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Relationship between degree of anemia and blood gases in cattle with theileriosis

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Abstract: This study was conducted to determine the relationship between the degrees of anemia and blood gases in cattle with theileriosis. It included 28 cattle with theileriosis and 7 healthy animals as the control group. The cattle with theileriosis were divided into 4 groups according to their hematocrit (Hct) values: cattle with Hct of >26 were considered as nonanemic (Group 1), Hct of 20–26 as mildly anemic (Group 2), Hct of 14–19 as moderately anemic (Group 3), and Hct of 10–13 as severely anemic (Group 4). The PCO₂, HCO₃, and TCO₂ levels in the study groups were lower than those of the control group. Moreover, reductions in HCO₃ and TCO₂ concentrations were significant only in the severely anemic group. Similarly, the lowest PCO₂ levels were also detected in the severely anemic group. There were no significant differences in the SO₂ levels between the control and study groups. On the other hand, the increase was significant in the severely anemic group when compared to Groups 1 and 2. The results seemed to indicate the trend of uncompensated metabolic acidosis in theileriosis cases with severe anemia; thus, veterinary surgeons should bear this in mind when evaluating the prognosis for such cases.

Key words: Anemia, blood gases, cattle, theileriosis

1. Introduction

Theileriosis is a protozoan disease in ruminants caused by the *Theileria* species transferred from ticks belonging to the family *Hyalomma*. The disease caused by *T. annulata* is also called tropical theileriosis or Mediterranean coast fever (1–3). Theileriosis causes major losses due to high mortality, decreased production, and reproductive problems. An increased risk of secondary infection can occur. While it appears in summer in subtropical regions, it can occur throughout the year in tropical regions (3–6).

The main reason for pathological change in theileriosis is progressive anemia and related disorders (3,7–9). Increased fragility and oxidative damage in erythrocytes, damage to erythrocytes by parasites in the reticuloendothelial system, autoimmune reactions, and intraerythrocytic piroplasms contribute to the development of anemia (7–9). The mortality observed in acute cases is directly related to the anemia process and the hematocrit (Hct) value can decrease below 10% within 4–5 days after the beginning of parasitemia in such cases (10). The clinical and biochemical changes observed in this disease are related to degree of anemia, the degree of parasitemia, and the severity of hypoxia (8,11,12). Although textural hypoxia formed by anemia starts compensatory mechanisms (13), the rapid disintegration of O₂- and CO₂-

carrying erythrocytes in the host and the simultaneous quick release of the contents of erythrocytes seem to cause changes in blood pH and electrolytes (10). Several researchers have investigated the acid-base and electrolyte situation of dogs with babesiosis, analyzing the compensation status and development of the disorder in detail (14,15). However, there are a limited number of studies concerning blood parasite diseases and the acid-base and electrolyte situations in cattle (10,16,17). Wright et al. (17) reported that in cattle with *Babesia bovis* metabolic and respiratory alkalosis is a constant feature in acute infections, and this condition may progress to death. In contrast, other researchers observed metabolic acidosis in *B. canis* cases in dogs (14,15), as well as in *Anaplasma marginale* (10), *Eperythrozoon wenyoni* (10), and *Theileria* spp. (16) infections in cattle. These studies did not evaluate the degree of anemia. Allen and Kuttler (10), although they did not classify anemia levels, compared calves still alive infected with *E. wenyoni* and both living and dead calves infected with *A. marginale*. They revealed that metabolic acidosis could not compensate for the effects of *Eperythrozoon* and calves died from *A. marginale* in anemic crisis. In the survivors, despite the development of metabolic acidosis, compensation occurred and thus anemic crisis was avoided.

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Considering the pathological effects of theileriosis, the clinical monitoring of cattle with theileriosis is of great importance for determining changes in electrolyte values and the acid-base situation according to the degree of anemia in order to identify the treatment strategy. To the best of our knowledge, little or no research related to the degree of anemia and changes in electrolytes and blood gases has been done in cattle infected with *Theileria*. This study was therefore designed to shed light on this issue.

