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Molecular Sequencing of *Theileria lestoquardi* in Iraqi Goats

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Abstract

This study was performed to molecular investigation of caprine theileriosis (*Theileria lestoquardi*), phylogenetic analysis of study isolates, and estimation association of infection to animal risk factors. Initially, venous blood was collected from totally 168 anaemic goats, tested by polymerase chain reaction (PCR), and the positive isolates were sequenced. Findings revealed 16.07% positive goats to *T. lestoquardi*, which increased significantly in goats aged ≥4 years compared to <1 and 1-3 years old, with lack of significance between female and male goats though risk in males was higher than females. Targeting *18S rRNA* gene, phylogenetic analysis of study isolates with the NCBI-BLAST *T. lestoquardi* isolates / strains detected a high percentage of identity ranged 96.61-100% and mutation/changes at 0.01%. However, the study isolates were shown a significant identity with the NCBI-BLAST Iraqi *T. lestoquardi* isolate (GenBank ID: MN704657.1) at 99.13-100% and mutation/changes at 0.000168%. In conclusion, this might represent the first study in Wasit province suggesting the importance of furthermore molecular phylogenetic studies to indicate *T. lestoquardi* isolates circulate in small ruminants.

Keywords: Caprine theileriosis, *18S rRNA* gene, Phylogenetic analysis, Risk factors, Wasit.

التسلسل الجزيئي لل *Theileria lestoquardi* في الماعز العراقي

مثنى نعمة كرم الطائي
قسم علوم الحياة , كلية العلوم , جامعة واسط , واسط , العراق

الخلاصة

أُجـريت هذه الدراسة للتحقق الجزيئي من داء الثيليريا في الماعز (*Theileria lestoquardi*) ، والتحليل التطوري لعـزلات الدراسة، وتقدير ارتباط العدوى بعوامل الخطر الحيوانية. في البداية ، تم جمع عينات الدم الوريدي من 168 ماعزًا مصابًا بفقر الدم ، وخضعت العينات للفحص باستخدام تفاعل البوليميراز المتسلسل (PCR)، ومن ثم خضعت العزلات الإيجابية للتسلسل الجيني . كشفت النتائج عن إصابة 16.07% من الماعز بـ *T. lestoquardi*، وقد زادت هذه النسبة بشكل ملحوظ في الماعز التي تبلغ أعمارها 4 سنوات فأكثر مقارنةً بالماعز التي تقل أعمارها عن سنة واحدة وتلك التي تتراوح أعمارها بين سنة واحدة و3 سنوات، مع عدم وجود فرق إحصائي بين الإناث والذكور، على الرغم من أن خطر الإصابة كان أعلى لدى الذكور منه لدى الإناث. وباستهداف جين *18S rRNA*، كشف التحليل التطوري لعزلات الدراسة باستخدام برنامج NCBI-BLAST عن نسبة تطابق عالية تتراوح بين 96.61% و100%، ونسبة طفرات/تغيرات بلغت 0.01%. ومع ذلك، أظهرت العزلات المدروسة تطابقًا كبيرًا مع عزلة *T. lestoquardi* العراقية (معرف GenBank: MN704657.1) في قاعدة بيانات NCBI-BLAST بنسبة 99.13-100%، ونسبة طفرات/تغيرات بلغت 0.000168%. وختامًا، قد تمثل هذه الدراسة الأولى من نوعها في محافظة واسط، مما يُشير إلى أهمية إجراء المزيد من الدراسات الجزيئية الوراثية لتحديد مدى انتشار عزلات *T. lestoquardi* بين المجترات الصغيرة.

1. Introduction

Theileria lestoquardi is one of the most pathogenic hemoprotozoan parasites, which causes malignant theileriosis in small ruminants throughout the tropical and subtropical areas [1]. The parasite belongs to the Piroplasmida order in the Aconoidasida Class under the phylum of Apicomplexa that composite a large group of intracellular protozoan parasites characterized by the presence of an apical complex involved in host-cell invasion [2-4]. The lifecycle of parasite includes two hosts, vertebrates as small ruminants and invertebrates as hard ticks. Throughout feeding ticks on parasitemic animals, gametogenesis and sexual reproduction occur, producing zygotes and kinetes that migrate to salivary glands where sporogony occurs, and generating sporozoites for transmission to another host. After entering lymphocytes and macrophages, sporozoites differentiate into schizonts that undergo repeated nuclear division and proliferation with occurrence of lymphoid hyperplasia and severe inflammatory responses. Subsequently, schizonts produce merozoites after rupture of infected cells to invade erythrocytes leading to hemolytic anemia and systemic manifestations [1, 5-7].

