Research Article

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Microscopic Identification of *Babesia* Spp in Sheep in Mosul City

Baydaa Y. Al-lahaibi, Eman G. Suleiman.

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Corresponding Author Email Address: baydaaum18@uomosul.edu.iq

ORCID ID: https://orcid.org/0000-0002-8535-5091

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Abstract

Babesia, one of the most important parasites transmitted by hard ticks (family Ixodidae), is responsible for high infection and mortality rates in sheep herds in various countries around the world. The current study included the diagnosis of the Babesia parasite in 260 blood samples collected from sheep in different areas of Mosul from November 2023 to June 2024. Thin blood smears were prepared from them, stained with the Giemsa stain, and examined by using the light microscope. The *Babesia* species was diagnosed in 112 blood samples, with a total infection rate of 43.07%. Two species of *Babesia* were diagnosed: the large-sized *Babesia* (*Babesia motasi*) with a measurement rate of (3.76±0.83) x (2.39±0.78) and a range of (2.5-5) x (1-5) µm, and the small sized *Babesia* (*Babesia ovis*) with a measurement rate of 1.81±0.58 x (1.1±0.47), with a range of (1-2.5) x (1.1-2.1) µm. The current study also included the possibility of using the fluorescent Acridine orange stain (AO) to stain 50 sheep blood samples. This stain was distinguished by its easily and speed in diagnosing infection with the *Babesia* parasite in blood smears within two minutes. This stain is useful in survey and epidemiological studies, but it is not useful in parasite specific identification.

Keyword: *Babesia* , sheep, blood, Giemsa , fluorescent Acridine orange .

Introduction

Babesia parasites are tick-borne diseases and obligate intraerythrocytic protozoa belonging to the phylum Apicomplexa (1). Wild and domestic animals are reservoir hosts for more than 100 *Babesia* spp. (2). Microscopic examination of blood smears stained with Romansky stains, especially the Giemsa stain, through the hundred years that have passed is the gold standard test for diagnosis. Many blood parasites and Rickettsia (3) *Babesia* spp. have a direct impact on productivity and economics for animals; these pathogens are transmitted by hard ticks (4). Microscopic examination by using Giemsa stain is considered the fast, sensitive which ranging from 10^{-5} -10^{-6} and non-expensive methods to diagnose *Babesia* species in different host (5).

One of these diagnostic alternatives involves using fluorescent dyes to stain nucleic acids, such as Acridine orange (AO). Fluorescent dyes penetrate cells and interfere with deoxyribonucleic acid DNA and ribonucleic acid RNA (via electrostatic attraction). AO dye also disrupts acidic components, including lysosomes. In slightly acidic conditions, this dye emits an orange light when excited by blue light (6). In 1942 both Hilbrich, Struggler described the first used acridine orange in diagnosis of microorganisms. Since then, the dye has been used to examine the bacterial content in soil and water to determine the degree of bacterial contamination in water (7). AO is used in the diagnosis of some parasites such as *Plasmodium* spp., *Trypanosoma* spp., *Trichomonas vaginals*, *Toxoplasma gondi*, *Babesia* spp. such as *B. bigemina* and *B, canis (*3,6,8-10).

This study aimed to detect *Babesia* spp. in sheep in Mosul city and study the morphological and biometrical characteristics of this parasite by using Giemsa and Acridine orang stain.

Materials and Methods

Collecting blood samples

A total of 260 blood samples were collected randomly from sheep through field visits to different areas of the city center of Mosul and from the fields of the College of Agriculture and Forestry / University of Mosul, as well as from disease cases received at the teaching hospital of the College of Veterinary Medicine / University of Mosul and animals slaughtered in the Mosul abattoirs for the period November 2023 to June 2024. Blood samples were collected from both sexes and different ages, from animals suffering from distinct clinical signs of disease, and from animals that appeared clinically healthy.

