Molecular Detection and Hematological Changes of Theileriosis in Sheep and Goats in Baghdad Province

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Abstract

Theileriosis is an important blood disease that affects most species of domestic and wild ruminants, especially in tropical and subtropical regions. Sheep and goats are often infected with *T. listoquardi* and *T. ovis* and cause significant economic losses. Because of the scarcity of data on the incidence of the disease in Baghdad governorate, this study was conducted to determine the presence of subclinical infection by microscopic, hematological and molecular examination (PCR). 200 blood samples were collected randomly from healthy sheep and goats, 100 samples from sheep (77 females and 23 males) and 100 samples from goats (83 females and 17 males), samples were collected from north and west Baghdad from October to December 2020. The results of the study showed the presence of *T. ovis* in sheep in a percentage of 9% by microscopic examination and 16% by conventional polymerase chain reaction (PCR). All sheep samples were negative for *T. lestoquardi* by both techniques and all the goat samples were negative for both species of *Theileria* by both tests.High infection rate of *Theileria* spp. was recorded in males 17.39% (4/23) compared with females 15.58% (12/77). High infection rate 17.18% (11/64) was recorded in the age group more than 12 months followed by 16.66% (2/12) in the age group under 6 months of age while the lower infection rate 12.50% (3/24) in the age group between 6-12 months old. In this study the RBC, Hb, HCT, MCH and MCHC showed no significant changes. The white blood cell count (WBCs) increased in positive cases of this study, the lymphocyte showed no significant increase, the monocyte value did not significant decreaseand the granulocyte showed significant increase.

Keywords: *Theileria listoquardi*, *Theileria ovis*, Polymerase chain reaction, Iraq

Introduction

Theileriosis is a tick-borne infection of small ruminant that is happened due to a type of blood parasite called *Theileria*. *Theileria* species that affect sheep and goats are *T. ovis*, *T. lestoquardi*, *T. separata*, *T. recondita* and (*T. uilenbergi* and *T. luwenshuni*) Chinese *theileria* (Schnittger *et al.*, 2000). Some of these species are highly pathogenic and cause high mortality and economic losses such as *T. lestoquardi* and Chinese *theileria* while other species are benign or less pathological such as *T. ovis* and *T. separata*. In the local Iraqi breeds, very virulent strains were recorded, causing a high mortality rate (Friedhoff, 1997).

Many new pathogenic species were recorded, in addition to the pathogenicity of some previously non-pathogenic species *T. ovis*, *T. separate* and *T. recondita* (Morrison, 2015). All stages of *Theileria* parasite develop inside red blood cells and white blood cells, so it is considered a blood parasite. The most pathogenic types of *Theileria* have the ability to multiply widely within white blood cells during the period of their development, which gives them the ability of pathogenicity.*Theileria* parasite has been recorded in various types of mammals, but the vast majority has recorded its presence in all types of large and small ruminants (Sivakumar *et al.*, 2014; Morrison, 2015). The way the parasite is transmitted by different types of ticks, the climatic influence plays an important role in the geographical distribution of the parasite around the world through the climatic conditions suitable for the tick, which is a vector medium (Morrison, 2015). The spread of *Theileria* infection is concentrated in the tropics and subtropics, including Africa, the Middle East, and even southern Europe (Razmi *et al.*, 2019).

Theleria species vary greatly in their virulence or ability to cause disease, some species are highly virulent and cause high morbidity and mortality, and others are benign. Some benign types may show mild clinical signs or may not show at all, but some types may show some clinical signs such as fever, anemia and hemoglobinuria, which may cause death in severe cases. Animals that recover from infection become carriers of the disease and become reservoirs of the parasite and its vector, the ticks (Abdullah and Ali, 2021). *Theileria lestoquardi* is a highly virulent species, as it affects the lymphatic system and causes significant economic losses, causing death in most cases unless treated in time. Domestic sheep are always at risk of infection, especially in areas where ticks are difficult to control or if they are moved from disease-free to disease-endemic areas (Tageldin *et al.*, 1992).