2. Materials and methods

2.1. Animal materials

This study comprised 28 cattle diagnosed with theileriosis based on clinical signs and microscopic examination and 7 healthy cattle as the control group from the Van region of Turkey. All animals included were aged between 3 months and 9 years and were from various breeds (Simmental, Brown Swiss, Holstein, and local black). The animals were allotted into groups based on breed and age in order to establish relative uniformity within each group. This study was approved by the Local Ethics Committee of Animal Experimentation of Yüzüncü Yıl University (Ref No.: 2012 / 05).

2.2. Study design

Systematic clinical examinations were performed in all animals. The clinical findings were recorded. For microscopic evaluation, blood smears were prepared from a puncture of the ear vein of each animal. The blood smears were stained with Giemsa to reveal piroplasms of *Theileria* in erythrocytes for diagnosis. The existence of piroplasm forms of *Theileria* was then determined using a light microscope (Olympus, Japan). Cattle showing evidence of piroplasm forms were considered positive for *Theileria*. Blood samples were taken from the vena jugularis into anticoagulant tubes (EDTA) from healthy and diseased cattle. The cattle with theileriosis were divided into 4 groups according to their Hct values (18): cattle having Hct of >26 were considered as nonanemic (Group 1), Hct of 20–26 as mildly anemic (Group 2), Hct of 14–19 as moderately anemic (Group 3), and Hct of 10–13 as severely anemic (Group 4). As for treatment, all infected animals received a single dose of 2.5 mg/kg of body weight (BW) of BQ (Butalex - Cevadif, Turkey) intramuscularly and 1 mL/20 kg BW of daily vitamin B combinations (Berovit B₁₂ - Cevadif, Turkey) intramuscularly for 5 days. In addition, 6% dextran (Macrodex 6%, Eczacıbaşı-Baxter, Turkey) solution with isotonic sodium chloride was given intravenously to the moderately and severely anemic animals.

2.3. Hematological analysis

Hct values, hemoglobin (Hb) concentration, and leukocyte (WBC) and platelet (PLT) counts were determined using a veterinary hematology device (QBC Vet Autoreader, Idexx, USA).

2.4. Blood gases analysis

Blood pH, partial carbon dioxide pressure (pCO₂), bicarbonate (HCO₃) concentration, anion gap (AnGAP), total carbon dioxide concentration (TCO₂), partial oxygen pressure (pO₂), and oxygen saturation (SO₂) levels were measured in heparinized blood samples of all animals using a veterinary blood gases device (VetStat Electrolyte and Blood Gas Analyzer, Idexx).

2.5. Statistical evaluation

Data from the control and infected animals were analyzed using an independent sample T test. Differences between the groups were tested by one-way analysis of variance (ANOVA). Abnormal data distribution in groups was tested using the Duncan test. For this purpose, a statistical package (SPSS Inc., USA) was used. Statistical significance was set at $P < 0.05$. All data were expressed as mean \pm standard error of the means (SEM).

3. Results

3.1. Clinical findings

Pyrexia, tachycardia, tachypnea, and swelling in superficial lymph nodes were determined in all cattle with theileriosis. Upon examination of the mucous membrane of conjunctiva, paleness was observed in the majority of infected animals, and hyperemia and petechial hemorrhages were observed in some animals. In addition, petechial hemorrhages in some of the animals were observed on the planum nasolabiale (n = 2) and perineum (n = 1). There were also some general clinical findings such as dyspnea, coughing, decrease in rumen movements and rumination, inappetence, and stillness. Furthermore, pseudopericarditis was detected in one severely anemic (Hct = 12.5) patient, as previously reported in cattle by Keles et al. (19).

3.2. Hematological findings

Comparisons of the hematological findings in this study are given in Table 1. It was detected that the WBC count was significantly high ($P < 0.05$) and Hct values, Hb concentrations, and PLT counts were significantly low ($P < 0.001$) in all infected animals as compared to the control group. The comparison revealed that there was a parallel decrease in Hct value and Hb concentration from Group 1 to Group 4. In addition, there was a statistically meaningful decrease in Hb concentration, except for Group 1. The lowest Hb concentration appeared in the severely anemic group (group 4). When compared to the control group, the PLT count was low in all groups ($P < 0.001$), but there were no differences among the diseased groups. Although there were no differences among the diseased groups in WBC counts, they increased gradually as the severity of anemia increased (Table 1).