Blood samples of 3-5 ml were taken from the jugular vein after sterilizing the area with 70% ethyl alcohol using wine-filled, sterile, single-use medical syringes. As for samples collected from sheep at the slaughterhouse, blood was also taken from the jugular vein directly during the slaughter process. The blood samples were kept in tubes containing the anticoagulant type EDTA and were stirred slightly to prevent

blood clotting. The samples were transported in cool boxes with ice packs to the Laboratory of Parasitology\ College of Veterinary Medicine\University of Mosul to conduct laboratory tests on them.

Microscopic examination of blood samples

From 260 blood samples, thin blood smears were made and stained with Giemsa stain.

1- Preparation of Giemsa stain 5%

A- Prepare a stock of Giemsa stain by mixing 0.75 g of Giemsa powder with 25 ml of glycerin in a ceramic mortar until it becomes a paste, then incubate for 30 minutes at 37 °C, mix well with 75 ml of absolute methyl alcohol, and incubate for 24 hour at 37 °C.

B- Prepare working Giemsa solution: by mixing 5 ml of the concentrated dye solution with 45 ml of distilled water, then filtered using filtering paper and storing in dark glass bottles to prevent light from entering until used (11).

C- Staining method: 260 thin blood smears were prepared. Fix the blood smears with absolute methyl alcohol for 3-5 minutes. The blood smears were stained with 5% Giemsa stain for 30-60 minutes. The smears were washed with water, left to dry, and then examined using a light microscope with 100X oil lens magnification to distinguish *Babesia* spp.(12).

2- Preparation Acridine Orange stain:

50 blood smears were selected randomly, fixed with absolute methyl alcohol, and stained with Ao according to (13).

A- prepare stock solution: It has been dissolved 50 mg of Ao powder (BDH Chemicals Ltd Poole England) Acridine orange in 10 ml of distilled water (0.5%) and placed in an opaque bottle. It was stored in the refrigerator for four weeks.

B-Prepare the working solution: Take 1 ml of Ao stock solution and 0.5 ml of glacial acetic acid, which add to 50 ml of distilled water. This solution is sufficient to stain eight glass slides. And the concentration becomes (0.01%). The PH of the working solution was measured using a PH meter (PH=3).

C-Staining method:

1- Blood smears fixed with absolute methyl alcohol
2- Placed after dry it completely in Ao Working solution (0.01%) for 2 minutes.
3- The blood smears were washed gently with water
4- The sample was completely dried in air and then examined using a fluorescence microscope at the College of Dentistry/University of Mosul. The type of fluorescent microscope used was the Italy/Optika -350B, equipped with a filter set Sequence (G BF B). The examination was conducted using a blue light on the candidate BF.

Results

This study recorded a total rate of infection with Babesia spp. in sheep by microscopic examination of Giemsa-

stained blood smears of 43.07% (112/260) in Mosul city (Table 1).

Table 1: Total rate of infection with *Babesia* spp. in sheep

Number of examined blood samples	Number of positive samples	Percentage%
260	112	43.07

Two types of Babesia were found in the blood smears. These were based on the shape and size of the bacteria, as well as where they were found in the erythrocytes. The large Babesia (B motasi) bacteria usually take up two thirds or more of the red blood cell and are pyriform. They can be found singly or in pairs, usually at an acute angle, and can be ring, oval, elongated, amoeboid, or subspherical (Fig 1). The average size of a large Babesia was 3.76±0.83 µm and its paired forms were usually at an acute angle on the edge of the red blood cells (Fig. 2). The average size of a small Babesia was 1.81±0.58 µm and its paired forms were usually 1.1±0.47 µm and their mean size was (1-2.5) ×(1.1-2.1) µm Table(2).

Table (2) : the characteristic features and measurements of the *Babesia ovis and Babesia motasi* diagnosed in sheep blood samples.