The disease is diagnosed by microscopic examination of blood smears or biopsies of lymph nodes and observation of the presence of schizonts

doi.org/10.31838/hiv23.01.23 Received: 15.09.22, Revised: 18.10.22, Accepted: 22.12.22

(Uilenberg, 1981). However, the diagnosis of theileriosis by microscopic examination in animals carrying the disease is inaccurate due to the low number of parasites in the blood, which often leads to an incorrect diagnosis, by microscopic examination, it is not possible to accurately diagnose or differentiate between species of *Theileria* especially in subclinical cases (Razmi *et al.,* 2006; Heidarpour *et al.,* 2010). To know the role and epidemiological risk of all types of *theileria*, we need a more specialized and sensitive examination, such as polymerase chain reaction (PCR), (Aktas *et al.,* 2005; Altay *et al.,* 2007a; HeidarpourBami *et al.,* 2009). Currently, due to the use of PCR, it has been possible to diagnose the parasite in disease-carrying animals, despite the small number of parasites (Almeria *et al.,* 2001). These high infection rates may be indicative of the presence of *T. Listoquardii* in Iraq, where severe symptoms have occurred in sheep and goats. These symptoms are not caused by *T. ovis* as previously thought, it is essential to use molecular diagnostics to ensure accurate diagnosis, treatment of infected animals and disease control. The disease has great economic importance, especially in countries that depend on sheep products for their national income, as the disease causes a high death rate, and even sheep that recover from the disease lose weight, reduce milk and reduce fertility. The diagnosis of the disease has been confirmed in different regions of Iraq, as well as in countries neighboring Iraq such as Turkey and Iran (Dhaim and Aziz, 2014). This study was designed to determent the percentage of theileriosis in sheep and goats in Baghdad governorate.

Materials and Methods

Study area and Blood sampling

One hundred sheep and one hundred goats in north of Baghdad were examined during the period from November 2020 to December 2020, clinically healthy animals with different sex, and age. Sheep and goats were examined clinically. The sheep and goats in the study were selected randomly.Blood was collected from the jugular vein the collected blood samples were kept in 5 ml glass tubes with the anticoagulant EDTA.

Hematological and blood smears

Two and half ml of blood in EDTA tube with gentle mixing was used for hematological and blood smears. Complete blood count (CBC) was performed on an automatic blood cell counter and thin blood smears were immediately prepared for Giemsa staining after fixing with methanol. The stained blood smears were examined under a microscope with the aim of immersion in oil to detect the parasitemia.

Molecular methods

A 2.5 ml blood sample was also collected from jugular vein of 100 sheep and 100 goats and stored at -20 °C until used for DNA extraction for PCR amplification. Genomic DNA from 50, 100 and 200 µl whole blood samples was extracted using the gSYNC™ DNA Extraction Kit according to the manufacturer's instructions. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 0.8% agarose gel.

Two sets of primers (Table 1) were used for PCR amplification of different gene segments of both of the *Theileria* species. The first pair of oligo-nucleotide primer was used to amplify 479 bp region of *18S rRNA* gene of *T. ovis* (Muhammed and Zahida, 2017). The second primer pair specific for *T. lestoquardi* was used for the amplification of *18S RNA* gene fragment 771 bp (Ahmed *et al.,* 2019).

Table (1) Primers used for the detection of genus Theileria, *T. ovis* and *T. lestoquardi* in sheep and goats

Target gene		Primer specificity (5'-3'	Product size (bp)	References
T.ovis	*18S rRNA*	F: TCGAGACCTTCGGGT R: AAAGACTCGTAAAGGAGCAA	479	Muhammed and Zahida, 2017
T. lestoquardi	*18S rRNA*	F: GTGCCGCAAGTGAGTCA R: GGAATGATGAGAAGACGATG	771	Ahmed *et al,* 2019

The PCR condition was optimized under following program: for *T. lestoquardi* specific primer was an initial denaturation at 95 C° for 5 minutes followed by 35 cycles, 30 s for denaturation at 95C°, 40 s for annealing at 54C° and 2 minutes for extension at 72C° and with a final extension step at 72C° for 5 minutes. The PCR conditions for the *T. ovis* specific primer were similar. The PCR products were run on 1.5% agarose gel, 10 µL of PCR product was mixed with 5 µL 3 X bromophenol blue dyes and the electrophoresis was done at constant voltage 100V for 40-50 minutes in 1X TAE buffer.

