Basrah Journal of Veterinary Research 2026; 25(1):101-115.

Print ISSN: 1813-8497
Online ISSN: 2410-8456
https://bjvr.uobasrah.edu.iq/

Molecular Characterization of Tropical Theileriosis Among Local and Imported Dairy Cattle in Duhok Governorate–Kurdistan Region/Iraq

Article Info.
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Article History

Received: Dec. 27, 2025
Accepted: Feb. 4, 2026
Published: March 31, 2026
Article type: **Research Article**

https://doi.org/10.23975/bjvr.2026.168166.1269

Abstract

Bovine tropical theileriosis, caused by *Theileria annulata,* was surveyed in dairy cattle herds of Duhok Governorate, Kurdistan Region, Iraq. In a cross-sectional study of 212 cattle, Giemsa-stained microscopy was compared to nested PCR (n-PCR), and the determinant risk factors and phylogenetic relationships were investigated. The microscopic and PCR prevalence of infection was 12.7 and 21.7, respectively (P≤0.0001). Age increased prevalence by a significant margin (P≤0.032): cattle 5 years old were almost four times more likely to be infected than calves. Poor body condition was a significant determinant as opposed to sex and breed effects, which were not significant (P≤0.0001). Risk was also predisposed by management variables, outdoor grazing, and infrequent use of acaricides (P≤0.0001). The phylogenetic analysis was done using 10 local 18S rRNA sequences (GenBank accessions OP435250-OP435259), and all Duhok isolates appeared in one clade, but some of them were extremely close to a strain present in the neighboring countries (99-100% identity). These results suggest regional genetic continuity and most likely cross-border spread. To sum up, *T. annualata* causes a significant epidemiological impact in dairy cattle in Duhok. The superior sensitivity of PCR, the strong influence of age, body condition, and management practice, and the evidence of regional strain circulation underscore the need for enhanced molecular surveillance, regimented acaricide programs, and targeted, risk-based control strategies to safeguard herd health and productivity.

Keywords:*Theileria annulata*, molecular characterization, phylogenetic analysis.

Publisher: University of Basrah,
College of Veterinary Medicine, Basrah, Iraq

Introduction

Tick-borne diseases affect millions of wild and domestic animals in tropical and subtropical regions around the world (1). One of the most prevalent tick-borne illnesses is theileriosis, which puts over 250 million animals at risk each year (2). With a high morbidity and death rate, theileriosis is a global disease that affects almost all ungulates and can induce latent or fatal infections (3). All species of *Theileria* are known to pose a significant threat to many vertebrate animals. However, *T. annulata* and *Theileria parva* are the most dangerous for cattle (4). The disease is mainly transmitted by various species of hard ticks from the Ixodidae family (5, 6).

Although certain *Theileria* species cause little or no noticeable symptoms in infected animals, others can trigger severe clinical conditions such as high fever, anemia, hemoglobinuria, and, in some cases, death (1, 7). Notably, animals tend to be in a chronic infection even after they have healed from the original illness. These animals that carry the parasite act as reservoirs, which help them transfer it further to ticks, who subsequently infect other hosts (1, 7). Several conditions come together to determine the prevalence of tick-borne diseases. Beyond fundamental ecological drivers and livestock management, the risk depends on host characteristics (species, sex, breed, health), habitat suitability, and the density of coexisting susceptible animals (8, 9). A central element in this equation is the abundance of ticks competent to carry and transmit the pathogens (10, 11). Theileriosis has been confirmed in animals across various regions of Iraq, as well as in neighboring countries such as Iran and Turkey (5). Because theileriosis causes weight loss and reduced milk production, it has a detrimental impact on the productivity of dairy and cattle animals, resulting in large losses for the livestock industry (6).

Microscopic blood smear analysis is the main test for the diagnosis of acute piroplasmosis, but it has difficulty finding the low-level, ongoing infections seen in asymptomatic carriers during the chronic phase (12). Since identifying these carriers is essential for outbreak prevention and epidemiology, molecular methods have become indispensable. PCR tests with amplicon sequencing have the required sensitivity, specificity, and accuracy information to be able to confidently identify various *Theileria* spp. in infected animals (13). Molecular techniques are becoming more significant in the accurate identification of *Theileria* species because of their high specificity and sensitivity. PCR is the most widespread technique in the detection of different species and genotypes of *Theileria* (14, 15). The *18S rRNA* gene has been especially useful in the identification and discrimination of the various species of *Theileria* (1). The DNA sequencing, especially of the *18S rRNA* gene, is crucial in the phylogenetic comparisons and evolutionary discrepancies of *Theileria* species in different geographical locations. The analysis of sequence variations provides researchers with an opportunity to evaluate genetic relationships between strains of *Theileria* and helps to understand their evolutionary trends better (16). Such a method enhances better identification of the species, and it also leads to a larger knowledge of epidemiology and global distribution of various *Theileria* genotypes.

