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Microscopical and Molecular investigation of Caprine Theileriosis: in Mosul and Erbil provinces -Iraq

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A B S T R A C T

This study was conducted to determine the prevalence of Theileria uilenbergi and Theileria luwenshuni in goats (Caprine Theileriosis) based on thin and thick Giemsa-stained blood smears and PCR techniques, with a total number of 150 goats (n=150) (in Mosul and Erbil province, between Oct. 2023, and March 2024). The total infection rates were 21.3 % (32 / 150) and 52.6 % (79 /150) for Theileria uilenbergi and Theileria luwenshuni, respectively. Clinical signs in infected goats includes enlarged of superficial prescapular lymph node, loss appetite, pyrexia, lameness, salivation, nasal discharge and lacrimation, dullness, pale yellowish and congested mucus membrane, loss of wight, cough, increased respiratory rate, abortion and rough hairy skin with tick infestations. There was no significant difference in infection rates between female (14%) and male (7.3%) goats, indicating that gender was not a major risk factor. Goats aged 1 to 5 years had a higher infection rate (11.3%) compared to other age groups. Imported goats exhibited the highest infection rate (10.6%), followed by imported Black goats (6%) and Merzi goats (4.7%). PCR analysis using 'catch-all' primers detected a 1098 bp band, confirming Theileria spp. infection. Specific primers for T. uilenbergi and T. luwenshuni detected bands at 878 bp and 812 bp, respectively, confirming the presence of both T. uilenbergi and T. luwenshuni. All goats that tested positive through microscopic examination were also confirmed positive by PCR. In conclusion, this study provides a comprehensive assessment of the prevalence of Theileria uilenbergi and Theileria luwenshuni in goats from the Mosul and Erbil provinces, utilizing both Giemsa-stained blood smears and PCR techniques. These findings highlight the need for targeted interventions and further research to manage and control caprine theileriosis in the region.

Introduction

Goats play a crucial socio-economic role globally, particularly in developing countries, providing various products and services to humans, with their significance in nutrition, health, and food security emphasized by the increasing global production and consumption of goat milk and meat [1,2]. Theileriosis, a tick-borne disease caused by apicomplexan parasites of the genus Theileria, poses a serious global threat to livestock production due to its high morbidity and mortality rates, resulting in significant economic losses in the goat industry and affecting the health and management of small ruminants [3]. *Theileria* is an obligate protozoal parasite living inside erythrocytes and lymphocyte and transmitted by ixodid tick specie vector ticks belonging to the genera *Hyalomma*, *Rhipicephalus* and *Haemaphysalis*. Identifying the specific *Theileria* species present in the host is crucial due to significant differences in pathogenicity among the species. Of the six *Theileria* species known to infect goats, *T. lestoquardi*, *T. luwenshuni*, and *T. uilenbergi* are highly pathogenic and can lead to high mortality. In contrast, the remaining three species—T. separata, T. ovis, and T. recondita—are less pathogenic in small ruminants [4,5].

The diagnosis of theileriosis typically relies on clinical manifestations such as fever, tachycardia, anemia and lymph node enlargement in goats, and it can be confirmed through the identification of schizonts in Giemsa-stained blood smears or lymph node aspirates during acute cases [6]. Various molecular diagnostic tools, including conventional PCR, multiplex PCR, nested PCR, and real-time PCR, have been developed to directly detect parasite infections in blood and assess genetic diversity and phylogenetic relationships among emerging hemoprotozoan species, offering reliable diagnostic capabilities beyond clinical observation [7,8]. No research has been conducted on the occurrence of *T. uilenbergi* and *T. luwenshuni* in goats in Erbil and Mosul governorates of Iraq. Hence, this study research is to assess the prevalence of caprine theileriosis using direct microscopic examination and molecular techniques.

Material and methods

Sample Collection and Processing:

A total of 150 samples of different breeds of goats of ages and both sexes were collected randomly from Mosul and Erbil province, Iraq between Oct. 2023, and March 2024, A comprehensive clinical examination, lymph node inflation, temperature, heart rate, mucous membrane status, and respiration were conducted on all infected animals [9].The blood samples were taken from the jugular vein with EDTA were taken in a 2.5 ml tube. Thin and thick smears were done then dried and fixed in absolute methanol for 5 minutes and stained with 30 minutes with 10% Giemsa. Then examined with 100X. The remaining amount of the blood samples were stored at-20 °C until it was used for DNA extraction.

