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SERO-SURVEILLANCE AND IMMUNIZATION IN SHEEP AND GOATS AGAINST PESTE DES PETITS RUMINANTS IN BANGLADESH

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ABSTRACT

The sero-surveillance and immunization studies on Peste des Petits Ruminants (PPR) in non-descriptive indigenous sheep and Black Bengal goats aged between 5 to 12 months were carried out in the district of Mymensingh during the period from June to November 2003. Sero-prevalence of PPR by using competitive enzyme linked immunosorbent assay (C-ELISA) was conducted on 100 sheep and 100 goats which were selected randomly from 10 different areas in the district of Mymensingh. Of the 100 sheep and 100 goats tested, 27% sheep and 25% goats had positive (≥ 50) antibody titre. This indicates that 27% sheep and 25% goats might have the capability to resist natural PPR infection and accordingly, sero-monitoring could be required to give a better indication of nation herd immunity. The immunization study against PPR with a commercial PPR VAC® was conducted on 10 sheep and 10 goats by inoculating @ 1.0 ml vaccine / animal subcutaneously. The clinical, haematological, biochemical and antibody levels were determined at pre-immunization and at 7th, 14th and 21st day of post-immunization. The mean antibody titre at 7th, 14th and 21st day of post-immunization in sheep (38.28 ± 4.34, 58.63 ± 3.60 & 68.27 ± 3.09) and goats (49.84 ± 4.37, 63.23 ± 3.64 & 76.60 ± 4.07) were significantly ($p < 0.01$) increased in comparison to the respective pre-immunization mean titre of sheep (20.0 ± 6.78) and goats (14.00 ± 6.96). Results of haematological examination showed that the PPR VAC® has no effects on haemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed celled volume (PCV) and total erythrocyte count (TEC). The TLC was markedly increased at all the stages of post-immunization in both sheep and goats but significantly ($p < 0.05$) increased at 21st day (10.08 ± 1.55 10^3 / mm^3) in sheep and at 14th day (14.76 ± 0.84 10^3 / mm^3) in goats in comparison to pre-immunization values of sheep (7.95 ± 0.97 10^3 / mm^3) and goats (9.00 ± 1.28 10^3 / mm^3). No distinct difference was observed on rectal temperature, pulse and respiratory rate between the pre- and post-immunization values which indicates PPR-VAC® has no clinical effects in vaccinated animals. No significant differences on calcium, albumin, total serum protein (TSP) and glucose levels were observed between the pre- and post-immunization values in both the sheep and goats. But the TSP in both the sheep 8.43 ± 0.69 g/dl) and goats (8.26 ± 0.50 g / dl) at 21st day of post-immunization increased insignificantly ($p > 0.05$) in comparison to the pre-immunization values of sheep (7.66 ± 0.79 g / dl) and goats (7.89 ± 0.92 g / dl). The results of humoral immune response produced by PPR-VAC® indicates the activity of the inoculated vaccine but challenge studies with virulent PPR virus would be required to confirm its efficacy.

Key words: PPR, sheep, goats, sero-surveillance, C-ELISA, PPR-VAC®, haematology, biochemistry

INTRODUCTION

Peste des Petits Ruminants (PPR) is the French name of a Rinderpest-like disease in sheep and goats first described in Ivory Coast, West Africa in 1942. Many others prefer the appellation of stomatitis-pneumonia-enteritis complex disease, pseudo-rinderpest of small ruminants and kata. But official instances like FAO and OIE use the French name PPR. It is an acute highly contagious and fatal disease of small ruminants, caused by *Morbillivirus* close to Rinderpest virus and characterized by fever, necrotic stomatitis, gastro-enteritis and pneumonia. In unprotected animals the morbidity can be up to 100% and mortality may be 20 to 90% and in severe outbreaks with 100% case fatality particularly in goats (Samad, 2008). PPR virus was considered a variant of Rinderpest virus, specially adapted for goats and sheep that had lost its virulence for cattle. It is now known that the two viruses are distinct though closely related antigenically. Goats and sheep are the natural hosts of PPR, but goats appear to be more susceptible and suffer a more severe clinical disease than sheep. In endemic areas, goats more than 4 months up to 24 months age are affected (Samad, 2008). PPR was once thought to be only an African problem, but the recent outbreaks in Middle East and Indian sub-continent causing

