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Identification of Blood Protozoa Infestation Transmitted by Vector Tikes among Awassi Sheep Herds in Kifri City, Kurdistan Region of Iraq

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ABSTRACT

Blood protozoan disease is a common disease among animals in the Kifri city, Kurdistan region of Iraq that this disease is mostly transmitted by ticks. Therefore, the present study aimed to investigate the level of blood protozoan and to identify vector ticks in the native breed sheep (Awassi sheep) in Kifri city. For this purpose, blood samples were taken from 150 sheep suspected suffering from protozoan infection according to their clinical symptoms. In the present study, we prepared blood slides from suspected sheep and stained with Giemsa staining, and then at the same time, hard ticks were collected from the sheep's body. Then, the protozoan type was diagnosed and the vector tick species were identified by microscopically. The obtained results were statistically analyzed by the chi-square test. The results showed that 35 (23.33%) of that samples were infected with *Babesia* protozoa as 25 samples (16.66%) were infected with *Babesia ovis*, seven samples (4.66%) with *Babesia mutasi*, and three samples (2%) with *B. ovis* and *B. mutasi*. No infestation with *Theileria* and *Anaplasma* species was found. *Rhipicephalus*, *Hyalomma*, *Dermacentor,* and *Haemaphysalis* ticks were isolated and identified from the studied sheep. The results showed that the presence of the *Rhipicephalus bursa* tick is significantly ($P < 0.05$) related to the existence of *Babesiosis* disease in sheep. This study concluded that most of the studied sheep in Kifri city are infected with *Babesia* protozoa, especially *B. ovis*.

Index Terms: *Babesia ovis*, *Babesia mutasi*, *Kifri*, *Rhipicephalus* bursa, Sheep

1. INTRODUCTION

The sheep population in Iraq in 2020 was about 7 million head [1]. Most of this population (99.9%) is owned by the private sector [2] and is distributed all over the Iraq. The native breeds include the Awassi, Arabi, Karadi, and Hamadni sheep. One of the important native species of sheep in Kifri region is the Awassi sheep, which is abundant in this region. The condition of herding in Kifri city and the presence of a large nomadic population in this area indicates that most of the sheep grazing is done in the pastures and the ranchers tried to make the most of it in the hot seasons. Because ticks spend a relatively short time of their life cycle on the host, and they spend a long time apart from the host on the surface of pastures. As the climate of the region becomes favorable for the growth and appearance of ticks during the period of livestock grazing in the pastures, various types of blood protozoa cause contamination and the sheep suffer from protozoan diseases, especially

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Babesiosis. Babesia ovis and *Babesia mutasi* are among the most common causes of *Babesiosis* in sheeps [3], [4]. *Babesia crassa* from Iraq, *Babesia foliata* from India, and *Babesia taylori* from Pakistan have been reported as non-pathogenic *Babesia* [5]. *B. mutasi* is found in Southern Europe, Southern Africa, the Middle East, Caucasus, Southeast Asia, Mediterranean coastal areas, and other regions with warm and moderate climates [6], [7]. Sheep and goats are considered the main hosts for them. *Haemaphysalis punctata, Rhipicephalus bursa, Rhipicephalus sanguineous,* and *Ixodes ricinus ticks* are vector parasites [8], [9]. Sheep and goats are the main hosts of *B. ovis*. This parasite is spread throughout the tropical and subtropical regions, as well as in southern Europe, the former Soviet Union, Eastern Europe, North Africa, the equatorial region, and western Asia [10], [11]. The vector of *Babesia ripe* is *Cephalus bursa* tick, which is a two-host tick [12]. The *Hyalomma anatolicum excavatum, I. ricinus, Rhipicephalus turanicus,* and *Rhipicephalus sanguineus* ticks were also reported as vectors of *B. ovis* [8]. *B. ovis* is the most important cause of Babesiosis in Europe [13]. *Theileria hirci* is the cause of malignant theileriosis in sheep and goats, and the ticks *C. bursa* and *Hyalomma anatomical* are its vectors. These protozoa are found in lymphocytes and red blood cells of small ruminants. *Theileria ovis* causes a mild disease in small ruminants and is transmitted by species of *C. bursa* tick. Based on the results of the studies, diagnosis of parasites is possible by preparing slides from blood and lymphatic glands [14].

The disease caused by *Anaplasma ovis* is called tropical anaplasmosis of small ruminants. The distribution of this parasite is related to the distribution of its most important carriers, including the *Rhipicephalus bursa* in the Mediterranean region and the *Rhip*icephalus *ortisi* in the tropical regions of Africa [6].

