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Effects of PPR Vaccine on Goat Haematology in Tangail District of Bangladesh

Ausraful Islam[a]*, Shankar Majumder[b], Amitav Singha[a] and Muhammad Kamrul Islam[c]

[a]Mennonite Central Comittee, Bangladesh

[b]Department of Agricultural Statistics, Faculty of Agricultural Economics and Rural Sociology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

[c]Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

[d]GPO Box 128, Dhaka - 1000, Bangladesh

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Abstract. The present study was conducted during the period May 26, 2007 to July 2, 2007 to observe the blood parameters of 18 she goats of the same age after vaccination with PPR vaccine after challenge with field virus. The within-subjects test indicated a significant ($P<0.01$) time effect on TEC. The interaction effect of vaccine on TEC over time was significant ($P<0.05$). The between subject effect of vaccine on TLC was significant ($P<0.01$). That is, the mean values of TLC at different doses of vaccine varied significantly. Double dose of vaccine reduced TLC to a significant ($P<0.05$) extent with respect to single dose and single dose boosting after 7 days of vaccination. Hb at the 1st, 2nd, 3rd and 4th observations were computed and the reduction of Hb estimation at each of 7 days duration was significant ($P<0.05$). The within-subjects test showed a significant ($P<0.01$) time effect on Hb of goats. Hb concentration declined cubicly over time. The interaction effect of vaccine on Hb over time was significant ($P<0.01$). The increment of ESR was recorded as significant ($P<0.05$) at 3rd and 4th observations. The within-subjects effects on ESR reveal that time had a significant ($P<0.01$) effect on ESR. The values of ESR changed in a quadratic pattern over time. The test of within-subjects effects showed a significant ($P<0.01$) time effect on the values of PCV of goats. The values of PCV changed in a linear form over time which reveals that the PPR vaccine affected the hematological parameters of the goats at different patterns over time.

Keywords: peste des petits vaccine, goats, heamatology, Bangladesh

Introduction

Goat is an economically valuable animal for the poor people of Bangladesh. At present the approximate number of goats in our country is 33.5 million (Samad, 2001). The local indigenous goat breed "Black Bengal" is highly prolific and a good survivor (Devandra, 1979). However, among many constraints of goat production in our country PPR (Peste des Petits Ruminants) is the most dangerous one (Kamaruddin and Islam, 2005). Outbreaks of PPR are common in South-East Asia including Bangladesh (Taylor *et al*., 1990). The prevalence of this disease is higher in young animals (65.58%) than in adults (45.92%) (Rajeswari *et al*., 2000). Prevalence of PPR is higher in Black Bengal goats (67-74%) than in Jamunapari goats (32.76%) (Mondal *et al*., 1995).

Hematological tests are widely used for the diagnosis of various animal diseases (Tibbo *et al*., 2004) that help in determining the nature of disease and the response of the defense mechanism of the patient (Schalm *et al*., 1975). Various workers have reported on the treatment, vaccine development and diagnosis of PPR of goats. But there is very limited study on the hematological values of animals vaccinated with different doses of PPR vaccine. Therefore, the present study was undertaken to observe the blood parameters of goats after vaccination and after challenge with field virus.

Materials and Methods

Study area and animal management. The study was conducted during the period May 26, 2007 to July 2, 2007 at Modhupur of Tangail district, Bangladesh. A total of 18 female, apparently healthy goats of the same age (4-6 months) were purchased from different places of Tangail district. The goats bought from each market were kept in separate houses and observed for at least 15 days. All the goats were identified by hanging a labeled tag around their neck. The feeding program was mainly intensive, where all the goats were supplied

*Author for correspondence; E-mail: rajibdvmpara@gmail.com

[d]Permanent address

with ample amount of water, enough grass and leaves and 100 g concentrate/goat/day. To avoid the effect of parasitism on normal hematological parameters, all the goats were dewormed with a potentiated anthelmintic containing levamisole and triclabendazole.

Study design and data collection. Goats were divided into six groups. Sampling was repeated for 4 times such as seven days pre vaccination, seven days post vaccination, 14 days post vaccination and seven days after challenge with PPR virus.

