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EVALUATION OF THE ERYTHROCYTES AND LEUCOCYTE ALTERATIONS IN COWS INFECTED WITH *THEILERIA ANNULATA*

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The percentages of anemia, parasitemia and leucocyte changes were investigated in bovine theileriosis in the Northwest of Iran. The hematological values in 91 cows with theileriosis were compared with 19 healthy ones. The disease was confirmed by the presence of parasites in the peripheral blood smear test. Five mLs of blood from the jugular vein was collected and mixed with EDTA. Hematological parameters and parasitemia were assessed by current laboratory methods. The mean for hematocrit (PCV), hemoglobin (Hb), total leucocytes counts (WBC) and erythrocyte count (RBCs) were 22.1%, 6.8 mg/dL, 7075/μL and $4x10^6$/μL, respectively. Mean total protein, absolute lymphocyte, neutrophil, eosiniphil counts, MCV, MCH and MCHC were 6.4 g/dL, 3898, 2746, 136/μL, 55.5 fl, 16.7 Pg and 30.5 g/dL, respectively. Mean concentrations for PCV, Hb, RBCs, and MCHC were significantly ($p<0.05$) lower and MCV was higher than the control group. To determine the severity of anemia in theileriosis, PCV was classified into cows without anemia (>24%), mild (20-23.99%), moderate (12-19.99%) and severe anemia (<11.99%). Therefore, the frequency and percentage of infected cows were 44 (48.4%), 14 (15.3%), 23 (25.3%) and 10 cases (11%), respectively ($p<0.01$). The percentages of parasitemia among 4 groups (1.23, 3.86, 9.64 and 34.2%, respectively) were also significantly ($p<0.01$) different. Among the indices under study, mean RBCs and Hb showed significant differences ($p<0.01$) in all types of anemia. The types of anemia were varied from normocytic hypochromic to macrocytic hypochromic. Negative correlations were found ($p<0.01$) between parasitemia/PCV ($r=-0.74$), parasitemia/Hb ($r=-0.64$) and parasitemia/RBCs ($r=-0.63$). It is concluded that anemia would be the main clinical sign in theileriosis. Type of anemia varied based on the severity of the anemia. Hematocrit, Hb, RBCs MCV and MCHC are the main indices affected in theileriosis. The majority of the infected cows were affected by moderate anemia except for 11%, in that they showed severe anemia with 34.2% parasitemia. Thus, the assessment of RBCs indices will lead to the prognosis and appropriate treatment strategies.

Key words: anemia, cow, Hb, parasitemia, PCV, RBCs, theileriosis, WBC

INTRODUCTION

Theileriosis is a tick-borne, intracellular protozoan, haemoprotozoan and lymphocytic disease in ruminants which is characterized by severe fever, haemolytic anemia, jaundice and digestive disorders (Izzo *et al*., 2010). The disease is transmitted by the Ixodes species of ticks during the spring to fall seasons. Economical effects of anemia due to theileriosis include low milk yield, unthriftiness, emaciation, infertility and treatment costs (Radostits *et al*., 2010). Anemia due to theileriosis is a reliable and common symptom, thus assessment of hematological parameters and mainly the rate of extravascular hemolysis, would be considered as useful tools in the diagnosis, prognosis and treatment of the disease (Shiono *et al*., 2003).

According to literature data, the dominant clinical sign of theileriosis is usually anemia and the severity depends on the level of parasitemia and types of theileria species (Stockham *et al*., 2001; Omer *et al*., 2002; Shiono *et al*., 2004). Anemia due to theileriosis is the result of schizonts, (lymphocyte) or piroplasm (erythrocytic merozoites) forms of infection and/or mixed infection of both forms (Radostits *et al*., 2010). The types of anemia in theileriosis are reported as macrocytic normochromic, macrocytic hypochromic and normocyctic normochromic (Mbassa *et al*., 1994; Omer *et al*., 2002), while no literature was found on microcytic hypochromic and hyperchromic.

Because of the variations in the etiology of the disease (*T. annulata, T. parva*), the types and severity of anemia due to theilerial infection, parasitemia and leucocyte changes, it is necessary to understand the relevant information in order to appropriately diagnose the disease, and give the prognosis and prevention suggestions for the disease in susceptible Holstein dairy cows in Urmia, Iran. The aims of the study were: 1) determination of the frequency and percentage of hematological indicators, mainly anemia and leucocytes in theileriosis, 2) evaluation of the severity and type of anemia occurring in infected cows, 3) assessment of the rate of parasitemia and its relationship to hematological indices, and 4) presentation of the main indices affected in theilerial infection.

