HAEMATOLOGICAL PROFILE FOLLOWING IMMUNOMODULATION DURING LATE GESTATION IN BUFFALOES (*BUBALIS BUBALUS*)

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ABSTRACT

Thirty-two adult riverine buffaloes (*Bubalis bubalus*) in their last trimester of pregnancy were selected and randomly divided into four groups. The buffaloes of group I served as control. Animals in group II, III and IV were treated twice (7 days apart) with levamisole hydrochloride (0.5mg/kg b.w. orally), Etosol (Vit E+Se, 10ml, I/m) and Bacilli Calmette Guerine (BCG) (0.5 ml/animal, s/c), respectively. Blood samples were collected at weekly intervals starting day 0 untill parturition. Total erythrocytic count and packed cell volume values were higher ($P<0.05$) in levamisole and vit E+Se treated group of buffaloes. Haemoglobin concentration was higher ($P<0.05$) in Vit E+Se treated group. MCV, MCH and MCHC remained unchanged among all the experimental groups. Total leukocyte count was higher ($P<0.05$) in levamisole treated group of buffaloes. Differential leukocyte counts (relative) revealed moderate lymphocytosis in all immunomodulated groups with significantly higher counts in Vit E+Se treated buffaloes. It was inferred that levamisole and vit E-se altered some haematological values, whereas BCG did not affect the haematological parameters.

Keywords: Haematological profile, immunomodulation, gestation, buffalo

INTRODUCTION

Haematological values vary according to physiological states, environmental factors and genetic differences. Advanced pregnancy, early lactation, raised concentration of progesterone throughout pregnancy and high level of corticosteroids around calving suppress the immune status of pregnant dam due to their lympholytic effect (Staples *et al.*, 1983; Lioyd, 1983; Tizard, 1987). Adjuvants and immunomodulators have attracted considerable interest in the field of immunotherapy. Various immunomodulators have been used in different animals to enhance their immune status with variable degree of success. Among these levamisole hydrochloride (Debowy *et al.*, 1988), vitamin E-Selenium (Larsen, 1988) and *Bacilli calmette-Guerin* (BCG) have been studied alone or in combination with vaccines for the enhancement of immunity (Qureshi *et al.*, 1999). However, there is paucity of information delineating the effect of these immunomodulators on the haematological profiles during gestation especially in buffaloes. Therefore, the present investigation was carried out to evaluate the comparative effect of three known immunomodulators, viz., levamisole hydrochloride, Vitamin E-Selenium and *Bacilli calmette Guerin* on haematological profiles during late gestation in Nili-Ravi buffaloes.

MATERIALS AND METHODS

Experimental animals and treatment schedule

Thirty-two adult Nili-Ravi buffaloes in their last trimester of pregnancy belonging to the Livestock Production Research Institute (LPRI), Bahadurnagar, Okara, Pakistan were selected. The buffaloes were randomly divided into four equal groups. All the experimental animals were kept under similar management conditions and were offered good quality seasonal green fodder (*Trifolium alexandrum* in winter and sorghum valgaral in summer) and water *ad libitum*. In addition, each animal was given 2.0 kg of concentrate daily. The animals in group I served as the control. Those in group II were treated orally with levamisole hydrochloride (Shahani Laboratories, Faisalabad) at the rate of 0.5 mg/kg body weight. Etosol (Aesculaap bv, Boxtel, Holland) 10ml containing 500 mg vit E and 15 mg selenium was administered intramuscularly to each buffalo in group III. Each buffalo in group IV was given 0.5 ml s/c of BCG (Pasteur Merieux, Lyon, France). Immunomodulators were given twice, one week apart, starting 80 days prior to the expected date of parturition which was counted as day 0.

Blood sampling and haematology

Blood was collected from all the experimental animals at weekly intervals starting from day 0 till parturition. Total leukocytic count (TLC) and total

erythrocytic count (TEC) were determined by the haemocytometer method. Haemoglobin (Hb) concentration was determined by the acid hematin method using Sahli's apparatus. Packed cell volume (PCV) was determined by using microhematocrit. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were calculated using the results of red blood cell (RBC) count, haemoglobin and packed cell volume. Erythrocyte sedimentation rate (ESR) was determined using Westergren tube method (Benjamin, 1978), after one hour of filling. Differential leukocytic count (DLC) was obtained on wright's stained blood films (Schalm *et al.*, 1975). The statistical differences between groups on weekly basis were calculated by the analysis of variance test using completely randomized design (Steel and Torrie, 1980). The significant means were compared with Duncan's Multiple Range Test (Duncans, 1955). The differences were considered significant at 5 per cent level ($P<0.05$).

