Article

Sero-surveillance and sero-monitoring of locally produced PPR vaccine in the field and experimental level

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Abstract: Peste des Petits Ruminants (PPR) is a highly contagious, economically important viral disease of goats with high morbidity and mortality. To control the disease effectively a live attenuated vaccine is available in Bangladesh which is produced by Livestock Research Institute (LRI), Mohakhali, Dhaka. The study was carried out to determine the immune status and immune response against PPR in field and experimental Black Bengal goats. Sero-surveillance of PPR was conducted by using c-ELISA in non-vaccinated 240 goats in Gazipur, Sirajgonj and Barisal. Out of the 240 goats tested, of which only 39 (20.31%) goats had positive level of PPR antibodies while 16.25% (13 out of 80 goats) in Gazipur, 28.75% (23 out of 80 goats) in Barisal and 3.75% ((3 out of 80 goats)) in Sirajgonj. In case of sero-monitoring of PPR, the result revealed that vaccinated goats from Rajshahi showed high positive result and have higher seroprevalence where 75% (60 out of 80 goats) were seropositive and only 25% (20 out of 80 goats) are seronegative. These result indicated that vaccinated Rajshahi goats is more resistant for PPR virus than non vaccinated goats. In experimentally to perform sero-monitoring, 10 seronegative goats were selected and divided into two equal groups (A and B). The immunization study against PPR with a commercial PPR vaccine was conducted on 5 goats of group A by inoculating @ 1.0 ml vaccine / animal subcutaneously and group B kept as non-vaccinated. The antibody titres against PPR in goats were determined at 0 day on vaccination and after 21DPV, 180DPV and 365DPV. The results found that 100% (5 out 5goats) seronegative in both vaccinated goats of group A and non-vaccinated goats of group B at 0 day on vaccination. The mean negative titres± SD were 79.285±13.921 and 76.707±9.265 in vaccinated group A and group B, respectively. The mean positive titers ±SD were 20.201±2.480, 8.630±4.970 and 11.382±1.419 at 21DPV, 180DPV an 365DPV, respectively in group A (100% seropositive). In case of non-vaccinated group B, the mean negative titres±SD were 74.258±7.793, 77.726±9.142 and 82.965±7.492 at 21DPV, 180DPV and 365DPV, respectively (100% seronegative). As it is observed, the antibody titres remain at the level over the period of time that indicates the immune response against PPR. From this finding, it is said that PPR vaccine could produce immune response in goats for about one year or 365 days.

Keywords: PPR; goats; sero-surveillance; sero-monitoring; c-ELISA; vaccine

1. Introduction

Peste des Petits Ruminants (PPR), which literally means "Plague of small ruminants", is an economically important viral disease of goats and sheep (Kamaruddin and Islam, 2005). The disease causes a huge loss of small ruminant production per year in Bangladesh. In Bangladesh first outbreak of PPR occurred in 1993 as Rinderpest like infection and later it was confirmed as PPR by the British reference laboratory (Sil *et al.,* 1995; Islam *et al.,* 2001). Vaccine efficacy is defined as percentage reduction in disease incidence in a vaccinated group compared to an unvaccinated group under optimal conditions. In 2000, the conventional live attenuated

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vaccine was developed against PPR by BLRI to control PPR in Bangladesh and is being used by the DLS. But, like other Morbilli virus vaccine, the main disadvantage of this vaccine is its poor thermal stability. For this PPR vaccine, a cold chain system is required that cannot be maintained properly in the field/village level and potency of the vaccine is seriously deteriorated. A question about its efficacy is being often raised by farmers and field veterinarians. PPR is seen occasionally in the vaccinated goats. In case of conventional PPR vaccine, it was found that 62% of the goats were sero-positive at 21 days post vaccination (DPV), which declined to 34.72 % at 180 DPV. In Bangladesh, total goat population is approximately 19.43 million (Economic Review, 2013-2014) at present. The outbreak of PPR in goats is occurred every year sporadically. PPR vaccine is used to control PPR in goats. This PPR vaccine is produced from Livestock Research Institute (LRI), Mohakhali, Dhaka. The production number of PPR vaccine is 45 lacks (approximately) doses per year. The nature of PPR vaccine is live attenuated vaccine and the dose and route of this PPR vaccine is 1ml per goat subcutaneously. Therefore, the present study was planned to determine the long duration immune response after vaccination and determine the immune status of post-vaccinated and non-vaccinated goats at field level with experimental level.

