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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (3): 1024-1026, 1999 Research Article

Effect of Experimentally Induced Coccidiosis on Some Blood Parameters of Buffalo Calves

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Abstract
The influence of the disease on various blood parameters was studied in 4-6 months old buffalo calves experimentally infected with coccidiosis. The oocysts of *Eimeria* species appeared in faeces on day 13th post infection with their peak counts on day 16th post infection. A significant decrease in TEC, PCV, Hb concentration, MCV, MCHC and lymphocytes and increase in ESR, TLC, eosinophils and neutrophils was observed as compared with control. No significant effect on eosinophils and monocytes was recorded.

Introduction
Coccidia infection cause diarrhoea, anaemia and high mortality in buffalo calves (Blood *et al*., 1983). There is a paucity of information on the high prevalence of coccidiosis and its effect on different blood parameters in buffaloes calves. The present study reports the haematological changes in buffalo calves infected with coccidiosis. The study will provide information both to the farmers as well as clinicians to institute appropriate supportive therapy in affected animals.

Materials and Methods
Collection and sporulation of oocysts: The coccidial oocysts were collected from the faeces of infected buffalo calves brought for treatment at various veterinary hospitals in and around Faisalabad. The infected faeces were processed for culture at the laboratory of Department of Veterinary Parasitology, University of Agriculture, Faisalabad. The sporulation of the coccidial oocysts for species characterization was carried out in 2.5 percent potassium dichromate ($K_2Cr_2O_7$) solution at room temperature. The sporulated oocysts were identified and stored at 4°C for further use as inoculum in the experiment.

Experimental animals: Twelve male buffalo calves (4-6 months old) having good physical condition with no history of illness were procured form the market. These calves were rendered free from any parasitic infection and maintained till used in the experimental studies. They were fed on green fodder and concentrate ration containing 65% wheat bran, maize oil cake, cotton seed cake, maize gluten, molasses, bone meal and 35 percent wheat straw. Fresh water was provided *ad libidum*. The animals were divided randomly into two groups viz. A and B comprising of six calves each. The group A was infected with $1x10^5$ sporulated oocysts orally and the group B served as uninfected control. Both the groups were provided with the similar environmental and managemental conditions. The faecal examination of the experimental calves was conducted daily upto day 30th post-infection (pi).

Haematological Parameters: Blood samples of both the groups were collected from jugular vein in 10 ml sterilized tubes containing heparin (Benjamin, 1978) at five different intervals during the experiment as under:

1. First day after infection.
2. First appearance of oocysts in faeces.
3. At the maximum counting of oocysts in faeces.
4. After coccidiostat therapy.
5. No appearance of oocysts in faeces.

Blood parameters included total erythrocytic count (TEC), haemoglobin estimation (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocytic count (TLC) and differential leukocyte count (DLC) were analyzed (Benjamin, 1978). The data was subjected to statistical analysis for two factor of analysis of variance and DMR test (Steel and Torrie, 1980).

Results
The results of haematological observations are depicted in Table 1. As judged by the results, a highly significant decrease ($p<0.001$) of TEC was observed in the infected calves from 7.68 to 4.86 million/µl by day 1 to 16 pi. At the peak of infection, when animals were treated with coccidiostat, the TEC started increasing and normalized (8.08 million/µl) on day 30th pi.

A highly significant ($p<0.001$) decrease in the concentration of Fib was observed in the infected calves from 11.22 to 5.93 gm/100 ml of blood, indicated hypochromic anaemia during the peak of infection (day 16th pi). After coccidiostat therapy, these values lightly improved (7.91 gm/100 ml) on day 30th pi. The PCV decreased significantly ($p<0.001$) from 37.16 to 28.83 percent in 16 days in the challenged calves. The PCV attained an increasing pattern after coccidiostat therapy and by day 30th pi, it resumed the normal position.

The ESR increased significantly ($p<0.001$) from 27.3 to 41.3 rem/hour at the peak of infection in infected calves

Table 1: Mean ± S.E. of various blood parameters at different stages of the experiment

