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Clinicopathological Changes Associated with Bovine Babesiosis and Trypanosomiasis in Cattle

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Abstract

Clinicopathological changes associated with blood protozoa in cows have been well-examined. However, these changes can be influenced over time due to the development of the animal immune system status and environmental changes. The current study aimed to assess clinical and pathological changes associated with blood protozoa infection in naturally infected cattle. A total of 80 cows were examined for evidence of babesiosis and trypanosomiasis using Giemsa-stained blood smears. Hematological parameters, clotting factors, and urinalysis were also estimated. Babesiosis and trypanosomiasis were identified in 16% and 33% of the examined stained blood smears, respectively. The study revealed a significant decrease ($p<0.05$) in values of hemoglobin concentration, packed cell volume, total erythrocytes count, mean corpuscular hemoglobin, and total platelet count in both groups infected with babesiosis and Trypanosomiasis. In contrast, a significant increase was recorded in total leukocytes count, clotting time, prothrombin time, fibrinogen time, and activated partial thromboplastin time, as well as platelet volume and platelet distribution width in the infected cattle compared with non-infected cattle. Urinalysis showed an appearance of urobilinogen, bilirubin, protein and glucose in the urine of some infected animals, while nitrate and ketones were not detected in any of the study animals. We concluded that hemoprtozoa exhibits considerable effect on clotting system which might be reflected in disseminated intravascular coagulation (DIC) abnormalities and changes in urine components.

Key words: Babesiosis, Trypanosomiasis, Clotting factor, Urinalysis.

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Introduction

Tropical and subtropical parasitic diseases affect the bloodstream of cattle in many countries around the world. Hemoprotozoan can affect up to 80% of cattle population globally, causing significant economic losses to livestock (1,2,3). Babesiosis and Trypanosomiasis are among major hemaprotozoa that greatly affect livestock health and productivity (1,4,5). They cause fever, anemia, jaundice, loss of appetite, weight loss, difficult breathing, migraines, neurological defects, and in per acute form of disease sudden death of animals may occur (6,7,8). Many studies have indicated that infection with bovine babesiosis and trypanosomiasis causes hematological changes observed in natural and experimental infections. These changes include simultaneous development of leukocytosis, as well as decrease in blood indices, abnormalities of clotting factor values and disseminated intravascular coagulation (DIC) defect (8,9) which may be considered a primary or secondary cause of death in infected animals (10,11). On the other hands, clear changes were also indicated in biochemical and urine parameters related to babesiosis and trypanosomiasis such as the toxic effect of *Babesia bigemina,* causing liver and kidney dysfunction in affected animals (12,13). The histopathological alterations of kidneys in naturally infected animals include degeneration, necrosis, detachment of renal tubular epithelial cells in proximal convoluted and hemoglobin casts (12). Urinalysis changes due to Babesiosis and Trypanosomiasis in cattle were reported in many studies around the world (14,15). The information from blood and urine biochemical parameters helps to identify the function of specific organs in the progression of the disease, providing a more effective therapeutic strategy to achieve better clinical outcomes (1,11). The aim of this study was to assess some clinicopathological changes in bovine naturally infected with babesiosis and trypanosomiasis in Mosul city, Iraq.

Materials and Methods

Study population

This study was conducted to examine 80 local cattle breeds with age between 2.5-6 years old and from both sexes. The study animals were admitted to the Veterinary Teaching Hospital, College of Veterinary Medicine, University of Mosul, Iraq. Suspected animals showed different clinical signs such as fever, anemia and weight loss during the period from July 2023 and January 2024. All cases were examined clinically and then confirmed infected with *Babesia* spp. and *Trypanosoma* spp. by scanning thin blood smears stained by Giemsa's stain.

Blood smear preparation and examination

All blood smears were air-dried, fresh smears directly prepared after the sampling, fixed with methyl alcohol, and then stained with Giemsa. Later, the smears were examined under the light microscope at a 1000 × magnification with immersion of oil (BX51, Olympus, Tokyo, Japan).