The current study highlighted the presence of *Theileria* parasites in cattle in Duhok Governorate, leading to the selection of molecular diagnostics and phylogenetic analysis to determine specifically which *Theileria* species were infecting cattle in the area. This methodology is not only useful in the correct identification of species but also serves the purpose of distinguishing *Theileria* from other related parasites, which is not easily done using the traditional microscopic techniques. The results will help enhance disease control measures by gaining a better insight into the local *Theileria* species.

Material and Methods

Sampling and Microscopic Examination:

The study used a cross-sectional sampling technique to identify data and the specimens of 212 dairy cattle of various ages and sexes in a high number of farms in Duhok Province, Iraq, from the beginning of March to the end of July 2025. Detailed clinical assessments were conducted, including the most necessary physiological values such as body temperature, respiratory rate, pulse rate, and the existence of any symptoms of overt diseases. Aseptically sampled blood samples (through the coccygeal vein) into vacuum tubes containing EDTA. A thin blood smear was obtained by placing a single drop of the sample on a clean glass slide to be investigated using the parasitological technique. After air drying, the smears were allowed to fix in absolute methanol (99%) for a period of five minutes, followed by staining with a 10 percent Giemsa solution for a defined time of half an hour. The ready-prepared slides were then studied under the microscope at a magnification of 100x under oil immersion to find and describe the morphological characteristics of *Theileria* spp., following the method described by Zajac *et al*. (17). Remaining blood aliquots were frozen at -20°C and stored to be used at a later time in molecular studies.

Data Collection

The standardized questionnaires were used to collect epidemiological data, both at the herd and individual animal level. All animals were taken to a detailed clinical examination in order to capture the health profile of the animal, such as age, sex, body condition score, and whether the animal had clinical signs or not. The questionnaire provided the necessary data concerning herd management practices and environmental conditions such as the composition of the cattle herd, the presence of mixed-species herds, the presence of tick infestation, the primary grazing system (confined barn vs. pasture), the acaricide application regimen (none, regular, or irregular), and the flooring type of housing facilities (cemented vs. non-cemented). Such variables were then subjected to statistical analysis to determine how much they could be used as risk factors determining the distribution of *Theileria* spp. infection in the dairy cattle population surveyed.

DNA extraction

DNA was extracted from blood samples containing *Theileria* using a commercial DNA extraction kit (Bio Korea). Following the manufacturer's instructions. A volume of 200 μl of whole blood

was processed for each extraction, yielding approximately 100 µl of eluted DNA at a concentration of ~6 *µg*/*µl*.and the extracted DNA was stored at -20°C for subsequent PCR analysis. The purity and concentration were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA). Nested PCR (n-PCR) was employed to detect the *18S rRNA* gene of *Theileria* spp. by targeting the *Tams-1* gene (Table 1).

Table 1: Nucleotide primers used for amplifying the *18S rRNA* and *Tams-1* genes of *T. annulata*.

Target gene	**Sequences 5'-3'**	Annealing of primers	**Expected size (*bp*)**	**References**
Theileria spp	5′-GAAACGGCTACCACATCT-3′ 5′-AGTTTCCCCGTGTTGAGT-3′	55 ºC	778	18
T. annulata	5′- TTAAACCTCTTCCAGAGT -3′ 5′- TCAGCCTTGCGACCATAC -3′		581	

The first-round PCR reaction was carried out in a 20 µl volume containing 2 µl of DNA template, 1 µl of each outer primer (10 µM), 10 µl Add Taq Master Mix (Addbio, Korea), and 6 µl distilled water. The nested PCR was also performed in a 20 µl reaction volume, consisting of 1 µl of each inner primer (10 µM), 10 µl Master Mix, 7 µl distilled water, and 1 µl DNA from the first-round PCR product as template DNA. Thermal cycling was performed using an Eppendorf thermal cycler with the following conditions: initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 94 °C for 45 seconds, annealing at 55 °C for 50 seconds, and extension at 72 °C for 1 minute, followed by final extension at 72 °C for 7 minutes (18).

