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TICKS AND HEMOPROTOZOANS PARASITIZING SHEEP IN BASRAH PROVINCE, SOUTH OF IRAQ

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Abstract

Seven hundred and twenty sheep from various areas in Basra province were examined for tick infestation during the period from October 2020 to September 2021. The examined sheep were found to be infested by six species of hard ticks, namely *Hyaloma anatolicum*, *Hyaloma exacavatum* and *Hyaloma scupense*, *Rhiphice palisturanicus*, *Rhiphice sanguineus* and *Rhiphice* (*Boophilus*) *annulatus*. At the meantime, blood smears samples from the same sheep were also examined by microscopy for hemoprotozoan pathogens. The examination revealed that those sheep were infected by *Babesia* sp. (5.83 %) and *Theileria* sp. (26.66 %) and the total prevalence was 32.5 %. For accurate identification of *Babesia* and *Theileria* species, Polymerase Chain Reaction Technique (PCR) was used, which showed that sheep and ticks were infected by *B. ovis* and three species of *Theileria* (*Theileria annulata*, *Theileria ovis* and *Theileria lestoquardi*).

Key words: *Hard ticks , sheep , Basrah ,Iraq, Hyaloma , Babesia, Theileria*

1. Introduction

Sheep are considered one of the most significant animals in Iraq due to their importance as a significant source of meat, leather, and wool (Akhtar *et al*., 2011). Sheep meat is one of the best meat for Iraqis and comes in the second place in consumption after cattle meat. Sheep are affected by various disease agents, among these parasitic pathogens, including ticks. Ticks are very harmful blood sucking protozoans and *Rickettsial* diseases in livestock and cause an adverse economy of the world (Kunwar *et al*., 2022), causing blood loss, damage to hides and udder, and paralysis. Accordingly, the role of ticks is very important economically in worldwide especially in the tropical and subtropical region (Norval *et al*., 1992). The prevalence of TBPs is usually higher in sheep and goats which effects health, production and welfare of small ruminants (Ghafar *et al*., 2020).

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Although Iraq climatic is suitable for the rapid development of various tick species, However, there are few research related to tick infestation in sheep of Iraq. Nevertheless, how tick species occur and infest the sheep population in Iraq is still ununderstood. The lack of studies related to prevalence, abundance and distribution of ticks is the main limitation to application of preventive methods. Therefore, the aims of the current study were (i) To identify the ticks parasitizing sheep in Basra Governorate and estimate the infection rates; (ii) Determining the role of ticks in transmit blood protozoans by shedding light on the percentage of infected sheep, and the species of ticks that transmit protozoan by diagnosing the protozoans they harbor by PCR technique.

2. Materials and Methods

Study area and Field Sampling

The study was conducted in sheep from Basra province, which is located in the south of Iraq at altitude of 30˚30′ and longitude 47˚48′,

and its climate is characterized by high temperatures in the summer, which may exceed 50 ºC, while the rest seasons are moderate in temperatures and temperatures do not drop below 10 ºC except for few days during winter season.

Sheep are raised in small herds that may not exceed 100 heads in many cases. A total number of 720 sheep were randomly sampled during the period from October 2020 to September 2021 every month from several sites located in Basrah Governorate, represented by City centre, Al-Haritha, Al-Qurnah, Shatt Al-Arab, and Abu Al-Khasib. All sheep without regular acaricide treatment.

Ticks collection

Ticks were manually collected from head, ears, udder, outlet and abdomen from randomly 720 male and female sheep. They were collected with alcohol pads surrounding the skin of sheep and removed by forceps and kept in labelling screw plastic tubes-containing ethanol. All collected ticks were counted and examined under the Stereomicroscope. Species, sex and state of feeding were recorded. Ticks identification was done according to Hoogstraal (1981). Some of ticks were frozen at -20 ºC for DNA extraction.

Blood samples collection

Blood samples were taken from Jugular vein from a total of 720 sheep using a 10 ml medical wine syringe under aseptic precautions. About 5 ml of blood was collected in evacuated tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) for DNA extraction and then stored in ice boxes at 4 ºC. The samples were transported to the parasitology laboratory at Basrah University, Education College for Pure Sciences, where blood smears were prepared and fixed by using methanol and stained by Giemsa, then examined under an oil immersion objective 100 X.