Statistical analyses

For statistical analysis animals were divided into three age groups: ≤ 6 month, 6-12 month and > 1 year, also divided according sex (male and female). Chi square and Fisher's exact test was used to statistically analyze the data regarding *Theileria* infection and possible risk factors responsible for the spread of *Theileria* species infection in studied population of sheep and goats. $P<0.05$ was accepted to be statistically significant (Al-gharban and Dhahir, 2015; Gharban and Yousif, 2021).

Results and Discussion

Two hundred blood samples (100 sheep and 100 goats) from apparently healthy animals were examined microscopically and by PCR method for both *T. ovis* and *T. lestoquardi*. All the goat samples were negative for both species of *Theileria* by both

tests. The sheep samples were positive for *T. ovis* by microscopic examination (Figures 1, 2) and PCR method (Figure 3, 4), but the results by PCR examination were higher than the microscopic method.

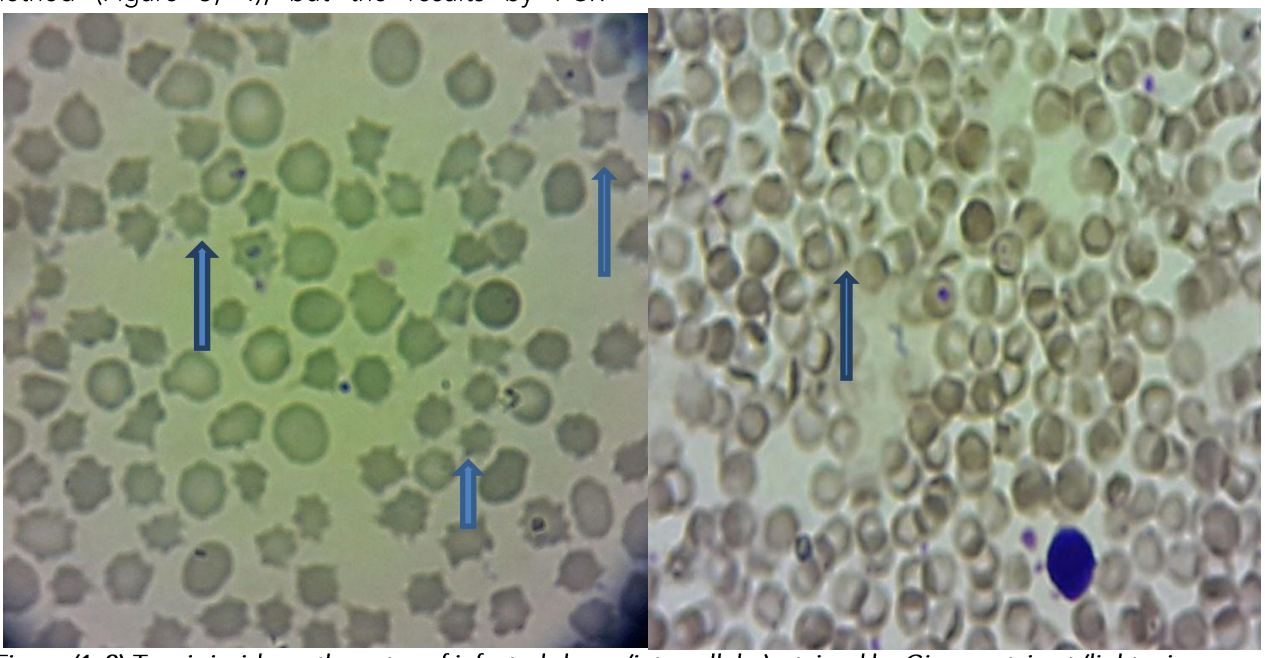

Figure (1, 2) T. ovis inside erythrocytes of infected sheep (intracellular), stained by Giemsa stain at (light microscope X100)