Gene sequence processing

PCR products from several positive samples were sent to Macrogen Inc. (Korea) for bidirectional sequencing using both forward and reverse primers. The *18S rRNA* gene was amplified using broad-range primers as described by Nehra *et al*. (16). Raw sequencing data were quality-checked, aligned, and assembled into consensus sequences using BioEdit software (v7.0.5.3). These consensus sequences were subsequently compared with the reference sequences from the GenBank database for identification.

Phylogenetic analysis

The obtained *18S rRNA* gene sequences were analyzed using the NCBI's BLAST tool (http://www.ncbi.nlm.nih.gov) to determine sequence similarity with known *Theileria* species. The MEGA version 11 software was used to construct phylogenetic trees with the Maximum Composite Likelihood algorithm, which is an algorithm that is built on distance matrices. A bootstrap test with 1,000 replicates was employed to evaluate the strength of phylogenetic grouping (19). The overall data of 10 isolates of *T. annulata* were put in the analysis to form the phylogenetic tree.

Statistical Analysis

To assess differences in prevalence rates among the studied groups, chi-square and Fisher's exact tests were applied. Furthermore, binomial logistic regression was performed using GenStat, 12th

Edition, to estimate the odds ratios along with their corresponding 95% confidence intervals. A p-value of less than 0.05 was regarded as the threshold for statistical significance (20).

Results

Theileria spp. identification was done by microscopic examination using a Giemsa-stained blood smear. Morphological polymorphism was intense in intra-erythrocytic stages that were oval, anaplasmosis, circular, and single, pear-shaped merozoite. This morphological hallmark was the most common (Figure 1), with the shape being the double pear. Cruciform (Maltese cross) schizonts were also sometimes reported, which is further cytological evidence of species-level identification.

Table 2 shows a significant difference in the detection of tropical theileriosis in dairy cattle when comparing microscopy and nested PCR. Using microscopic examination, 27 out of 212 samples were positive, giving a prevalence of 12.7%. In contrast, nested PCR detected 46 positive cases, corresponding to a higher prevalence of 21.7%. The difference between the two diagnostic methods was statistically significant ($P \leq 0.0001$), indicating that nested PCR is significantly more sensitive than microscopy for detecting *Theileria annulata* infections in dairy cattle.

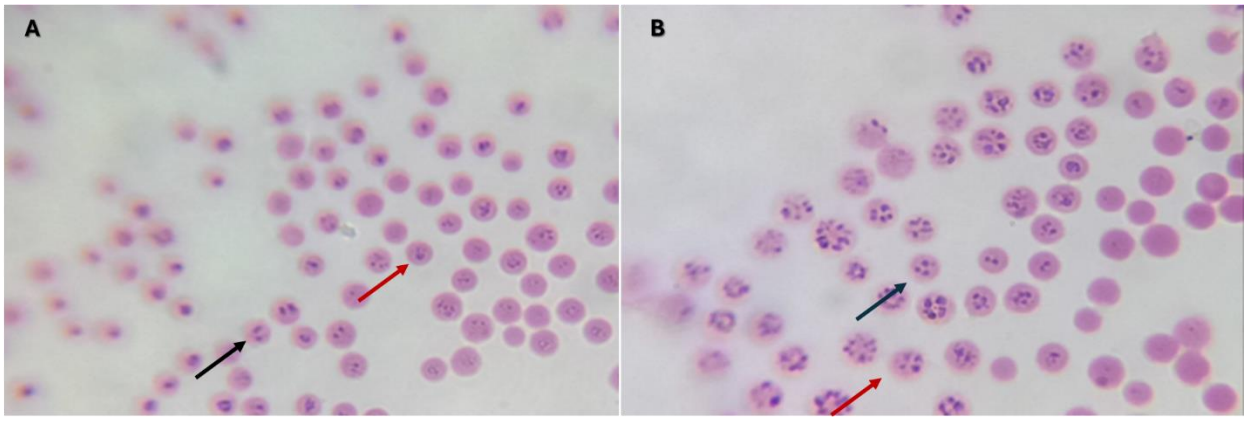

Figure 1. Giemsa-stained erythrocytes displaying intraerythrocytic morphological forms of *Theileria* spp.; (A) Single pear-shaped merozoite (red arrowhead) and paired pear-shaped merozoites (black arrowhead); (B) Maltese cross–shaped arrangement of merozoites (red arrowhead) and three rounded merozoites (black arrowhead), as visualized under a light microscope at 100× magnification.