Parasite	Characteristic features	Measurements µm			
		Length		width	
		Mean	Range	Mean	Range
B.ovis	small *Babesia* mostly oval or pyriform, paired forms usually at an obtuse angle at the margin of the red blood cells	1.81±0.58	1-2.5	1.1±0.47	1.1-2.1
B.motasi	as large *Babesia* occupying two thirds or more of the red blood cell ,usually pyriform ,singly or in pairs generally at an acute angle; ring, oval, elongated, amoeboid , sub spherical	3.76±0.83	2.5-5	2.39±0. 78	1-5

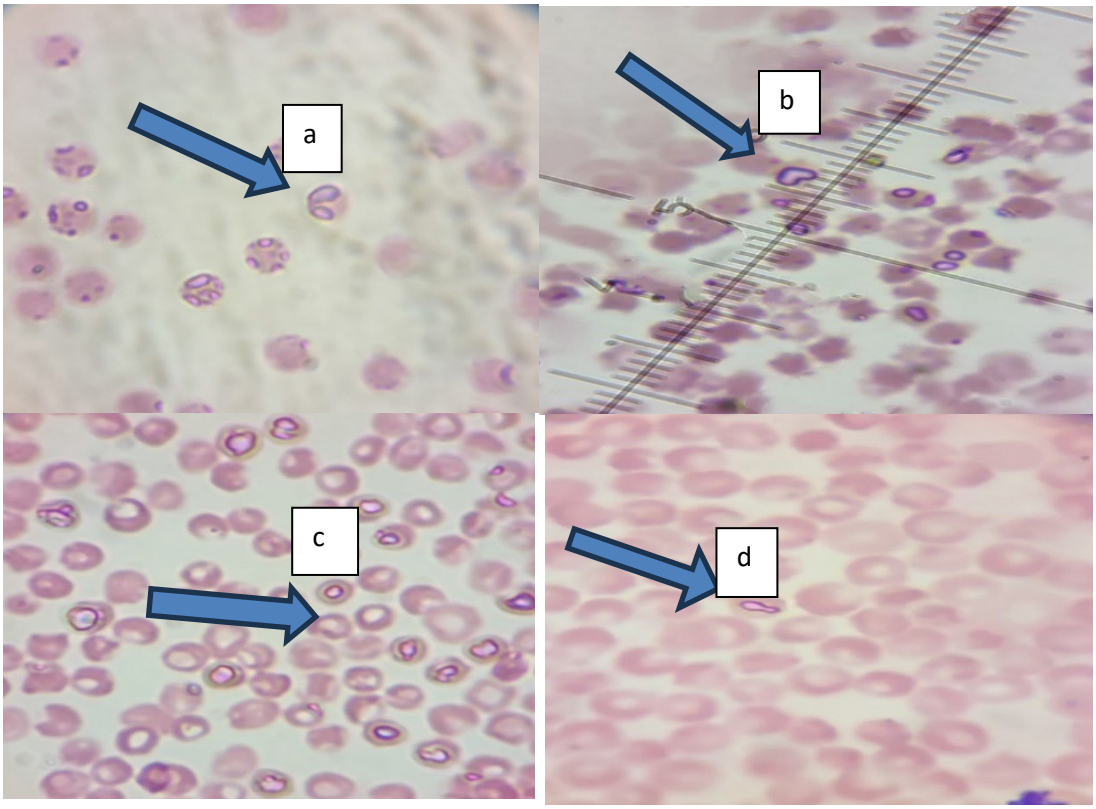

Fig 1: *Babesia . motasi* in different forms (a, b- pyriform in acute angle ,c- round form, d-amoeboid form) X100 stained with Giemsa stain and by using digital camera