alarming losses of animals especially goats. Outbreaks of PPR are now known to be common in India, Nepal, Bangladesh, Pakistan, Bhutan and Afghanistan. PPR virus first reported in India in 1987 from an outbreak in Tamil Nadu and since then the disease has been reported from all over the country. The outbreaks of a Rinderpest-like disease later confirmed by World Reference Laboratory to be PPR have been occurring in goats since 1993 in Bangladesh (Barrette *et al*., 1997). The seroprevalence of PPR has been reported to be 36.0% in sheep, 49.17% in goats and 19.05% in cattle from Bangladesh (Razzaque *et al*., 2004). The outbreaks of PPR caused 74.13% morbidity and 54.83% mortality in Black Bengal goats in Bangladesh (Islam *et al*., 2001, Das *et al*., 2007). This paper describes the sero-surveillance and effects of immunization in sheep and goats immunized against PPR in Bangladesh.

MATERIALS AND METHODS

The study on PPR was carried out on sero-surveillance and immunization in sheep and goats during the period from June to November 2003.

Sero-surveillance of PPR

The sero-surveillance study on PPR was conducted on randomly selected indigenous 100 sheep and 100 Black Bengal goats, of either sex, aged between 5 to 12 months with history of no earlier vaccination against any diseases. These animals were selected from 10 different localities in the district of Mymensingh (Table 1).

Collection of blood

Blood (about 5.0 ml / animal) was collected from jugular vein of each of the selected 100 goats and 100 sheep in separate sterilized test tubes with and without adding any anti-coagulants. The tubes containing blood without any anti-coagulants were kept in refrigerator overnight and sera were separated as per conventional method by centrifugation at 5000 rpm for 15 minutes. Sera were transferred to the small glass vial and stored at – 20 ^{0}C until tested.

Screening of the sera samples

Each of the randomly selected sera samples of 100 goats and 100 sheep was screened with the help of commercial competitive enzyme linked immunosorbent assay (C-ELISA) kit (IAEA and BDSL, UK) as described by Sil *et al.* (2001).

Immunization studies

Ten non-descriptive indigenous sheep (6 male and 4 females) and 10 Black Bengal goats (6 male and 4 females), aged between 5 to 12 months were purchased from the local market and maintained in the houses of the Bangladesh Agricultural University (BAU) Veterinary Clinic. These experimental animals were allowed to graze day-time in the field and some wheat bran was supplied at night with water supply *ad libitum.* After acclimatization of these goats in the new environment, parasitological examination of faecal samples, examination of rectal temperature, pulse and respiratory rate, antibody titre against PPR and certain haemato-biochemical parameters were determined.

Each of the experimental 10 sheep and 10 goats was immunized with PPR-VAC® (LRI, Mohakhali, Dhaka) @ 1.0 ml / animal subcutaneously. Venous blood from each of the experimental animal was collected in duplicate tubes, one contained double oxalate used for haematology and the other without adding any anti-coagulant used for determination of antibody and biochemical constituents at pre-immunization and at 7th, 14th and 21st day of post-immunization.

Haematological studies

Haemoglobin (Hb) concentration (Acid haematin method with Sahli and Hellige hemometer), erythrocyte sedimentation rate (ESR) by using Wintrobe's hematocrit tube, packed cell volume (PCV) by using Wintrobe's hematocrit tube, total erythrocyte count (TEC) by using Thomas's red blood cell pipette with Hayem's solution and total leukocyte count (TLC) by using Thomas's White blood cell pipette with N/10 hydrochloric acid were determined at pre-immunization and at 7th, 14th and 21st day of post-immunization as described by Samad (2001).

Antibody determination

Each serum was subjected to test for PPR antibodies by using C-ELISA test kit as described by Sil *et al.* (2001).