As the restraining of animals affects the hematological values, blood was collected from the animals at rest, with minimum excitement or disturbance (Jain, 1993). This was done by allowing the animals to rest for at least 4 min of an adaptation time before sampling. The sampling time was fixed at 9.00 a.m. to avoid the effect of diurnal variation (Tibbo *et al*., 2004) and sampling following the order of same serial number each time.

5 mL of blood was drawn from the external jugular vein by venipuncture of the 18 goats. The blood samples from each goat were taken into a dry, clean, vacuum and labeled tube containing anticoagulants.

Method of vaccination. One hundred dose vial of freeze-dried PPR vaccine (LRI, Mohakhali) was reconstituted in 100 mL of sterile refrigerated solution supplied with the vaccine. The reconstituted vaccine was injected into a goat subcutaneously in the neck region, with a sterile needle following all the instructions of the manufacturer.

Laboratory analysis. Packed Cell Volume (PCV) and Hemoglobin estimation (Hb) were determined by microhaematocrit and cyanomethemoglobin methods, respectively (Ghai, 1999). Total Leukocyte Count (TLC) was performed according to the method described by Schalm *et al*. (1975). ESR (Erythrocyte Sedimentation Rate) and TEC (Total Erythrocyte Count) were determined following the procedures described by Ghai (1999).

Challenge with virus. After 14 days of post vaccination all the goats were challenged with PPR virus collected from research Institute. One hundred dose vial of freeze-dried PPR virus was reconstituted in 100 mL of sterile refrigerated solution. 3 mL of the reconstituted virus was injected into a goat subcutaneously in the neck region, with a sterile needle.

Statistical analysis. Repeated Measures Analysis of Covariance (RMANCOVA) in Completely Randomized Design (CRD) was performed to investigate the effect of different doses of vaccine on various blood parameters of Black Bengal goats (Zar, 2002). Body weight of the goat was incorporated in the analysis as the covariate for adjusting the effects of the unequal body weights on the hematological parameters viz., TEC, TLC, Hb, ESR and PCV (Table 1). In the analysis, the dependent variables were these parameters. The hematological parameters were considered as the within subjects factors because they were repeatedly measured on the experimental goats. Vaccine (independent variable) was treated as the between subjects factor. The sphericity test was done to identify which one was the most appropriate: the MANOVA or the univariate test. The sphericity assumption states that the variance of the difference scores in a within subjects design (S_d^2 in a paired t-test) are equal across all the groups. That is, the variances of each of these sets of difference scores are not statistically different from one another. Mauchly's test of sphericity was performed in this study (Table 2). According to this test, if the significant level of any parameter is greater than 0.05, the sphericity assumption is met and hence univariate test results can be accepted for repeated measures ANOVA. In Mauchly's test of

Table 1. The mean value* (adjusted for unequal body weights) with its standard error of TEC, TLC, Hb, ESR and PCV at different doses of vaccine

Parameters	Single dose	Double dose	Single dose boosting after 7 days
TEC	8.87 ± 0.121	9.06 ± 0.118	9.03 ± 0.119
TLC	1005.63^{a} ± 8.692	974.17^{b} ± 8.533	1017.29^{a} ± 8.593
Hb	8.62 ± 0.113	8.71 ± 0.111	8.60 ± 0.112
ESR	4.97 ± 0.180	5.18 ± 0.176	5.23 ± 0.178
PCV	20.47 ± 0.506	20.88 ± 0.497	21.01 ± 0.501

*Mean values were compared by Least Significant Difference (LSD) test. Mean values having different superscripts differed significantly at 1% level of probability.