MATERIAL AND METHODS

Ninety one Holstein dairy cows at different ages up to 5 years of age which were infected naturally with theileria (*Theileria annulata*) were studied in Urmia, Iran. The disease was confirmed by the presence of parasites in the peripheral blood smear test. The results were compared with 19 healthy cows as the control group. Five mL of blood from the Jugular vein was collected and mixed with EDTA. Hematological parameters and parasitemia were assessed by current laboratory methods. The hematocrit (PCV) was assessed by microhaematocrit, hemoglobin (Hb) by cyanomethemoglobin method, total leucocytes (WBC), erythrocyte counts (RBCs) by hemocytometry, MCHC, MCH, MCV by calculation, and total plasma protein (TP) in a spectrophotometer using commercial protein kits. The parasitemia was calculated in percentage by dividing the infected RBCs to 200 RBCs observed in the blood smear test. Differential counts were carried out in

percentages of neutrophils, lymphocytes, eosinophils, monocytes and basophils by counting about 100 leucocytes in blood smears and then by multiplying by the total leucocyte counts.

Data were analyzed by SPSS statistical program and Means ± SEM were determined for the studied parameters. One-way ANOVA and student t-test were carried out to find the differences in the parameters. Pearson's correlation test was used to evaluate the relationships between parasitemia and the hematological indices. Chi-Square test was used to evaluate the dispersal and percentages of leucocytes and hematological indices in cows.

RESULTS

Mean±SEM for blood hematological parameters and the percentage of parasitemia in healthy and infected cows were shown in Table 1. Mean concentrations for PCV, Hb, RBCs and MCHC in cows with theileriosis were significantly ($p<0.05$) lower and MCV was higher than that of healthy cows. There were no significant differences in WBC, lymphocytes, neutrophiles and MCH between the two groups.

To determine the level of anemia in theileriosis, PCV was classified into >24%, 20-23.99%, 12-19.99% and <11.99% as affected cows without anemia, mild, moderate and severe anemia, respectively. Therefore, the frequencies and percentages of infected cows were 44 (41.4%), 14 (15.4%), 23 (25.3%) and 10 cows (11%), respectively. The differences were significant ($p<0.01$). Student t-test showed significant differences in RBCs, Hb and MCHC among the different groups ($p<0.05$).

The percentages of parasitemia among four groups classified as 44 cows with 1.23% and without anemia, 14 cows with 3.86% and mild anemia (normocyctic, normochromic), 23 cows with 9.64% and moderate anemia (normocyctic hypochromic) and 10 cows with 34.2% and severe anemia (macrocytic hypochromic).

Increment of the severity of anemia in theileriosis causes a significant decrease ($p<0.05$) in mean concentrations of PCV, Hb, RBCs and MCHC, while no differences were observed in WBC, lymphocytes, neutrophiles and TP between anemic cows (Tables 1, 2). Meanwhile, mean concentrations for Hb, RBCs, neutrophiles, eosinophils and MCHC in affected cows without anemia were significantly lower and MCV was higher than in healthy cows ($p<0.05$).

The strong significant correlations between blood hematological parameters in affected cows were reasonably expected (Table 3). The major correlations were obtained among RBCs indices, while there were no such correlations among WBC and absolute differential counts. Significant negative correlations were observed between percentages of parasitemia and PCV, Hb, RBCs, MCHC and MCH, in which the highest correlations ($p<0.01$) were found between parasitemia/PCV ($r=-0.74$), parasitemia/Hb ($r=-0.64$) and parasitemia/RBCs ($r=-0.63$), respectively.

Table 1. Mean±SE of blood hematological parameters in healthy cows, cows without anemia, mild, moderate and severe anemia (n=91)

Parameters	Healthy cows	Cows without anemia	Mild anemia	Moderate anemia	Severe anemia
PCV	26.3±1.1	28.6±0.7	21.8±0.3	15.1±0.5	8.6±0.6
Hb	9.7±0.2	8.84±0.24	6.85±0.25	4.75±0.2	3.00±0.7
RBCs	6.0±0.2	5.2±0.17	4.25±0.32	2.8±0.13	1.6±0.13
MCV	48.6±1.5	56.3±1.45	55.5±2.8	54.6±1.5	53.5±1.5
MCH	16.0±0.5	17.2±0.34	7.1±0.64	16.4±0.54	15.0±0.61
MCHC	22.0±0.1	31.0±0.47	31.5±0.96	30.2±0.68	28.0±0.95
WBC	6642±416	7122±565	8392±1424	7634±791	6860±1039
Lymphocytes	4062±297	3931±276	4029±519	4702±459	4382±899
Neutrophils	2098±162	3085±335	2575±528	2256±300	3388±924
Eosinophils	229±28	141±31	89±16	319±129	305±33
Total Protein	5.9±0.19	6.8±0.23	7.00±0.48	6.35±0.36	5.8±0.27
%Parasitemia	----------	1.23±0.06	3.86±0.49	9.64±1.15	34.2±3.77