Biochemical studies

The biochemical constituents of serum of sheep and goats collected at different stages of immunization were determined with the help of Spectronic® Genesys TM-5 (Spectronic Instrument Inc., Rochester, NY, USA) as per method of the diagnostics kits (Human Gesellschaft fur Biochemica and Diagnostica mbH, Max-Planck-Ring 21, D-65205 Wiebaden, Germany).

The serum calcium, serum albumin, total serum protein (TSP) and serum glucose were estimated by using complete test kits Cat No. 10011 at 570 nm, Cat No. 10560 at 546 nm, Cat No. 10570 at 546 nm and Cat No. 10260, 10121 and 10123 at 580 nm, respectively as described by the manufacturer and Diagnostica mbH, Germany).

Statistical analysis

Results were analyzed statistically with the help of Student's `t' test for significance as described by Gupta (1982).

RESULTS AND DISCUSSION

Peste des Petits Ruminants (PPR) is a highly contagious disease affecting small ruminants in African countries situated in a wide-belt between the Sahara and Equator, Middle East and Indian sub-continent (Samad, 2008). 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), 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 sero-surveillance and immunization studies in sheep and goats against PPR with a commercial PPR-VAC® would help to assess the feasibility to use vaccine to control PPR in small ruminants under field conditions.

Sero-surveillance

Sero-prevalence of PPR by using C-ELISA was conducted in non-vaccinated 100 sheep and 100 goats of 10 different areas in the district of Mymensingh. Out of the 100 sheep and 100 goats tested, of which only 27% sheep and 25% goats had positive level of PPR antibodies (Table 1). The sero-prevalence results of this study could be compared with earlier findings of Razzaque *et al.* (2004) who reported sero-prevalence of PPR in 36% sheep, 49.17% goats and 19.05% cattle from the district of Mymensingh. These results indicate that a certain percentage of sheep (27%) and goats (25%) in the district of Mymensingh have natural positive level of antibody titre against PPR which might protect against natural PPR challenge, as the humoral antibodies have shown to have a protective value in PPR (Bidjeh *et al*., 1999, Islam *et al*., 2003). Maternally derived antibodies against PPR in young animals could be detected up to 6 months of age but fall below the protection threshold level at 3.5 and 4.5 months in lambs and kids, respectively. Therefore, it is suggested that kids and lambs from immunized or exposed dams should be vaccinated at 4 and 5 months of ages, respectively (Awa *et al*., 2002). Similarly, the antibodies due to naturally exposed to PPR infection might also interfere the efficacy of vaccines. In addition, Anderson and Macky (1994) reported that antibodies against PPR virus and Rinderpest virus are both cross-neutralizing and cross-protective against each other. Accordingly, PPR in small ruminants has primarily been controlled by using tissue culture Rinderpest vaccine in Africa (Taylor 1979, Samad 2008). Therefore, monitoring of antibodies would be required before mass vaccination against PPR, especially in enzootic areas.

Clinical response

The rectal temperature, pulse and respiratory rates of sheep and goats were recorded at pre-vaccinated and at 7^{th}, 14^{th} and 21^{st} day of post-vaccinated period and the results are presented in Table 2. No significant differences were observed on rectal temperature, pulse and respiratory rates between the pre- and post-immunization values.

Table 1. Sero-surveillance of Peste des Petits Ruminants (PPR) in sheep and goats