Table 1. Comparison of hematological findings in cattle with theileriosis.

Parameters	Control (n = 7)	Total infected cattle (n = 28)	Group 1 (n = 7)	Group 2 (n = 7)	Group 3 (n = 7)	Group 4 (n = 7)
Hct (%)	35.47 ± 1.86 ^a	20.03 ± 1.64 [*]	30.47 ± 1.19 ^b	24.55 ± 0.81 ^c	16.01 ± 0.64 ^d	11.01 ± 0.31 ^e
Hb (g/dL)	11.73 ± 0.65 ^a	7.16 ± 0.68 [*]	9.96 ± 0.66 ^{ab}	8.40 ± 0.57 ^{bc}	6.98 ± 1.64 ^c	3.83 ± 0.12 ^d
WBC (×10 ⁹ /L)	10.26 ± 0.48	12.95 ± 1.24 [‡]	9.98 ± 1.47	11.00 ± 2.77	13.10 ± 1.23	16.89 ± 3.39
PLT (×10 ⁹ /L)	517.4 ± 68.8 ^a	216.8 ± 29.5 [*]	218.1 ± 32.2 ^b	228.7 ± 78.2 ^b	229.3 ± 66.0 ^b	196.3 ± 71.9 ^b

Statistical importance between cattle with theileriosis and control group: [‡]P < 0.05, ^{*}P < 0.001.

a, b, c, d, e: Means within in the same row with different letters are statistically significantly different (P < 0.01).

3.3. Blood gases and electrolyte findings

Comparisons of blood gases and electrolyte balance in this study are given in Table 2. PCO₂ (P < 0.01), HCO₃ (P < 0.01), TCO₂ (P < 0.01), and Na (P < 0.05) concentrations decreased significantly in infected animals in total when compared to the control group. However, differences in pH, AnGAP, PO₂, SO₂, K, and Cl levels were not significant (Table 2).

Analysis of the diseased groups and the control groups indicated that PCO₂ pressure was lower in the groups with theileriosis except for Group 2, and the decrease in the severely anemic group (Group 4) was significant (P < 0.01). Decreases in HCO₃ and TCO₂ concentrations occurred in all groups with theileriosis, but this decrease was statistically significant only in the severely anemic animals (P < 0.05) (Group 4). There was no difference in the levels of SO₂ between theileriosis groups in total and the control group, but the differences among the diseased groups were statistically significant. These values gradually increased from Group 2 to Group 4, and only the increase

in the severely anemic group (Group 4) was statistically significant compared to Groups 1 and 2 (P < 0.05) (Table 2).

4. Discussion

Other researchers have reported changes in hematological parameters in cattle with theileriosis (20,21). The changes observed in hematological parameters and blood gas levels were only studied by Gökçe et al. (16), but there seems to be no study that observed the relationship between the degree of anemia and changes in venous blood gases in cattle with theileriosis. For this reason, in this study the discussion will be focused on the relationship between degree of anemia and changes in venous blood gases in cattle with theileriosis. In this study, the development of primarily compensated metabolic acidosis was detected in all infected animals with theileriosis, but metabolic acidosis could go into uncompensated form (lowest HCO₃, TCO₂, and PCO₂ compared to the other groups) in the severely anemic group.

Table 2. Comparison of blood gases and electrolyte findings in cattle with theileriosis