Parameters	Animal groups	Days post-infection				
		1	13	16	22	30
TEC (million/µl)	A	7.68 ± 0.154[a]	5.76 ± 0.203[b]	4.86 ± 0.295[c]	5.53 ± 0.233[b]	8.08 ± 0.070[a]
	B	7.63 ± 0.209[a]	7.95 ± 0.171[a]	8.01 ± 0.187[a]	8.10 ± 0.021[a]	8.16 ± 0.095[a]
Hb (gm/100 ml)	A	11.227 ± 0.210[a]	7.133 ± 0.313[e]	5.933 ± 0.099[f]	6.600 ± 0.231[c]	7.917 ± 0.091[d]
	B	10.167 ± 4.458[b]	8.333 ± 0.246[cd]	8.567 ± 0.289[c]	8.400 ± 8.179[cd]	8.783 ± 0.138[c]
PCV (%)	A	37.167 ± 1.014[abc]	27.500 ± 0.099[bc]	28.833 ± 10.261[bc]	27.167 ± 0.833[c]	40.500 ± 0.619[a]
	B	37.167 ± 1.167[abc]	37.500 ± 1.384[abc]	36.500 ± 1.176[abc]	38.000 ± 1.183[abc]	39.000 ± 1.155[ab]
ESR (%)	A	27.333 ± 0.882[e]	34.333 ± 1.174[de]	41.333 ± 1.054[de]	38.833 ± 0.601[d]	36.333 ± 1.406[c]
	B	25.167 ± 1.447[de]	28.333 ± 1.406[c]	27.667 ± 1.333[a]	29.167 ± 1.046[ab]	29.333 ± 0.667[bc]
MCV (fl) A	A	48.360 ± 0.820	48.257 ± 3.512	38.512 ± 2.172	49.598 ± 2.928	49.998 ± 1.034
	B	40.755 ± 0.316	47.195 ± 1.588	45.747 ± 1.399	46.940 ± 1.521	47.777 ± 1.460
MCH (Pg)	A	14.628 ± 0.444[a]	12.473 ± -0.784[bc]	12.437 ± 0.876[bc]	11.553 ± 0.894[cd]	9.795 ± 0.143[d]
	B	13.530 ± 0.422[ab]	10.497 ± 0.352[d]	10.695 ± 0.366[d]	10.375 ± 0.281[d]	10.760 ± 0.232[d]
MCHC (g/dl)	A	30.285 ± 0.975[ab]	26.165 ± 1.756[cd]	32.390 ± 1.618[a]	24.485 ± 1.426[de]	19.560 ± 0.286[f]
	B	27.852 ± 1.111[bc]	22.458 ± 1.423[ef]	23.598 ± 1.147[de]	22.163 ± 0.567[ef]	22.630 ± 0.866[ef]
TLC (000/cu mm)	A	9666.66 ± 493.73[b]	12321.66 ± 392.71[a]	7116.66 ± 465.77[c]	11550.00 ± 502.49[a]	9566.66 ± 313.75[b]
	B	9533.33 ± 492.55[b]	8533.33 ± 328.54[b]	8691.66 ± 316.86[b]	8816.66 ± 311.35[b]	8613.33 ± 260.08[b]

p value = 0.001; Different superscript letters indicate difference

Table 2: Mean ± S.E. of differential leukocyte count (DEC) at different stages of the experiment

Parameters	Animal groups	Days post-infection				
		1	13	16	22	30
Neutrophils(%)	A	38.33 ± 1.96[b]	54.33 ± 2.20[a]	58.33 ± 1.62[a]	53.83 ± 2.08[a]	36.16 ± 1.01[b]
	B	38.83 ± 1.19[b]	35.66 ± 1.56[b]	37.50 ± 1.25[b]	36.16 ± 1.35[b]	36.50 ± 1.50[b]
Segmented	A	32.16 ± 0.98[c]	49.83 ± 2.07[b]	55.50 ± 1.25[a]	50.33 ± 1.85[b]	31.83 ± 1.22[c]
neutrophils (%)	B	31.16 ± 1.07[c]	31.83 ± 1.01[c]	32.16-I-1.35[c]	32.00 ± 1.06[c]	32.33 ± 1.14[c]
Eosinophils (%)	A	7.50 ± 0.80	5.83 ± 1.01	6.16 ± 0.98	4.50 ± 0.56	5.00 ± 0.57
	B	6.00 ± 0.85	5.16 ± 1.19	5.16 ± 0.83	4.33 ± 0.88	5.83 ± 0.01
Lymphocytes (%)	A	51.50 ± 1.31[a]	36.33 ± 2.92[b]	29.16 ± 1.49[c]	37.50 ± 2.02[b]	54.83 ± 1.49[a]
	B	50.66 ± 2.04[a]	55.83 ± 2.08[a]	52.33 ± 1.72[c]	55.33 ± 1.40[a]	53.50 ± 1.23[a]
Monocytes (%)	A	4.66 ± 0.76	3.66 ± 0.71	6.33 ± 1.08	4.16 ± 0.60	4.00 ± 0.51
	B	4.16 ± 1.01	3.50 ± 0.76	5.00 ± 1.06	4.16 ± 0.60	4.16 ± 0.60
Band	A	6.16 ± 1.07	4.50 ± 0.76	2.832 ± 0.60	3.50 ± 0.42	4.33 ± 0.66
neutrophils OM	B	7.66 ± 0.88	3.83 ± 1.04	5.33 ± 1.28	4.16 ± 0.60	4.16 ± 0.47