DNA extraction

The DNA was extracted from blood and ticks samples using DNA extraction kit (ADDBIO Company, Korea) according to the manufacture instructions. The extracted DNA were tested by Nano drop Spectrophotometer (Gel documentation system) at wave length 260/280 nm.

Polymerase Chain Reaction (PCR)

For the molecular diagnosis of *Babesiaovis* and *Theileria annulata*, *T. ovis* and *T. lestoquardi* in sheep and ticks, PCR technique was performed using specific primers Table 1.

Table - 1: Primers used in PCR technique

Parasite	Target gene	Primers	Sequences	Size bp	Tm (°C)	Reference
T. annulata	Tms92	F	GAGACAAGGAATATTCTGAGTCC	547	55	Habibi *et al*. (2020)
		R	TTAAGTGGCATATAATGACTTAAGC			
T. ovis	To	F	GTAGGGCTAATACATGTTCGAGACCTTC	121	53	Habibi *et al*. (2020)
		R	TGATACATCGCATCCGAAGAC			
T. lestoquardi	SLAg1	F	ATCAGCGGCAACACAACC	400	50	Habibi *et al*. (2020)
		R	TTCCTGGTCATGAGAACCG			
B.ovis	Bo92n	F	TAATTTGACTCAACACGG	256	50	Habibi *et al*. (2020)
		R	ATCACAGACCTGTTATTGC			

Statistical analysis

The prevalence of ticks was calculated by dividing the total numbers of tick positive sheep by the total numbers of examined sheep.

The intensity of ticks per sheep was calculated by dividing the total number of ticks in all infested sheep by the total number of tick positive sheep. Chi-squire (X^2) test was used to

analyse the significance difference between two or more variables and p-value less than 0.05.

3. Results

Prevalence, intensity and diversity of ticks

The results revealed that out of 720 sheep, 267 sheep (37.08 %) were infested with six species of hard ticks. No significant differences were recorded in tick infestation between male and female sheep (X^2 = 0.00015 P=0.536). The average intensity of ticks per sheep was 2.33. The current study reported three species of *Hyaloma* (*H. anatolicum anatolicum*, *H. exacavatum* and *H. scupense*), three of *Rhiphicepalis* (*R. turanicus*, *R. sanguineus* and *R.* (*Boophilus*) *annulatus*). On the other hand, *H. anatolicum* recorded the highest prevalence of infestation with significant differences from the rest of the detected species (X^2 = 102.3, P = 0.00) (Table – 2).

Table 2. Diversity and prevalence of detected ticks

Species of ticks	No. of infected sheep	Prevalence	No. of collected ticks	Mean of Intensity
H. a.anatolicum	89	12.36	335	3.76
H. a. exacavatum	66	9.17	254	3.84
H. scupense	9	1.25	21	2.33
R. turanicus	47	6.53	189	4.02
R. sanguineus	31	4.31	115	3.7
R. (B.) annulatus	25	3.47	96	3.84
Total	**267**	**37.08**	**1010**	**3.78**

Blood Smears Examination

Microscopic examination of 720 of blood smears of both male and female sheep from different ages showed that 234 (32.5 %) were infected by *Babesia* sp. and *Theileria* spp. with an infection rate of (5.83 %) and (26.66 %), respectively. According to the sex of sheep, however, there were significant differences in the prevalence of infection (X^2 = 0.07997, P = 0.777) (Table - 3).

Table - 3: Results of Microscopic diagnosis of sheep blood about Haemoprotzoans.

Samples of examined blood	Number of specimens infection rate %	Samples of infected sheep with *Babesia*	Samples of infected sheep with*Theileria*
720	(32.5) 234	(5.83%) 42	(26.66%) 192

Polymerase Chain Reaction (PCR)

PCR examination of 40 sheep blood samples showed that 14 (35%) of them were infected with *Theileria annulata*, 12 (30 %) with *T. ovis*, 11 (27.5 %), *T. lestoquardi* and 5 (12.5 %) with *Babesia ovis* (Table - 4) (Fig. 1, 2, 3 and 4).

