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Sero-surveillance of peste des petits ruminant viral antibody in goats at different areas of Bangladesh

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Abstract: Peste des petits ruminants (PPR) is a devastating and killer disease of domesticated small ruminants particularly goats. The present study was conducted on the serosurveillance of PPR in goats in different areas of Bangladesh. Blood samples were collected apparently from different years of age of goats in different areas of Bangladesh. All sera were subjected to serological test by competitive ELISA. For sero-surveillance of PPR virus total 200 serum samples were collected at different areas of Bangladesh such as Bogra 18/60 (30%), Sirajganj 26/30 (86.67%), Mymensingh 5/50 (10%) and Rangpur 7/60 (11.67%). Among those 56 were seropositive and 144 were seronegative. The mean positive antibody titer \pm SD of goat serum samples was 26.03 ± 16.04 and the mean negative antibody titer was 77.76 ± 11.22 . The highest antibodies was detected in Sirajganj that was vaccinated flock whereas the lowest antibodies was found Mymensingh that was non vaccinated goat. It may be concluded that, in absence of vaccination, the presence of PPRV antibodies indicated that PPR viruses were circulating among the goats population but the vaccinated goat's have protective level of antibodies against PPRV. So that preventive measures should be taken in goats against PPR virus by vaccination with proper maintain of cool chain.

Keywords: goat; PPR viral antibody; cELISA

1. Introduction

Livestock are very important for both the subsistence and economic development of Bangladesh. They provide a flow of essential food products throughout the year. In some countries they are a major source of government's revenue and export earnings. They also sustain the employment and income of millions of people in rural areas, contribute draught energy and manure for crop production and are the only food and cash security available to

many Africans (Swai *et al.*, 2009 and Brumby, 1990). In Nigeria, as in other African countries, small ruminants (sheep and goats) contribute a substantial proportion of the nation's meat supply. Many health problems are encountered to put some obstacles and constraints in the front of developing productivity of small ruminants. Peste des Petits Ruminants (PPR) is considered the most important single cause of morbidity and mortality for sheep and goats, in Bangladesh. In Bangladesh, sheep and goats contribute a major proportion of the nation's meat supply and they are also a source of government revenue and export earnings. Infection with peste des petits ruminant's virus (PPRV) results in an acute, highly contagious disease of small ruminants particularly in sheep and goat (Awa *et al.*, 2002 and Anderson *et al.*, 1994). It was first described in cote d'Ivoire in West Africa in 1942. Gradually, it was realized that several clinically similar diseases occurring in other parts of West Africa shared the same cause. The virus now called Peste des petits ruminants virus (PPRV). Investigators soon confirmed the existence of the disease in Nigeria, Senegal and Ghana. For many years, it was thought that it was restricted to that part of the African continent until a disease of goats in Sudan, which was originally diagnosed as rinderpest in 1972, was confirmed to be PPR. The disease is endemic in Bangladesh since 1993 (Banil *et al.*, 2008). Generally 100% morbidity and 80-90% mortality were recorded in goat (Dhar *et al.*, 2002 and Hamdy *et al.*, 1976). The etiological agent of PPRV is a member of the genus morbillivirus (Haroun *et al.*, 2002) under the family of the paramyxoviridae. Other members of the genus are rinderpest, measles, canine distemper, seal distemper and dolphin distemper viruses. PPRV although serologically related to rinderpest virus but can be differentiated using cDNA probes (Diallo *et al.*, 1989), monoclonal antibodies (Anderson *et al.*, 1994 and Zohari *et al.*, 2008) and blocking ELISA (Saliki *et al.*, 1993). It has low resistance in the environment, chemical and pH. The PPR virus is mostly spread by aerosol and probably only small amount of virus is required to infect susceptible individuals. Cattle may be infected by contact with the PPR virus, but will not exhibit any symptoms and respond to rinderpest vaccination (Anderson *et al.*, 1991). The clinical signs of PPRV infection are always associated with high fever (106°-107.7°F), discharges (nasal, ocular and oral), erosive stomatitis and excessive salivation. The oculo-nasal discharges become mucopurulent followed by pneumonia accompanied with coughing, pleural rales and abdominal breathing. A watery blood stained diarrhea is common in the later stage of infection, which is followed by death. During disease cycle, severe immuno-deficiency is common and this is thought to contribute susceptibility to secondary infections that accounts for most of the mortality associated with PPR infection (Ozkul *et al.*, 2002 and Hilan *et al.*, 2006). Epidemiological data of PPR in sheep in Bangladesh is limited. Razzaque *et al.* (2004) found the sero-prevalence of PPR is 36% in sheep in Mymensingh district. Bangladesh Livestock Research Institute (BLRI) was carried out a study on sero-surveillance in sheep during the period from June to November 2008 in Naogaon, Tangail and Noakhali regoin and found overall sero-prevalence of 33.34% in Bengal sheep. Keeping the above in mind, the experiment was conducted Sero-surveillance of PPR antibody of goats in selected areas of Bangladesh.