Based on the preparation method, genomic DNA was extracted from 150 goat blood samples using the Primer™ Genomic DNA Isolation Kit (Genet Bio, South Korea). Two types of PCR reactions were performed: first, to detect positive Theileria spp. infections, universal "catch-all" primers (Favorgen Biotech Corporation, Taiwan) were utilized in a conventional PCR assay. Second, both positive and negative blood samples were further processed using reverse primers and the forward primer set (989-F and 990-R) to detect T. luwenshuni and T. uilenbergi in the PCR assay [10,11] (Table 1).

In short, a PCR reaction was conducted using 25 µl of a mixture containing 2 µl of template DNA, specific reverse, and forward primers for Thieleria, water, PCR master-mix, and Taq DNA polymerase [12]. The PCR cycling conditions included denaturation, annealing, and extension steps. Gel electrophoresis was then performed to visualize the amplified DNA bands of *Thieleria*. Positive and negative controls were used to validate the results [13].

Specifically, the PCR products were analyzed using different sets of primers (987-R and 500-F for *T. uilenbergi*, and T170-F and T670-R for *T. luwenshuni*). The band sizes obtained to confirm the presence of *T. uilenbergi* and *T. luwenshuni* in the samples. Positive controls were prepared from infected goat blood, while a piroplasm-free goat DNA served as a negative control [14,15].

After amplification, gel electrophoresis was performed using 1.5% agarose and visualized under UV light to detect distinct bands of Thieleria [16]. In conclusion, the PCR results using specific primers confirmed the presence of *T. uilenbergi* and *T. luwenshuni* in the samples, with distinct band sizes observed for each species [17,18].

Statistical analysis

The differences between different parameters were evaluated using a computerized database structure (SPSS program), The analysis of data of the current study was carried out by using Chi-square and P value at <0.05.

Results

The total rate of infection with Thieleria parasite in goat by Giemsa-stained blood of smears and conventional PCR technique of 150 samples were 21.3% (32 out of 150) and 52.6 % (79 out of 150), respectively (Table 2). *Theileria* species were determined based on physical features of the merozoite in infected RBCs. *Theileria* spp. appeared as a few parasites single round and double pyriform with acute or obtuse angle, and also it appeared in different morphological forms inside the RBCs

including spherical, oval, pyriform, ring, dot, tail, rounded, small rod, anaplasmoid, single pear and double pears shape was one the most prominent shapes as in (Fig. 1).

The main clinical signs found in clinically infected goats were suffered from anorexia, affecting 20.6% of the goats, followed by enlargement of the prescapular lymph node and dehydration, each affecting approximately 19% of the goats. Other signs included pale mucous membranes, fever, yellowish soft diarrhea, edema, and respiratory signs (Table 3).

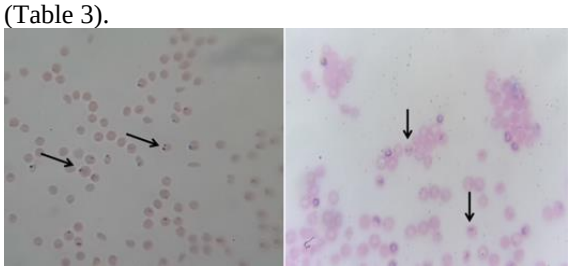

Figure. 1. Blood smear stained with Giemsa showed, A) several blood cells infected with *Theileria* spp. which appear as double pear acute and an obtuse angle, single pear and round. B) *Theileria* spp. appear shape pyriform (a pair of joints) (at magnification of x1000).

In the present study, no significant differences in the prevalence of *Theileria* spp. were observed between genders or age groups of the goats. Female recorded 14 % (21/93) *T. uilenbergi* and 20.6 % (31/93) *T. luwenshuni* and male recorded 7.3 % (11/57) *T. uilenbergi* and 10.7 % (16/57) *T. luwenshuni* infection rates, respectively, without significant difference P≤ 0.05. Higher rate of infection with caprine theileriosis was recorded in age group 1-5 compared with two other groups without significant differences P≤ 0.05 (Table 4).

The infection rate of theileriosis in caprine populations examined in this study appeared that the highest rate of infection was observed among imported goats (10.6 %) *T. uilenbergi and* (12.7 %) *T. luwenshuni*, whereas the second highest rate of infection (6.0 %) *T. uilenbergi and* (11.3 %) *T. luwenshuni* was among local Black goats and the ratio among Merzi goats was (4.7%). *T. uilenbergi and* (7.3 %) *T. luwenshuni*. it is apparent that the imported goats had the highest percentage of both species compared with two other groups without significant differences P≤ 0.05 (Table 5).

