats, 36.0% in sheep and 19.1% in cattle. The seroprevalence of PPR in goats is 37.5% in St. Martin's Island of Bangladesh in one of our previous studies<sup>9</sup>. The overall prevalence of PPR in goats was found 20.57% in Rajshahi in a study conducted by Sarker and Islam<sup>10</sup> which showed a minor difference (12% less) than the findings of this study. This might be due to the geographical location of both districts in Bangladesh (Sylhet is a northeastern district, whereas Rajshahi is a northwestern district). An overall seroprevalence of 17.90% in goats in NE India, where serum samples were collected from 28 Districts in 7 states including Meghalaya and Assam of North-East India by Balamurugan *et al.*<sup>11</sup> that almost in agreement with present findings. Another study revealed an overall prevalence rate of 13.18% in goats where samples were collected from five districts of Assam in Northeast India by Begum *et al.*<sup>12</sup>. Blood collected from five districts of Meghalaya and eLISA was performed by Karam *et al.*<sup>13</sup> that revealed 9.81% seroprevalence of PPR in goats. All the findings are very close to the findings of the present study. The seroprevalence of goats in this study (overall 32.5% and district-wise 35 and 30% in Sylhet and Jhenaidah Districts, respectively) was also in accordance with the findings stated by Khan *et al.*<sup>14</sup> (overall 43.33 and 39.02% in Punjab) in a study in Pakistan. The presence of PPR viral antibodies in the serum of animals indicates the natural circulation of the PPR virus in those areas. In the present study, in the case of cattle, 30 and 20% of serum samples from Sylhet and Jhenaidah Districts respectively were found positive which was 36% in Jessore (adjacent district of Jhenaidah) in another study conducted by Haque *et al.*<sup>15</sup>. On the other hand, in the case of goats, 35% of serum samples from Sylhet and 30% from Jhenaidah were found positive which were 52 and 48%, respectively in the same study. These differences might be due to time gaps (2004 to 2022) when mass awareness and PPR vaccination of animals increased and even Rinderpest disease has been eradicated. Seroprevalence is somewhat higher in both cattle and goats in the Sylhet district than Jhenaidah district, though both are bordered areas of the country, where animal trespass (both legal and illegal) plays an important role in virus dissemination, particularly in the case of transboundary diseases like PPR. These minor differences might be due to increased farmers' mass awareness and vaccination coverage. As few low land area of Sylhet district lies far away from the local livestock office and Veterinary Hospital, having poor communication, therefore did not come under the PPR vaccination program. This may also play a positive role in this case. The presence of PPR viral antibodies indicates the natural circulation of the PPR virus in the study animals. Seropositive cattle also indicated that the PPR virus might have extended its host range. However, for this advanced study including other species of animals like Horses, Pigs, Sheep and Buffalos are essential. Therefore, contemplating the zoogeography of the country, the inadequacy of border security and the deficiency of PPR vaccination, it is suggested that, to control PPR, Bangladesh should follow Office International des Epizooties (OIE) guidelines as well as should formulate a new PPR control and prevention strategy, particularly for bordered districts of the country.

## CONCLUSION

This study revealed that, overall 25% of cattle and 32.5% of goats are seropositive in the studied bordered areas of the country despite no PPR vaccination history. The district-wise seroprevalence was found in the case of cattle 30 and 20% in Sylhet and Jhenaidah Districts and in the case of goats 35 and 30% in Sylhet and Jhenaidah, respectively. From these findings, it is concluded that the natural status of the circulating PPR virus is much higher in the bordered areas therefore, it is essential to formulate a PPR control strategy at the national level emphasizing bordered areas. Seropositive cattle also indicate that, the PPR virus may be extending its host range, but to assure this a large-scale study including other animals like Horses, Buffalos and Pigs is recommended.

## SIGNIFICANCE STATEMENT

Since Peste des petits ruminants (PPR) is endemic in Bangladesh and country shares a wide bordered area with the neighboring countries and there is huge illegal animal trespass through borders, which might play a key role in virus dissemination. On the other hand, for formulation of a PPR control strategy in the country, bordered areas should be included and as a prerequisite, the status of the naturally circulating PPR virus in the bordered area must be explored. Therefore, this study was conducted and the overall seroprevalence was found 25% in cattle and 32.5% in goats, which are much higher in these PPR viral antibodies indicating the natural circulation of the PPR virus in study animals of the study-bordered area. Seropositive cattle also indicate that the PPR virus might have extended its host range.

## ACKNOWLEDGMENT

We acknowledged the University Grants Commission (UGC), Government of the People's Republic of Bangladesh via the Sylhet Agricultural University Research System (SAURES), Central Disease Investigation Laboratory (CDIL), Livestock offices under the Department of Livestock Services and Jhenaidah Government Veterinary College, Jhenaidah, Bangladesh for all sorts of support.

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