Clinically, infected goats can develop various forms of disease (acute, subacute and chronic) with many non-specific symptoms that may overlap with other hemoparasitic diseases. Additionally, the disease severity depends on parasite strain, host immunity, age, and parasite burden, several environmental and management-related factors have been demonstrated to influence the disease incidence such as heavy tick infestation, poor hygiene and husbandry, warm and humid climates, mixed grazing systems, as well as stress and malnutrition [8-10].

In laboratory, microscopic examination of Giemsa-stained blood smears remains one of the oldest diagnostic techniques that can identify *T. lestoquardi* as ring-shaped, comma-shaped, or rod-like inclusions that morphologically similar to other *Theileria* species necessitating the need to other advanced diagnostic techniques for accurate differentiation [11, 12]. Globally, a number of serological assays have been developed for surveillance and detection of antibodies against parasite or its antigens; however, cross-reactivity with other related-species, with reduced sensitivity and specificity represent the main limitations of these tools [13, 14]. In last decades, molecular techniques such as PCR have been demonstrated insightful advantages involving high sensitivity and specificity, detection of carrier animals, and capability to differentiate various close-related species [15]. As well, parasite genome sequencing has revolutionized molecular parasitology, providing significant advantages for combating parasitic diseases and understanding evolution [16, 17].

In Iraq, though several studies have been conducted to investigate *T. lestoquardi* in sheep [18-20], only recent few reports have been detected in goats of Erbil provinces [21, 22]. Therefore, this study was aimed to molecular investigation of theileriosis caused by *T. lestoquardi* in goats in Wasit province (Iraq) with estimation their association to animal risk factors (age and sex) and sequencing of study isolates to identify its relationship to the NCBI-GenBank isolates / strains.

2. Materials and Methods

2.1 *Samples*

Totally, 168 suspected anemic goats were selected randomly based on their clinical from various private field and veterinarian clinics located in Wasit province during July-September (2025). Under aseptic condition, approximately 1ml of venous blood were obtained from the marginal ear vein into EDTA-coated plastic tube, transported under cooled conditions and frozen until be examined molecularly. Additional information as age and sex of study goats were reported as risk factor data.

2.2 *Molecular Examination*

After preparation of blood samples at room temperature, Presto^{TM} Mini gDNA Bacteria Kit (Geneaid, Taiwan) was served for extraction of DNAs that tested furthermore for concentrations and purity. Targeting *18S rRNA* gene, one set of primers (HaF: 5′-AGG AAC AGT TGG GGG CAT TC-3′ and HaR: 5′-TCC ACC AAC TAA GAA CGG CC-3′) designed based on NSBI-GenBank Iraq isolate (ID: MW735693.1) in addition to DNA samples and the AccuPower® PCR Pre Mix Kit (Bioneer, Korea) were utilized to preparation the MasterMix tubes at 25μL. For DNA amplification, PCR reaction was performed in the Thermal Cycler system as described by Al-Shaeli et al. [23], and the resultants were subjected to Agarose-gel (1.5%) electrophoresis at 100Volt and 80mA for 90minutes. The positive isolates were identified under the UV transilluminator at 433bp.

2.3 *Sequencing*

DNAs of all positive study isolates to *T. lestoquardi* were sequenced by the modified Sanger method (Macrogen, South Korea), and the received data by private email were submitted in the NCBI-GenBank database, and analyzed by multiple sequence alignment (MSA), phylogenetic tree analysis and homology sequence identity (%) in the MEGA-11 software and NCBI MSA Viewer [24].

2.4 *Statistical Analysis*

Chi-square (x^2) and one-way ANOVA in the GraphPad prism software were utilized to indicate significant differences between the positive values of study groups at $p<0.05$ (*) and $p<0.0001$ (****) with 95% confidence interval (95%CI). Whilst, MedCalc Statistical software was served for determining the risk of *T. lestoquardi* infection through detection values of Odds Ratio (OR) and Relative Risk (RR), [25].