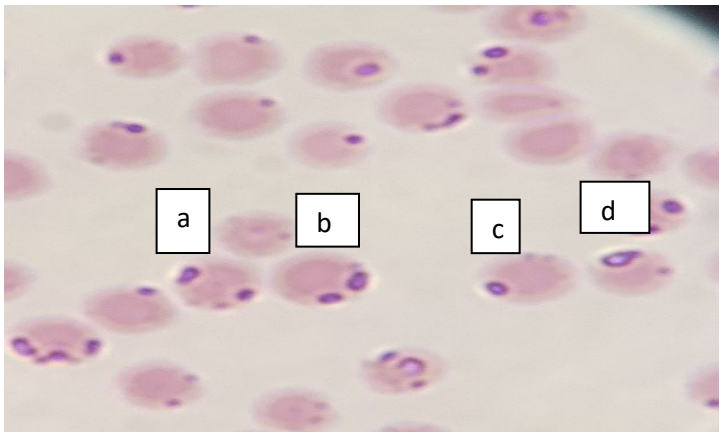

Fig 2: *Babesia ovis* with different form (a-b-pyriform with obtuse angle, c- ring form, d- single pyriform) X100 stained with Giemsa stain and by using digital camera

The present study used fluorescent stain (AO) to examine 50 blood smears. The stain revealed the presence of Babesia in the infected red blood cells, with the nucleus fluorescing in a bright orange to yellow color, while the uninfected red blood cells displayed a green color, giving the smear a green background. The AO stain diagnosed the Babesia infection in less than two minutes, utilizing low magnification at 4X, 10X, and 40X. (Fig3 ,4).

Fig3: Blood smear stained with AO showing *Babesia* parasite at 4X by using digital camera ,

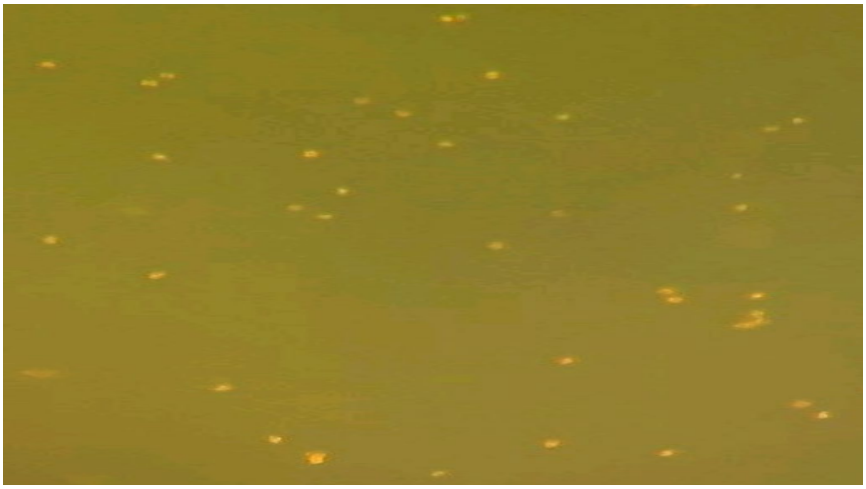

Fig4: Blood smear stained with AO showing *Babesia* parasite at 10X by using digital camera

Discussion

The study found that the total infection rate of Babesia spp. in sheep in Mosul city was 43.07%. This high percentage may be attributed to the potential endemicity of Babesiosis in Mosul city. In addition to the high infection rate, it could also be attributed to the climatic conditions that facilitate the growth of vector ticks. Numerous studies have examined the prevalence of Babesia spp. infection in sheep across various Iraqi governorates, including Mosul city, Dohuk, Sulaimaniya, and Baghdad, as well as neighboring countries like Turkey and Iran, and Arab nations like Egypt, India, and Pakistan. These studies have documented varying infection rates in both sheep and goats, which range from 4% to 90% (14–22).

Recording different, high or low rates of infection with the *Babesia* parasite in different regions of the world may be due to several factors, including, the difference in management systems, the difference in diagnostic methods used sample size, climatic conditions, animal breeds, the distribution of ticks that carry the *Babesia* parasite, and methods of combating (16, 17, 21).