Blood samples collection

7.5 ml of blood and urine samples were collected from each animal with a smear tested positive for *Babesia* spp. and *Trypanosoma* spp. Blood samples were divided into (i) EDTA-added part for

assessment of complete hematological profile assessment using hematological coulter from GENES, USA (ii) Trisodium citrate-added for (using plasma) estimating clotting factor parameters. Samples from negative animals were considered as control group for comparison of the hematological parameters' changes.

Estimating blood parameters and clotting factors

In this study, the coulter counter (GENES, USA) was used for the analysis of the total erythrocytes count (RBC), hemoglobin (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), as well as total leukocyte counts (TLC), total Platelet count (TP), mean platelet volume (MPV) and platelet distribution width (PDW). Clotting factors (including prothrombin time, activated partial prothrombin time and Fibrinogen time) were estimated according to manufacture instructions, using automatic coagulation analyzer, Biolabo Solea 100 (Biolabo SAS, Maizy, France). The clotting time was calculated according to (16).

Urinalysis

Urine samples were collected immediately while animal urination. The first portion of the urine was discarded, and the late excretion was collected in a container for urinalysis by Urinalyzer URYXXON®Relax (MACHEREY NAGEL company, Germany) for detecting glucosuria, ketonuria, urobilinogen, bilirubin, protein (proteinuria) and nitrate. Centrifuged urine sediment was microscopically examined to determine the red blood cells (RBCs), white blood cells (WBCs), and bacteria. Glucosuria was recorded as negative, 1+ or mild (50 milligram/ deciliter), 2+ or moderate (100 milligram/ deciliter), 3+ or severe (150 milligram/ deciliter). Ketonuria was reported as negative, 1+ or mild G(25 milligram/ deciliter), 2+ or moderate (100 milligram/ deciliter), 3+ or severe (300 milligram/ deciliter). Urobilinogen was reported as negative, 1+ or mild (4 milligram/ deciliter), 2+ or moderate (8 milligram/ deciliter), 3+ or severe (12 milligram/ deciliter). Bilirubin was reported as negative, 1+ or mild (1 milligram/ deciliter), 2+ or moderate (2 milligram/ deciliter), 3+ or severe (4 milligram/ deciliter). Protein was reported as negative or $\geq$ 30, 1+ or mild (30 milligram/ deciliter), 2+ or moderate (100milligram/ deciliter), 3+ or severe (500 milligram/ deciliter). Nitrate was reported as negative or positive. All these categories were according to manufacturer instructions of the urinalyzer URYXXON®Relax (MACHEREY NAGEL company, Germany).

Statistical analysis

SPSS (Version 17; SPSS Inc., Chicago, USA) were used for data analysis. The value of $P<0.05$ was considered for the statistical significance.

Results

The results reveal that infection rate of Trypanosomiasis and babesiosis represent around 33% (26/80) and 16% (13/80) respectively. Both protozoa observed in their known forms (*Babesia* spp. were identified as pear-shape, while *Trypanosoma* spp. was identified as eel-like cell) in the Giemsa-stained blood smears (Figure 1). The infected cattle showed variations in blood and urine parameters compared with the non-infected

group (Tables 1, 2, 3). The hematological analysis of cattle infected with babesiosis and trypanosomiasis are summarized in Table1. The respective mean values of hemoglobin, packed cell volume and total erythrocyte count were decreased, while total leukocytes count were increased significantly ($P<0.05$) as compared to that of non-infected cattle.

The mean values of blood indices, i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of affected cattle revealed significant ($P<0.05$) changes compared to healthy animals (Table1), the gender (males 78.6%), and stray dogs (56%, Table 1).