2. Material and Methods

The study was conducted in the SAARC Regional Leading Diagnostic laboratory for PPR, Bangladesh Livestock Research Institute (BLRI) during the period of January/2017 to July/2017. The detailed outline of materials and methods are given below:

2.1. Study area

The study was conducted on sero-surveillance of Peste Des Petits Ruminants (PPR) virus specific antibodies in goats. For this purpose, sera were collected from different areas of Bangladesh namely Bogra (n=60), Sirajganj (n=30), Mymensingh (n=50), Rangpur (n=60) those area was selected as non vaccinated areas where as Sirajganj (n=30) was selected as vaccinated area. The animals with no clinical sings of PPR virus and with no report of vaccination by PPR vaccine were chosen as for experimental animals except Sirajganj. Total numbers of experimental goats were 200 (two hundred).

2.2. Blood sample collection and serum separation

The blood of goats was collected in early morning. After controlling the goat vein was detected. (2-3) ml of fresh blood sample was collected from each of the animal aseptically by puncturing jugular vein after swabbing with 70% alcohol or iodine swab in a gentle manner. The loaded syringe was remaining in inverted condition at (30-40) minutes for blood clotting. In that way blood sample was taken from different aged goats. After clotting of blood, serum was separated into eppendorf tube, numbering and packaging was done remain in freeze condition (2-8)°C for further use.

2.3. Design of the experiment

The main aim of these experiments is to study the seroprevalence of Peste Des Petits Ruminants virus (PPRV) specific antibodies in goat. For this experiment, sera samples were collected from 200 non-vaccinated goat's and vaccinated goat's 45 (age ranging from 6 months to >2 years) from selected goat farms in different area of Bangladesh. The sera samples were tested by c-ELISA (ID vet. Innovative Diagnostics, France) according to the Instruction.

3.4. Test procedure of cELISA

All the reagents were allowed to come to room temperature ($21^{\circ}\text{C} \pm 5^{\circ}\text{C}$) before use. Homogenized all reagents by inversion or vortex. 25 μl of dilution buffer 13 were added to each well of the ELISA micro plate. Then 25 μl of the positive control were added to wells (A1 and B1) and 25 μl of the negative control were added to wells (C1 and D1). 25 μl of the each sample was added to test to the remaining wells. Then the plate was incubated at 37°C and waited for 45 ± 4 minutes. The plate was washed 3 times with approximately 300 μl of the wash solution and to avoid drying of the wells between washings. After washing then the conjugate was prepared 1X by diluting the conjugate 10X to 1/10 in dilution buffer 4 and again 100 μl of the conjugate 1X was added to each well. Again the plate was incubated at 21°C and waited for 30 ± 3 minutes. Then the plate was washed 3 times with approximately 300 μl of the wash solution and to avoid drying of wells between washing. 100 μl of the substrate solution was added to each well. Then the plate was incubated at 21°C in the dark place and waiting for 15 ± 2 minutes. 100 μl of the stop solution was added to each well in order to stop the reaction. Finally the micro 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 data sheet of Microsoft[®] Excel program and saved in the computer hard disc with specific identification name.

