Science Journal of University of Zakho
DOI: https://doi.org/10.25271/sjuoz

PREVALENCE OF *Theileria annulata* AND THE FIRST REPORT OF *Theileria sinensis* IN CATTLE FROM ERBIL PROVINCE, IRAQ

Musa Adil Wahab[1], and Khalid Jabar Aziz[2, *]

[1]Department of Animal Resources, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, Kurdistan Region, Iraq.
[2]Department of Basic Sciences, College of Veterinary Medicine, Salahaddin University-Erbil, Kurdistan Region, Iraq.

[*]Corresponding author email: khalid.aziz1@su.edu.krd (Tel.: +9647504524256)

Received: 02, Jul, 2025
Accepted: 01, Aug, 2025
Published: 10, Jan,2026

ABSTRACT

Bovine theileriosis is a significant tick-borne protozoan disease of cattle, primarily caused by species of the genus *Theileria*. This study investigated the prevalence of bovine theileriosis in cattle using both microscopic examination and conventional PCR (c-PCR). Microscopic analysis of Giemsa-stained blood smears identified an infection rate of 51/236 (21.6%), while c-PCR revealed a significantly higher prevalence of 106/236 (44.9%). *Theileria* species were characterized morphologically and confirmed via molecular methods. PCR analysis identified *Theileria annulata* and *Theileria sinensis* as the causative agents, with *T. annulata* showing the highest rate of single infections 89/106 (84%) compared to *T. sinensis* 17/106 (16%). Mixed infections were detected in 13/106 (12.3%) of cases. Age, sex, breed, housing conditions, acaricide use, and tick infestation were identified as key risk factors influencing infection rates. Management and tick-related factors significantly influenced *Theileria* prevalence, with higher infection rates observed in cattle with tick infestation 54/102 (52.9%), irregular or no acaricide use 58/115 (50.4%) and 27/47 (58.7%) respectively, non-cemented floors 72/125 (57.6%), and tick presence in barns 64/127 (50.4%). Phylogenetic analysis demonstrated genetic similarities between the identified *T. annulata* and *T. sinensis* sequences and strains reported from other geographical regions. Notably, this study provides the first report of *T. sinensis* in Iraq, highlighting its emergence and underscoring the need for enhanced surveillance and control measures against *Theileria* infections in the country. The high prevalence of the disease underscores the urgent need for enhanced surveillance, effective tick control measures, and the implementation of integrated strategies to mitigate the impact of bovine theileriosis on cattle health and productivity.

KEYWORDS: Molecular; Epidemiology, *Theileria annulata*; *Theileria sinensis*; Cattle.

1. INTRODUCTION

Bovine theileriosis is a tick-borne haemoprotozoan disease of cattle, classified under the kingdom Protista. It is transmitted by hard ticks of the genera *Hyalomma, Rhipicephalus,* and *Haemaphysalis* and leads to major economic losses in the cattle industry. The tropics and subtropical regions are where the disease is most prevalent (Ismael & Omer, 2021; Larcombe *et al.*, 2022; Shahedi *et al.*, 2022).

Bovine theileriosis is globally distributed, and it poses a serious risk to livestock production. *Theileria annulata*, *T. orientalis*, *T. velifera*, *T. sergenti*, *T. mutans,* and *T. sinensis* are major species, the latter of which is very closely related phylogenetically to *T. orientalis*, and has recently been discovered as a causative agent of bovine theileriosis in China and Malaysia (Kamani *et al.*, 2023; Liu *et al.*, 2010; Qin *et al.*, 2016). The host's immune status, particularly its acquired immunity, along with the *Theileria* species, determined the virulence and impact of infections. *Theileria orientalis* and *Theileria sinensis* have been reported to be associated with non-symptomatic or benign infections (Liu *et al.*, 2010; McFadden *et al.*, 2011). While varying degrees of pathogenicity and eliciting serious clinical signs in cattle, such as anemia, and jaundice, are found in *T. orientalis* genotypes such as Ikeda, Chitose, and Buffeli infections (Oakes *et al.*, 2019; Schnittger *et al.*, 2022; Lakew *et al.*, 2023). Highly pathogenic tropical theileriosis was recorded in *T. annulata* infection (McFadden *et al.*, 2011; Ota *et al.*, 2009). The availability of susceptible hosts and environmental parasite vectors is factored as an important factor in the prevalence, intensity, and transmission of theileriosis. Chronic carriers typically serve as reservoirs for the disease. Additionally, the prevalence of *Theileria*