Table - 3: Results of Molecular diagnosis by PCR of sheep blood about Haemoprotzoans.

Number of blood samples	Number of specimens infected with *Theileria annulata*	Infection rate %	Number of specimens infected with *Theileria ovis*	Infection rate %	Number of specimens infected with *Theileria lestoquardi*	Infection rate %	Number of specimens infected with *Babesia ovis*	Infection rate%
40	14	35	12	30	11	27.5	5	12.5

In the current study, it was found that *H. a. anatolicum* and *H. a. exacavatum* were infected by *T. annulata, T. ovis* and *T. lestoqardi*, while the *R. (B.) annulatus* was found to be infected by the same previous species, as well as *Babesia ovis. R. turanicus* had been infected by *T. ovis* and *T. lestoquardi*. No, infection was recorded in *H. scupense* and *R. sanguineus* (Fig. 1, 2, 3, 4).

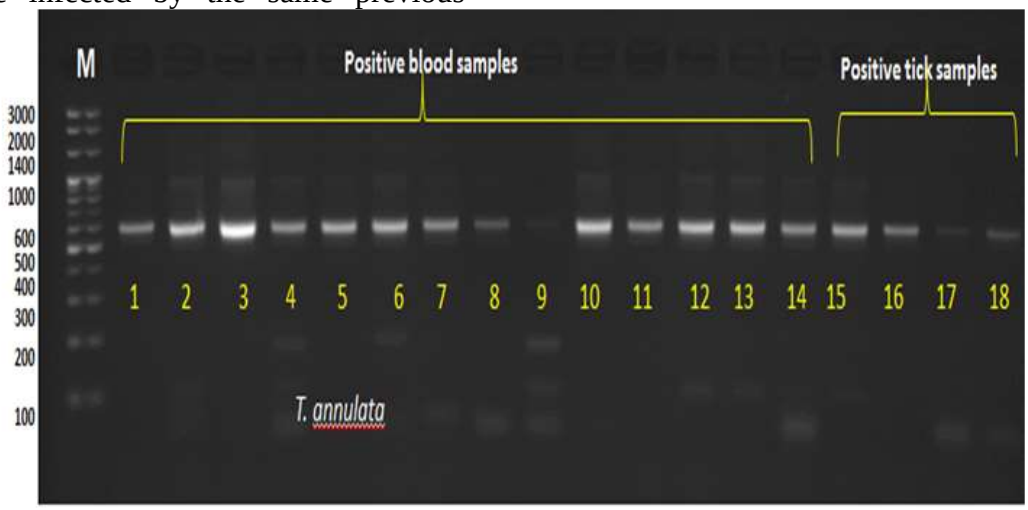

Figure - 1: Electrophoresis of PCR technology results about *T. annulata* in sheep blood and the ticks (*Hyalomma a. anatolicum, Rhipicephalus* (*Boophilus*) *annulatus* and *H. a. exacavtum,* Agarosose 2% (547). M= DNA marker (100 -3000), (1 - 14) = infections of in sheep blood, (15-18) = infections of tick