A significant difference was recorded in the values of blood clotting factors in animals infected with babesiosis and trypanosomiasis, i.e., a significant decrease ($P<0.05$) in the rates of the total platelets count compared to cows in non-infected group, On the other hand, elevation in the rates platelet volume, platelet distribution width, clotting time, prothrombin time and activated partial thromboplastin time and fibrinogen time was indicated in infected cattle as shown in Table (2):

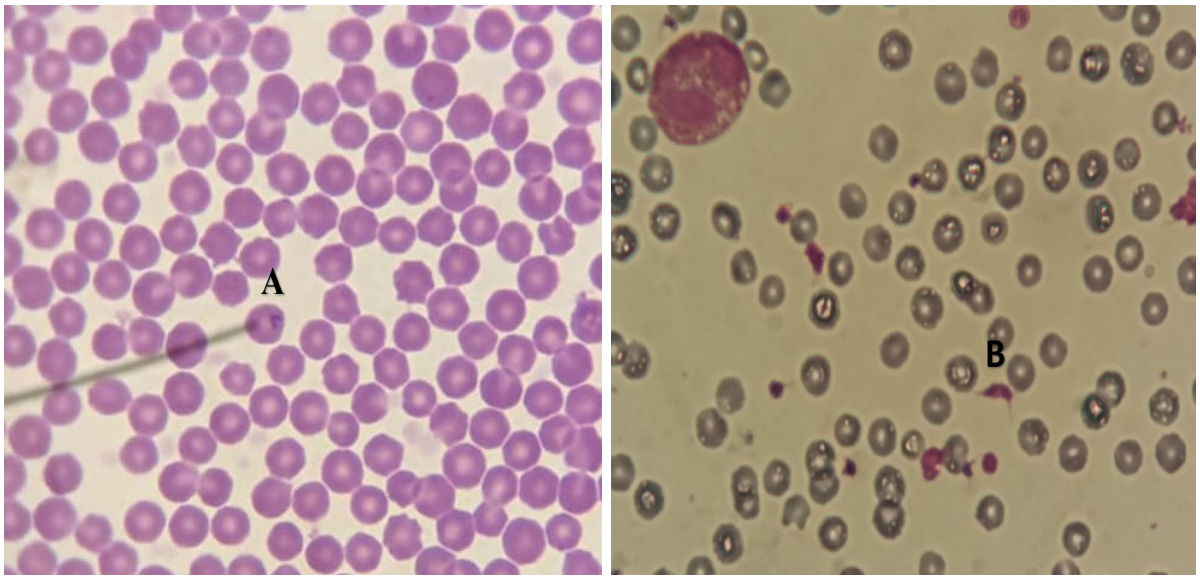

Figure 1: Microscopic examination of blood smears: A- *Babesia spp., B- Trypanosoma spp.* Giemsa stained (1000X).

Table 1: Hematological analysis of infected/non-infected animal with bovine babesiosis and trypanosomiasis

Haematological Parameter	MEAN±SE		
	Babesia spp.	*Trypanosoma* spp.	Non-Infected group
Hb g/dl	5.78± 0.24[c]	9.56 ± 0.98[b]	12.05±0.23[a]
WBC /µl	6.34 ± 0.52[b]	7.21 ± 0.36[a]	5.35±0.42[c]
RBC /µl	4.32± 0.74[c]	5.54 ± 0.23 [b]	6.33±0.20[a]
PCV%	26.25± 4.32[b]	25.75± 0.64[c]	30.14±0.09[a]
MCV fl	53.21± 0.83[a]	46.27± 0.45[b]	42.01±1.02[c]
MCH pg	12.08± 0.34[c]	14.69± 0.23[b]	16.5±0.34[a]
MCHC g/dl	25.18±0.42[c]	27.08±0.32[b]	31.5±0.26[a]
MCHC g/dl	25.18±0.42[c]	27.08±0.32[b]	31.5±0.26[a]

Different small letters refer to the presence of significantly different at (P<0.05).