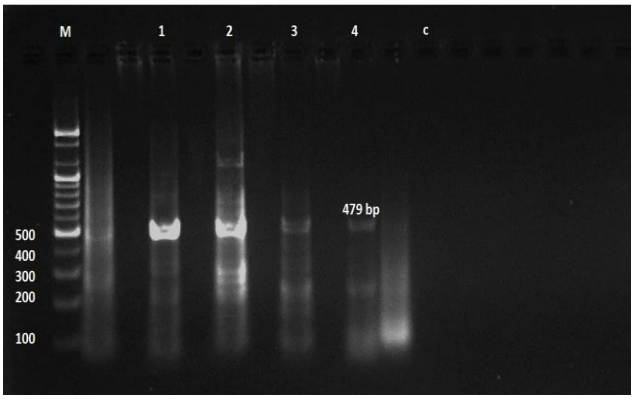

Figure (3) Agarose gel electrophoresis with red safe stain showing amplification of 479bp fragment of 18S rRNA gene (TO and TL primers), numbers 1-4 cases positive of T. ovis

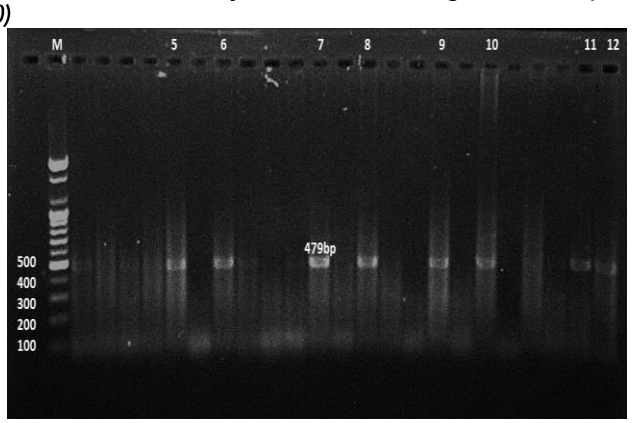

Figure (4) Agarose gel electrophoresis with red safe stain showing amplification of 479bp fragment of 18S rRNA gene (TO and TL primers), numbers 5-12 cases positive of T. ovis

All sheep samples were negative for *T. lestoquardi* by both techniques. The results of sheep we mentioned for *T. ovis* according to the PCR results. Microscopic examination of blood the smear from sheep reveled 9 slides positive for the *Theileria* parasites. The nine sheep for the positive smear were later positive for *T. ovis* by PCR test such a result was also mentioned by Radwan and Kelesh (2009) whom mentioned that the microscopic result is less than the PCR result.

Our study mentioned that the males (17.39) were infected more than the female (15.58), (Table 2). Dhaim and A'aiz (2013) mentioned that the male infection rate was 23% while the female infection rate was 22.8% which are nearly the same, similar results were found by Hegab *et al.* (2016) who detected (15.9%) for male and (15.34%) for female. The differences between our results and the above two researcher could be due to few number of males (23) examined in our study.

Table (2): Infection rate of *T. ovis* in sheep according to sex

Sex	No. of samples examined	Positive	Percentage (%)
Males	23	4	17.39
Females	77	12	15.58
Total	100	16	16
χ^2	23*		

The age groups should differences as shown in Table (3) the highest was in the above 12 months of age (17.18%) and lowest in the 6-12 months (12.5%). A similar result was recorded by Dhaim and A'aiz (2013) whose highest result was above 12 months (26.6 %) but their lowest was less than 6 months (15.7%) also Durrani *et al.* (2012) agreed with our result that high infected age was more than one year.