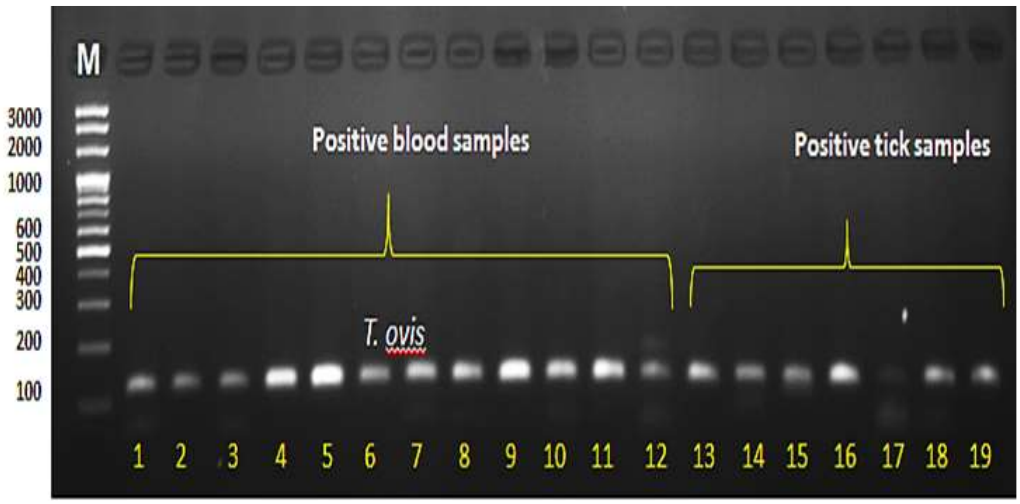

Figure - 2: Electrophoresis of PCR technology results about *T. ovis* in sheep blood and the ticks (*Hyalomma a. anatolicum, Rhipicephalus* (*Boophilus*) *annulatus* and *H. a.exacavtum,* Agarose 2% (112). M = DNA marker (100 - 3000), (1 - 12) = infections in sheep blood, (13 - 19) = infections of ticks.

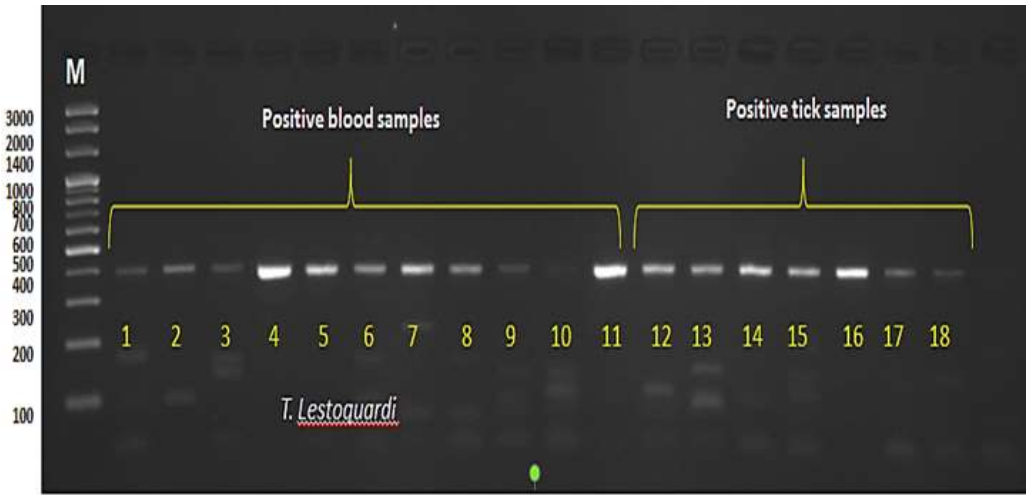

Figure - 3: Electrophoresis of PCR technology results about *T. lestoquardi* in sheep blood and the ticks (*Hyalomma a. anatolicum*, *Rhipicephalus* (*Boophilus*) *annulatus* and *H.a. exacavtum*, Agarose 2 % (400). M = DNA marker (100 - 3000), (1 - 11) = infections in sheep blood, (12 - 18) = Infections of ticks