3. Results and Discussion

Among totally 168 blood samples tested conventionally by PCR assay, 27 (16.07%) anemic goats were shown a positive result to *T. lestoquardi* infection; whereas, 141 (83.93%) goats were found negatives. These findings confirmed the circulation of this pathogenic hemoprotozoan parasite among caprine populations in the study area. This finding indicates that caprine theileriosis remains an important tick-borne disease affecting goat health and productivity in Iraq. The detected prevalence may reflect continues exposure of goats to infected tick vectors, particularly *Hyalomma* spp., which are widely distributed in tropical and subtropical environments as Iraq. Also, the occurrence of *T. lestoquardi* in current study emphasizes the epidemiological importance of goats as susceptible hosts and potential reservoirs contributing to maintenance of infection cycles in the endemic regions. In comparison with other national studies, Tawfeeq and AlBakri [21] documented markedly the prevalence rates of caprine theileriosis microscopically and *T. lestoquardi* molecularly in Erbil province were 29.3% and 65.3%, respectively. Similarly, Aziz and Hamadamin [22] identified *T. lestoquardi* as a predominant species infecting goats in Erbil province with a prevalence rate reaching 34.8% using PCR assay suggesting that the ecological conditions that characterized by suitable humidity and grazing systems favoring tick propagation and facilitate transmission of disease. Globally, Ahmed et al. [26] reported that 33.33% of study goats were molecularly positive to *T. lestoquardi* infection; while in Pakistan, Riaz et al. [27] recorded significant higher prevalence of *T. lestoquardi* infection in sheep (19.4%) rather than goats (8.4%). In Iran, several studies have been done with variable prevalence rates of *T. lestoquardi* infection in goats ranging from 6.25 [28] to 11.7% [29]. However, differences in prevalence rates of *T. lestoquardi* infection are expected because of variations in geographical locations, climate conditions, sampling period, diagnostic techniques, targeted gene, management systems, tick burden, host immunity and sample size.

According to age factor, significant elevation (p<0.0146, p<0.0001) in values of positivity and risk (OR, RR) of *T. lestoquardi* infection was detected in study goats aged ≥4 years (28%, 3.1410, and 2.2043, respectively) whereas lower values were observed in goats aged <1 year (4.35%, 0.1764, and 0.2450, respectively) when compared to 1-3 years (15.28%, 0.9016, and 0.9277, respectively). Relation to sex factor, though no significant differences (p<0.0678) were seen in prevalence rats of positive *T. lestoquardi* infection between female (15.24%) and male (17.46%) goats, the risk (OR, RR) *T. lestoquardi* infection was markedly higher (p<0.0001) in males (1.1767, 1.1242) more than females (0.8498, 0.8896), (Table 1).

Table 1. Distribution of positivity and risk of *T. lestoquardi* infection among study goats (No.168)

Factor	Total No.	Positive	OR	RR	NNT	95%CI
Age (Year)						
<1	46	2 (4.35%)	0.1764	0.2450	7.788 (Benefit)	4.176 (Benefit) to ∞ to 57.748 (Benefit)
1-3	72	11 (15.28%)	0.9016	0.9277	96.833 (Benefit)	11.402 (Harm) to ∞ to 9.228 (Benefit)
≥4	50	14 (28%) *	3.1410 ****	2.2043 ****	8.367 (Harm)	56.674 (Harm) to ∞ to 4.517 (Harm)
p-value		0.0146	0.0001	0.0001	-	-
95% CI		13.53 to 45.28	2.433 to 5.245	1.345 to 3.596	-	-
Sex						
Female	105	16 (15.24%)	0.8498	0.8896	60.912 (Benefit)	11.982 (Harm) to ∞ to 8.599 (Benefit)
Male	63	11 (17.46%)	1.1767 ****	1.1242 ****	60.912 (Harm)	8.599 (Harm) to ∞ to 11.982 (Benefit)
p-value		0.0678	0.0001	0.0001	-	-
95% CI		-2.246 to 30.45	1.064 to 3.090	0.4835 to 2.497	-	-

Relation to animal risk factors, significant elevation in values of positivity and risk of *T. lestoquardi* infection was detected in study goats aged ≥4 years whereas lower values were observed in goats aged <1 year when compared to 1-3 years. These observations suggest that age represents an important epidemiological factor influencing susceptibility and exposure to infected tick vectors over time, prolonged grazing activity, and increased opportunities for contact with endemic transmission cycles. Adult animals generally experience repeated exposure to *Hyalomma* ticks throughout their lifetime, which increases the probability of acquiring infection compared with younger goats that have had shorter environmental exposure period [8, 11, 30]. In agreement with our results, Tawfeeq and AlBakri [21] reported that the high prevalence rates of caprine theileriosis was shown in adult goats aged >5 years (32.5%) and 1-5 years (29.5%) compared with younger < 1year (25%), but in contrast, Kebzai et al. [31] reported that age did not significantly correlated with the presence of *T. lestoquardi* infection. However, Riaz et al. [27] mentioned that young animals often exhibit higher infection rates because of immature immune responses; whereas, stressed or malnourished animals experience increased vulnerability to parasitic diseases. Furthermore, animals raised under extensive pastoral systems generally have greater exposure to tick vectors that intensively managed goat.