Table (3): Infection rate of *Theileria* spp. in sheep according to age			
Age/ Months	No. of sheep examination	Positive	Percentage
< 6	12	2	16.66
6-12	24	3	12.50
> 12	64	11	17.18
Total	100	16	16
χ^2	23*		

Results of hematological examination will include Red blood cell count (RBC), Hemoglobin concentration (Hb), hematocrit value HCT, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) as shown in Table (4). In this study the RBC showed no significant changes when compared to the normal while it increased in the studies by Khaki *et al.* (2015) and decreased by Al-Fetly (2012), Mahmoud *et al.* (2019). Hemoglobin concentration showed no significant increase in this study while in Khaki *et al.*, (2015) it was variable, it decreased according to Al-Fetly (2012) and Mahmoud *et al.* (2019). Hematocrit value did not show significant increase in our study and we read mentioned by other researchers Al-Fetly (2012), Mahmoud *et al.* (2019) and Khaki *et al*, (2015).The mean corpuscular volume of our study also did not show significant increase while it was decreased as mentioned by Khaki et al., (2015) increased by Al-Fetly (2012) and Mahmoud *et al.* (2019).The mean corpuscular hemoglobin did not increase in our study while it decreased by Mahmoud *et al.* (2019) and no significant changes by Al-Fetly (2012). The mean corpuscular hemoglobin concentration did not show significant decrease in our study but it was decreased by Mahmoud *et al.* (2019) and Khaki *et al.* (2015) and no significant changes by Al-Fetly (2012). The above level of erythrogram were variable differing from research could be depending on the physical states of the animal, presence of anemia and the debility condition in the animals body.

Table (4): Erythrogram values of the infected and non-infected sheep with *T.ovis*			
Parameters	Positive mean ±SE	Negative mean ±SE	t-test
RBC10^{12}/l	5.59 ± 0.46	6.09 ± 0.7	0.43 NS
HGB g/dl	8.46 ± 0.27	8.18 ± 0.53	0.35 NS
HCT %	22.79 ± 1.47	20.64 ± 1.85	0.64 NS
MCV fl	36.37 ± 0.66	35.37 ± 0.93	0.62 NS
MCH pg	16.49 ± 1.2	16.47 ± 1.45	0.0075NS
MCHC g/dl	44.58 ± 2.6	45.63 ± 3.01	0.18 NS

The results of the leukogram shown in Table (5) include white blood cell count, lymphocyte, monocyte value, granulocyte value, lymphocyte percentage, monocytes percentage and granulocyte percentage and are as follow. The white blood cell count increased in positive cases of this study, it was more than in this study as mentioned by Khaki *et al.* (2015) and less as mentioned by Al-Fetly (2012) and Mahmoud *et al.* (2019). The lymphocyte in this study showed no significant increase which in the studies they were increased in both researcher Mahmoud *et al.* (2019) and Khaki *et al.* (2015). The monocyte value did not significant decrease in this study while the granulocyte value was increased. The lymphocyte percentage in our study did not show significant decrease is will were increased by Mahmoud *et al.* (2019) and Khaki *et al.* (2015). The monocyte percentage of this study shows no significant increase and the granulocyte percentage in this study showed no significant increase and not performed by other studies. Such difference could be due to other viral, bacterial or parasite infection in the studied animal because there were only clinically healthy and not experimental to test for many other diseases.

Table (5): Leukogram values of the infected and non-infected sheep with *T. ovis*			
Parameters	Positive mean ±SE	Negative mean ±SE	t-test
WBC 10^9/l	9.09 ± 0.63	6.37 ± 0.57	2.26*
LYM 10^9/l	3.69 ± 0.39	3.28 ± 0.46	0.48 NS
MON 10^9/l	1.11 ± 0.19	1.18 ± 0.47	0.10 NS
GRA 10^9/l	4.28 ± 0.33	2.3 ± 0.36	2.86**
LYM%	40.2 ± 3.49	40.7 ± 4.86	0.05 NS
MON%	11.92 ± 1.6	11.47 ± 1.26	0.15 NS
GRA%	46.2 ± 3.27	38.42 ± 4.4	1.01 NS

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