Table 2. Mauchly's test of sphericity

Parameters	Within subject effect	Mauchly's W Chi^2	Approx.	df	Sig	Epsilon	
						Greenhouse-Geisser	Huynh-Feldt
TEC	Time	0.603	6.425	5	0.268	0.759	1
TLC	Time	0.593	6.653	5	0.249	0.729	1
Hb	Time	0.672	5.056	5	0.410	0.820	1
ESR	Time	0.573	7.081	5	0.126	0.795	1
PCV	Time	0.814	2.614	5	0.76	0.891	1

sphericity, the value of epsilon should be greater than 0.75 (1.00 indicate perfect sphericity). Where the value of epsilon was less than 0.75, the results of the Huynh-Feldt corrections of univariate tests were used (Max and Onghena, 1999). All of the multiple comparisons of the various parameters were performed by least significant difference (lsd) test after adjustment for unequal body weights of the experimental goats.

Results and Discussion

Effect on TEC. The within-subjects test indicates that there was a significant (P<0.01) time effect on TEC of goats and TEC significantly (P<0.05) decreased over time at a cubic pattern (Table 3-4). The 3rd and 4th observations exhibited a significant (P<0.05) reduction in the mean values of TEC with respect to 1st and 2nd observations (Table 5). The interaction effect of vaccine on TEC over time was significant (P<0.01) and it acted quadratically (Table 3 and 4). According to Devireddy *et al*. (1999) PPR virus contains a protein which causes lysis of erythrocytes. It might be occurred due to the cause that the values of TEC decreased after vaccination and after challenge with the virus as the PPR vaccine contained live attenuated viruses. Besides, the PPR antigen might attach to erythrocyte membrane making the cells more susceptible to phagocytosis by macrophages resulting in decreased number of RBC count (Aikhuomobhogbe and Orheruata, 2006).

Effect on TLC. Pairwise comparison of TLC for different doses of vaccine has been shown in Table 6. The between-subjects effects of vaccine on TLC was significant (P<0.01) (Table 7). It is evident from the Table 1 and 7 that there existed a significant (P<0.01) difference among the effects of different doses of vaccine and double dose of vaccine reduced TLC to a significant (P<0.05) extent with respect to other two doses. The reduction in leukocyte numbers occurred possibly due to their response to vaccination (Aikhuomobhogbe and Oreruata, 2006). This also might be due to the immunosuppressive effect of the PPR virus (Haroun *et al*., 2005; Heaney *et al*., 2002) of the vaccine. This finding is similar to that of Rajak *et al*. (2005).

Effect on Hb estimation. The within-subjects test implies that there existed a significant (P<0.05) time effect on Hb concentration of goats (Table 3). Table 5 demonstrates that the reduction of Hb concentration at each of 7 days duration was significant (P<0.05). Hb concentration declined cubicly over time (Table 4). Hb concentration decreased after vaccination and after challenge with virus. According to Benjamin (1978) hemoglobin concentration might be decreased if there was reduction in the size of RBC rather than number. But in this case it can be hypothesized that the hemoglobin concentration decreased due to the reduction in the number of RBC. In another way it can be said that as TEC decreased, the Hb concentration decreased as well.

Table 3. Tests of within-subjects effects on the hematological parameters TEC, TLC, Hb, ESR and PCV

Source		F-values				
		TEC	TLC	Hb	ESR	PCV
Time	Sphericity assumed	9.17**	-	6.041**	43.409**	14.069**
	Huynh-Feldt	-	2.879*	-	-	-
Time * Body weight	Sphericity assumed	1.828	3.780*	3.599*	43.409**	1.259
Time * Vaccine	Sphericity assumed	3.156*	2.468*	0.536	0.727	1.567

**indicates significant difference at 1% level of probability; *indicates significant difference at 5% level of probability

Table 4. Tests of within-subjects contrasts for TEC, Hb, ESR and PCV[a]

Parameter	Source	TIME	F
TEC	Time	Cubic	5.662*
	Time*Vaccine	Quadratic	4.237*
Hb	Time	Cubic	8.495*
	Time*Body weight	Cubic	9.350*
ESR	Time	Quadratic	31.686**
PCV	Time	Linear	50.651**

**indicates significant at 1% level of probability; *indicates significant at 5% level of probability; [a]Other parameters were not mentioned because of insignificant effect\patterns on them.