Access this article online
https://doi.org/10.25271/sjuoz.2026.14.1.1665
Printed ISSN 2663-628X;
Electronic ISSN 2663-6298
Science Journal of University of Zakho
Vol. 14, No. 01, pp. 60 –69, January-2026

infections is also found to be determined by biological factors like sex, breed, management systems, habitat type, and tick infestation (Riaz & Tasawar, 2017; Larcombe *et al.*, 2022).

In warm or temperate climates, ixodid ticks serve as vectors for the transmission of *Theileria* species by injecting the pathogen into bovine blood (Payne *et al.*, 2009; Zangana and Naqid, 2011). *Hyalomma anatolicum,* the three-host tick, plays a significant role in *Theileria* spp. since it possesses the capability of transstadial transmission. Likewise, *H. dentritum*, *H. marginatum*, *H. asiaticum,* and *Rhipicephalus turanicus* are crucial vectors for *Theileria* spp. Due to their transstadial transmission capability (Zangana *et al*., 2013). Apart from the cattle breed and the pathogen, additional determinants of risk, such as intensity of tick infestation and age of animal, influence the severity of the illness (Al-Saeed & AL-Badrani, 2014; Li *et al.*, 2020; Gharbi *et al.*, 2020; Kozhabaev *et al.*, 2023; Norouzi *et al.*, 2023).

The most common symptoms of tropical theileriosis include fever, extensive ocular and nasal discharge, hyper-salivation, swelling of superficial lymph nodes, respiratory distress, icterus, and, in severe cases, death due to asphyxiation (Omer *et al.*, 2003). The most common lesions in infected cattle are pulmonary emphysema, lymph node enlargement, splenomegaly, and subcutaneous and intramuscular hemorrhages. Hepatomegaly is also extremely common, followed by pleural and pericardial fluid excessive accumulation (Ma *et al.*, 2020).

Traditional diagnosis of theileriosis is based on microscopy and clinical presentation, wherein lymph node smears are stained to see the schizont stage and blood smears are stained to see piroplasm's (Aulakh & Singla, 2006). Parasites cannot be seen by these traditional methods during subclinical and chronic phases of disease due to extremely low parasitemia (Tuli *et al.*, 2015). Thus, genetic and serological tests have been considered to be more sensitive methods for diagnosis of tropical theileriosis (TP) (Aktas *et al.*, 2006; Liu *et al.*, 2008; Tuli *et al.*, 2015).

Given the limited data on the prevalence of bovine theileriosis in Erbil Province, the present study aims to investigate the presence of *Theileria* species in cattle using both microscopic and molecular diagnostic techniques. Additionally, the study seeks to identify potential risk factors and management practices associated with the prevalence of disease, and to characterize the molecular epidemiology of *Theileria* in the region.

2. MATERIAL AND METHODS

Sample Collection and Study Design:

This cross-sectional study was conducted in Erbil city, north Iraq, between June and October 2024. In total, 236 (97 from males and 139 from females) blood samples were collected randomly from the coccygeal vein of cattle. Clinical signs such as raised body temperature, weakness, lymph node swelling, rapid respiration, and nasal discharge were observed at the time of sampling. Diagnosis was achieved by clinical examination and laboratory confirmation through Giemsa staining of blood smears. Besides sample collection, a previously designed questionnaire was used to acquire information on the farms and animals, including location, sex, age, breed, and risk factors such as acaricide use, floor types, tick presence in barns, and tick infestation. Multivariable analysis was performed to assess the association between these risk factors and *Theileria* infection rates.

Examination of Blood Stain:

Aseptically, blood samples were collected from the coccygeal vein of every animal and placed in EDTA tubes. Then, thin and thick blood smears were prepared, air-dried, fixed with 70% methanol for 5 minutes, and stained with 10% of Giemsa stain for 30–40 minutes. The smears were then examined using a microscope at 100X oil immersion magnification to identify and examine the morphological characteristics of *Theileria* species and the estimation of parasitemia according to the protocol described by Zajac *et al.* (2021). The remaining blood samples were stored at −20°C for molecular work.