RESULTS

The mean ± SE values for erythrocytic indices in control and immunomodulated groups are presented in Table 1. The total erythrocyte count (TEC) and packed cell volume (PCV) were higher ($P<0.05$) in levamisole hydrochloride and vitamin E-Se treated buffaloes. Haemoglobin (Hb) concentration was higher ($P<0.05$) in vit E-Se treated group compared to rest of the three groups. The values of MCV, MCH and MCHC remained unaltered among all experimental groups. Erythrocyte sedimentation rate was higher ($P<0.05$) in control and levamisole treated group of buffaloes compared to vit E-Se and BCG treated buffaloes.

The results of leukocytic indices (Table 2) indicated that the TLC was higher ($P<0.05$) in levamisole treated group of buffaloes compared to rest of the three groups. Eosinophil percentage was higher ($P<0.05$) in vit E-Se and BCG treated group of buffaloes. Monocyte percentage was higher ($P<0.05$) in control group of buffaloes followed by levamisole hydrochloride treated group of buffaloes. Lymphocyte percentage was higher ($P<0.05$) in vit E-Se treated group of buffaloes, compared to control group. Neutrophils and basophils counts did not differ among control and treatment groups.

DISCUSSION

The investigations regarding the effect of immunomodulators on the haematological parameters in bovines are limited in non-pregnant water buffalo (Verma and Joshi, 1994) and Sahiwal cows (Asif *et al.*, 1995). Generally, the packed cell volume of pregnant animals has been reported to be higher than that of non-pregnant ones (Jain *et al.*, 1982). This increase during pregnancy was described due to increased requirement of oxygen by the foetus (Coles, 1987). A higher PCV might result either from increased red blood cell (RBC) count or increased mean corpuscular volume. In the present study, a higher ($P<0.05$) PCV in levamisole and vit E-Se treated buffaloes was also accompanied by the higher ($P<0.05$) RBC count in these groups suggesting that increased RBC count and not the corpuscular volume was responsible for higher PCV. However, Verma and Joshi (1994) reported a slight increase in PCV and RBC following levamisole administration (2.5mg/kg b.wt) co-administered with *Haemmorhagic Septicemia* vaccine in non-pregnant buffaloes. The difference could be attributed to the different physiological states, as the later study was conducted on non-pregnant animals. Asif *et al.* (1995) also reported a significant increase in PCV accompanied by increased RBC count after 24 hours following levamisole administration in Sahiwal heifers. Hb concentration in present study was also higher ($P<0.05$) in vit E-Se treated group of buffaloes suggesting that this immunomodulating substance had an acceleratory effect on haemopoietic activity. Sodium selenite is a known immunomodulator and increases the PCV, Hb and RBC count in goats (Ahmed *et al.*, 1988). Vitamin E is also known to increase the RBC and WBC number in prolonged treatment (Hardie *et al.*, 1990). Similarly, the haemopoiesis enhancing effect of levamisole hydrochloride in the present study is also in accordance with Renoux and Renoux (1973).

Haemoglobin is known to decrease in the later half of pregnancy (Morris, 1994). An increase in Hb level in the last trimester especially in vit E-Se treated group in the present study may be of significance in enabling the dam to cope with parturition stress. The MCV of all experimental groups in the present study showed non-significant difference. Variation in haemoglobin synthesis is reflected by increase or decrease in MCH and MCHC values (Benjamin, 1978). An overall non-significant difference in MCH and MCHC of different groups in the present study suggest that immunomodulation did not affect the Hb synthesis. The present observations on MCH and MCHC differed from those of Verma and Joshi (1994), who reported a significant increase in the MCH and MCHC in non-pregnant buffaloes. It may be possible that mechanism of action of immunomodulators in pregnant and non-pregnant animals is different.