Table 2: Comparison of Tropical Theileriosis*:* Prevalence in Dairy Cattle Using Microscopy and nested PCR

Status	Total Sample Screened	No. Positive Sample	Percentage	P- value
Microscopy	212	27	12.7%	<0.0001
nested-PCR		46	21.7%	

Risk factor analysis identified age and body condition score (BCS) as significant predictors of *Theileria annulata* infection (Table 3). Cattle over 5 years showed the highest prevalence by c-PCR (29.6%), significantly higher than calves under 1 year (10%, 3/30; OR=3.79, 95% CI: 1.05–13.70). Sex and breed were not significantly associated with infection, although females and crossbreeds had slightly higher rates. Poor BCS was strongly associated with infection by microscopy (43.9% vs 5.3%; P≤0.0001; OR=14.0) and showed a non-significant trend with c-PCR (31.7% vs 19.3%; p=0.083). These results highlight age and nutritional status as key risk factors in *T. annulata* infection.

Table 3: Analysis of Tropical Theileriosis associated risk factor in Dairy Cattle

Factor	No. of examined cattle	Microscopic examination			c-PCR		
		N. (%)	OR (95%CI)	*p*	N. (%)	OR (95%CI)	*p*
Age group							
>1	30	2 (6.7)	1	0.174	3 (10)	1	0.032
1--5	101	11 (11)	1.72 (0.37–8.00)		19 (18.8)	2.09 (0.57–7.60)	
<5	81	14 (17.3)	2.91 (0.62–13.68)		24 (29.6)	3.79 (1.05–13.70)	
Sex							
Male	41	2 (4.8)	1	0.112	6 (14.6)	1	0.196
Female	171	25 (14.6)	3.34 (0.76–14.72)		41 (23.9)	1.84 (0.72–4.68)	
Breeds							
Indigenous	98	11 (11.2)	1	0.538	21 (21.4)	1	
Crossbreed	114	16 (14.1)	1.29 (0.57–2.91)		25 (21.9)	1.03 (0.53–1.98)	0.93
B.C.S.							
Good	171	9 (5.3)	1	<0.0001	33 (19.3)	1	0.083
Poor	41	18 (43.9)	14.0 (5.66–34.60)		13 (31.7)	1.94 (0.91–4.15)	
Total	212	27 (12.7)			46 (21.7)		

BCS: Body score condition, N: Number of positive samples, CI: Confidence interval, OR: Odds ratio

Management-related factors significantly influenced the prevalence of tropical theileriosis (Table 4). The prevalence was significantly higher in cattle kept mixed with other animals (29.3%, OR = 1.79, P ≤ 0.05) compared to those kept only with cattle (18.8%). Grazing cattle had a markedly higher prevalence than barn-kept animals (35.2% vs. 14.9%, OR = 3.12, P ≤ 0.003). Acaricide application was strongly associated with infection: cattle receiving no acaricides had the highest prevalence (36.9%, OR = 5.74, P < 0.001), followed by those with irregular application (29.4%, OR = 4.10), whereas regular application maintained a low prevalence (9.2%).

Table 4: Analysis of Tropical Theileriosis in Dairy Cattle: Prevalence Based on Management Strategy Scores via c-PCR

