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IMMUNE RESPONSE OF GOATS TO THERMOSTABLE PPR VACCINE IN BANGLADESH

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ABSTRACT

To control peste des petits ruminants (PPR) in Bangladesh a live attenuated conventional PPR vaccine was developed by Bangladesh Livestock Research Institute (BLRI) and currently being used in the country. Farmers and field veterinarians often raise the question about the efficacy of that vaccine. The poor efficacy of PPR vaccine in rural situation was thought to be due to break in cold chain system during transportation. To address to this problem, a thermostable preparation of the former PPR vaccine has been made by BLRI. The present study was carried out to assess the efficacy of both the vaccines on the basis of the humoral immune response. A total of 190 goats more than 3 months of age of both sexes were selected for this experiment. Sera collected from experimental goats were analyzed with a competitive enzyme linked immunosorbent assay (cELISA). Percent inhibition (PI) value greater than 50% was considered as positive. 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. On the other hand, in case of thermostable PPR vaccine, 70% serum samples were found positive at 21 DPV and that decreased slightly to 60% at 180 DPV. Kruskal-Wallis test and paired *t*- test revealed no significant difference in the antibody response of the thermostable vaccine kept at 25, 30, 35 and 40^{o} C temperatures for 7 -14 days.

Keyword: cELISA, immune response, thermostable PPR vaccine, storage time, temperature

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INTRODUCTION

Peste des petits ruminants (PPR) is an acute, highly contagious viral disease of sheep and goats is characterized by fever, anorexia, ulcerative necrotic stomatitis, diarrhoea, purulent ocular and nasal discharges and respiratory distress which may be associated with coughing, pneumonia and death (Lefevre and Diallo, 1990). In non-endemic areas morbidity and mortality may vary depending upon susceptible population and in severe cases that may reach up to 100 % and 90%, respectively (Hussain *et al*., 1998). The causative agent of this economically important disease of small ruminants is a Morbillivirus, the peste des petits ruminants virus (PPRV), under the family Paramyxoviridae of order Mononegavirales (Murphy *et al*., 1999). The virus is closely related to rinderpest virus (RPV), another member of Morbillvirus genus, which causes similar disease in large ruminants (Couacy-Hyman *et al*., 1995). The disease has been reported from many countries of the world including most parts of Africa, Middle East, the Arabian Peninsula and southern Asia (Abu-Elzein *et al*., 1990 Shaila *et al*., 1996 and Lefevre *et al*., 1991). In Bangladesh, PPR virus was identified during a severe outbreak in 1993 (Sil *et al*., 1995). Previously, locally produced tissue culture based live attenuated rinderpest vaccine (TCV), as well as imported rinderpest vaccine were used against PPR in this country, but failed (Sil *et al.*, 2001). To overcome the problem, conventional live attenuated vaccine was developed against PPR in nineties by BLRI to control PPR in Bangladesh and is being used by the Department of Livestock Services (DLS). But, like other Morbillivirus 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. To respond to this problem, a thermostable preparation of the former PPR vaccine has been developed experimentally by BLRI (Chowdhury *et al*., 2004). Previous efficacy study on this thermostable PPR vaccine asserted that this can be kept at normal room temperature (25^{o}-30^{o}C) as long as 14 days without loss of its potency (Siddique *et al*, 2006). Since, the environmental temperature in Bangladesh fluctuates with seasons and it rises up to 40^{0}C, it is therefore necessary to test the immune efficacy as well as shelf life of the vaccine stored at different temperatures and its shelf life. The present study was carried out to determine the potency in terms of antibody response of newly developed thermostable PPR vaccine kept at gradient temperature of 25^{0}, 30^{0}, 35^{0} and 40^{0}C for 7 and 14 days as well as immuno-efficacy of conventional PPR vaccine currently being used in Bangladesh.

MATERIALS AND METHODS

Vaccine: Two types of PPR vaccine, conventional PPR vaccine developed by BLRI and produced by Livestock Research Institute (LRI), Mohakhali, Dhaka and thermostable vaccine experimentally prepared by BLRI, Savar, Dhaka were used in this study. Conventional PPR vaccine was collected from LRI from a freshly prepared batch. Proper cold chain was maintained during transportation of the vaccine.