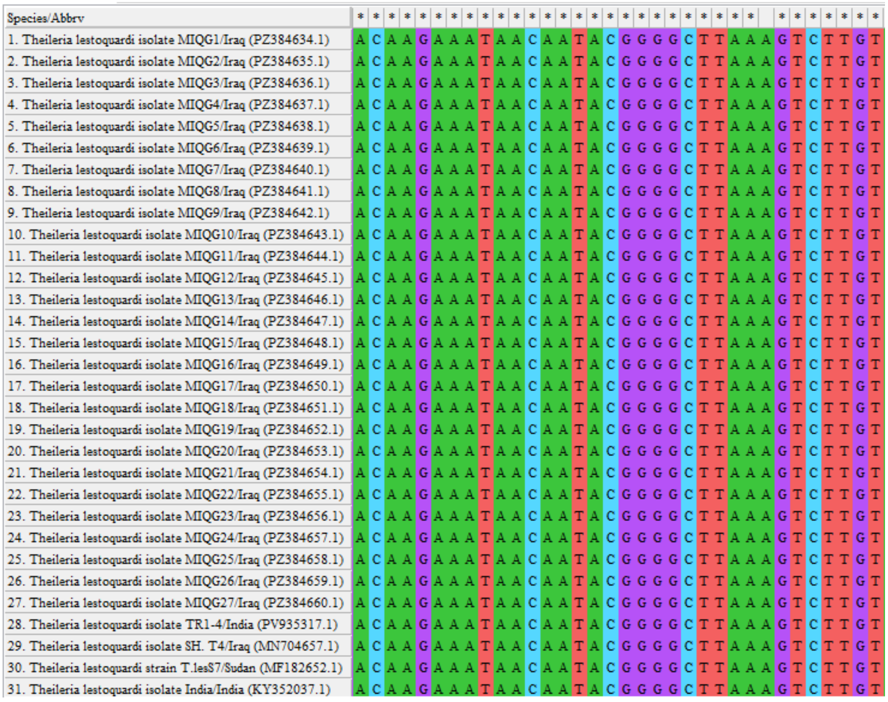
Relation to sex factor, though no significant differences were seen in prevalence rats of positive *T. lestoquardi* infection between female and male goats, the risk *T. lestoquardi* infection was markedly higher in males more than females. Comparatively, our findings were compatible with the results of Mohammadi et al. [28]; but in contrast, Tawfeeq and AlBakri [21] who reported that female goats having a higher rate of theileriosis (32.9%) than males (25%). However, Riaz et al. [32] detected that *Theileria* infection is more often significantly in male (22.8%) than female (14%) goats attributing these findings to the level of exposure and variable physiological position during pregnancy, parturition and lactation. Also, number of tested males compared to females (sample size) might reflect on our data since low number could lead to ambiguous, misleading or non-reproducible results.

Initially, sequencing data of study isolates were named as MIQG1 to MIQG27, submitted in the NCBI-GenBank database to get their specific access numbers that being as respectively as PZ384634.1 to PZ384660.1. Targeting *18S rRNA* gene, phylogenetic analysis of study isolates with the NCBI-BLAST *T. lestoquardi* isolates / strains detected a high percentage of identity (%) ranged 96.61-100% and mutation/changes at 0.01%. However, the study isolates were shown a significant identity with the NCBI-BLAST Iraqi *T. lestoquardi* isolate (GenBank ID: MN704657.1) at 99.13-100% and mutation / changes at 0.000168% as shown in Table 2, Figures 2 and 3.

Table 2- Homology Sequence identity (%) for local and NCBI-BLAST *T. lestoquardi* isolates / strains

Local isolate		**NCBI-GenBank isolate**				
Name	**Access No.**	**Access No.**	**Isolate**	**Host**	**Country / Region**	**%**
MIQG1	PZ384634.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.65
MIQG2	PZ384635.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.40
MIQG3	PZ384636.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.49
MIQG4	PZ384637.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.15
MIQG5	PZ384638.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.15
MIQG6	PZ384639.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.17
MIQG7	PZ384640.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.52
MIQG8	PZ384641.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.77

MIQG9	PZ384642.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.88
MIQG10	PZ384643.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.17
MIQG11	PZ384644.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.33
MIQG12	PZ384645.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.13
MIQG13	PZ384646.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.30
MIQG14	PZ384647.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	100
MIQG15	PZ384648.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.56
MIQG16	PZ384649.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.82
MIQG17	PZ384650.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.86
MIQG18	PZ384651.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.95
MIQG19	PZ384652.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.96
MIQG20	PZ384653.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.97
MIQG21	PZ384654.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.99
MIQG22	PZ384655.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.45
MIQG23	PZ384656.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.36
MIQG24	PZ384657.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.93
MIQG25	PZ384658.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.81
MIQG26	PZ384659.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.74
MIQG27	PZ384660.1	MN704657.1	SH.T4	Sheep	Iraq/ Sulaymaniyah	99.53