Factor	No. of examined cattle	Microscopic examination			c-PCR		
		N. (%)	OR (95%CI)	*p*	N. (%)	OR (95%CI)	*p*
Animals in a stable							
Only cattle	154	14 (9.1)	1	0.01	29 (18.8)	1	<0.05
Mixed with others	58	13 (22.4)	2.87 (1.28–6.45)		17 (29.3)	1.79 (0.90–3.57)	
Management							
In barn	141	15 (10.6)	1	0.196	21 (14.9)	1	0.003
In grazing	71	12 (16.9)	1.71 (0.75–3.88)		25 (35.2)	3.12 (1.60–6.07)	
Tick infestation							
Absent	133	13 (9.8)	1	0.097	27 (20.3)	1	0.48
Present	79	14 (17.7)	1.99 (0.87–4.54)		19 (24)	1.24 (0.64–2.40)	
Acaricides application							
No	46	6 (13.1)	3.55 (0.95–13.2)	0.081	17 (36.9)	5.74 (2.29–14.4)	<0.001
Regular	98	4 (4.1)	1		9 (9.2)	1	
Irregular	68	17 (25)	7.83 (2.48–24.7)		20 (29.4)	4.10 (1.74–9.67)	
Floor							
Cemented	115	11 (9.6)	1	0.2	22 (19.1)	1	0.32
Non cemented	97	16 (16.5)	1.86 (0.81–4.29)		24 (24.7)	1.39 (0.73–2.63)	
Total	212	27 (12.7)			46 (21.7)		

N: Number of positive samples, CI: Confidence interval, OR: Odds ratio

The nPCR results using universal primers (Macrogen Inc., South Korea) revealed a DNA band of 778 bp in the first reaction, confirming the presence of *Theileria* spp. (Figure 2A). The second reaction, using *T. annulata*-specific primers, produced a 581 bp band, indicating specific detection of *T. annulata* (Figure 2B)

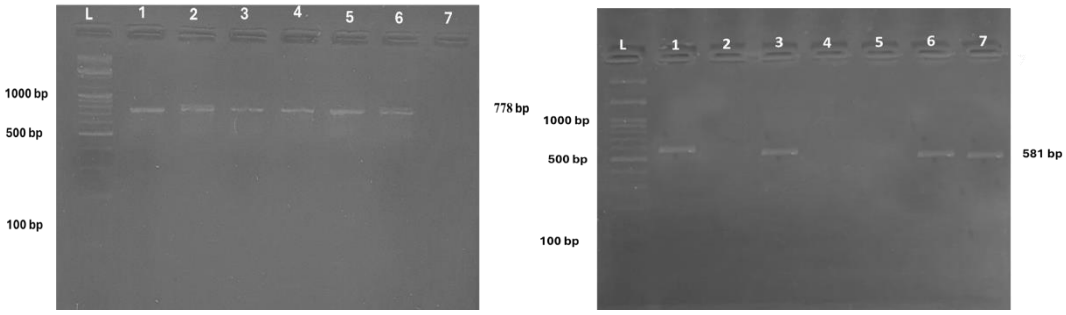

Figure 2: Agarose gel electrophoresis (1.5% agarose) of PCR products targeting *Theileria*. Lane L contains the 100 bp DNA ladder. (A) Lanes 1–6 show positive results for *Theileria* spp., with bands appearing at approximately 778 bp. (B) Lanes 1, 3, 6, and 7 show positive results for *T. annulata*, with bands at approximately 581 bp.

This study analyzed the phylogenetic relationships of *T. annulata* isolates obtained from five districts in Duhok Governorate, as previously reported by Farhad (2023).
All sequences that had been obtained during this investigation were submitted to the GenBank database under the accession numbers OP435250-OP435259. The phylogenetic analysis conducted on the *18S rRNA* genes showed that the Duhok isolates were tightly clustered in one clade (Fig. 1), which shows a high level of genetic similarity. This clustering notwithstanding, some of the Duhok isolates clustered with reference strains of geographically diverse regions, such as Iraq, Iran, Turkey, and Egypt, and had 99-100% sequence identity. Interestingly, an isolate of one of the local breeds had 100% identity with an isolate of Iran (KF429800.1) and 99% identity with an isolate of Turkey, indicating perhaps a regional spread or common ancestry (Fig. 3).

Discussion

Theileria annulata is the causative agent of bovine tropical theileriosis, a disease that poses significant economic constraints due to its considerable impact on dairy production, especially in tropical and subtropical areas that are endemic (21, 22). This study not only determined the prevalence of the infection but also explained the epidemiological risk factors that relate to *T. annulata* infection in dairy cattle flocks of the Duhok Governorate, Iraq. The prevalence as found in diagnostic assessment was 12.7% through microscopic examination and 21.7% through PCR, which is consistent with the findings in Ahmed *et al*. (23) of Sulaimani City that reported 27.5 and 31.25 as the prevalence rate through microscopy and PCR, respectively. Identified inter-study variation in prevalence is probably multifactorial, due to variations in geographic locale, animal movement patterns, acaricidal application regimes, diagnostic sensitivity, antiparasitic treatment protocols, husbandry practices, sampling chronology, land utilization, ecology of vector distribution, and cattle access to tick-infested environments (9, 24, 25).