Parameters	Control (n = 7)	Total infected cattle (n = 28)	Group 1 (n = 7)	Group 2 (n = 7)	Group 3 (n = 7)	Group 4 (n = 7)
pH	7.47 ± 0.01	7.44 ± 0.02	7.45 ± 0.01	7.44 ± 0.01	7.42 ± 0.05	7.46 ± 0.02
PCO ₂ (mmHg)	40.43 ± 1.44 ^a	34.20 ± 0.93 [†]	35.29 ± 1.70 ^b	37.75 ± 1.25 ^{ab}	35.43 ± 1.23 ^b	29.86 ± 1.68 ^c
HCO ₃ (mmol/L)	26.41 ± 1.16 ^a	21.29 ± 0.81 [†]	21.69 ± 0.65 ^{ab}	23.42 ± 1.02 ^{ab}	21.47 ± 2.17 ^{ab}	19.50 ± 1.71 ^b
AnGAP (mmol/L)	14.40 ± 0.54	15.88 ± 1.16	11.63 ± 1.59	15.55 ± 3.44	18.67 ± 2.22	17.53 ± 1.91
TCO ₂ (mmol/L)	27.24 ± 1.26 ^a	22.28 ± 0.82 [†]	23.04 ± 0.79 ^{ab}	24.05 ± 0.86 ^{ab}	22.49 ± 2.18 ^{ab}	20.31 ± 1.76 ^b
PO ₂ (mmHg)	33.57 ± 2.11	35.20 ± 0.97	36.43 ± 2.28	33.75 ± 0.63	36.43 ± 1.95	33.57 ± 1.77
SO ₂ (%)	61.28 ± 1.73 ^{ab}	60.44 ± 1.24	57.14 ± 2.87 ^a	55.50 ± 1.71 ^a	61.29 ± 1.78 ^{ab}	65.71 ± 0.92 ^b
Na (mmol/L)	142.9 ± 1.33	137.5 ± 1.11 [‡]	136.1 ± 1.28	135.5 ± 0.65	140.7 ± 3.14	137.0 ± 1.93
K (mmol/L)	4.23 ± 0.13	4.12 ± 0.10	4.10 ± 0.20	4.07 ± 0.29	4.39 ± 0.18	3.89 ± 0.16
Cl (mmol/L)	105.7 ± 1.70	105.9 ± 1.13	108.4 ± 1.48	106.7 ± 2.59	105.0 ± 2.99	103.9 ± 1.82

Statistical importance between cattle with theileriosis and control group: [‡]P < 0.05, [†]P < 0.01.

a, b, c: Means within in the same row with different letters are statistically significant (P < 0.05).

In this study, clinical findings in cattle with theileriosis were consistent with those reported by other researchers (2,3,21). As reported by others (2,3), petechial hemorrhage was observed on the planum nasolabiale and inner parts of the pinna in one patient. The results of these studies indicated that theileriosis was related to progressive anemia and correspondingly growing defects (1–3,5,7).

Findings showed that WBC counts were significantly high ($P < 0.05$) in all infected animals in this research (Table 1). These results were parallel to those of other researchers (21,22), indicating that inflammatory responses (22) increased due to the disease or that lymphocyte proliferation (23) occurred in lymphoid organs to fight against protozoa invading the organism. There was no difference between the anemia groups as far as WBC levels concerned (24). There was a reduction in the number of leukocytes, which were lowest in the severely anemic group of animals with theileriosis (25). A gradual increase in the level of WBC counts was detected in this study (Table 1); however, no statistically significant relationship was detected between WBC count and degree of anemia levels, similar to the results reported by Ramin et al. (24). This implies that the increase in leukocyte levels can be considered as an indicator of disease progression or the deterioration of prognosis (26).

Thrombocytopenia and coagulopathy are important hematological findings observed in theileriosis (8,20). The PLT counts in this study were significantly low in infected animals in total ($P < 0.001$) and in all groups with theileriosis in comparison to the control group ($P < 0.01$), confirming the findings of other researchers (8,20). However, the PLT count did not differ among the diseased groups (Table 1). This may indicate that the decrease in the number of platelets might not be a prognostic indicator of the deterioration of the disease. The thrombocytopenia defined in this study can be explained by disseminated intravascular coagulation development (20) or bone marrow suppression (8) due to the disease.

Metabolic acidosis was defined as venous pH of 7.25 or below, bicarbonate ions (27), and total carbon dioxide concentration (28) of 20 mmol/L. Normal blood pH, abnormal PCO_2 , and HCO_3 were reported as the compensations for acid-base abnormalities (29), and close or normal (or low-normal) blood pH, low plasma bicarbonate, and low PCO_2 were expressed as compensated metabolic acidosis (30). In this study (Table 2), there was no difference among all groups of animals with theileriosis in terms of pH. However, this study has shown that PCO_2 , HCO_3 , and TCO_2 concentrations were significantly low ($P < 0.01$) in all infected animals, thus indicating metabolic acidosis development in this study in parallel to results reported by many other researchers (10,15,16). However, Wright et al. (17) reported, in contrast, that metabolic and respiratory alkalosis can be observed in cattle with