Other studies suggested that the distribution of *B. mutasi* was reported to be limited to the northwestern regions of Iraq [15]. Mosqueda *et al.* also believe that sheep *Babesiosis* caused by *B. ovis* is spread all over Iraq and is considered an acute disease in Iraqi sheep [16]. Survey of seroepidemiology of *B. ovis* in sheep in climatic regions of Iraq using indirect brilliant antibody test shows that 36% of sheep had a positive serum titer [17]. Considering the economic losses due to protozoan diseases, especially *Babesiosis* in sheep, paid for this. For this reason, the present study was conducted to investigate the contamination of blood protozoa and to identify the vector ticks in Awassi sheep in Kifri region.

2. MATERIALS AND METHODS

This study was conducted in the summer of 2020 in the villages of Kifri City, Kalar, Kurdistan region of Iraq. Sampling carried out on 150 Awassi sheep (39 male and 111 female sheeps) that were suspected of protozoan infestation and had the disease symptoms. General clinical examinations were performed on the sheep introduced by the owner. Sampling was collected only from the sheep that had symptoms of illness such as depression, anorexia, high fever (40–41°C) or had jaundice, and urine nails and also had respiratory symptoms such as tachypnea and tachycardia. After sampling, one slide was prepared from each sample. The slides were dried in the air and sent to the laboratory. In the laboratory, the slides were stained with Giemsa's stain and then examined. If objects were observed in the desired slide, the parasites were measured in microns with a calibrated optical micrometer. To collect the tick sample, the target sheep was laid on the ground. Then, first, the area below and around the tail were visually inspected, and in the second step, in the side, chest, around the chest, back of the legs, and ears, respectively. The ticks were collected by the angle they were attached to the host so that their oral appendages remain intact. Then, they were transferred to the sampling container containing 10% formalin and the containers were labeled. During sampling, animal characteristics such as the area, the date of sampling, the animal owner, the number of samples and clinical symptoms, the presence or absence of jaundice, and blood from the animal's urine were recorded in the sampling handbook. In this study, *Babesia* and Ticks species were identified morphologically based on the guidelines of William *et al.* [18] and Zajac and Conboy [19]. The data of the present study were analyzed using SAS software.

3. RESULTS

The results of the present study showed that 35 (23.33%) the samples were infected with *Babesia* protozoa and that 25 samples (16.66%) were infected with *B. ovis*, seven samples (4.66%) with *B. mutasi*, three samples (2%) with *B. ovis,* and *B. mutasi* (Fig. 1). In this study, the samples infected with *Babesia theileria* and *Babesia anaplasma* were not found. Based on the results of our findings, *B. mutasi* is pear-shaped, 2.5–4 microns long and two microns wide, and *B. ovis* is mostly round and has 1–1.5-micron red blood cells on the sides. There is a hole in the center of the parasite, and thus, it takes the shape of a ring. Pear-shaped bodies are relatively rare and are seen as pairs with open angles in the margin of red blood cells (Figs. 2 and 3).

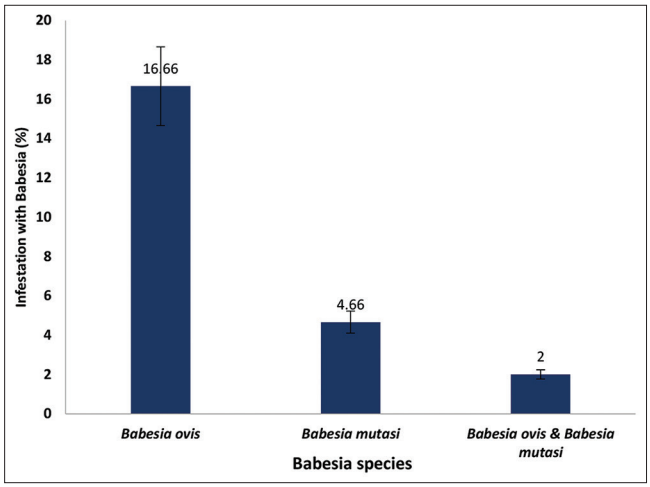

Fig. 1. The rate of infection of Babesia protozoa among native sheep in Kifri city.