Experimental animals and immunization: To determine the immunoefficacy of thermostable PPR vaccine, a total of 90 goats over 3 months of ages of both sexes were selected randomly. All the animals were kept under same housing and management conditions at BLRI animal shed. The animals were treated against gastrointestinal helminths with Albendazole at 15 days pre-vaccination. The animals were divided into nine groups comprising of 10 goats each. To maintain storage time and temperature gradients the vaccines were kept in 4 different incubators in Department of Pathology, Bangladesh Agricultural University, Mymensingh and BLRI, Savar, Dhaka (Table 1).

Table 1. Storage time and temperature of experimental thermostable PPR vaccine

Temperature	Days in the shelf	
25^0C	7 days	14 Days
30^0C	7 days	14 Days
35^0C	7 days	14 Days
40^0C	7 days	14 Days

Goats of group 1-8 were vaccinated sub cutaneously (SC) with a dose rate of 1ml (4 Log_{10} $TCID_{50}$ ml^{-1}) per animal of the thermostable PPR vaccine kept at different temperatures and storage times while goats of group 9 were kept as un-vaccinated control. The vaccinated animals were monitored for the occurrence of any disease up to 30 days post-vaccination. Blood samples without anticoagulant were collected through jugular vein puncture with sterile disposable plastic syringes from all the groups at pre-vaccination and at 21 and 180 days post vaccination. The sera were separated from blood and stored at -20^0C until tested.

Similarly, to determine the immunoefficacy of non thermostable PPR vaccine, 100 unvaccinated healthy goats reared by the farmers as in the traditional farming condition were selected from three villages (Chattarpur, Boira and Digharkana) under Mymensingh Sadar Upazila, Mymensingh and tagged individually. The selected

goats were treated against gastro intestinal helminthes 15 -20 days before administration of vaccine. Vaccine was provided subcutaneously (S/C) at the neck region with the dose rate of 1 ml (4 Log_{10} $TCID_{50}$ ml^{-1}) per goat and observed similarly as described above. Blood samples were collected from jugular vein with sterile disposable plastic syringe at pre-vaccination and at 21 and 180 days post vaccination. The sera were separated from blood and stored at -20^0C until tested.

Competitive ELISA (c ELISA): PPR c-ELISA kit for PPRV antibody detection was obtained from Biological Systems Department, CIRAD, France Competitive ELISA test was performed as the method developed by Libeau *et al*. (1995) and strictly as per the protocol outlined in the manual supplied with the kit. Briefly, PPR antigen was diluted in coating buffer (PBS- 0.01 M, pH 7.4); each well of micro titer plate was charged with 50µl diluted antigen, incubated for 1 h at 37°C on a thermo mixer. After 3 washing with washing buffer and blotting dry, 45 µl of blocking buffer (PBS with 0.05% between 20 and 0.5 negative lamb serum) was added to all wells. Five µl of test sera were added to all the test wells, 5 µl of blocking buffer to monoclonal control wells and 55 µl of blocking buffer to the conjugate control wells. Strong positive, weak positive and negative control sera were added to the corresponding wells. Monoclonal antibody (Mab), diluted (1/150) in blocking buffer, was added at the rate of 50 µl to all the wells except the conjugate control. Then the plate was incubated for 1 hour at 37°C on thermo mixer. After 3 washings and blotting to dryness, 50 µl of anti-mouse conjugate were added to all wells. After 1 hour incubation and 3 washings, 50 µl of the chromogen per substrate mixture (OPD/H_2O_2) was added to all wells. After 10 minutes of incubation at room temperature, colour development was stopped by adding 50 µl of stop solution (H_2SO_4, 1M) to all wells. Optical density (OD) values were read at 492 nm with absorbance microplate reader (EL×800, Bio Tek Instruments, USA.).