S/N	Area	Species	No. of animals tested	No. of animals with antibody titre								Positive (titre ≥ 50) No. (%)
				1-10	11-20	21-30	31-40	41-50	51-60	61-70	71 - 80	
①	Shesmor	Sheep	10	4	3	2	1	0	0	0	0	0 (00.00)
		Goat	10	3	5	2	0	0	0	0	0	0 (00.00)
②	Digarkanda	Sheep	10	1	0	1	0	1	2	3	2	7 (70.00)
③	Chatrapur	Sheep	10	0	1	0	1	2	2	3	1	6 (60.00)
		Goat	10	0	0	1	2	3	3	1	0	4 (40.00)
		Goat	10	1	0	2	1	4	2	0	0	2 (20.00)
④	Boyra	Sheep	10	2	1	0	3	2	0	2	0	2 (20.00)
		Goat	10	0	2	1	2	3	1	1	0	2 (20.00)
⑤	Baparipara	Sheep	10	2	0	1	2	3	1	0	1	2 (20.00)
		Goat	10	1	0	1	3	2	2	1	0	3 (30.00)
⑥	Iswargonj	Sheep	10	0	2	2	3	2	0	1	0	1 (10.00)
		Goat	10	1	0	1	2	1	3	2	0	5 (50.00)
⑦	Atherobari	Sheep	10	2	0	1	2	3	1	1	0	2 (20.00)
		Goat	10	2	0	0	3	4	1	0	0	1 (10.00)
⑧	Khalbala	Sheep	10	1	1	2	3	1	2	0	0	2 (20.00)
		Goat	10	1	0	2	3	2	1	1	0	2 (20.00)
⑨	Shivbari	Sheep	10	0	2	1	2	3	1	1	0	2 (20.00)
		Goat	10	1	3	0	2	2	2	0	0	2 (20.00)
⑩	Parangonj	Sheep	10	1	1	0	3	2	1	2	0	3 (30.00)
		Goat	10	0	1	2	1	2	3	1	0	4 (40.00)
	Total	Sheep	100	13	11	10	20	19	10	13	4	27 (27.00)
		Goat	100	10	11	12	19	23	18	07	0	25 (25.00)

Humoral immune response

The antibody titre against PPR in sheep and goats were determined at pre-immunization and at 7^{th}, 14^{th} and 21^{st} days of post-immunization and presented in Table 2. The occurrence of low level of natural antibodies against PPR in sheep (20.0 ± 6.78) and goats (14.0 ± 6.96) at pre-immunization status indicates that these experimental animals were appropriately selected for immunization study against PPR. The mean antibody titre at 7^{th}, 14^{th} and 21^{st} day of post-vaccination in sheep were 38.28 ± 4.34, 58.63 ± 3.60 and 68.27 ± 3.09 and in goats were 49.84 ± 4.37, 63.23 ± 3.64 and 76.60 ± 4.07, respectively. These findings indicate that the antibody titre in both the sheep and goats at post-immunization increased significantly ($p < 0.01$) up to the protective level (≥ 50). Although these immunized goats could not be challenged due to lack of virulent challenge PPR virus in the stock. The detection of protective level of antibody against PPR in the PPR-VAC® inoculated sheep and goats indicates that the vaccine has actively stimulated the immune system in the inoculated animals. This findings support the earlier observation of Das *et al*. (2007) who reported the efficacy of PPR-VAC® against natural PPR infection.

Haematological studies

To study the haematological effects of PPR-VAC® in the vaccinated small ruminants, haemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC) were determined at pre-immunization and at 7^{th}, 14^{th} and 21^{st} day of post-immunization (Table 2). No significant differences between the pre-and post-immunization values of Hb, ESR, PCV and TEC was observed in both the sheep and goats but insignificantly ($p > 0.05$) increased TLC was observed at 7^{th}, 14^{th} and 21^{st} day of post-immunization in comparison to pre-immunization values (Table 2). These results indicate that the PPR-VAC® antigen has actively stimulated the leukocytes for the production of humoral immune response.

Table 2. Clinical and haemato-biochemical responses (Mean ± SD) in sheep and goats immunized against PPR