babesiosis. The development of metabolic acidosis was accompanied by a reduction in the concentration of O_2 and Hb in the tissues (18) due to anemia. Erythrocyte clumps in the capillaries caused anemic infarcts (1,17), leading to common tissue anoxia (17) and severe liver and kidney dysfunctions; consequently, anaerobic metabolism supersedes aerobic metabolism as especially the production of lactic acid from other products of anaerobic metabolism increases (14,17,31). These facts might explain our findings regarding metabolic acidosis, especially for Groups 3 and 4 (Table 2).

In the present study, metabolic acidosis developed in all infected animals, which might be considered as the primary form of compensation, as reported earlier (29–31). It was reported that in response to metabolic acidosis, PCO_2 started to drop in a short time, and in primary acid-base disturbance, bicarbonate and PCO_2 changed in the same direction (31). There were no significant changes in pH values in this study. However, PCO_2 , HCO_3 , and TCO_2 concentrations were significantly low ($P < 0.01$) in infected animals in total (Table 2). However, metabolic acidosis, which developed in the severely anemic group (Group 4), might be indicator of a trend of the uncompensated form. This may be because this group had the lowest HCO_3 (especially), TCO_2 , and PCO_2 levels as compared to the other groups (Table 2). Similarly, HCO_3 and TCO_2 concentrations in groups with theileriosis gradually decreased. However, only the decrease in the severely anemic group (Group 4) was significant ($P < 0.05$), quite similar to PCO_2 (Table 2). This finding was in agreement with that of Allen and Kuttler (18), but differed from those of other researchers (15,16). Allen and Kuttler (18) explained that metabolic acidosis developed in animals that died of *A. marginale* infection due to anemic crises and those with *Eperythrozoon* infection. They also reported that metabolic acidosis was compensated for in those that survived *A. marginale* infection, but not compensated for in those that died of *A. marginale* infection.

Blood parasite diseases have been reported to affect blood pH levels (16); they may remain the same (15), or become higher in survivors but lower in those that die (10). In this study, pH did not differ among the diseased groups, and similar results were reported by Leisewitz et al. (15). In contrast, differences were observed in other studies, but this may be related to differences in the etiologic agent involved, the period of the disease, or its compensation status.

In this study, although a statistical difference in AnGAP levels was not determined between the anemia groups and all animals with theileriosis, there was a slight increase in AnGAP level in proportion to increased anemia intensity. However, the difference was not significant among the diseased groups, as reported by other researchers (10). This slight increase occurring in AnGAP in this study could be related to an increase in lactic acid, although it was not measured in this study.

No statistical difference was seen in venous blood PO₂ and SO₂ levels between the control and infected animals and among the diseased groups, except for an increase SO₂ in Group 4. This could be attributed to physiological mechanisms such as a reduction in O₂ consumption and an affinity developed for tissue hypoxia related to anemic hypoxia, or increased O₂ tissue perfusion, cardiac pulse, and pulmonary function, as reported by earlier researchers (2,32). Although tachypnea and tachycardia were observed in all cattle with theileriosis in this study, the absence of changes in venous blood PO₂ and SO₂ levels was in accordance with the results of Gülseli (32). The partial removal of anemia impressions is due to an almost normal amount of oxygen transportation to tissues caused by the acceleration of blood flow resulting from increased cardiac output. This could also be an indicator of a compensatory response developed in cattle with theileriosis.

Chronic rising in H⁺ ions can increase the oxygen affinity of hemoglobin by the inhibition of 2,3-diphosphoglycerate formation (33). The gradual increase observed in SO₂ values from Group 2 to Group 4 (Table 2) and especially the SO₂ increase observed in the severely anemic group (P < 0.05) compared to other groups (Groups 1 and 2) could be explained as chronicity of theileriosis, as Beers and Berkow also reported similar findings (33). There is a progressive development of anemia in theileriosis (1–3,5,7,21), and severe anemia can be associated with the duration of disease. The increase in oxygen saturation is probably dependent on the shift in oxygen dissociation curve to the left (34) as a result of the methemoglobin formations, which increase parallel to anemia severity (7,35).

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