Molecular detection of *Thieleria* spp using PCR:

The results of the amplified PCR products using general or universal 'catch-all' primers (Macrogen Inc, South Korea) revealed DNA bands of 1098 bp in size for the first reaction, indicating that the samples were positive for *Theileria* spp. (Figure 2). While for the second reaction using specific primer for *T. uilenbergi*, visualized that the DNA bands size was 818 bp, and using specific primer for *T. luwenshuni* visualized that the DNA bands size was 812 bp meaning that the samples were positive for *T. uilenbergi* and *T. luwenshuni*. The results showed that 21.3% (32/150) of the goats were infected with *Theileria* by Giemsa stain, and 52.6% (79/150) were confirmed by PCR. All samples that tested positive by microscopic examination were also positive by PCR.

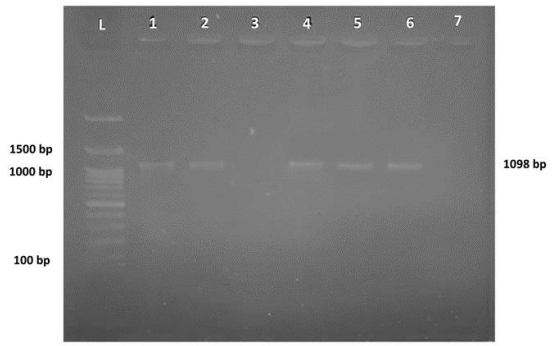

Figure 2. Gel electrophoresis image showing; PCR detection of *Theileria* spp. with a pair of universal primers ((Macrogen Inc, South Korea): Lanes L) 100 bp ladder DNA marker; Lane 1,2,4,5,6) *Theileria* spp. in approximately band size 1098 bp; lane 3 and 7) negative control.

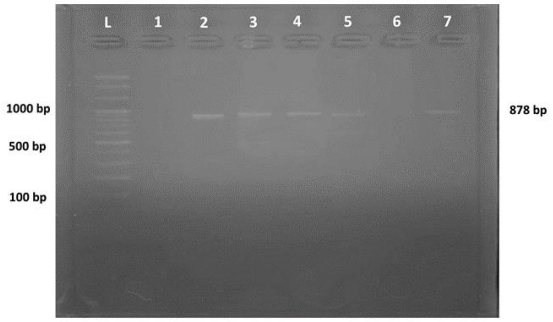

Figure 3. Gel electrophoresis image showing; PCR detection of *T. uilenbergi.* with a pair of specific primers (Macrogen Inc, South Korea): Lanes L) 100 bp ladder DNA marker; Lane 3-5 *of T. uilenbergi* in approximately band size 878 bp; lane 4-7).

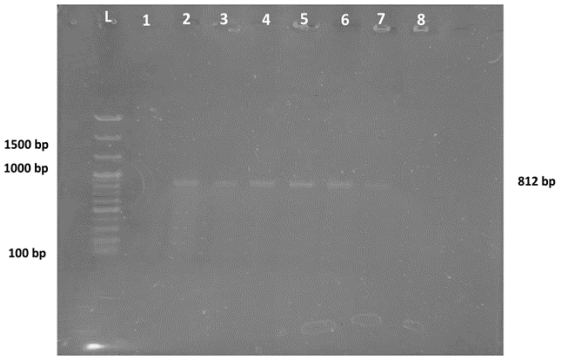

Figure 4. Gel electrophoresis image showing; PCR detection of *T. luwenshuni*. with a pair of specific primers (Macrogen Inc, South Korea): Lanes L812 bp ladder DNA marker.

Discussion

Little information is available regarding caprine theileriosis in Iraq, and there is no data on the prevalence and incidence of *T. uilenbergi* and *T. luwenshuni* in goats in the northern region [19,20]. Therefore, the objectives of this study were to identify caprine theilerioses using direct and indirect

methods to detect both species including Giemsa-stained blood smears [21,22].

In the current investigation, the infection rates were 21.3% (32 out of 150) and 52.6 % (79 out of 150), by Giemsa-stained blood smears and the conventional PCR technique, respectively. The lower infection rate observed through microscopic methods is attributed to their limited sensitivity and specificity [23,24], particularly in detecting infections during the latent or carrier stage with low parasitemia [25,26]. Molecular techniques, known for their high sensitivity and specificity, have been widely employed for detecting and differentiating caprine theileriosis, particularly in carrier animals [27,28].

The clinical symptoms observed in this study align with those reported by Sandhu et al. (1998) and Radostits et al. (2000). Additionally, the anorexia caused by prolonged fever and lymphoid hyperplasia in young, infected goats may explain the development of superficial lymph nodes. The corneal opacity observed in infected goats could be attributed to the infiltration of white blood cells [29,30].