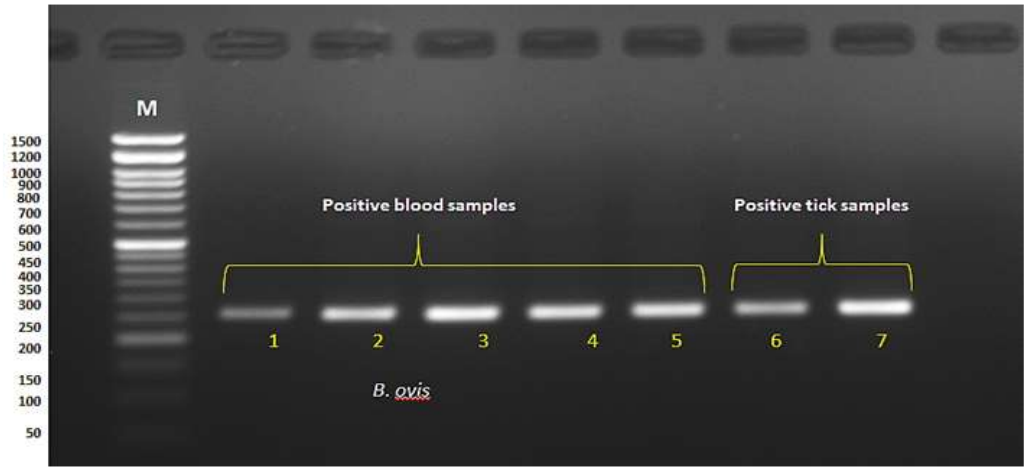

Figure - 4: Electrophoresis of PCR technology results about *B. ovis* in sheep blood and the ticks (*Hyalomma a. anatolicum*, *Rhipicephalus* (*Boophilus*) *annulatus* and *H.a. exacavtum*, Agarose 2% (265). M= DNA marker (50 - 1500), (1 - 5) = infections in sheep blood, (6 - 7) = infections of ticks

4. Discussion

The development of livestock in Iraq faces several challenges, foremost of which are diseases, including parasitic diseases, which are caused by endo or ectoparasites. Ticks play an important role in causing serious effects to animal health, as well as economic losses as a result of the death of infested animals and their lack of productivity and the money spent on controlling them and reduce the diseases it transmits. The prevalence (37.08 %) of ticks in present study was lower than reported in previous studies conducted in sheep of central Iraq in which the rates of infestation were 48 %, 54.3 %, 57 %, and 48.6 % (Aktas *et al*., 2006; Tuama *et al*., 2007; AL-Ramahi, 2011; Shubber *et al*., 2014) respectively. The work of Hatem and AL-Asadi (2020) showed that *Hyalomma anatolicum* affects livestock in Basrah at high rates, including sheep. A study by Hatem (2020) pointed out that *R. sanguineus* is distributed among sheep in Basrah province.

The difference and variation in the rates of tick infestation in sheep may be attributed to many factors, including environmental factors, such as temperature, humidity and rain fall that affect the life cycle of ticks (Ibrahim *et al*.,

2006). The study areas and their vegetation were influenced in tick infestation. As other conditions that effect in ticks occurrence were conditions of methods of studies and the availability of hosts and using of acaricides (Salib *et al*., 2013).

As in previous studies conducted in Iraq, it was noted that *H. a.anatolicum*is the most dominant and abundant ticks in all seasons in the sheep of Basrah province. This population dynamic pattern may be explain by the fact that hot and dry weather is conductive for the development of *H.a. anatolicum* ticks (Ghafar *et al*., 2020).

In current study microscopic examination of thin blood smears stained with Giemza was used, despite its low sensitivity, but it is traditionally widely used in epidemiological studies due to it is an easy method, does not time consuming and allows examining the large number of samples (George *et al*., 2015).

According to the findings of the current study, heamoprotozoans (*Babesia* and *Theileria*) were detected in 234 sheep (32.5 %). The prevalence of *Babesia* and *Theileria* were higher in females compared to those in in male, this result may be explain due to the hormonal influences as well as stress factors during pregnancy and lactation which cause a reduced resistance to pathogen infection. Prevalence of *Babesia ovis* was 5.83 % while prevalence of *Theileria* spp. is 26.66 %. The prevalence rates (32.5 %) of heamoprotozoans in current study was lower than reported in Turkey (Inci *et al*., 1998; Inci *et al*., 2002; Sarayli *et al*., 2006; Inci *et al*., 2010; Aktas *et al*., 2007; Ceylan *et al*., 2020), while Aydın and Coskun (2019) found the infection in other regions ranges between 17 - 61.4 %.

The difference may be due to the difference seasons, the epidemiology of ticks, availability of reservoir hosts and geography. Species of hard ticks are capable to spreading the Babesiosis and Theileriosis from one host to another (Shayan, 2005). In Australia, (Robson *et al*., 1968). It was noted that the Babesiosis is spread by the tick *R.B. annulatus* while Aktas *et al*. (2007) was found that the *H. anatolicum* is the main transmission of Theileriosis diseases in some areas of Iraq, followed by *R.* (*B.*) *annulatus*, in infection rates, then *R. sanguineus*. In Basrah, the study of AL-Mayahand Abdul-Karim (2020) revealed to Piroplasmida detection in Cattle, they showed that cattle are infected by *Babesia* spp. (27.14 %) and *Theileria* spp. (19.52 %).

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