Research Article

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Detection of Babesia spp by using of Acridine Orange and Giemsa Stain with Study of its Effect on Blood Picture in Local Breed Cows

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Abstract

This study was conducted to diagnose of *Babesia spp* by using fluorescent microscope and light microscope, the study also included evaluating of Hemogram (RBC, HB, PCV, MCV, MCHC, Total Leukocyte Count, Differential Leukocyte Count) in cows infected with *Babesia spp* which cause piroplasmosis. The study included examination of 60 local breed Cows from many districts of Mosul city from the beginning of February 2018 until the end of July 2018, 17 were clinically infected with piroplasmosis and the infection was confirmed by using Giemsa and Acridine orange staining blood smears, and 10 cows were clinically intact and served as control. the results indicated that the percentage of the infection with *Babesia spp* was 28.33%, and the percentage of parasitemia ranged between (5-23%) with mean of (12.57%). The Results showed a statistical significant decrease ($P<0.05$) in RBC, Hb, and PCV values of diseased animals, Normocytic Normochromic type of anemia was found, the results also indicated a significant decrease in total Leukocyte count and neutrophil, with significant increase in lymphocytes number. the results also indicated non-significant changes in Eosinophil, Basophil and Monocyte numbers.

Keywards: *Babesia*, Heamatology, Cow

الكشف عن طفيلي البابيزيا باستخدام صبغة الاكردين البرتقالية وصبغة كيمزا مع دراسة تاثيره على الصورة الدموية في الابقار المحلية

الخلاصة

اجريت هذه الدراسة لتشخيص طفيلي البابيزيا باستخدام المجهر المتألق Fluorescent microscope وكذلك المجهر الضوئي, كما تضمنت الدراسة تقييم الصورة الدموية للأبقار المصابة بطفيلي البابيزيا حيث شملت عدد كريات الدم الحمراء RBC , تركيز خضاب الدمHB, حجم خلايا الدم المرصوصة PCV, معدل الحجم الكروي MCV معدل تركيز خضاب الدم الكروي MCHC،العدد الكلي لخلايا الدم البيضTLC ، العدد التفريقي لخلايا الدم البيض DLC . شملت الدراسة فحص 60 راسا من الابقار المحلية من مناطق مختلفة من مدينة الموصل منذ بداية شباط 2018 وحتى نهاية تموز 2018 تضمنت17 بقرة مصابة اظهرت علامات سريرية بداء الكمثريات وتم تأكيد اصابتها عن طريق أخذ المسحات الدموية التي صبغت بصبغة الكيمزا وصبغة الاكردين البرتقالية, و 10 بقرات سليمة سريريا عدت كمجموعة سيطرة. اظهرت نتائج الدراسة ان نسبة الاصابة بطفيلي البابيزيا كانت (28,33) وبنسبة تطفل بلغت(5-23%) وبمعدل (12.57%) وكما اظهرت التغيرات الدموية للأبقار المصابة وجود انخفاض معنوي ($P<0.05$) في عدد كريات الدم الحمراء وكمية خضاب الدم بالإضافة الى حجم الخلايا المرصوصة وكان فقر الدم من النوع ذي الكريات سوية الحجم وسوية الصباغ Normocytic Normochromic anemia, كما سجل انخفاض معنوي ($P<0.05$) في العدد الكلي لخلايا الدم البيض وكذلك بالنسبة للعدلات في حين سجل ارتفاع معنوي ($P<0.05$) في عدد الخلايا اللمفية ولم يسجل أي اختلاف معنوي في عدد الحمضات والقعدات ووحيدة النواة

Introduction

Babesia spp is one of the most virulent protozoa that affect cows, causing so-called bovine piroplasmosis , Texas fever or red water disease , The parasite replicate within the red blood cells and the most important species that infected cows are *Babesia bovis* , *Babesia bigemina, Babesia divergens* (1, 2).

The parasite is transmitted by a hard tick, *Babesia bovis* and *Babesia bigemina* is widespread in Asia and Africa, parts of Australia, while *Babesia divergens* is widespread in parts of Europe and North Africa (3).

Babesia bigemina appears within the red blood cells in the form of double pear shape, with a sharp angle appear between them and is characterized by its large size and can also appear in the form of a single pear in addition to the oval shape and irregular shape (4).

While *Babesia bovis* appears inside the red blood cells in the form of double pear, between them a separate angle and its located near to the surface of the red blood cells and has a nuclear mass in one of the poles and is characterized by small in size and it is more virulent than *Babesia divergens, Babesia bigemina*. Giemsa stain has been used since a long period of time in detection of *Babesia spp* in blood smear , Despite the efficiency of the Giemsa stain in the diagnosis of the parasite where reliable in determining the form of the parasite such as pears or oval, However the acridine orange stain is simply applied and takes about 2-4 minutes in the diagnosis of *Babesia spp* using a fluorescent microscope compared to Giemsa stain which process of pigmentation takes more time (30-60) minutes using a normal light microscope (5).