Present study revealed that immunomodulation with levamisole hydrochloride increased the total leukocytic count. These results differed from those of Verma and Joshi (1994) who reported that TLC was not affected by immunomodulation with levamisole in non-pregnant animals. Sodium selenite has been reported to increase TLC in goats (Ahmad *et al.*, 1988) but in the present study the effect of vitamin E and selenium was non-significant. A difference in the dose of selenium, route of administration and specie difference may be among the possible causes/factors.

Table 1: Mean ± SE values of erythrocytic indices after immunomodulation with levamisole hydrochloride, Vit. E-Se and BCG in pregnant Nili Ravi buffaloes.

Parameters	Experimental groups			
	Control	Levamisole	Vit. E-Se	BCG
Total erythrocytic count (10^{12}/L)	5.3 ± 0.22 b	6.4 ± 0.23 a	6.1 ± 0.15 a	5.6 ± 0.12 b
Hemoglobin (g/dL)	10.7 ± 0.22 b	10.7 0.15 b	11.8 ± 0.17 a	10.8 ± 0.2 b
Packed cell volume (PCV)	27.2 ± 0.68 b	29.10 ± 0.39 a	29.9 ± 0.6 a	26.4 ± 0.57 b
MCV (fl)	50.1 ± 2.22	51.4 ± 1.81	49.8 ± 1.50	43.8 ± 1.17
MCH (pg)	22.2 ± 1.16	19.6 ± 0.76	20.0 ± 0.56	19.7 ± 0.49
MCHC (g/dL)	39.8 ± 1.08	37.5 ± 0.72	40.6 ± 0.78	37.4 ± 1.32
ESR (mm/hr)	99.0 ± 4.05 a	96.6 ± 4.50 a	74.7 ± 1.2 b	86.1 ± 3.99 b

Values sharing same letter in a row do not differ significantly (P<0.05).

Table 2: Mean ± SE values of leukocytic indices after immunomodulation with levamisole hydrochloride, Vit. E-Se and BCG in pregnant Nili-Ravi buffaloes.

Parameters	Experimental groups			
	Control	Levamisole	Vit. E-Se	BCG
Total leukocyte count (10^3/mm^3)	8.9 ± 0.33 b	11.8 ± 0.60 a	7.3 ± 0.16 b	5.6 ± 0.12 b
Neutrophils (%)	23.7± 0.65	25.0 ± 0.96	23.3 ± 0.85	25.9 ± 0.84
Eosinophils (%)	3.4 ± 0.33 b	2.4 ± 0.23 b	4.8 ± 0.44 a	4.4 ± 0.35 a
Basophils (%)	0.51 ± 0.07	0.51 ± 0.07	0.46 ± 0.06	0.40 ± 0.07
Monocytes (%)	9.1 ± 0.48 a	7.3 ± 0.39 b	3.4 ± 0.27 c	4.7 0.32 c
Lymphocytes (%)	63.4 ± 0.78 b	66.0 ± 0.81 ab	67.6 ± 1.03 a	64.7 ± 1.02 ab

Values sharing same letter in a row do not differ significantly (P<0.05).

BCG treatment in the present study did not result in any significant increase in TLC (Table 2). In cats administration of immunoregulin (*propinobacterium acne*) resulted in increased TLC (Cox, 1988). Among the circulating leukocytes, the lymphocytes are responsible for humoral and cellular immune responses. An increase in their number in blood may be a good indicator of an immunomodulatory response. Present study showed an increase in lymphocytes in the all immunomodulated groups. However, the increase was significant (P<0.05) only in the animals treated with vit E-Se as compared with that of control. An increased TLC has also been reported following administration of vit E-Se in goats (Ahmad *et al.*, 1988) and Chicken (Bashir *et al.*, 1994). In all the immunomodulated groups, monocyte percentage in circulating blood significantly lowered as compared with control. One possible reason may be an enhanced lymphocyte percentage in these groups. A relative decrease in monocytes and non-significant changes in neutrophils is an indication of an absolute increase in lymphocyte number. Information about effect of immunomodulation upon eosinophil is scanty. Verma and Joshi (1994) reported eosinophilia in non-pregnant buffaloes treated with levamisole. Results of present study indicated a slight increase in eosinophil number in animals treated with vit E+Se and BCG but not in those treated with levamisole hydrochloride. The exact mechanism by which eosinophil number is increased by immunomodulators is not clear but it can be postulated that vit E-Se and BCG might have induced liberation of histamine in body which subsequently resulted in increased number of eosinophils.

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