Table 2. Mean comparison (t-test) of blood hematological indicators between cows with different types of anemia and without anemia in theileriosis (n=91)

Parameters	df	Hb	RBCs	MCHC	MCH	Total pro-tein	Eosino-phils
Without anemia X mild anemia	58	**4.17	**2.7	0.5	0.14	0.42	----
Without anemia X moderate anemia	67	**10.7	**9.3	0.98	1.3	1	1.1
Without anemia X severe anemia	54	**9.3	**9.7	**2.7	**2.7	**1.96	**1.95
Mild anemia X moderate anemia	37	**6.4	**4.9	1.14	0.84	1.1	1
Mild anemia X severe anemia	24	**5.7	**6.4	**2.5	**2.3	**1.89	**2.1
Moderate anemia X severe anemia	33	**3.1	**5.5	*1.8	1.57	0.93	1.17

**=p<0.01, *=p<0.05

Table 3: Correlations among parasitemia and hematological indicators in theileriosis (n=91)

Parameters	PCV	Hb	RBCs	MCH	MCHC
Hb	**0.93				
RBCs	**0.85	**0.89			
MCH	*0.30	*0.24	-0.09		
MCHC	*0.26	**0.47	**0.53	0.06	
Parasitemia	**-0.74	**-0.64	**-0.63	*-0.30	*-0.27

**=p<0.01*=p<0.05

DISCUSSION

The 50% of infected cows showed different types of anemia. The previous findings emphasized anemia as the main and most reliable sign in theileriosis, irrespective of the reference to the severity and types of anemia (Omer *et al*., 2002; Shiono *et al*., 2003), while in an individual survey of this study anemias were clearly clarified. The presence of significant differences in some of the blood hematological parameters between the infected and healthy groups indicates that erythrocytes indices were the main targets in theileriosis. In previous studies the degree of contamination in the infected cows was also unclear while in this study 50% of infected cows were certainly in the initial step of the disease without signs of anemia. Other factors related to the severity of anemia included theileria species, genetics, immunity and breeding factors which require further consideration (Omer *et al*., 2002; Stockham *et al*., 2000).

Mean PCV in infected cows was 24% less than in healthy ones. The same results were reported by Sandhu *et al*. (1998), Hasanpour *et al*. (2008), El-Deeb and Younis (2009). On the basis of the PCV result, the majority of infected cows (24.5%) revealed moderate anemia and the least (11%) severe anemia in which blood transfusion was probably necessary. However, these findings were quite lower than those reported by Asri and Dalir-Naghadeh (2006) in that 34% was for severe anemia and 66% for mild and moderate anemia. The reason for these discrepancies was probably related to the severity and stage of disease. Thus, indeed the peripheral blood smear test, PCV and RBC evaluation will be necessary to understand the severity and stage of the disease (Issi *et al.,* 2010). This was the reason for the differences in the erythrocyte counts up to three fold in different types of anemia observed for this study.

The pathogenesis of anemia in theileriosis is expected due to the extravascular hemolysis via enzymatous, immune-mediated, mechanical, toxic, erythroid hyperplasia and enhanced hemaglutinine mechanisms (Stockham *et al*., 2000; Shiono *et al*., 2003). Other factors would include protease enzymes (Somerville, *et al*., 1998; Stockham *et al*., 2000; Shiono *et al.,* 2004), oxygen radicals, increased oxidative proteins in the RBCs membranes and oxidative damage (Shiono *et al*., 2002; 2003). The main pathogenesis of *T. annulata* and

T.serjenti were reported by damaging effects on RBCs (Asri and Dalir-Naghadeh, 2006). Other erythrocytic abnormalities include Howell-Jolly bodies, basophilic stippling and Heinz bodies. In severe anemia, Hb and RBCs decrease to the least values and blood transfusion is necessary to compensate the anemia.

The results of total protein and leucocytes indices showed non-significant differences between healthy and infected groups. This means that these parameters were not influenced following theileriosis, whereas some referred to leucopenia and lymphopenia (Sandhu *et al.*, 1998) and others to lymphocytosis, leukocytosis and hypoproteinemia due to hypobilirubinemia (Stockham *et al*., 2000; Radostits *et al*., 2007). Lymphocytosis was due to intralymphocytic theilerial parasites transforming to host cells, leading to clonal growth of lymphocytes (Yamagushi *et al*., 2010). In this study WBC was not influenced by the various types of anemia, but in an individual survey 17 cases (18%) showed mild leukopenia. This was in agreement with the findings in that lymphopenia was mentioned as the clinical finding in tropical areas (Osman and Al-Gaabary, 2006). However, cows suffering from theileriosis will appear with chronic and severe leucopenia as the lowest WBC was observed in severe anemic cows. Eosinopenia in theileriosis was not yet identified and one hypothesis refers to pseudoeosinopenia due to their accumulation in the reticuloendothelial system (Omer *et al*., 2002).