S/N	Parameters	Species	No. of animals tested	Pre-immuni-zation	Post-immunization (days)		
					7	14	21
(1)	Temperature (^{0}F)	Sheep	10	101.90 ± 0.91	102.39 ± 0.38	102.18 ± 0.15	102.76 ± 1.28
		Goat	10	102.13 ± 0.54	102.46 ± 0.40	102.36 ± 0.45	102.58 ± 0.39
(2)	Pulse (/ minute)	Sheep	10	075.60 ± 3.86	078.60 ± 4.43	075.60 ± 3.09	072.00 ± 1.09
		Goat	10	076.80 ± 4.73	076.80 ± 3.79	077.40 ± 4.42	076.80 ± 4.73
(3)	Respiration (/ minute)	Sheep	10	018.30 ± 3.30	020.70 ± 2.31	010.80 ± 2.15	018.20 ± 1.99
		Goat	10	026.00 ± 2.21	027.60 ± 3.71	028.20 ± 3.58	027.70 ± 2.40
(4)	Antibody titre	Sheep	10	020.00 ± 6.78	038.28* ± 4.34	058.63* ± 3.60	068.27* ± 3.09
		Goat	10	014.00 ± 6.96	049.84* ± 4.37	063.23* ± 3.64	076.60* ± 4.07
(5)	Haemoglobin (Hb, g %)	Sheep	10	012.52 ± 1.08	013.06 ± 1.25	013.33 ± 1.15	013.46 ± 1.16
		Goat	10	012.41 ± 1.03	012.87 ± 0.99	013.09 ± 0.92	013.39 ± 0.90
(6)	ESR (mm / in 1st hr.)	Sheep	10	000.80 ± 0.26	001.87 ± 1.31	001.05 ± 0.25	001.12 ± 0.29
		Goat	10	000.75 ± 0.23	000.90 ± 0.26	001.02 ± 0.36	000.95 ± 0.28
(7)	PCV (%)	Sheep	10	032.52 ± 2.83	033.78 ± 3.35	033.90 ± 3.20	032.96 ± 7.13
		Goat	10	024.90 ± 4.19	026.54 ± 1.62	026.60 ± 1.51	026.92 ± 1.34
(8)	TEC (10^6/ mm^3)	Sheep	10	010.04 ± 1.41	011.25 ± 1.81	011.72 ± 1.79	011.95 ± 1.83
		Goat	10	012.61 ± 1.45	013.18 ± 1.14	014.78 ± 3.46	014.18 ± 0.98
(9)	TLC (TLC, (10^3 / mm^3)	Sheep	10	007.95 ± 0.97	008.77 ± 1.57	009.47 ± 1.54	010.08 ± 1.55
		Goat	10	009.00 ± 1.28	009.92 ± 1.15	014.76 ± 0.84	011.50 ± 1.05
(10)	Calcium (mg /dl)	Sheep	10	006.16 ± 0.75	006.07 ± 0.70	006.28 ± 0.64	006.21 ± 0.46
		Goat	10	006.13 ± 0.18	006.14 ± 0.23	006.15 ± 1.11	005.91 ± 0.97
(11)	Albumin (g / dl)	Sheep	10	003.25 ± 0.46	003.11 ± 0.17	003.19 ± 0.56	033.51 ± 0.52
		Goat	10	003.18 ± 0.28	003.43 ± 1.02	003.11 ± 0.41	003.18 ± 0.71
(12).	TSP (g / dl)	Sheep	10	007.66 ± 0.79	007.71 ± 1.04	007.75 ± 1.09	008.43 ± 0.64
		Goat	10	007.89 ± 0.92	007.47 ± 0.95	006.97 ± 2.34	008.26 ± 0.50
(13)	Glucose (mg / dl)	Sheep	10	071.37 ± 19.10	067.80 ± 11.41	072.21 ± 12.41	071.96 ± 9.13
		Goat	10	072.69 ± 15.61	069.80 ± 10.81	066.82 ± 12.68	071.49 ± 13.51

*Significantly difference at ($p < 0.01$).

Biochemical studies

The serum calcium, albumin, total serum protein (TSP) and glucose were determined in sheep and goats at pre-immunization and at 7th, 14th and 21st day of post-immunization with PPR-VAC® and the results are presented in Table 2. No significant difference was observed in any of the investigated biochemical constituents in sheep and goats due to immunization against PPR. But the TSP in both the sheep (8.43 ± 0.69 g / dl) and goats (8.26 ± 0.50 g/dl) at 21st day of post-immunization increased insignificantly ($p > 0.05$) in comparison to the pre-immunization values of sheep (7.66 ± 0.79 g/dl) and goats (7.89 ± 0.92 g /dl) which might be due to humoral immune response for the production of immunoglobulin antibodies against inoculated PPR virus antigens.

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