Data expression: Microplates readings were used in two types of data analysis:

1) Percent inhibition (PI) values which were used for the Quality Assurance (QA) acceptance. These PI values were calculated as follows:

$$PI = 100 - \left[\frac{\text{Replicate OD of each control}}{\text{Mean OD of Mab control (Cm)}} \times 100\right]$$

2) Percent inhibition (PI) values which were used for acceptance of replicate values for test sera and diagnostic interpretation. These PI values were calculated as follows:

$$PI = 100 - \left[\frac{\text{Replicate OD of Test Serum}}{\text{Mean OD of Mab control (Cm)}} \times 100\right]$$

The test was accepted when the OD values of Cm and PI values of different controls fall within the accepted limits written in QA Fact Sheet of the supplied ELISA Kit. Sera showing PI greater than 50% were considered positive.

Statistical analysis: Mean Percent Inhibition (PI) values and standard deviation were determined from the obtained PI values of the serum samples in each vaccinated and control groups. Kruskal-Wallis test were carried out to compare the immuno-efficiency of vaccine kept at various temperatures and for different time period. Paired *t*- test was carried out to determine the significance of variation of PI values among the different groups (including control group) at pre and post vaccination (Montgomery, 2004 and Islam, 2008). Statistical test were carried out by using the software SPSS Version 11.5 and Minitab 13.

RESULTS

Both thermostable and conventional vaccines used in this study provided partial sero- conversion. In case of thermostable PPR vaccine, 70% of the vaccinated goats were sero-positive (PI values >50%) at 21 DPV. At 180 DPV, the number of sero-positive cases slightly decreased to 60% in all groups except the group that received the vaccine treated at 40^0C for 14 days (30%) (Table 2). The average PI values for all groups also decreased by 8-20% (Table 2) at 180 DPV. On statistical analysis, no significant differences of immuno-efficiency of the vaccine (P-value<0.01) were observed after the vaccine kept at different temperatures and for different time periods (Table 3 and 4). No sero-positive goat was found in case of control group (PI < 50%).

Table 2. Percentage of sero-positive goats and average PI values (mean±SD) following vaccination with thermostable PPR vaccine treated at different temperatures and for different time period

Temperature	7 days group			14 days group			Control group		
	Day 0	Day 21	Day 180	Day 0	Day 21	Day 180	Day 0	Day 21	Day 180
25°C	[a]0% [b]27.7±3.8	70% 63.5±6.0	60% 53.8±2.6	0% 32.5±13.1	70% 56.4±1.3	60% 52.3±2.2	0% 38.5±8.6	0% 37.7±6.3	0% 36.4±7.6
30°C	0% 27.5±2.6	70% 63.6±6.6	70% 53.5±2.7	0% 34.9±7.1	70% 56.6±2.4	60% 52.7±3.7	0% 38.5±8.6	0% 37.7±6.3	0% 36.4±7.6
35°C	0% 22.6±9.0	70% 64.0±7.8	70% 52.6±1.2	0% 37.2±8.9	70% 55.2±2.6	60% 54±2.2	0% 38.5±8.6	0% 37.7±6.3	0% 36.4±7.6
40°C	0% 25.1±6.8	70% 63.3±5.6	70% 54.3±2.5	0% 32.6±5.2	70% 53.4±1.7	30% 53±1.7	0% 38.5±8.6	0% 37.7±6.3	0% 36.4±7.6

[a] % of positive reactor [b]Mean PI values ± standard deviation

Table 3. Summary of immuno-efficiency of vaccine kept at 25, 30, 35 and 40^{o} C temperatures

Duration of inventory at 25, 30, 35 and 40^{o} C	Days of post vaccination (dpv)	Value of Kruskal-Wallis test	P-value *	Remarks
7 days	21	0.55	0.997	No significance relationship between temperature and vaccine efficacy
	180	0.79	0.852	Ditto
14 days	21	2.06	0.560	Ditto
	180	3.46	0.326	Ditto

* If P-value <0.01, the hypothesis that "vaccine efficacy 25, 30, 35 and 40^{o} C temperatures are equal" to be rejected.