Species/Abbrv	* * * * * * * *
1. Theileria lestoquardi isolate MIQG1/Iraq (PZ384634.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
2. Theileria lestoquardi isolate MIQG2/Iraq (PZ384635.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
3. Theileria lestoquardi isolate MIQG3/Iraq (PZ384636.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
4. Theileria lestoquardi isolate MIQG4/Iraq (PZ384637.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
5. Theileria lestoquardi isolate MIQG5/Iraq (PZ384638.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
6. Theileria lestoquardi isolate MIQG6/Iraq (PZ384639.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
7. Theileria lestoquardi isolate MIQG7/Iraq (PZ384640.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
8. Theileria lestoquardi isolate MIQG8/Iraq (PZ384641.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
9. Theileria lestoquardi isolate MIQG9/Iraq (PZ384642.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
10. Theileria lestoquardi isolate MIQG10/Iraq (PZ384643.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
11. Theileria lestoquardi isolate MIQG11/Iraq (PZ384644.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
12. Theileria lestoquardi isolate MIQG12/Iraq (PZ384645.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
13. Theileria lestoquardi isolate MIQG13/Iraq (PZ384646.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
14. Theileria lestoquardi isolate MIQG14/Iraq (PZ384647.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
15. Theileria lestoquardi isolate MIQG15/Iraq (PZ384648.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
16. Theileria lestoquardi isolate MIQG16/Iraq (PZ384649.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
17. Theileria lestoquardi isolate MIQG17/Iraq (PZ384650.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
18. Theileria lestoquardi isolate MIQG18/Iraq (PZ384651.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
19. Theileria lestoquardi isolate MIQG19/Iraq (PZ384652.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
20. Theileria lestoquardi isolate MIQG20/Iraq (PZ384653.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
21. Theileria lestoquardi isolate MIQG21/Iraq (PZ384654.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
22. Theileria lestoquardi isolate MIQG22/Iraq (PZ384655.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
23. Theileria lestoquardi isolate MIQG23/Iraq (PZ384656.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
24. Theileria lestoquardi isolate MIQG24/Iraq (PZ384657.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
25. Theileria lestoquardi isolate MIQG25/Iraq (PZ384658.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
26. Theileria lestoquardi isolate MIQG26/Iraq (PZ384659.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
27. Theileria lestoquardi isolate MIQG27/Iraq (PZ384660.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
28. Theileria lestoquardi isolate TR1-4/India (PV935317.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
29. Theileria lestoquardi isolate SH. T4/Iraq (MN704657.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
30. Theileria lestoquardi strain T.lesS7/Sudan (MF182652.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T
31. Theileria lestoquardi isolate India/India (KY352037.1)	A C A A G A A A T A A C A A T A C G G G G C T T A A A G T C T T G T

Figure -2 MSA of local study and global NCBI-GenBank *T. lestoquardi* isolates / strains using the MEGA-11 software