Acridine orange stain is one of Fluorescent dyes which can be penetrate the cell wall and binds to DNA and RNA and this binding lead to emitting an orange color under the Fluorescent microscope So the parasite appears orange or yellow inside the red blood cell, while the red blood cell appears pale color because it does not contain DNA (5). Fever , pale or icteric mucus membrane, Hemoglobinuria and sometime abortion were the main characteristic signs showed by diseased cows (6).

The *Babesia sp* after entering the blood stream through the tick bite begins to attack red blood cells and then begins to divide and multiply within them, which increases the pressure inside the red blood cell and then break down of red blood cells, then parasite is released into the plasma to attack new erythrocytes and thus break down or lysis a large number of erythrocytes, causing a decrease in the number of erythrocytes and release of Hemoglobin into the blood (hemoglobinemia), which finally leads to hemoglobinuria (7). decrease in RBC, HB, PCV are recorded by (4).

The type of anemia in infected cows depends on the severity of infection and virulence of the parasite, some studies shows Normocytic Normochromic Anemia with non-significant changes in Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC) (8), and another study shows microcytic hypochromic anemia (9). The parasite also has an effect on the number of white blood cells, leukocytosis with lymphocytosis and monocytosis are recorded by (10), while leukopenia are seen by (11).

Materials and Methods

The study was conducted on 60 local breed cows, with different ages from many districts of Mosul city (Hawi Al-Kanesa ,ALGawssiat ,AL Quba, Telkif) . The period of study was from the beginning of February 2018 until the end of July 2018. Seventeen out of sixty local cows were infected with *Babesia spp.* The infection was confirmed by using blood smears staining by Giemsa and acridine orange stain, and 10 cows

clinically intact served as control.

Blood sample collection:

Blood were drained from The infected and control cows by using a sterile syringe from the jugular vein .3ml of blood are drained and mixed with anticoagulant(EDTA) to evaluating of Hemogram (erythrocyte count (RBC), hemoglobin (HB), packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), total leukocyte counts (TLC) by using Beckman (automatic digital cell counter), differential leukocyte counts (DLC) are estimated by using blood smears stained by Giemsa stain (12).

Detection of *Babesia spp* in blood smears:

blood smears were prepared by puncture of ear vein and a drop of blood was placed on glass slide then spread by other slide, blood smear was dried and fixed by a solute methanol (3-5 minute). Then blood smears were stained with Giemsa stain and acridine orange stain.

Staining of blood smears by Giemsa stain: Giemsa stain was added on fixed blood smears and left for 30 minute, blood smears were washed with tap water and examined under light microscope, the percentage of parasitemia in red blood cells was determined according to the following equation (13):

$$\frac{\text{Number of infected RBC}}{\text{Number of calculated RBC}} \times 100$$

Staining of blood smears by acridine orange stain:

The acridine orange stain were prepared by dissolving 25 mg of stain in 5 ml of distilled water and then placed in an opaque glass bottle and kept in the refrigerator for four weeks, 1ml of stain were mixed with 0.5ml of glacial acetic acid and 50ml of distal water, then the stain become ready to use, the stain was added to fixed blood smears for 2 minute and blood smears were washed with tap water and examined under fluorescent microscope (14).

Statistical analysis: Results were statistically analyzed using SPSS statistical program and T test when comparing between diseased and healthy cows (15).

Results and Discussion

The diseased cows showed several clinical signs (fever, loss of appetite, pale and in other cows icteric mucus membrane, hemoglobinuria (coffee color like urine), and the infection was confirmed by using Giemsa and Acridine orange staining blood smears, Where the parasite appeared inside red blood cells in various forms, including the single pear shape and double pear shape between them sharp or obtuse angle in addition to irregular forms, and the parasite appeared in different sizes (Figure 1,2). It was noted that Giemsa Stain was more accurate in the diagnosis of the parasite depending on the form, but the continuous filtering of the stain to prevent the stain deposits on the surface of red blood cells was one of the disadvantages of this Stain, in addition the staining time lasted 45 minutes, while the staining method with acridine orange characterized by quickly and easily and the period of staining was only 2 minutes and The uninfected erythrocytes was observed in a pale color ,while the parasite-infected erythrocytes appeared in bright orange or yellow, (Figure 3). The study also showed that 17 of the 60 cows were infected with *Babesia spp*, percentage of infection was (28.33) Table (1) .