2. Materials and Methods

This PPR research work was carried out on sero-surveillance and sero-monitoring in collaboration of Department of Pathology, Faculty of Veterinary Science (FVS), Bangladesh Agricultural University (BAU), Mymensingh-2202 and SAARC Regional Leading Diagnostic Laboratory (SAARC-RLDL) for PPR at the Animal Health Research Division, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341, Bangladesh.

2.1. Collection of blood

Approximately 5.0 ml blood/animal was collected from jugularvein of each of the selected 320 goats (non-vaccinated goats: n=240 and vaccinated goats: n=80) in separate sterilized syringe without adding any anti-coagulants. After (30-40) minutes of blood collection and then sera were separated into a small glass vial and stored at -20^0C until tested by c-ELISA.

2.2. Sero-surveillance and sero-monitoring of PPR at field

The sero-surveillance study was conducted in Gazipur, Sirajgonj and Barisal. From the mentioned area about 240 goat serum samples were collected from both sexes and aged between 0 to more than 24 months [Gazipur: n=80, Barisal: n=80, and Sirajgonj: n=80] with no earlier history of vaccination against of any diseases (Table1). The sero-monitoring observation was conducted in Rajshahi where about 80 PPR vaccinated goat serum samples were collected from both sexes and aged between 0 to more than 24 months (Table1).

2.3. Sero-monitoring in experimental trial

For sero-monitoring of PPR vaccine at experimental level, a total 10 number of Black Bengal PPR seronegative goats were selected from goat farm of BLRI. The total number of 10 goats was divided into two equal groups A and B (Group A as a vaccinated group and Group B as a non-vaccinated group). The animal (goats) was kept in separate room and management practice in same procedure. The goats of Group A were vaccinated by PPR vaccine at the dose rate of 1ml per goat subcutaneously. The sera samples were collected in both groups A and B at 0 day of vaccination, after 21 days, 180 days and 365 days of post vaccination (DPV) and tested by c-ELISA.

2.4. Detection of antibody by c-ELISA

The diagnostic kit is designed to detect antibodies against the nucleoprotein of the Peste des Petits Ruminants (PPR) virus. The test was performed by ID Screen® PPR competition test kit (ID. Vet, CIRAD-EMVT, Montpellier, France).

2.5. Calculation of c-ELISA reading

The competition percentage was calculated using the following formula:

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

At first the saved c-ELISA reading data from the computer was copied in working sheet of Microsoft® Excel programme. Mean OD of PC (positive control) well as calculated by dividing the sum of PC of two wells by 2. Mean OD of NC (negative control) as calculated by dividing the sum of NC of two wells by 2. Competition percentage (S/N %) as calculated by OD sample divided by OD negative then multiplied by 100. Sera showing S/N % less than or equal to 50% were considered to be positive.

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3. Results and Discussion

Peste des Petits Ruminants (PPR) is a devastating and killer disease of domesticated small ruminants especially in goats. The disease causes 100% morbidity and 80-90% mortality in goats (Hamdy *et al.,* 1976). Outbreaks of the disease have been reported to be associated with high morbidity and mortality in unprotected goats and the disease is now considered endemic in Bangladesh (Barrette *et al.,* 1997; Islam *et al.,* 2001; Das *et al.,* 2007). Sero-prevalence (Razzaque *et al.,* 2004), sero-surveillance and immunization studies (Banik *et al.,* 2008), evaluation of C-ELISA (Sil *et al.,* 2001), pathological investigation (Khan *et al.,* 2005) and evaluation of antibiotic combined hyperimmune serum therapy (Islam *et al.,* 2003) for PPR infected goats have been reported from Bangladesh. The present study was aimed to know the immune status in the field and immune response in experimental level in both vaccinated and non-vaccinated goats.

3.1. Sero-surveillance and sero-monitoring

Sero-prevalence of PPR was conducted by using C-ELISA in non-vaccinated 240 goats in Gazipur, Sirajgonj and Barisal. Out of the 240 goats tested, of which only 39 (20.31%) goats had positive level of PPR antibodies while 16.25% (13 out of 80 goats) in Gazipur, 28.75% (23 out of 80 goats) in Barisal and 3.75% (3 out of 80 goats) in Sirajgonj (Table 1). The sero-prevalence results of this study could be compared with the findings of sero-prevalence of PPR in goats of Mymensingh district; 49.17% and 25% reported by Razzaque *et al*. (2004) and Banik *et al*. (2008) respectively. Furthermore, the prevalence of PPR in goats of Rajshahi district was at 20.57% that reported by Sarker & Islam (2011).

