SEROPREVALENCE OF BABESIOSIS IN CATTLE IN MOSUL CITY, IRAQ

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ABSTRACT

Babesia bigemina is considered as one of the most remarkable blood protozoa in cattle and mainly transmitted via arthropod. This study was conducted on a random group of cows, they numbered 180 local cows who ranged in age from 3-7 years old, from different localities in Mosul city north Iraq, comprising both clinically healthy (n=162) and clinically suspected infected animals (n= 18). In this study, indirect-enzyme immunosorbent Assay (I-ELISA) was used to detect of babesia antibodies known as *B. bigemina* in the blood serum. Then, the blood and biochemical information that existed from both groups was analyzed, so that the two of them are compared with the control group (n=15). The result showed that the overall seroprevalence of *B. bigemina* in cows was 74/180 (41.1%) for clinical and subclinical cows were 10% and 31.1% respectively. The subclinical infected cows was statistically higher than that of clinically infected ($P<0.05$). Clinically infected cows were suffering from acute onset of the disease includes fever, anorexia, emaciation, drooping in milk yield, jaundice and hemoglobinuria,, with significant hematological and biochemical parameters alterations. While, subclinically infection cows appeared healthy with absence of changes in blood and biochemistry tests as compared to control groups. It has been concluded that significant cases were diagnosed suffering from acute infection with the *B. bigemina* with higher prevalence of subclinical cases in Mosul city, Iraq.

INTRODUCTION

Bovine Babesiosis is list B disease according to OIE classification for the most common blood parasitic disease of mammals after trypanosome, the disease implicated as a substantial economic losses due to high morbidity and mortality rates, decrease in animals production and the cost of treatment (1). It is intraerythrocytic protozoan and belongs to the Babesiidae family (the genus Babesia, order Piroplasmida, phylum Apicomplexa and subclass piroplamsia), which responsible to infect domestic animals, wild animals and human (2). However, the term piroplasmas is considered one of the commonly used terms due to its pear-like form, a form that expresses the presence of the merozoites present as small parasites within the red blood cells of the mammals (3, 4).

It is worth noting that there is a major type that acts as a vector for this babesia: The *Rhipicephalus (formerly Boophilus*) ticks (5). There are more common types belonging to this family and found in cows, and they are: *B. bigemina, B. divergens, B. bovis* and B. major. It should be noted that *B. bigemina* and *B. bovis* are among the most influential species on livestock health and productivity, especially those in tropical and subtropical countries such as Africa, Asia, Australia, and the United States of America (6, 7, 8) In these species, the transmission of *Babesia* species is through hard ticks and transovarially passes by the egg from one generation to the following, and these ticks are: *Rhipicephalus microplus* and *Rhipicephalus annulatus* (9).

On the other hand, there are some symptoms that accompany the infection of babesia ,which include: anorexia, pale mucus membrane, dyspnea, anemia, fever, hemoglobinuria. Mostly, these clinical signs affect animals that produce low-level parasitemia in a way that they become carrier(10, 11), Despite this, the livestock may remain infected and have no clinical symptoms, and this is the main transmission factor (3, 5).

In this regard, there are many techniques that are used to diagnose and detect the presence of different types of babesia, among which are: microscopic examination of blood film, serological tests and molecular assays. It must be clarified that a blood smear test is followed in

severe cases and this technique is classified as a standard technique for routine diagnosis of babesiosis, and it is rate by some as a gold standard (12).

Besides, serological tests including enzyme-linked immunosorbent assay (ELISA) are considered able to detect the presence of antibodies in pregnant animals, for later use for monitoring surveillance and export certification (13, 14, 15). Studies of *B. bigemina* in Mosul City, Iraq are very scarce and little information has been reported. The therefore, the aims of this study were to determine the prevalence of *B. bigemina* and concurrent infection in clinically and subclinically infected equids. Moreover, to investigate the clinical ,hematological and biochemical parameters alterations of clinical and subclinical infection cows.

MATERIAL AND METHODS

Animals and samples collection: From October 2018 to April 2019, the study was conducted on 180 native cows, 3-7 years old, from different farms in Mosul city north Iraq, comprising both clinically healthy (n=162) and clinically suspected infected animals (n= 18). Hematological and biochemical parameters were analyzed from blood and serum samples from clinical and subclinical cows in comparison to healthy cows (n=15) as control group. Information regarding age, origin, clinical status and clinical signs of the animals were recorded during sample collection. Ten-milliliter (ml) blood samples were collected from each cow via jugular vein puncture using 18G needle into two sterile vacutainers® tubes (5ml each), one with anti-coagulant ethylene diamine tetraacetic acid (EDTA) for hematological analysis. Serum was separated from the second tube without anti-coagulants for detection *B. bigemina* antibodies and for biochemical analysis.