2.5. Results interpretation

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

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

Sample presenting a S/N%:

- Less than or equal to 50% are considered positive
- Greater than 50% and less than or equal to 60% are considered doubtful.
- Greater than 60% are considered negative.

Result	Status
S/N % \leq 50 %	POSITIVE
50 % < S/N % \leq 60%	DOUBTFUL
S/N % > 60%	NEGATIVE

3. Results and Discussion

A total of 200 goat's serum samples were collected from different localities of Bangladesh namely Bogra (n=60, non vaccinated), Sirajganj (n=30, vaccinated), Mymensingh (n=50, non vaccinated) and Rangpur (n=60, non vaccinated) and tested by c-ELISA. Among those 56 were seropositive and 144 were seronegative. The mean positive antibody titer \pm SD of goat serum samples was 26.03 ± 16.04 and the mean negative antibody titer was 77.76 ± 11.22 (Table 1). The highest average seropositive S/N values were seen in the goats of Rangpur and the lowest average seropositive PI values were seen in those goat of Mymensingh (Table 1). The highest seroprevalence (86.67%) was found in goat of Sirajganj, followed by Bogra (30%), whereas Rangpur and Mymensingh showed a very minimal seroprevalence of 11.67% and 10% respectively (Table 2).

Table 1. Serosurveillance of PPR virus antibodies in goats based on cELISA.

SL No.	Location/Area	Seronegative S/N value (%) (Mean \pm SD)	Seropositive S/N value (%) (Mean \pm SD)
1	Bogra (n=60)	89.00 \pm 5.97	13.07 \pm 3.80
2	Sirajganj (n=30)	73.09 \pm 16.45	38.46 \pm 7.02
3	Mymensingh (n=50)	84.68 \pm 7.36	11.30 \pm 2.14
4	Rangpur (n=60)	64.28 \pm 6.98	41.30 \pm 3.85
5	All (n=200)	77.76 \pm 11.22 (n=146)	26.03 \pm 16.04 (n=54)

Table 2. Comparative study of PI (S/N) values of different sources sera samples.

SL No.	Source of Sample	No. of Samples	No. of Positive Samples	No. of Negative Samples	Mean PI (S/N) value	Remarks
1.	Bogra	60	18	42	66.22	Highest antibody titer was 20.99% and about 30% goats were Seropositive
2.	Sirajganj	30	26	04	45.38	26 samples were Seropositive (86.67%)
3.	Mymensingh	50	05	45	77.84	Highest antibody titer was 13.61% and about 10% Seropositive to PPR
4.	Rangpur	60	07	53	78.82	Average 11.67% goat were seropositive

The present study investigated the serosurveillance of PPRV antibodies under natural condition in non-vaccinated 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. 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.

This research provided valuable data on the serologic status in the goat with respect to PPRV. On the basis of vaccination, the highest seroprevalence (80%) was found in sirajganj Farm and the second highest prevalence (30%) was found in Bogra (Table 2). The overall seroprevalence of PPR was 26.03% in selected areas in Bangladesh. Whereas Razzaque *et al.*, 2004 and Banik *et al.*, 2008 who found the seroprevalence of PPR is 36% in goat and BLRI, 2008 were found the seroprevalence of PPR is 33.34% in sheep. The main cause of this low seroprevalence evidence showed that those were well non organized farm and no history of vaccination.

In case of non vaccinated goats sera shows large negative value contains a small amount of antibody that indicates to attack by PPR virus at any time. On the other hand, vaccinated goats have high positive value and shows high immunity against PPR virus.

4. Conclusions

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. Vaccination should be done regular basis with proper maintain of cool chain.

Conflict of interest

None to declare.

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