Table 2: Clotting factor of infected/non-infected cattle with bovine babesiosis and trypanosomiasis

Clotting factor parameter	MEAN±SE		
	Babesia spp.	*Trypanosoma* spp.	Not-Infect group
Total platelet count × 10^3	297.15± 42.34[c]	309.64 ± 53.65[b]	501.42 ± 49.05[a]
Mean platelet volume / fl	11.49± 4.54[c]	15.35 ± 3.26[a]	10.4 ± 2.35[c]
Platelet distribution width /%	17.94± 3.65[b]	19.32 ± 1.22[a]	12.65 ± 1.08[c]
Clotting time / Sec	5.72± 1.54[a]	4.01 ± 1.65[b]	3.67 ± 1.89[c]
Prothrombin time / Sec	19.22± 2.35[b]	22.531 ± 4.12[a]	13.67 ± 3.26[c]
Activated partial thromboplastin time / Sec	62.09±5.37[a]	61.51± 4.79[b]	54.34±5.72[c]
Fibrinogen time / Sec	37.16± 6.98[a]	35.38± 7.15[b]	12.63 ± 5.42[c]

Different small letters refer to the presence of significantly different at (P<0.05)

Urine examination revealed a coffee-colored urine (hemoglobinuria) in animals infected with severe babesiosis, while cloudy urine observed in babesiosis and trypanosomiasis groups. Urinalysis revealed a varying degree of renal injury arranged from mild (1+) to severe (3+) in the urine of some infected animals.

urinalysis of Cattle infected with severe babesiosis recorded positive (3+) for urobilinogen, bilirubin, protein and glucose, on the other hand, all animals were negative for nitrate and ketone as shown in Table (3):

Table 3: Urinalysis of cattle infected with bovine babesiosis and trypanosomiasis

Urine parameters	Category	Hemoprotozoan	
		Babesia spp. %	*Trypanosoma* spp. %
Urobilinogen mg/dl	Negative	38.5	100
2	1+	23	0
4	2+	23	0
8	3+	15.4	0
12	4+	0	0
Bilirubin mg/dl	Negative	30.8	96.2
1	1+	30.8	3.8
2	2+	23	0
4	3+	15.4	0
Total Protein mg/dl	Negative ≥ 30	0	26.9
30	1+	23	50
100	2+	61.5	23
500	3+	15.4	0
Nitrate mg/dl	Negative	100	100
	+	0	0
Ketone mg/dl	Negative	100	100
25	1+	0	0
100	2+	0	0
300	3+	0	0
Glucose mg/dl	Negative	46.2	88.5
50	1+	23	7.7
150	2+	30.8	3.8
500	3+	0	0

Discussion

In the current research, 39 samples were positive for Trypanosomiasis and babesiosis out of 80 animals (total infection rate 48.8%) which represents around 33% (26/80) and 16% (13/80) respectively. Infected animals showed significant decrease in the values of blood indices (Hb, PCV, RBC, and MCHC) compared to healthy animals. These findings are concurrent with other studies (11, 17, 18). Hemolysis of RBCs could be mediated by an immunological reaction that is related to erythrophagocytosis.

The piroplasm-infected erythrocytes can be released from the reticuloendothelial system into the blood-stream, which then may lead to intravascular hemolysis (17). Symptoms of regenerative anemia, such as anisocytosis, polychromasia, and basophilic stippling, are associated with severe babesiosis and trypanosomiasis (1). Leukocytosis is observed in the infected cattle due to the capability of the protozoan to activate the lymphoid system which is associated with increased white blood cell counts during infection. (19). The detected hematological changes can be closely matched with the previous reports (14,19).

In this study a significant variation in clotting factors has been indicated in the