Laboratory processing: In order to perform the direct diagnosis process, these slides were repaired using methanol70% and were stained with Giemsa for 30 mints to be observed under the optical microscope (Olympus BX 51, America, Inc.).

The blood was examed to calculate (CBP) : total erythrocyte counts (TRBC), packed cell volume (PCV), hemoglobin concentration (Hb), total and differential leukocyte counts (TLC), thin and dried blood smears stained with Giemsa were allocated to calculate the number of differential leukocyte counts (16).

In addition, there are a number of Serum biochemical parameters were analyzed using special cassettes for each in a Chemistry analyzer (Vet Test, Arachem/ USA), which includes: alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALKP), blood urea nitrogen (BUN), total protein, creatinine, glucose and calcium

Indirect enzyme linked immunosorbent test (I-ELISA): In this study, an Indirect ELISA examination was performed in order to detect the presence of *B. bigemina* antibodies present in the serum .Samples selected in the study, and this was done according to the techniques provided by (Savona-Sweden). However, absorbance was measured at 405 nm through the use of a micro-plate reader known as the BioTek EL-800 micro plate reader. In order to obtain a set of values for Optical density (OD), these values are then transferred to a Microsoft Excel spread-sheet. Of these values, Percent sero-positivity (PP) was calculated according to the manufacturer's instructions equation below, and only when both versions gave PP ≥ 40 an animal was considered as positive.

$$PP = \frac{\text{Mean OD Samples}}{\text{Mean OD Positive control}} \times 100$$

The interpretation of results : PP ≤ 25 = Negative , PP 26-39 = Doubtful , PP ≥ 40 = Positive

Statistical analysis: For data analysis, IBM-SPSS statistics (ANOVA) program-version 21 was used, from which several tests were performed, including: two-sided Chi-square and 95% confidence interval. In order to compare between the control group and the experimental group selected in this study, ($P < 0.05$) was determined in order to identify all the important differences.

RESULTS

In the current study, based on indirect ELISA analysis of *B. bigemina* in cow was 74/180 (41.1%) for the clinical and subclinical cows were 10% and 31.1% respectively. The subclinical infected cows was statistically higher ($P<0.05$) than that of clinically infected (Table1).

The clinically infected cows with *B. bigemina* were suffering from acute form of the disease and exhibited fever, anorexia, emaciation, drooping in milk yield, jaundice and hemoglobinuria with different frequency percentages of each signs (Figure 1). While subclinical infected cows appear healthy with no clinical signs. Further, a statistically significant increase ($P<0.05$) in the body temperature (40.5C°), respiratory rate (58.3 /min), heart rate (89.8 /min) (Table 2).

Table 1: Clinical status of *B. bigemina* in cows using indirect ELISA test (n=180).

Clinical Status	***B. bigemina* No. Positive (%)**
Clinically infected	18(31.1)[a]
Subclinical infected	56(10)[b]
Total	74(41.1)

Values significantly different ($P < 0.05$) between cows status are labelled with the vertically different letters (a, b).