Table 4. Summary of immuno-efficiency of vaccine kept at 7 days and 14 days

Temperature in storage time 7 and 14 days	**Days of post vaccination (dpv)**	**Value of paired –t**	**P-value ***	**Remarks**
25^{0}C	21	0.971	0.357	The efficacy of vaccine does not influenced by stock time
	180	1.202	0.260	Ditto
30^{0}C	21	0.901	0.391	Ditto
	180	0.713	0.494	Ditto
35^{0}C	21	1.130	0.288	Ditto
	180	0.701	0.501	Ditto
40^{0}C	21	2.084	0.067	Ditto
	180	2.736	0.023	Ditto

* If P-value<0.01, the hypothesis that "the efficacy of vaccine in different storage time period are equal" to be rejected.

On the other hand, in case of conventional PPR vaccine that is still being used by DLS to control PPR exhibited 62% sero-conversion at 21 DPV and declined to 34.72

% at 180 DPV. It means that about 38 and 65.27% goats remained sero-negative at day 21 and 180 DPV, respectively. The average PI values at 180 DPV were decreased by 15% (Table 5).

Table 5. Antibody response of goat after vaccination with conventional PPR vaccine

Sampling (Day post vaccination)	Total number of goats	Number sero-positive PI (Mean±STD)	
		No. Positive	% Positive
0	100	10 59.68±9.64[a]	10
21	100	62 68.36±9.20	62
180	72[b]	25 53.17±7.87	34.72

[a] Mean PI values ± standard deviation, [b] 28 goats were sold by the farmer.

In both the cases (for thermostable and conventional vaccine), Paired *t*- test suggested that PI values of sero-positive cases of post vaccination period is greater than the pre-vaccination period at 1% level of significance.

DISCUSSION

Both conventional and thermostable PPR vaccines induced a partial sero-conversion and in both cases the sero-positivity also declined with time. Normally, homologous PPR vaccine attenuated after 63 passages in Vero cell has been reported to have produced a solid immunity in 98% of the vaccinated animals for the whole economic life around 3 years (Diallo *et al*., 1995). The PPRV homologous vaccine was found to be safe under field conditions even for pregnant animals (Diallo *et al*., *1995*). However, in this study, at 21 DPV 70% goats in case of thermostable vaccine and 62% goats in case of conventional vaccine were found sero-positive and even some of the sero-positive goats became sero-negative at 180 DPV. Average PI values of the serum samples for both the vaccines decreased at least by 10% at 180 DPV. Nutrition or management factors would have affected some of the goats in both the experimental conditions which would have some reflection on the immuno-efficiency of goats. The reasons that more number of sero-positive goats became sero-negative at 180 DPV with conventional vaccine is not clear. However, two experiments were conducted in two experimental conditions; conventional vaccine

was tested in actual field condition, thermostable vaccine was tested in intensive condition and it is likely that some unknown stress condition might have prevailed in the field condition. The goats were vaccinated at a dose rate of 1 ml (4 Log_{10} $TCID_{50}$ ml^{-1}) per goat in both the experiments. The minimum titer of vaccine recommended by Office International des Epizooties (OIE) is 2.5 Log_{10} $TCID_{50}$ ml^{-1} (OIE, 2008). Initially poor immunity of PPR vaccine in rural situation was thought to be due to not maintaining a cold chain during transportation. Using thermo-stable vaccine, on statistical analysis, no significant differences of effectiveness of the vaccine have been observed after the vaccine kept on the shelf at different temperatures and for different time periods that also induced the almost similar results (70% immuno-efficiency). This needs to be investigated if the low efficiency was due to less homology between the vaccine and field viruses or more attenuation of the vaccine virus. This may even happen if the seed virus is not maintained properly.

CONCLUSION

PPR vaccine produced in Bangladesh provided about 70% sero-conversion that declined with time. The reasons of low immunity by PPR vaccine remained unidentified. Immune response of thermostable PPR vaccine kept at different storage temperature gradients and for time being revealed no significant differences indicating that thermosatble preparation of PPR vaccine may be used without maintaining a cold chain.

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