infected cattle in comparison to non-infected cattle. The decline of the total platelet count may be due to the destruction of megakaryocytes and the decrement of platelet production by megakaryocytes as well as the raised depletion of the platelet cells in the periphery, or functional shortage of the thrombocytes which leads to an increase in platelet volume and Platelet distribution width (20). The results of this study agree with (21) who recorded clear differences in the standards of blood clotting factors during the hemaprotozoan infection, which indicated an increase in clotting time. Prothrombin time is known as extrinsic pathway of the clotting process, and this time increases in the liver's efficiency in producing it weakens. Others added (21- 23) that variation in the values of clotting factors, especially in the infected animals refers to a malfunction in the blood clotting mechanism inside the blood vessels of infected animals, producing hemorrhagic diathesis, causing a large and effective consumption of clotting factor values, forming fibrin clots that are deposited in the blood vessels, which may be a cause of vascular thrombosis (10,11). These changes in clotting factors were closely matched to the previous studies (20- 22). Urinalysis results revealed that bilirubin, urobilinogen, protein and glucose were reported in varying degrees arranged from mild to severe specially in cattle infected with babesiosis. While a few cases infected with trypanosomiasis showed an appearance of protein and glucose (mild to moderate) and bilirubin (mild), the variation in the intensity of these parameters were dependent on severity of infection, level of parasitemia and degree of renal injury. (14,15). The presence of bilirubin and urobilinogen could be due to excessive hemolysis and the inability of the liver to convert the whole amount of the bilirubin. The results of urinalysis are concurrent with other studies (15,24,25).

Conclusion

We concluded that hemoprtozoa reveals an obvious effect on the clotting system which might be reflected in disseminated intravascular coagulation (DIC) abnormalities and changes in urine components Therefore, clinicopathological changes associated with babesiosis and trypanosomiasis should be evaluated periodically to evaluate animal health status.

Acknowledgments

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

The Research Ethical Committee approves this work.

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التغييرات السريرية المرضية المرافقة للإصابة بداء الكمثريات والمثقبيات في الابقار.

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الخلاصـــــــــــة

ان التغيرات السريرية والمرضية المرتبطة بالاوالي الدموية لدى الأبقار قد تم فحصها جيدًا، إلا أن هذه التغيرات قد تتأثر بمرور الوقت نتيجة لعوامل مختلفة مثل كتطور الجهاز المناعي للحيوانات وبعض التغيرات البيئية. ان الهدف من هذه الدراسة هو تقييم التغيرات السريرية والمرضية المرتبطة بعدوى الاوالي الدموية في الأبقار المصابة بشكل طبيعي. إذ فحصت 80 بقرة للكشف عن الكمثريات والمثقبيات باستخدام المسحات الدموية المصبوغة بصبغة كيمزا, فضلا عن تقدير التغيرات في معايير الدم وعوامل تخثرها وتحليل البول. اذ تم تشخيص داء البابيزيا وداء المثقبيات بنسبة إصابة كلية بلغت 16٪ و 33٪ على التوالي في المسحات الدموية المصبوغة. كما أوضحت الدراسة وجود انخفاض معنوي في مستوى تركيز خضاب الدم وحجم خلايا الدم المرصوصة والعدد الكلي لكريات الدم الحمر الكلي ومعدل تركيز خضاب الكرية والعدد الكلي للصفائح الدموية في الأبقار المصابة. في حين، تم تسجيل زيادة معنوية في عدد خلايا الدم البيض الكلي، وزمن التجلط وزمن سابق الخثرين وزمن منشئ الليفين وزمن حرك الخثرين الجزئي المنشط، وكذلك حجم الصفائح الدموية ومعدل انتشارها في الابقارالمصابة مقارنة بالحيوانات السليمة. وأظهر تحليل البول وجود مولد الصفراوين، والصفراوين، والبروتين الكلي والكلوكوز في بول بعض الحيوانات المصابة، استنتج من هذه الدراسة بان الإصابة بالكمثريات والمثقبيات كان له تأثير واضح على معايير الدم وعوامل تخثرها في الابقار المصابة مما قد يؤدي الى حدوث اضطراب في الية تخثر الدم فضلا عن الإصابة بفقر الدم وظهور بعض التغييرات في البول لذا يجب تقييم التغيرات السريرية المرضية المرتبطة بالطفيليات الدموية بشكل دوري لغرض تقييم حالة الحيوان الصحية.

الكلمات المفتاحية: داء الكمثريات ، داء المثقبيات ، عوامل التخثر، تحليل البول .