In this study, the prevalence of *T. uilenbergi* and *T. luwenshuni* rates were not differed significantly between the goats' genders and their ages; these findings are in line with that reported by [31]. The study found no significant differences within goat genders and age categories, suggesting that caprine theileriosis is widespread in Erbil. This could be attributed to physical stressors temporarily compromising the immune system, rendering animals more susceptible to infection [32,33].

In this study, molecular study of goat blood samples from several regions in Erbil governorate, Iraq, revealed that (79/150) of clinical goats were infected with *Theileria*. *Theileria* spp. had an infection rate of 52.6%. The significantly high incidence identified in this study might be due to the extensive tick vector population. Previous studies in Duhok reported prevalence rates of 20.8% for *Theileria* species [49], and in Baghdad, infection rates were reported at 26.6% for *Theileria* species [50]. Higher prevalence rates were noted in Sulaimani city (71.7% by PCR) [33,34] and Mosul city 22.7% by microscopic examination and 52.4% by PCR) [31,35].

PCR emerged as an important method in epidemiological research, allowing the identification of both carrier and diseased animals. Based to the molecular investigation, *T. luwenshuni* is considered the main species infecting goats in Erbil city. Future studies on caprine theileriosis should focus on aspects related to the vector (ticks), aligning with findings from previous research [30,32 34].

T. luwenshun and, T. ulenbergi are considered the main species that infect goat in current study (28,31,33). Further studies on caprine theileriosis should be more focused on aspects associated to the vector (ticks) [34,36,37].

Conclusion:

Specific and progressive molecular techniques were able to diagnose (caprine theilerioses) for the first time in Erbil city caused by *T. luwenshuni*, *T. ulenbergi* which is evidence of emergence of diseases. From this PCR method that was designed in this study. Furthermore, investigation and monitoring will be needed to expand superintendence and control politics, such as full vaccination coverage, improvement of traditional diagnostic tools.

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Competing Interests

The authors declares that there are no competing interests.

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Table 1. Primers used for the detection of genus *Theileria* spp, *T. uilenbergi*, and *T. luwenshuni*.

Primers	Sequence of nucleotides	Target gene	Product size (*bp*)	References
PIRO-A PIRO-B	5'- AGTTTCTGACCTATCAG -3' 5'- TTGCCTTAAACTTCCTTG -3'	*Theileria* spp.	1,098	30
BAB1 F BAB4 R	5'- TGACACAGGGAGGTAGTGAC -3' 5'- CTCCCGCACCCTATTTAGCA -3'	*T. uilenbergi*	878	7
BAGIF BAGIR	5' ATTGGAGGGCAAGTCTGGTG 3' 5' CGATCACGGGACAGCAAAAG 3'	*T. luwenshuni*	812	31

Table 2. Total infection rate *Theileria* spp. in goat using microscopic and PCR examination.

Diagnostic methods	number examined	Positive	(%)
Microscopic Ex.	150	32	21.3%
PCR		79	52.6%

Table 3. Clinical signs of infected goat with *Thieleria* parasite.

Clinical signs	No. of infected Goats	%
Enlargement of prescapular lymph node	29	19.3
Pale mucous mm.	26	17.3
Fever	19	12.6
Yellowish soft diarrhea	15	10.0
Dehydration	27	18.0
Edema	6	4.0
Respiratory signs	25	16.6
Anorexia	31	20.6

Table 4. Prevalence of *Theileria spp.* in goat according to sex and age using c-PCR technique.

Factor	No. of animals	No. Positive (%)	
		T. uilenbergi (%)	*T. luwenshuni* (%)
Gender			
Female	93	21 (14.0)	31 (20.6)
Male	57	11 (7.3)	16 (10.7)
P- value		0.27	0.45
Age group			
< 1 year	29	6 (4.0)	11 (7.3)
1—5 years	82	17 (11.3)	25 (16.7)
> 5 years	39	9 (6.0)	11 (7.3)
P- value		0.09	0.79
Total	150	32 (21.3)	47(31.3)

Table 5. The prevalence of *Theileria* spp. in goats according to the breeds using c-PCR technique.

Type	No. of animals	*T. uilenbergi* (%)	*T. luwenshuni* (%)
Merzi goats	33	7 (4.7)	11 (7.3)
Black goats	76	9 (6.0)	17 (11.3)
Imported goats	41	16 (10.6)	19 (12.7)
P-value		10.39	7.193
Total	150	32 (21.3)	47 (31.3)