Table (1) Total percentage of infection in cows

Number of examined cows	Number of infected cows	Percentage of infection
60	17	**28.33**

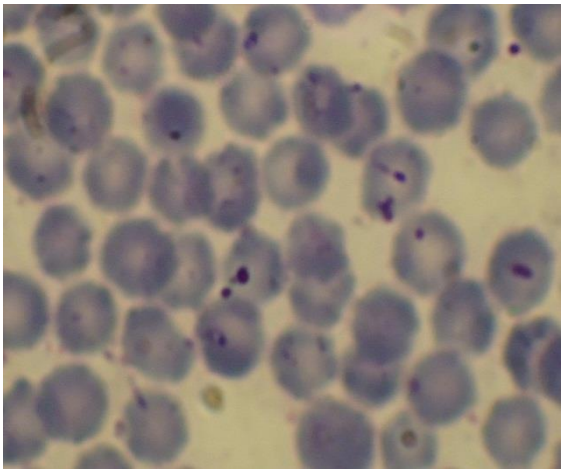

Figure:1 irregular form of *Babesia* inside erythrocyte

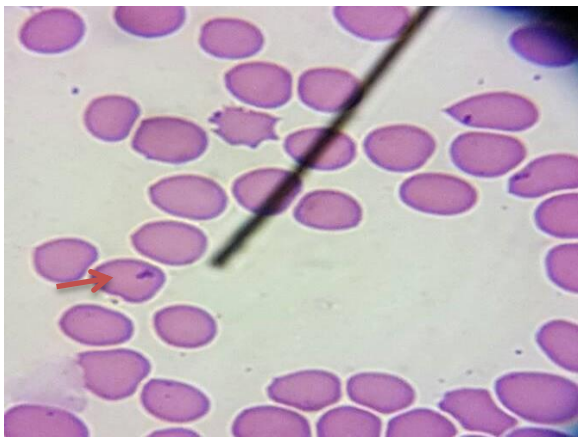

Figure:2 double pear form of *Babesia* inside erythrocyte

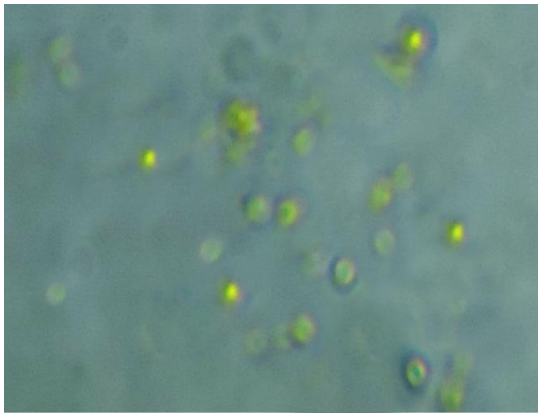

Figure: 3 brilliant yellow appearance of *Babesia* inside erythrocyte

The results of the study showed significant decrease ($P < 0.05$) in the number of erythrocytes, hemoglobin concentration and the volume of packed cell volume in infected cows compared with healthy cows. The decrease of erythrocytes may be due to the direct effect of the parasite to the infected erythrocytes and decrease life span of RBCs in addition to suppression of hemopoitic system and the result was agreement with (7)(16), Auto immunity and the anti erythrocytic auto antibodies lead to increasing of erythrophagocytosis and bone marrow depression was recorded by (11) as the main causes of anemia and decrease of erythrocytes.

No significant changes was observed in Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration between infected cows and control group, So the type of anemia was Normocytic Normochromic anemia, the result was agreement with (16). The percentage of parasitemia ranged between (5-23%) with mean of (12.57%), while The parasitemia of control cows was 0% Table (2). A significant decrease was showed in total leukocytes count as well as in neutrophils count in infected cows, some studies were recorded decrease of Total leukocytes count and another studies were showed increase of its counts and this may be depending on the stage of disease, where decrease of total leukocytes count was reported by (17)(18) in early stage of disease, on the other hand increase of its counts were showed by (16) in advance stage of disease. Total leukocytes count was decreased in current study and the cause may be due to the direct effect of parasite on bone marrow and inhibit its production, similar result was seen by (11)(17) , While the study showed a significant increase ($P < 0.05$) in the number of lymphocytes in infected cows compared with the control cows and this may be attributed to the response of the immune system to produce antibodies against of *babesia spp* through the increase of lymphocytes, and the results were agreed with (9), non-significant changes in Eosinophil ,Basophile and Monocyte numbers were seen in this study Table(3).

Table (2) Blood parameters of infected cow with Babesiosis and controls

Parameters	Mean ± Standard Error	
	Control cows	infected cows
TRBc ($\times 10^6/\mu L$)	9.20 ±0.44	5.70±0.39*
HB (g\dL)	0.67 ± 13.08	8.51±0.38 *
PCV(%)	36.90 ± 1.41	26.10±0.78*
MCV/fl	40.37±2.19	44.78 ± 3.40
MCHC (g\dL)	35.44 ± 1.66	33.16±1.46
Parasitemia (%)	00000000	21.57±6.81

(P<0.05) significant values

Table (3) Total and Differential Leucocytes Count of infected cow with Babesiosis

Parameters	Mean ± standared error	
	Control cows	infected cows
TLC ($\times 10^3\ \mu L$)	10.43±0.58	8.47±0.55*
Neutrophils (%)	35.77± 0.16	22.40 ±0.28 *
Lymphocytes) (%	54.36 ± 0.11	70.88 ±0.31*
Eosinophils (%)	5.14±0.08	5.80 ± 0.02
Basophils (%)	0.07 ± 0.04	0.09 ±0.01
Monocytes (%)	4.32 ± 0. 40	4.65±1.86

(P<0.05) significant values*

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