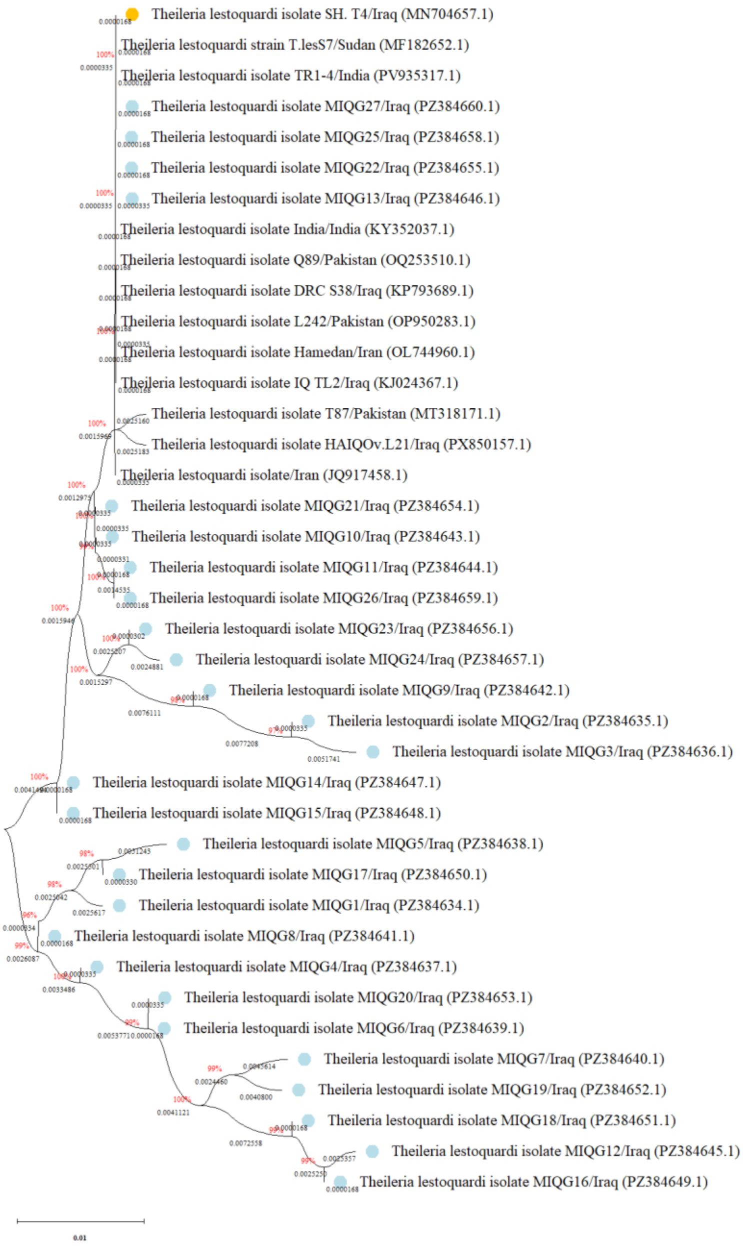


Figure -3 Phylogenetic tree analysis of local study and global NCBI-GenBank T. lestoquardi isolates / strains using the MEGA-11 software.

Targeting *18S rRNA* gene, phylogenetic analysis of study isolates with the NCBI-BLAST *T. lestoquardi* isolates / strains detected a significant identity with the NCBI-BLAST Iraqi *T. lestoquardi* isolate at 99.13-100%. Worldwide, *18S rRNA* gene has become one of the most widely used molecular markers for the detection and characterization of *T. lestoquardi* in goats because of its high sensitivity, specificity, conserved structure, and phylogenetic utility [7, 33]. In recent years, molecular diagnostic techniques targeting the *18S rRNA* gene have largely replaced conventional microscopic methods in epidemiological investigations of caprine theileriosis, particularly in endemic regions where subclinical and chronic infections are common [34, 35]. The application of *18S rRNA*-based PCR assays has significantly improved the accuracy of diagnosis and contributed substantially to understanding the epidemiology, genetic diversity, and evolutionary relationships of *T. lestoquardi* in goats and other small ruminants [26, 36, 37].

4. Conclusion

This might represent the first study in eastern Iraqi goats which provided a valuable molecular data about the prevalence rate and risk of *T. lestoquardi* in goats with phylogenetic information for the isolates circulating in study areas suggesting the importance of furthermore molecular phylogenetic studies to indicate *T. lestoquardi* isolates circulate in small ruminants. However, phylogenetic data indicates that only one genotype might be circulated in Iraqi goats necessitating moreover phylogenetic works to investigate *T. lestoquardi* strains found in small ruminants.

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Ethical Approval

This study was licensed by the Scientific Committee in the Department of Biology (College of Science, University of Wasit) and certificated by Ethical Guidelines and Welfare Standards in the College of Veterinary Medicine / University of Wasit (WU-CVM.CS-BD-6 in 10/5/2026).

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

[1] B. Esmaeilnejad, F. Malekifard, S. J. Hasani, S. K. M. Mofidi, A. Samiei, and A. Ghorbani, “*Theileria lestoquardi* and malignant theileriosis in small ruminants: An updated review,” *Tropical Animal Health and Production*, vol. 57, no. 8, p. 393, 2025.

[2] L. Schnittger, S. Ganzinelli, R. Bhoora, D. Omondi, A. M. Nijhof, and M. Florin-Christensen, “The Piroplasmida *Babesia*, *Cytauxzoon*, and *Theileria* in farm and companion animals: Species compilation, molecular phylogeny, and evolutionary insights,” *Parasitology Research*, vol. 121, no. 5, pp. 1207–1245, 2022.

[3] M. Florin-Christensen, D. Sojka, S. Ganzinelli, P. Šnebergerová, C. E. Suarez, and L. Schnittger, “Degrade to survive: The intricate world of piroplasmid proteases,” *Trends in Parasitology*, vol. 39, no. 7, pp. 532–546, 2023.

[4 H. A. Gharban, H. N. Al-Ghuraibawi, Z. A. Al-Rubaye, H. A. Jahlol, A. A. Al-Zergany, and G. J. Al-Abedi, “Most clinically detected viral diseases in field animals of Wasit Province, Iraq,” *Annals of the Romanian Society for Cell Biology*, vol. 27, no. 1, pp. 154–168, 2023.

[5] S. J. Clift, N. E. Collins, M. C. Oosthuizen, J. C. Steyl, J. A. Lawrence, and E. P. Mitchell, “The pathology of pathogenic theileriosis in African wild artiodactyls,” *Veterinary Pathology,* vol. 57, no. 1, pp. 24–48, 2020.