In case of sero-monitoring of PPR, the result revealed that vaccinated goats from Rajshahi showed high positive result and have higher seroprevalence where 75% (60 out of 80 goats) were seropositive and only 25% (20 out of 80 goats) was seronegative (Table1). Mahmudul Hasan (2012) found that the sero-prevalence was 67.7% in goat with overall average of about 55.1% cases within vaccinated flock and about 16.8% cases found sero-positive in case of non-vaccinated flock. From the data non vaccinated goats showed very high seronegative result and lower antibody level. These result indicated that vaccinated Rajshahi goats is more resistant for PPR virus than non-vaccinated goats.

Table 1. Results of c-ELISA between vaccinated (V) and non vaccinated (NV) goats at different region.

Area (Immune Status)	No. of Animal (Goats)	No. of animals with antibody titre		
		S/N % ≤ 50 % (Seropositive, %)	50 % < S/N % ≤ 60% (Doubtful, %)	S/N % > 60% (Seronegative, %)
Gazipur (NV)	80	13 (16.25%)	0	67 (83.75%)
Barisal (NV)	80	23(28.75%)	0	57 (71.75%)
Sirajgonj (NV)	80	3 (3.75%)	0	77 (96.25%)
Rajshahi (V)	80	60 (75%)	0	20 (25%)

3.2. Sero-monitoring in experimental trial

The antibody titres against PPR in goats were determined at 0 day on vaccination and after 21DPV, 180DPV and 365DPV. The results found that 100% (5 out of 5 goats) seronegative in both vaccinated goats of group A and non-vaccinated goats of group B at 0 day on vaccination. The mean negative titres ± SD were 79.285±13.921 and 76.707±9.265 in vaccinated group A and group B, respectively (Table2). The mean positive titers ±SD were 20.201±2.480, 8.630±4.970 and 11.382±1.419 at 21DPV, 180DPV an 365DPV, respectively in group A (100% seropositive, 5 out 5) (Table2). In case of non-vaccinated group B, the mean negative titres±SD were 74.258±7.793, 77.726±9.142 and 82.965±7.492 at 21DPV, 180DPV and 365DPV, respectively (100% seronegative, 5 out 5 goats) that shown in Table 2. As it is observed, the antibody titres remain at the protection level over the period of time that indicates the immune response against PPR (Figure 1). From this finding, it is said that PPR vaccine could produce immune response in goats for about one year or 365 days.

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Table 2. Sero-monitoring of antibodies against PPRV based on c-ELISA.

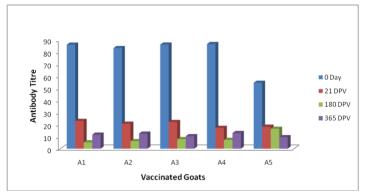| Days of c-ELISA test	Goats group	No. of positive sample	No. of negative sample	Seropositive CP(S/N%) value (Mean±SD)	Seronegative CP(S/N%) value (Mean±SD)
0 DPV	Group A (n=5)	-	5	-	79.285±13.921
	Group B (n=5)	-	5	-	76.707±9.265
21DPV	Group A (n=5)	5	-	20.201±2.480	-
	Group B (n=5)	-	5	-	74.258±7.793
180DPV	Group A (n=5)	5	-	8.630±4.970	-
	Group B (n=5)	-	5	-	77.726±9.142
365DPV	Group A (n=5)	5	-	11.382±1.419	-
	Group B (n=5)	-	5	-	82.965±7.492

Figure 1. Screening of PPR antibody through sero-monitoring based on c-ELISA.

In our experiment we found 100% seropositive CP values in vaccinated experimental goats through c-ELISA after at 21 DPV, 180DPV and 365 DPV whereas Rahman *et al*. (2011) found 34.72% and 60% seropositive goats in cases of conventional and in thermostable PPR vaccine after at 180 DPV. Similarly Anderson and McKay in 1994 found that about 60-70% animals were sero-positive for PPRV due to RP vaccination (tested with c-ELISA). The challenge test with PPR virus after at 180 DPV showed that LRI produced live attenuated PPR vaccine protected all goats (100%) of group A while all the non-vaccinated goats were not protected after challenge showing clinical signs of PPR/or mortality that similar with the findings of Siddique *et al.* (2006).

4. Conclusions
Sero-surveillance revealed that the non vaccinated goats are more susceptible of PPR virus than vaccinated goats in the field level. The locally produced vaccine is highly protective for the goat population against the PPR disease with proper maintenance of cold chain, dose and route of vaccination. In case of field level study, some goats of different region were found antibody of PPR virus may be due to natural outbreaks and maternal antibody. So, it is needed to clarify the natural history of outbreaks, risk factors and should be vaccinated all the goat population of the country.

Conflict of interest
None to declare.

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