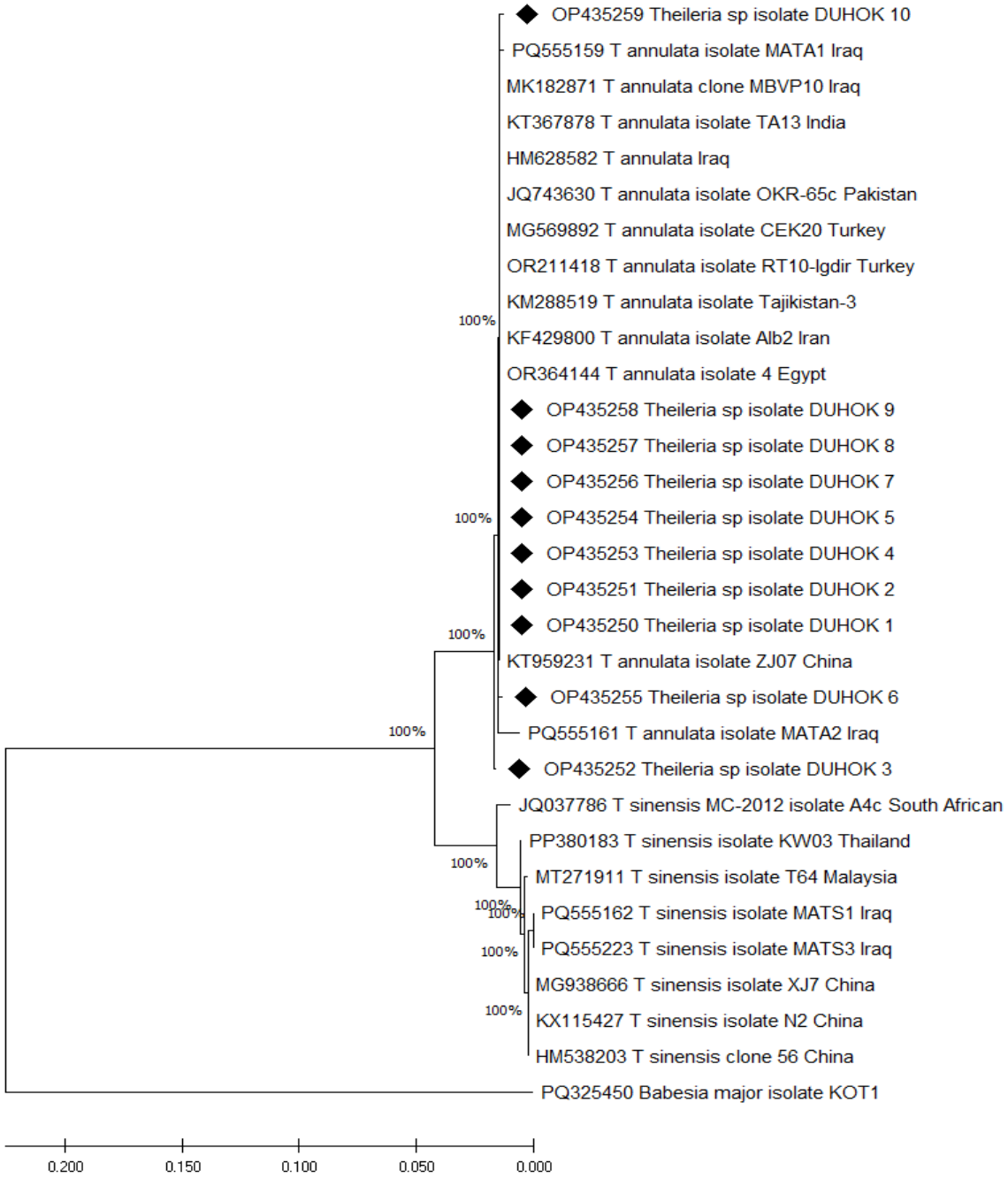

Figure 3: Phylogenetic tree of *Theileria* spp. sequences obtained in this study alongside reference sequences from various countries retrieved from the NCBI database. The black diamond marks the sequences generated in this study. *Babesia major* was used as the outgroup. Numbers at the nodes represent bootstrap values.