[6] S. Tajeri and G. Langsley, “*Theileria* secretes proteins to subvert its host leukocyte,” *Biology of the Cell*, vol. 113, no. 4, pp. 220–233, 2021.

[7] R. Elseadawy et al., “Prevalence of piroplasm infection and associated risk factors in goats from Upper Egypt, with emphasis on *Theileria ovis 18S rRNA* gene diversity,” *Journal of Parasitic Diseases*, pp. 1–11, 2025.

[8] Y. Demessie and S. Derso, “Tick borne hemoparasitic diseases of ruminants: A review,” *Advances in Biological Research*, vol. 9, no. 4, pp. 210–224, 2015.

[9] H. A. Gharban, “Clinical and serological diagnosis of bovine hypodermosis in Wasit Province,” *Revista Electronica de Veterinaria*, pp. 457–466, 2022.

[10] A. J. Gharban, S. J. Al-Shaeli, G. J. Al-Abedi, Z. R. Abbas, and A. F. Jassim, “Microscopic investigation of bovine haemoparasites in Wasit Province, Iraq,” *Annals of the Romanian Society for Cell Biology,* vol. 26, no. 1, pp. 1143–1159, 2022.

[11] H. Kiara, L. Steinaa, V. Nene, and N. Svitek, “*Theileria* in ruminants,” in Parasitic Protozoa of Farm Animals and Pets. Cham, Switzerland: Springer International Publishing, 2017, pp. 187–213.

[12] A. H. Sray, A. H. Al-Shabbani, and H. A. Gharban, “Serological and molecular estimation of *Theileria equi* infections in horses of Baghdad, Al-Qadisiyah, and Wasit Provinces, Iraq,” *Annals of RSCB*, vol. 23, no. 2, pp. 276–289, 2019.

[13] O. A. Alkefari, H. A. Al-Gharban, and T. H. Ahmed, “Microscopic, serological and molecular detection of *Babesia bigemina* in buffaloes (*Bubalus bubalis*) in Wasit Province, Iraq,” *Al-Qadisiyah Journal of Veterinary Medicine Sciences,* vol. 16, no. 1, pp. 123–130, 2017.

[14] R. Pienaar, B. B. Maboko, and B. J. Mans, “*Theileria* diagnostics and species definition,” in East Coast Fever: The Disease, Its Impact and Control, pp. 157–186, 2026.

[15] E. M. Al-Eodawee, T. K. Abdulwahed, G. J. Al-Abedi, and H. A. Gharban, “Molecular identification of *Eimeria* spp. and *Eimeria bovis* in water buffaloes, Iraq,” *Journal of Global Innovative Agricultural Sciences*, vol. 11, pp. 363–369, 2023.

[16] S. Kumar, S. Gupta, A. Mohmad, A. Fular, B. C. Parthasarathi, and A. K. Chaubey, “Molecular tools: Advances, opportunities and prospects for the control of parasites of veterinary importance,” *International Journal of Tropical Insect Science,* vol. 41, no. 1, pp. 33–42, 2021.

[17] H. A. Gharban, “First genotyping confirmation of Pichia kudriavzevii in subclinically mastitic cows in Iraq,” *Revista de Ciências Agroveterinárias,* vol. 23, no. 3, pp. 417–424, 2024.

[18] A. A. Faraj and D. H. Assi, “Microscopically and molecular detection of *Theileria* species in sheep in Baghdad Province, Iraq,” *Al-Anbar Journal of Veterinary Sciences*, vol. 15, no. 2, pp. 7–18, 2022.

[19] M. M. Hamid and Q. T. Al-Obaidi, “Prevalence of ovine theileriosis in Mosul City, Iraq,” *Iraqi Journal of Veterinary Sciences*, vol. 37, no. 1, pp. 205–211, 2023.

[20] M.A.A. Al-Shabbani, H. N. Rufaish, H. H. Naser, M. Abbas, and H. Almhanna, “Genotyping study for infections of *Theileria* and *Babesia* in cattle and sheep in Al-Najaf Governorate, Iraq,” *Journal of Animal Health and Production*, vol. 13, no. 1, pp. 216–222, 2025.

[21] D.A. Tawfeeq and H. S. AlBakri, "Clinical, microscopical and molecular detection of caprine theileriosis," *Iraqi Journal of Veterinary Sciences*, vol. 38, no. 3, pp. 693–699, 2024.

[22] K. J. Aziz and B. Q. Hamadamin, "Epidemiological and molecular study of Theileria spp. in sheep and goats in Erbil, Iraq," *Tropical Animal Health and Production*, vol. 57, no. 2, p. 80, 2025.