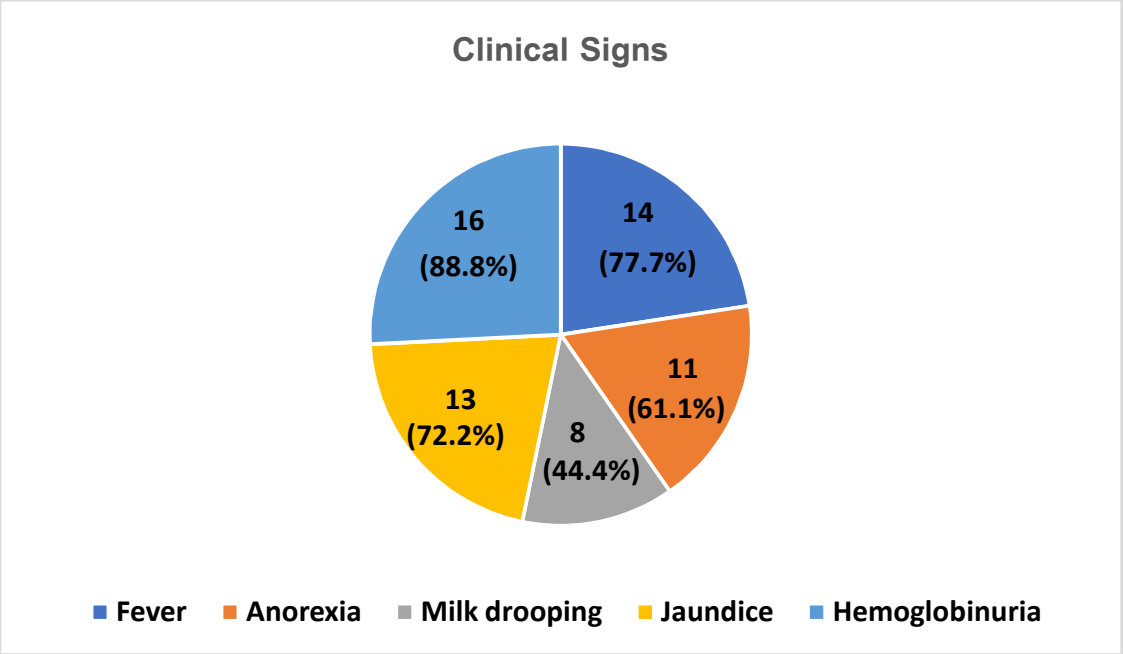

Figure 1: Frequency and percentage of clinical signs in cows clinically infected with *B. bigemina* (n=18).

Table 2:Clinical parameters of cows clinically infected with compared with subclinical infected and healthy group.

Parameters	**Healthy group (n=15)** Mean ± S.E	**Subclinical infected cows (n=56)** Mean ± S.E	**Clinically infected cows (n=18)** Mean ± S.E
Body temperature °C	37.8 ± 0.22	37.5 ± 0.13	40.5 ± 0.73*
Respiratory rate /1 min	30.5 ± 3.27	31.5 ± 4.45	58.3 ± 5.45*
Heart rate/1 min	57.5 ± 4.33	58.3 ± 5.21	89.8 ± 4.43*
Rumen contraction / 5min	5.82 ± 0.43	5.92 ± 0.23	2.87 ± 0.22*

Mean values ± standard error (S.E.), * ($P < 0.05$) a significantly different between cows clinical status.

In the present study the haemo gram of cows clinically infected with *B. bigemina* reflected to statistical significant decrease in TEC, Hb, and PCV, along with significant increase in total TLC, due to neutrophilia and lymphocytosis ($P < 0.05$), while no statistically significant alterations of these hematological parameters in the subclinically infected cows compared with the healthy group (Table 3).

In addition, serum biochemistry analysis of clinically infected cows showed significant increase in ALT, AST, ALKP, blood urea nitrogen BUN, creatinine and significant decrease in total protein, while no significant changes were found in the glucose and calcium levels ($P<0.05$). No statistically significant alterations of these biochemistry parameters in the subclinical infected cows compared with the healthy group (Table 4).

Table 3: Hematological values in cows clinically infected with *B. bigemina* compared with subclinical infected and healthy group

Parameters	**Healthy group (n=15)** Mean ± S.E	**Subclinically infected cows (n=56)** Mean ± S.E	**Clinically infected cows (n=18)** Mean ± S.E
Total erythrocytes counts $\times 10^6$ µl	7.21 ± 0.84	6.92 ± 0.54	5.29 ± 0.43*
Hemoglobin, mg/ 100ml	11.88 ± 1.64	12.11 ± 1.23	7.83 ± 1.72*
Packed cell volume %	33.25 ± 3.78	32.15 ± 2.67	26.22 ± 2.23*
Total leukocyte counts $x10^3$µl	11.72 ± 1.43	10.98 ± 1.31	13.54 ± 0.9*
Neutrophils $x10^3$µl (%)	46.34 ± 3.22	46.25 ± 5.12	(47.62 ± 2.32)*
Lymphocytes $x10^3$µl (%)	44.62 ± 3.13	44.12 ± 2.24	(45.94 ± 2.12)*
Monocytes $x10^3$µl (%)	3.63 ± 1.01	3.23 ± 1.11	3.73 ± 3.23
Eosinophils $x10^3$µl (%)	3.22 ± 1.13	3.13 ± 1.22	3.52 ± 1.22
Basophils $x10^3$µl (%)	1.21 ± 0.14	0.91 ± 0.12	0.87 ± 0.14

Mean values ± standard error (S.E.), * ($P < 0.05$) a significantly different between cows status

Table 4: Biochemical values in cows clinically infected with *B. bigemina* compared with subclinical infected and healthy group

Parameters	**Healthy group (n=15)** Mean ± S.E	**Subclinically infected cows (n=56)** Mean ± S.E	**Clinically infected cows (n=18)** Mean ± S.E
ALT U/L	40.52 ± 11.83	42.56 ± 12.19	65.18 ± 12.68*
AST U/L	54.55 ± 19.66	57.15 ± 20.15	95.81 ± 22.04*
ALKP U/L	54.35 ± 31.12	56.33 ± 21.11	67.88 ± 23.13*
BUN mg/dL	18.60 ±5.21	19.10 ± 6.23	27.54 ± 7.42 *
Total protein g /dl	7.47 ± 0.22	7.07 ± 0.12	3.27 ± 0.58*
Creatinine mg/dl	0.67 ± 0.19	0.98 ± 0.17	1.87 ± 0.22 *
Glucose mg/dL	58.46 ± 11.86	58.14 ± 10.45	57.21 ± 7.67
Calcium mg/dl	9.14 ±1.88	9.01 ± 1.48	8.9 ±1.72