It was found that there are significant epidemiological correlations between *Theileria* infection and a number of host and management factors, such as age, sex, breed, frequency of acaricide application, status of tick infestations, floor types, and commingling activities (25, 26). There was a statistically significant correlation with age ($P \leq 0.032$), in which age groups of bovines of 1-5 years and above showed significantly higher rates of infection than the younger groups. Such an age-related susceptibility gradient could be due to a low exposure to vectors in calves because the two main vectors, *Rhipicephalus* (*Boophilus*) *annulatus* and *Hyalomma anatolicum*, are mostly active in pasturelands occupied by older cattle (8, 27). Moreover, the cattle with more than 1 year of experience are characterized by rapid somatic growth, lactation and gestation cycle initiation, longer grazing periods in pastures with vectors, and selective management stressors, and a combination of these factors increases exposure and immunological susceptibility to vectors (22, 28, 29). Although the prevalence was statistically higher in female cattle, the difference in genders was not significant. The trend might be caused by periparturient endocrine alterations, temporary immunosuppression in gestation/lactation, and, possibly, increased ectoparasite loads (29, 30). The breed did not play a major role, but indigenous breeds always have a lesser prevalence compared to crossbreds and exotic genotypes. The stability of long-term enzootic, tick resistance, and regulated macrophage cytokine responses on acute-phase proteins is postulated to play a role in this resilience (31). Another way improved local environmental acclimatization can increase resistance to immunocompromising stressors is enhanced resistance to immunomodulators. The presence of common grazing resources probably contributes to the spread of the ticks and the risk of diseases (25, 32, 33). Animals that were in poor body condition had a greater infection prevalence, implying that being immunocompromised makes them vulnerable to *Theileria* infection (22, 30).

Dairy cattle kept in grazing systems were found to be considerably higher compared to their counterparts in stall feeding systems, which is consistent with other studies that noted that free-range behavior exposed cattle to a higher probability of contact with infected vectors and infected reservoir hosts, and confinement lowered exposure (9, 25, 32). On the same note, the prevalence was higher, but statistically not significant, among cattle that were co-mingling with other animals and cattle that showed tick infestations. The geographical persistence of *Theileria* is inherently correlated with the high competence of the vectors. Importantly, inconsistent or a lack of acaricidal treatment correlated with a higher rate of incidence ($P \leq 0.001$), and the critical role of using ticks as the sole vectors of piroplasmosis and confirming the consistent use of acaricides as a core herd control measure (9, 25). There was also a non-significantly higher prevalence in animals on non-cemented flooring substrates. This tendency can probably be explained by the impact of compromised drainage and sanitation, in which surface defects may retain the developmental stages of parasites and allow the survival of tick eggs, which increases the risk of transmission (29, 31). The lack of meaningful difference between cattle having or not having concurrent tick infestations has possibly been brought about by previous exposure history, because the incubation period of theileriosis is variable or by recently applied acaricides before sampling.

Phylogeny analysis revealed that all the isolates used in this study were too close and indicated a possibility of cross-transmission between the local and imported feedlot bulls. *Theileria* and other parasites may infect a veterinary environment in several different ways, mostly through ticks, which are biological vectors. Ruminants, even recovering ones, can have some *Theileria* species for as long as months or even years.

Transplacental transmission has also been demonstrated in a number of *Theileria* species, such as *T. annulata*, and the prevalence of vertical transmission depends on whether the mother is a carrier or acutely infected. Research has shown that *T. annulata* is more effective in the process of its transmission when infected hosts and the appropriate tick vectors are in high occurrence. Naturally, the tick *Hyalomma anatolicum* contributes to the spread of *T. annulata* among cattle in the nearby areas, and the parasite was already confirmed on both carriers and cattle.

This high level of genetic similarity in the *T. annulata* isolates of Duhok and other neighboring countries, coupled with the high sensitivity of detection through PCR, reveals the imperative of concerted regional surveillance and control measures to curb the spread of the disease to other regions. The presence of sequence data uploaded to GenBank (accession numbers OP435252-OP435259) also allows using the source as a successful point of reference in a future comparative analysis in order to gain a deeper insight into the genetic diversity of the parasite, its evolutionary processes, and its possible pathogenicity.