[23] S. J. Al-Shaeli, A. M. Ethaeb, and H. A. Gharban, "Molecular and histopathological identification of ovine neosporosis (*Neospora caninum*) in aborted ewes in Iraq," *Veterinary World*, vol. 13, no. 3, p. 597, 2020.

[24] G. J. K. Al-Abedi, E. M. M. Al-Eodawee, S. Khalili, and H. A. J. Gharban, "First molecular genotyping of Cryptosporidium felis in cattle, Iraq," Archives of Razi Institute, vol. 77, no. 6, p. 2345, 2022.

[25] H. A. Gharban, E. A. Ajaj, and H. A. Mohammad, "Molecular detection, seroprevalence, and phylogenetic analysis of *Brucella suis* in wild pigs (Sus scrofa) in Iraq: Implications for One Health surveillance," *Veterinary World*, vol. 18, no. 9, p. 2733, 2025.

[26] R. A. Ahmed, S. L. Mahmmod, N. A. Kakarash, and M. O. Baba Shekh, "Conventional and molecular diagnosis of theileriosis (Theileria annulata) in cattle in Sulaimani Province, Northern Iraq," *Passer Journal of Basic and Applied Sciences*, vol. 3, no. 2, pp. 150–155, 2021.

[27] M. Riaz et al., "Molecular epidemiology and phylogeny of *Theileria ovis* and *Theileria lestoquardi* in sheep and goats from Southern Punjab, Pakistan," *Vector-Borne and Zoonotic Diseases*, vol. 24, no. 10, pp. 656–665, 2024.

[28] S. M. Mohammadi, B. Esmaeilnejad, and G. Jalilzadeh-Amin, "Molecular detection, infection rate and vectors of *Theileria lestoquardi* in goats from West Azerbaijan Province, Iran," *Veterinary Research Forum*, vol. 8, no. 2, p. 139, 2017.

[29] A. Rehman, A. M. Nijhof, C. Sauter-Louis, B. Schauer, C. Staubach, and F. J. Conraths, "Distribution of ticks infesting ruminants and risk factors associated with high tick prevalence in livestock farms in the semi-arid and arid agro-ecological zones of Pakistan," *Parasites & Vectors*, vol. 10, no. 1, p. 190, 2017.

[30] R. Bishop, A. Musoke, R. Skilton, S. Morzaria, M. Gardner, and V. Nene, "*Theileria*: Life cycle stages associated with the Ixodid tick vector," Cambridge University Press, vol. 1, pp. 308–324, 2008.

[31] F. Kebzai, K. Ashraf, M. U. Rehman, H. Akbar, M. Avais, and M. Khan, "Molecular detection and assessment of risk factors for *Theileria lestoquardi* in sheep from Balochistan, Pakistan," *Parasitology Research*, vol. 122, no. 12, pp. 2957–2965, 2023.

[32] M. Riaz et al., "Molecular epidemiology and prevalence of *Theileria lestoquardi* and *Theileria ovis* infection in goats infested with tick vectors from Multan, Pakistan," *Journal of Medical Entomology*, vol. 56, no. 3, pp. 844–848, 2019.

[33] B. A. Celik, M. Kara, A. Ayan, M. A. Selcuk, and O. Y. Celik, "Molecular prevalence and phylogenetic analysis of *Theileria ovis* and *Theileria lestoquardi* in small ruminants in Siirt Province, Türkiye," *Veterinary Medicine and Science*, vol. 11, no. 5, Art. no. e70522, 2025.

[34] T. Kamal, "Prevalence of gastrointestinal parasitic infections, haemoprotozoan diseases and their associated risk factors in sheep and goat in selected hilly areas of Bangladesh," Ph.D.

dissertation, Chattogram Veterinary and Animal Sciences Univ., Chattogram, Bangladesh, 2020.

[35] O. Ceylan, M. A. Selcuk, C. Ceylan, and F. Sevinc, “Sequence-based analysis of *Theileria annulata* reveals high Tams1 gene diversity and conservation of *18S rRNA* in Türkiye,” *BMC Veterinary Research*, vol. 21, no. 1, p. 578, 2025.

[36] J. Zeb, B. Song, M. U. Aziz, S. Hussain, R. Zarin, and O. Sparagano, “Diversity and distribution of *Theileria* species and their vectors in ruminants from India, Pakistan and Bangladesh,” *Diversity*, vol. 14, no. 2, p. 82, 2022.

[37] M. C. Ulucesme, M. Aktas, and S. Ozubek, “Mitochondrial genome analysis of *Babesia ovis* (Apicomplexa: Babesiidae) endemic in sheep in Türkiye,” *Veterinary Sciences*, vol. 11, no. 11, p. 554, 2024.