Mean values ± standard error (S.E.), * ($P < 0.05$) a significantly different between cows status

DISCUSSION

This study showed that there is a an increase in the prevalence *of B. bigemina* infection in the city of Mosul, which is up to approximately 42%. The reason for this is that there is a large distribution of types of carrier ticks, as well as the presence of transporting animals that are either carrier or sub-clinical, in addition to environmental conditions, including farm managements that have a significant impact on the occurrence of the disease. This is consistent with what was found in the (17), as found that the main problem in the presence of babesiosis is the presence of animals that recover from the disease will become chronic carriers that act as a source of infection for other animals and ticks.

Which means that Bovine Babesiosis occurs mostly in the tropics, subtropics and temperate regions of the world, and this is what affects many livestock with this microbe (18). This is consistent with many previous studies that touched on the extent of Bovine Babesiosis prevalence of this was 27.27% (19), whereas, the prevalence of babesia in Qadisiyah Governorate reached 47.91%, in Iran 7.10% (20), and in Turkey it was 14.8% (21).

On the other hand, the current study showed that cows infected with *B. bigemina* was experiencing fever, anorexia, emaciation, drooping in milk yield, jaundice and hemoglobinuria. Besides, other symptoms are high body temperature, respiratory rate, heart rate and decreased in rumen contractions in infected cattle. These results are consistent with the results of some previous studies who mentioned that the symptoms of infection with this microbe are represented in pale mucus membranes, jaundice, increased respiratory rate, hemoglobinuria, and fever (22, 23, 24). It is noted when performing a haemogram that the number of erythrocytes and hemoglobin decreased, while there was a decrease in packed cell volume and total leukocytic count; Especially when both the infected group were compared to the healthy group. This is consistent with the results of some previous studies (6, 25, 26).

Regarding the results of the biochemistry of the affected group of cattle, There is a significant increase in the percentage of AST, ALT, ALKP, BUN, total protein and creatinine, on the contrary, there were no significant changes in the levels of glucose and calcium in the blood; This is consistent with the results of some previous studies (24, 25, 26).

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الانتشار المصلي لداء البابيزيوسز في الابقار في مدينة الموصل، العراق

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الخلاصة

يعد طفيلي *Babesia bigemina* احد اكثر طفيليات الدم التي يمكن ملاحظتها في الماشية، وينتقل الطفيلي بشكل رئيس عن طريق المفصليات. أجريت الدراسة بصورة عشوائية على مجاميع الابقار المحلية ١٨٠ بقرة، وبأعمار تراوحت بين ٣-٧ سنوات ومن مناطق مختلفة في مدينة الموصل شمال العراق. تضمنت ١٦٢ حيوان سليم سريرياً و ١٨ حيوان مشكوكة الاصابة. تم اجراء اختبار الممتز المناعي المرتبط بالأنزيم المباشر I-ELISA للكشف عن الأجسام المضادة لطفيلي *B. bigemina* في مصول الدم، وكما وتم اجراء الفحوصات الدموية والكيموحيوية على كلا المجموعتين ومقارنة النتائج مع حيوانات السيطرة ١٥ بقرة. أظهرت نتائج الدراسة ان النسبة الكلية لانتشار الطفيلي في الابقار ١٨٠/٧٤ (41.1%) ، منها ابقار مصابة سريريا و بقار مصابة تحت السريري (١٠٪ و ٣١.١٪) على التوالي، هذا يعني ان نسبة الاصابة التحت السريرية في الأبقار كانت اعلى معنويا من الابقار المصابة سريريا ($P<0.05$). عانت الأبقار المصابة سريريًا من الشكل الحاد للمرض شملت الحمى وفقدان الشهية والهزال وتدهور إنتاج الحليب واليرقان وبيلة الهيموغلوبينية ، مع وجود تغييرات في بعض اختبارات الدم والاختبارات الكيموحيوية، في حين ظهرت الأبقار المصابة بالشكل التحت السريري بصحة جيدة مع عدم وجود تغييرات في اختبارات الدم والاختبارات الكيموحيوية عند مقارنة النتائج مع مجموعة السيطرة. استنتج من الدراسة تشخيص الشكل الحاد من الاصابة بطفيلي *B. bigemina* مع انتشار العالي للحالات التحت السريرية في مدينة الموصل، العراق.

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