This research work has some significant implications regarding the genetic diversity of *T. annulata* in Duhok and the significance of the above molecular diagnostic techniques in identifying the parasite. In addition, the phylogeny of the Duhok isolates with the neighboring areas highlights the significance of cross-boundary cooperation in the surveillance and control of the *T. annulata* infection, especially in the regions involved in the active livestock movement and the presence of vectors.

Conclusion

This study demonstrates that bovine tropical theileriosis (*Theileria annulata*) is prevalent among dairy cattle in Duhok Governorate and is significantly associated with host-related factors, particularly older age (>5 years), poor body condition, and specific management practices, including outdoor grazing and irregular acaricide application. Phylogenetic analysis showed a high degree of genetic similarity between local and imported isolates, indicating the circulation of closely related *T. annulata* strains within the region and genetic clustering with isolates from neighboring countries. These findings highlight the importance of strengthening surveillance programs and improving farm-level management and tick control strategies to reduce the impact of this economically important transboundary parasite.

Acknowledgments

The authors are grateful to all staff of the Duhok Research Center at the College of Veterinary Medicine.

Ethical approval

The study's methodologies and procedures were all authorized and carried out according to the rules set out by the University of Duhok's College of Veterinary Medicine's Scientific Ethical Committee on Animal Experimentation (CVM2025/1004UOD).

Conflicts of interest

The author declares that there is no conflict of interest.

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التوصيف الجزيئي لداء الثيليريا الاستوائي بين أبقار الألبان المحلية والمستوردة في محافظة دهوك - إقليم كردستان / العراق

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الخلاصة

تمت دراسة داء الثايليريا الاستوائي في الأبقار ، الذي تسببه طفيلية *Theileria annulata*، في قطعان الأبقار الحلوب في محافظة دهوك، إقليم كردستان، العراق. أجريت دراسة مقطعية على 212 رأسًا من الأبقار، تمت فيها مقارنة التشخيص باستخدام صبغة جيمسا المجهرية مع تفاعل البلمرة المتسلسل المتداخل (nPCR) ، كما جرى تحليل عوامل الخطورة الوبائية والعلاقات الوراثية التطورية. بلغت نسبة انتشار الإصابة 12.7% باستخدام الفحص المجهري مقابل 21.7% باستخدام c-PCR (P ≤0.032). تضمنت العلامات السريرية النموذجية في الحيوانات الإيجابية بالـ PCR الحمى، الاكتئاب، واليرقان. ارتفعت نسبة الانتشار بشكل معنوي مع التقدم بالعمر(P ≤0.032) ، حيث إن الأبقار الأكبر من 5 سنوات كانت أكثر عرضة للإصابة بمعدل يقارب أربعة أضعاف مقارنة بالعجول. لم يكن للجنس والسلالة تأثير معنوي، بينما كان سوء حالة الجسم عاملاً محددًا رئيسيًا (P≤0.0001).كما أن العوامل الإدارية، خصوصًا الرعي الخارجي والاستخدام غير المنتظم لمبيدات القراد، زادت من خطر الإصابة .(P≤0.0001) أظهر التحليل الوراثي لعشر سلاسل محلية من جين 18S rRNA برموز GenBank: OP435250-OP435259) أن جميع عزلات دهوك تنتمي إلى سلالة واحدة، إلا أن عددًا منها تجمّع بشكل وثيق (بنسبة تطابق 99-100%) مع سلالات من دول مجاورة؛ إذ كانت إحدى العزلات مطابقة تمامًا لسلالة إيرانية. تشير هذه النتائج إلى وجود استمرارية جينية إقليمية واحتمالية انتقال عبر الحدود. في الختام، يشكل *T. annulata* عبئًا وبائيًا ملحوظًا في أبقار الحليب في دهوك. إن الحساسية العالية لتقنيةc-PCR ، والتأثير القوي لكل من العمر، وحالة الجسم، والممارسات الإدارية، إلى جانب الأدلة على تداول السلالات إقليميًا، تؤكد الحاجة إلى تعزيز المراقبة الجزيئية، وتنفيذ برامج منتظمة لمكافحة القراد، واعتماد استراتيجيات سيطرة مستهدفة قائمة على تقييم عوامل الخطورة لحماية صحة القطيع وإنتاجيته.

الكلمات المفتاحية: *Theileria annulata*، التوصيف الجزيئي، التحليل التطوري.