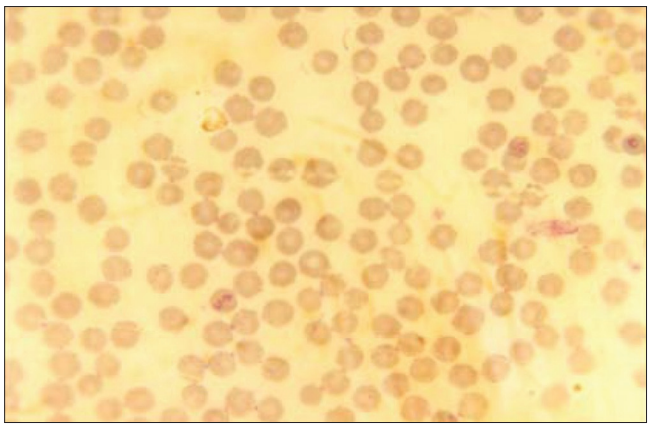

Fig. 2. The blood film of sheep stained with Giemsa contains the trophozoite of *Babesia mutasi* (×100).

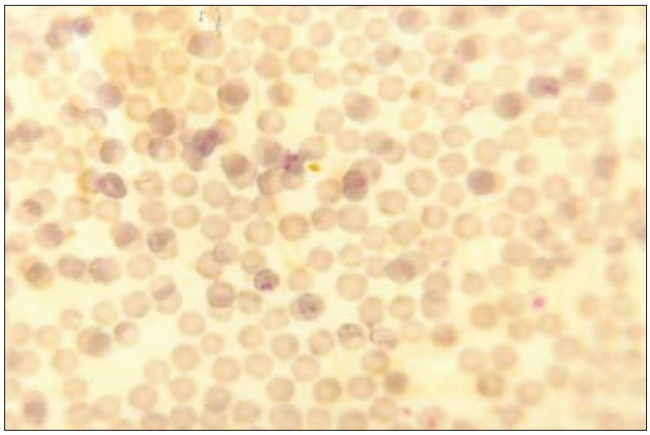

Fig. 3. The blood film of sheep stained with Giemsa contains the trophozoite of *Babesia ovis* (×100).

Out of 150 samples infected with *Babesia* protozoa, 39 samples were from male sheep (26%), and 111 samples were from female sheep (74%) (Table 1). Out of 39 samples of male sheep infected by *Babesia* protozoa, seven samples (4.66%) were infected with *B. ovis*. Out of 111 samples of female sheep infected by *Babesia* protozoa, 24 samples (68.58%) were infected with *B. ovis*, one sample (2.58%) with *B. mutasi*, and three samples (8.57%) with *B. ovis* and *B. mutasi* (Table 1).

Out of 150 samples of infected sheep in this study, 96 samples of sheep were infected with ticks, and a total of 204 ticks were isolated from them. Out of this number, 130 *Rhipicephalus* ticks (63.72%) were found among hard ticks, and the highest percentage of sheep infection with ticks in Kifri city is attributed to *Rhipicephalus* ticks. In addition to *Rhipicephalus* tick, other species of ticks were detected on the infected sheep that their infection percentages are as follows: *Hyalomma* tick 51 samples (25%), *Dermacentor* tick 13 samples (6.37%), and *Haemaphysalis* tick 10 samples (4.9%) (Fig. 4). Out of 130 samples of Rhipicephalus ticks, 112 samples of R. bursa, 17 samples of R. sanguineus, and one sample of R. turanicus were identified. Thirteen samples of *Dermacentor* tick belonged to the species *Dermacentor marginatus* and ten samples of *Haemaphysalis* tick belonged to the species *Haemaphysalis punctata*. Out of 51 *Hyalomma* ticks, 26 samples were *Hyalomma asiaticum asiaticum*, 17 samples were *H. anatolicum anatolicum*, seven samples were *Hyalomma marginatum* and one sample was *Hyalomma atatolicum exquatum*. The mean of intensity of ticks on each head of the sheep in Kifri city was 1.36 ticks, and the mean of intensity of ticks on each head of the sheep infested with *Babesia* protozoa was 2.7 ticks.