Effect on ESR. The within-subjects effects on ESR revealed that time had a significant (P<0.001) effect on ESR (Table 3). Time influenced ESR in a quadratic pattern (Table 3-4). ESR increased as the time duration increased after vaccination. The increment was recorded as significant (P<0.05) in 3rd and 4th observations (Table 5). But ESR increased to a great extent in the 4th observation (Table 5). In this study, no prevaccination sedimentation of erythrocytes was observed. But after vaccination and after challenge with the vaccine settling of RBC was observed. The virus caused hemolysis of RBC (Devireddy *et al*., 1999) which led to an increased ESR. According to Jain (1986), ESR increases in inflammatory conditions and in acute generalized infection, with change in concentration of various proteins in blood. It can also be stated that increased ESR was due to the decreased TEC. This finding was similar with that of Hayat *et al*. (1999).

Table 5. Pairwise comparisons[b] of TEC, TLC, Hb, ESR and PCV at different observations

(I)	(J)	Mean difference[a] (I-J) for the parameters				
Time	Time	TEC	TLC	Hb	ESR	PCV
1	2	0.022	-34.722*	0.311*	-0.333	4.667*
	3	1.588*	12.500	0.867*	-1.528*	6.889*
	4	2.434*	-75.000*	2.044*	-18.639*	25.083*
2	1	-0.022	34.722*	-0.311*	0.333	-4.667*
	3	1.566*	47.222*	0.556*	-1.194*	2.222*
	4	2.413*	-40.278*	1.733*	-18.306*	20.417*
3	1	-1.588*	-12.500	-0.867*	1.528*	-6.889*
	2	-1.566*	-47.222*	-0.556*	1.194*	-2.222*
	4	0.847*	-87.500*	1.178*	-17.111*	18.194*
4	1	-2.434*	75.000*	-2.044*	18.639*	-25.083*
	2	-2.413*	40.278*	-1.733*	18.306*	-20.417*
	3	-0.847*	87.500*	-1.178*	17.111*	-18.194*

[a]The mean difference is significant at 5% level of probability; [b]Multiple comparisons: LSD test.

Effect on PCV. It could be inferred from the test of within-subjects effects that there existed a significant (P<0.001) time effect on the values of PCV of goats and PCV value decreased over time following a linear trend pattern (Table 3-4). Table 5 reveals that PCV value reduced significantly (P<0.01) at each observation. The PCV values reduced at each observation that is after starting of vaccination. The attachment of PPR antigen to erythrocyte membrane rendered the cells more susceptible to phagocytosis by macrophages, thus resulting in decreased PCV values (Aikhuomobhogbe and Oreruata, 2006).

Table 6. Pairwise comparisons[b] of TLC for different doses of vaccine

Vaccine(I)	Vaccine (J)	Mean differenc[a] (I-J)	Standard error (SE)	P-value	95% Confidence interval for difference	
					Lower bound	Upper bound
Single dose	Double dose	31.466*	12.265	.022	5.161	57.772
	Boosting after 7 days	-11.657	12.390	0.363	-38.231	14.918
Double dose	Single dose	-31.466*	12.265	0.022	-57.772	-5.161
	Boosting after 7 days	-43.123*	12.053	0.003	-68.974	-17.272
Boosting after 7days	Single dose	11.657	12.390	0.363	-14.918	38.231
	Double dose	43.123*	12.053	0.003	17.272	68.974

Body weights were adjusted for multiple comparisons: LSD; [a]The mean difference is significant at 5% level of probability.Pairwise comparisons of the other parameters were not presented because of their insignificant mean differences at different doses of vaccine.

Table 7. Tests of between-subjects effects on TEC, TLC, Hb, ESR and PCV (adjusting unequal body weights)

Source	F-values with the levels of significance for the parameters				
	TEC	TLC	Hb	ESR	PCV
Vaccine	0.247 (0.737)	6.865 (0.008)	0.283 (0.758)	0.577 (0.575)	0.375 (0.694)

In conclusion, results of this study reveal that the PPR virus and PPR vaccine affected the goat haematology. Therefore, it is necessary to vaccinate the goats in order to protect them from the disease.

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