The current study's findings demonstrated the presence of Babesia species in sheep's thin blood smears, appearing as a ring, round, amoeboid form, double, and single pyriform within the red blood cell, and stained with blue color. This result was agreement with (17, 21, 23- 25,). According to the morphological and biometrical characters of the *Babesia* and their location inside the erythrocytes, the examined blood smears revealed identification of two *Babesias* species; large *Babesia* (*Babesia motasi)* and small *Babesia*(*B. ovis).* In Mosul city (14) reported four *Babesia* species in goats, which were: *B. ovis, B. motasi ,B. taylori , B. foliata* in blood smears , in Sulaimani city (17) described four species of *Babesia* are *Babesia motasi ,B. ovis , B. taylori and B. foliate* in small ruminants and in the Northwest of Iran (26) identified large *Babesia* on the blood smear of 20 naturally infected sheep. (27-29) revealed that babesiosis in domesticated small ruminants is due to at least three species they are: *Babesia motasi*, *Babesia crassa* and *Babesia ovis*. This study used the Acridine orange stain to diagnose babesia in sheep blood. This stain demonstrated high sensitivity for diagnosing this parasite at microscopic magnifications of 4X, 10X, and 40X. This stain stood out for its ease and speed in identifying infection with the Babesia species, as the staining and examination time did not exceed 5 minutes, and a large number of samples were examined in a very short time, which makes this stain very useful in epidemiological

studies in which the number of samples is very large. Despite these advantages, it does not facilitate the identification and study of the parasite's qualitative and morphological characteristics, and it requires pH adjustment. Apart from the fluorescence microscope, which may not be accessible in all diagnostic laboratories, these results align with references (3, 5, 30- 32).

Conclusion

Giemsa stain is the gold standard test for diagnosing many blood protozoa and studying the qualitative and morphological characteristics of the Babesia species. The Acridine orange stain has high sensitivity in diagnosing Babesia species at microscopic magnifications of 4X, 10X, and 40X, and this stain is very useful in epidemiological studies in which the number of samples is large.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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التشخيص المجهري لطفيلي الكمثريات في الضأن في مدينة الموصل

بيداء يونس محمود، ايمان غانم سليمان

فرع الأحياء المجهرية ، كلية الطب البيطري، جامعة الموصل، العراق .

الخلاصة

يعد طفيلي الكمثرية واحدا من اهم الطفيليات التي تنتقل بواسطة القراد الصلب وهو مسوؤل عن حدوث نسب اصابات وهلاكات عالية في قطعان الضأن في مختلف دول العالم . تضمنت الدراسة الحالية تشخيص طفيلي الكمثريات في 260 عينة دم جمعت من الضأن من مناطق مختلفة من مدينة الموصل وحضرت منها مسحات دموية خفيفة وصبغت بصبغة الكميزا وفحصت باستخدام المجهر الضوئي . تم تشخيص طفيلي الكمثري في 112 عينة دم وبنسبة اصابة كلية 43.07 % وتم تشخيص نوعين من الكمثريات وهما الكمثرية الكبيرة الحجم وبمعدل قياس بلغت (78 .0±2.39)×(0.83±3.76)مايكرون

وبمدى تراوح ((5-1)× (5-2.5مايكرون والكمثرية الصغيرة الحجم وبمعدل قياس (0.47±1.1)×(0.58±1.81) مايكرون وبمدى تراوح (2.1-1.1)× (2.5-1) مايكرون. كما تضمنت الدراسة الحالية امكانية استخدام صبغة الاكريدين البرتقالية المتفلورة في صبغ 50عينة دم اغنام وتميزت هذه الصبغة بسهولتها وسرعتها في تشخيص الاصابة بطفيلي البابسية في مسحات الدم خلال دقيقتين وان هذه الصبغة تفيد في الدراسات المسحية والوبائية ولكنها لا تفيد في التحديد النوعي للطفيلي .

الكلمات المفتاحية: الكمثرية ، ضأن ،الدم ،الكميزا، الاكريدين البرتقالية