4. DISCUSSION

B. ovis is highly pathogenic, especially in sheep and causes a severe infection that is characterized by fever, anemia, icterus, and hemoglobinuria with mortality rates ranging from 30% to 50% in the susceptible host during field infections [20], [21]. Due to its severe effect on the homeotic system, it has caused significant losses among small ruminants, especially sheep in Kifri city. Therefore, the present study aimed to investigate the infestation of blood protozoa and to identify the vector ticks in Awassi sheep in Kifri region. The results of the present study showed that the sheep in Kifri region are mostly infected with *B. ovis* species (16.66%), and the highest percentage of infection with external hard ticks is

Table 1: Distribution of absolute and relative frequency of sheep infected with *Babesia protozoa,* separated by species of sheep and *Babesia species*

The number of samples (male and female animal)	Babesia species	Infected male sheep		Infected female sheep	
		Number	%	Number	%
150	*Babesia ovis*	7	4.66	24	68.58
	Babesia mutasi	-	-	1	2.58
	Babesia ovis and *Babesia mutasi*	-	-	3	8.57

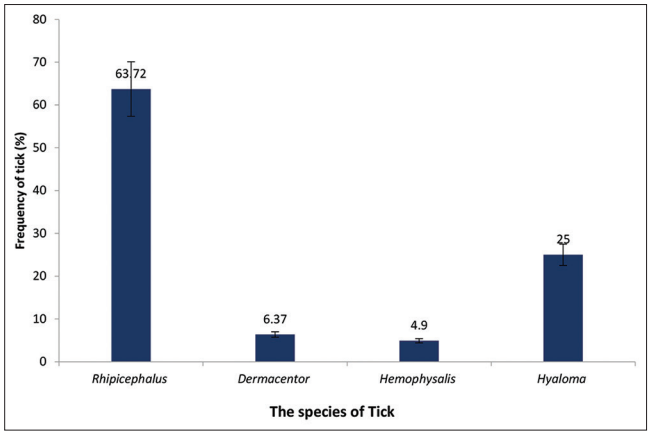

Fig. 4. Frequency of hard ticks identified from infected sheep in the present study.

related to *Rhipicephalus* (63.72%). The results of the present study indicate the predominance of *B. ovis* species in sheep infected with *Babesia* protozoa in the Kifri area. These results are consistent with the results of Tousli and Rahbari [22], which reported that 41.6% of sheep in the Kurdistan region of Iran were infected with *B. ovis*. Infestation with *B. ovis* is severe in some areas. The infection of sheep in Greece with *B. ovis* was reported to be 52% [23]. Furthermore, 72% of sheep in the Samson region of Turkey were infected with *B. ovis* [24]. As mentioned, the results obtained from this research are consistent with the results reported from Iran and Turkey, and the dominant species of this protozoan in these regions is *B. ovis*. One of the main reasons for this issue is the neighborhood of these areas. Due to the closeness of these areas, there are a lot of transfers and sales of sheep between ranchers. Paying attention to the fact that the information obtained from this research, from a statistical point of view, is mostly qualitative data. Hence, if we calculate the probability of disease transmission by all the hard ticks found in the area in comparison with the disease transmission by the statistical population of *Rhipicephalus* species by the chi-square test, there is a significant difference between the transmission of *Babesiosis* disease by the *Rhipicephalus* tick compared to its transmission by all other ixodidae ticks (*Dermacentor, Haemaphysalis*, and *Hyalomma* ticks) in the region ($P < 0.05$). Considering that the transmission of *Babesia* disease by ticks has been proven, it can be assumed that the sheep that are infected with *Babesia* and are tick-free; there is a possibility that the tick was separated from the host after feeding. Furthermore, in cases where the animal shows the symptoms of the disease, but the protozoa have not been isolated from its blood, such a case cannot be a negative reason for *Babesiosis* disease in this sheep. This probably indicates the presence of a small number of *Babesia* protozoa inside the sheep erythrocytes, which makes their identification difficult at this stage. In this case, it is better to repeat the sampling with a longer time interval.

There are different opinions about the severity and pathogenicity of the *Babesia* species. The reason for these reports is probably the long-term contamination of livestock in the region and finally the creation of relative immunity against some strains of protozoa. Therefore, there are strains with less intensity than any of the species of B. mutasi and *B. ovis* in different regions. However, in case of double infestation (*B. mutasi* and *B. ovis*), the disease will appear in a more severe form Iqbal *et al.* [17]. The investigations carried out at the time of sampling as well as the results obtained in the present study showed that the seasonal abundance of ticks on sheep starts from the end of January and reaches its peak in the middle of March. It seems that due to the warm weather in the Kifri region, the activity time of ticks is shorter and the maximum infection with *Babesia* in sheep is in February. In totally, babesiosis in sheep specially caused by *B. ovis* can be considered as an emerging disease in Kifri city.

5. CONCLUSION

Our finding showed that the common blood protozoan that causes sheep infection is *B. ovis* in the Kifri area. Furthermore, the predominant tick among infected sheep in the study area is Rhipicephalus tick, and the infection rate of the sheep with the tick was higher than *Babesiosis* species in Kifri area.

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