The results of MCV, MCH and MCHC in theileriosis revealed that MCV increased, while MCHC decreased. These indices relatively depend on the rate of parasitemia in which the severity of the infection is indicated. As the parasitemia rate increases in infected cows, RBCs, PCV, and Hb significantly decrease, resulting in an increase in the MCV and MCHC (Nazifi *et al*., 2009). Based on these results macrocyctic hypochromic anemia in severe form, normocyctic normochromic in theileriosis without anemia and normocyctic hypochromic in a moderate form were observed as demonstrated in other studies, too (Mbassa *et al*., 1994; Stockham *et al*., 2000; Omer *et al*., 2002). Microcyctic hypochromic and hyperchromic anemia were not reported in the literature.

Overall, half of the infected cows revealed anemia, 11% of which showed severe anemia with 34.2% parasitemia in which blood transfusion may be considered necessary. Erythrocyte indices were predominant parameters that were influenced in theileriosis. Leucocyte counts were not changed during the disease. The types of anemia varied from normocytic hypochromic and macrositic hypochromic. It is concluded that the evaluation of PCV and RBCs indices is recommended in order to obtain the optimal prognosis and treatment in theileriosis.

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ISPITIVANJE ALTERACIJA ERITROCITA I LEUKOCITA KRAVA INFICIRANIH SA *THEILERIA ANNULATA*

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SADRŽAJ

U ovom radu su izneti rezultati ispitivanja anemije, parazitemije i promena na leukocitima kod goveda inficiranih sa *Theileria Annulata* na severozapadu Irana. Hematološki parametri 91 krave, inficirane sa *Theileria Annulata* su upoređivani sa vrednostima registrovanim kod 19 zdravih krava. Bolest je potvrđivana nalazom parazita u razmazu periferne krvi

Srednja hematokritska vrednost (PCV), koncentracija hemoglobina (Hb), ukupan broj leukocita (WBC) i eritrocita (RBCs) su iznosile 22,1%, 6,8 mg/dl, 7075/μl i 4x10^6/μl respektivno. Srednje vrednosti koncentracije ukupnih proteina, apsolutnog hematokrita, eozinofila, neutrofila, MCV, MCH i MCHC su bile 6,4g/dl, 3898, 2746, 136μl, 5,55fl, 16,7pg i 30,5g/dl, respektivno. Srednje vrednosti PCV, Hb, RBCs i MCHC su bile značajno niže ($p<0,05$) u grupi inficiranih krava, dok je vrednost MCV bila viša nego u kontrolnoj grupi.

Da bi se utvrdio stepen anemije kod tajlerioze na osnovu vrednosti za PCV izvršena je sledeća klasifikacija: krave bez anemije (>24%), blaga anemija (20-23,99%), umerena anemija (12-19,99%) i ozbiljna anemija (<11,99%). Frekvenca i procenat inficiranih krava je bila 44 (48,4%), 14 (15,3), 23 (25,3%) i 10 slučajeva (11%), respektivno, a razlike između grupa su bile statistički značajne ($p<0,01$). Procenat parazitemije u 4 grupe (1,23, 3,86, 9,64 i 34,2%, respektivno) se takođe značajno razlikovao ($p<0,01$). Među indeksima koji su ispitivani, srednja vrednost broja eritrocita i koncentracija hemoglobina su se značajno razlikovale ($p<0,01$) u svim tipovima anemije. Tipovi anemije su varirali od normocitne hipohromne do makrocitne hipohromne. Negativna korelacija je utvrđena između parazitemije/PCV (r=-0,74), parazitemije/HB (r=-0,64) i parazitemije/RBCs (r= 0,63). Zaključeno je da je stepen anemije glavni klinički znak kod tajlerioze, a vrsta anemije je bila zavisna od težine simptoma. Glavni parametri koji su promenjeni kod tajlerioze su hematokritska vrednost, koncentracija hemoglobina, broj eritrocita, MCV i MCHC. U našem ispitivanju, većina inficiranih krava je imala umerenu anemiju, osim 11% kod kojih se javila ozbiljna anemija sa 34,2% parazitemije. Procena parametara crvene krvne slike uspešno vodi postavljanju realne prognoze i određivanju odgovarajuće strategije u lečenju.