p value = 0.001; Different superscript letters indicate difference

when compared with non-infected calves. This trend, after coccidiostat therapy, returned to normal. (36.3 mm/hour) on day 30th pi. MCV was found significantly low ($p<0.001$) at peak of infection in the experimentally induced coccidiosis when compared with the control animals. The similar situation as in MCV was also observed with MHC in the infected calves It was observed that during the onset of infection, the MCHC values decreased significantly ($p<0.001$) from 30.28 to 26.16 gm/dl from day 1st to 13th pi. These values increased to 32.90 gm/dl at the peak of infection. The TLC was found to be increased significantly ($p< 0.001$) from 9666.66 to 12321.66/cu mm on day 13th pi. At the peak of infection, the count decreased significantly ($p<0.001$) to 7116.66/cu mm. After coccidiostat therapy, TLC increased to 11550.00/cu mm on day 22nd pi and resumed the normal position till the termination of experiment.

The results of DLC are presented in Table 2. The percentage of neutrophils increased significantly ($p<0.001$) from 38.33 to 58.33 percent on day 16th pi. After medication, the count decreased and became normal in the remaining two weeks. The count of segmented neutrophils increased significantly ($p<0.001$) form 32.16 to 55.50 percent in 16 days pi and after the medication, it started decreasing and normalized on day 30th pi. A non-significant decrease ($p>0.001$) in eosinophils was noted on day 13th pi after the onset of the infection, but after the coccidiostat administration, the count resumed the normal values. A significant decreased ($p<0.001$) in lymphocyte count was encountered which followed the same pattern as that of neutrophils. The monocytic count did not differ significantly ($p>0.001$) during the different stages of the experiment. The band neutrophils did not differ significantly ($p>0.001$) when compared with the count of the control animals. However, a significant decrease ($p<0.001$) in the number of band neutrophils was observed on day 16th pi.

Discussion

The decrease in different haematological values has been reported by several workers, for instance, Shommein and

Osman (1980), Begum (1981), Anwar *et al*. (1982), Sanyal and Ruprah (1984), Rama *et al*. (1978) and Malik (1987) observed a considerable fall in TEC in coccidiosis. The results of the present investigation are in accordance with the findings of these workers. This considerable decrease in TEC could be due to the blood loss from the haemorrahic intestinal mucosa and bloody diarrhoea. Hb and PCV values decreased because both are dependent upon erythrocytic count and with its decrease, the haemoglobin and packed cell percentage has decreased proportionally. Similar observations are made by Svanbaev and Gorbunova (1969), Fitzgerald and Mansfield (1984), Rama *et al*. (1978) and Begum (1981). The decrease in Hb and PCV values of the studies is not in line with the results of Pout (1965) in ovine coccidiosis with no significant change in both the parameters. This difference may be due to the low severity of the disease induced by a less pathogenic species of *Eimeria cranclallis* as compared to more pathogenic mixed species used in the present investigation. An increase in ESR due to hypoproteninaemia resulting from blood loss and digestive disturbances of the present studies are accordance with those of Anwar *et al*. (1982) and Malik (1987) who confirmed increased ESR in lambs and sheep infected with coccidiosis.

The decrease in MCV and MCHC can be attributed to some deficiencies of hemopoietic factors. MCV became normal after coccidiostat therapy showing macrocytic anaemia which is in agreement with the results of Shommein and Osman (1980) and Malik (1987) who have indicated significant increase in mean corpuscular volume. This increase in mean corpuscular volume may be due to the production of immature RBCs of larger size. The decrease in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration showing hypochromic anaemia in infected buffalo calves may be due. to loss of blood and low level of haemoglobin in erythrocytes. These results are in accordance with the results of Malik (1987).

Similar to the present investigation, the increase in TLC because of their phagocytic role have been reported by Rama *et al*. (1978). Among DLC, an increase in neutrophils and a decrease in the eosinophils count along with marked lymphopenia was found. Similar pattern has been observed by Gretillat (1976), Rama *et al*. (1978) and Begum (1981). This increase in eosinophils may be related to its important role in neutralizing histamine, which is released from damaged intestinal cells and foreign substances, detoxified by eosinophils and then phagocytized. Marked lymphopenia was observed at day 13th pi and lowest at the peak of the infection. This situation has also been reported by Gretillat (1976), Rama *et al*. (1978) and Begum (1981). The decrease in lymphocytes could be attributed to its supply of globulins, which is under the control of adrenocortical hormones upon lymphoid tissues and lymphocytes, resulting in increase rate of cytoplasmic budding and dissolution of cells during the disease. Monocytes are usually increased in chronic infections but the coccidiosis is an acute infection, so monocytes did not increase in number as observed presently. On the other hand, significant decrease in band neutrophils at the peak of infection was noted which is similar to the findings of Begum (1981). The findings of prepatent and patent period of experimentally produced coccididsis can be used as a useful tool for pricking up field infections and will be a useful guide to clinician in the treatment of coccidiosis.

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