DNA Extraction and PCR Amplification:

DNA was extracted from the blood samples using a commercial DNA isolation kit (AddBio, South Korea), as indicated by the manufacturer. Genomic DNA concentration (ng/µL) was measured using a NanoDrop spectrophotometer (IMPLEN, Germany). The extracted genomic DNA was stored at -20°C until polymerase chain reaction (PCR) analysis. The *Theileria annulata* merozoite surface antigen 1 (Tams1) and 18S rRNA genes were amplified by species-specific PCR using primers specific to *T. annulata* and *T. sinensis,* respectively (Table 1). PCR amplification was performed with a gradient thermal cycler (BioRad, Canada) as described by (Albakri *et al.*, 2024). The cycling conditions for *Theileria* spp., *T. annulata* and *T. sinensis* are begin with DNA denaturation at 95ºC for five minutes. Following this, DNA from all species was denatured for one minute at 94ºC. Next, the primer annealing occurs at 55ºC for 45 seconds for *Theileria* spp., 60ºC for *T. annulata*, and 56ºC for 1 minute for *T. sinensis*. The extension step was performed for one minute at 72ºC for all species. To amplify the DNA, steps 3 through 5 are repeated 35 times. Finally, a 7-minute extension was carried out at 72ºC, after which the samples were stored at 4ºC until removed. After amplification, the PCR products were run on a 1.5% agarose gel and visualized using a gel documentation system.

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665

Table 1: Primers used to amplify the 18S rRNA and Tams genes of *Thieleria* spp.

Amplified gene	Sequences 5'-3'	Binding Temperature	Product size (*bp*)	References
989F	5'- AGTTTCTGACCTATCAG -3'	55ºC.	1098	d'Oliveira *et al*., 1995
990R	5'- TTGCCTTAAACTTCCTTG -3'			
Tams1F	5'- ATGCTGCAAATGAGGAT -3'	60ºC	785	Kirvar *et al*., 2000
Tspms1R	5'- GGACTGATGAGAAGACGATGAG -3'			
Tsin-F	5'- CACTGCTATGTTGTCCAAGAGATATT -3'	56ºC	887	Liu *et al*., 2010
Tsin-R	5'- AATGCGCCTAAAGATAGTAGAAAAC -3'			

989F: *Theileria* spp. Universal forwards primers, 990R: *Theileria* spp. Universal reverse primers, Tams1F: *T. annulata* - targeted forward primer, Tspms1R: *T. annulata* - targeted reverse primer, Tsin-F: *T. sinensis* - targeted forward primer, Tsin-R: *T. sinensis* - targeted reverse primer.

DNA Sequencing and phylogenetic Analysis:

In the present study, three sets of PCR primers targeting universal *Theileria* spp., *T. annulata*, and *T. sinensis* that yielded positive amplification via conventional PCR were submitted to a commercial sequencing facility (Macrogen Inc., South Korea) for purification and Sanger dideoxy sequencing. The resulting sequences were compared against existing entries in the GenBank database using the BLAST algorithm. Multiple sequence alignments were conducted with MEGA X software (http://www.megasoftware.net, July 2016), employing the MUSCLE algorithm to align sequences alongside reference entries from NCBI GenBank (Edgar, 2004). A phylogenetic tree was subsequently constructed using the Neighbor-Joining method, and the robustness of the branching patterns was assessed through 1,000 bootstrap replicates (Tamura *et al.*, 2021).

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 (Payne *et al.*, 2009).

3. RESULTS

Giemsa-stained blood smears were employed to determine the prevalence of bovine theileriosis, revealing an infection rate of 21.6% (51/236). *Theileria* sp, were identified based on the physical characteristics of merozoites observed in infected red blood cells (RBCs). The parasites predominantly appeared as small, double pyriform structures with acute or obtuse angles. Additionally, as illustrated in Figure 1, they exhibited diverse morphological forms, including oval, spherical, and the distinctive double pear shape. The infection rate was 21.6% (51/236) by microscopic examination and 44.9% (106/236) by molecular analysis, as summarized in Table 2.

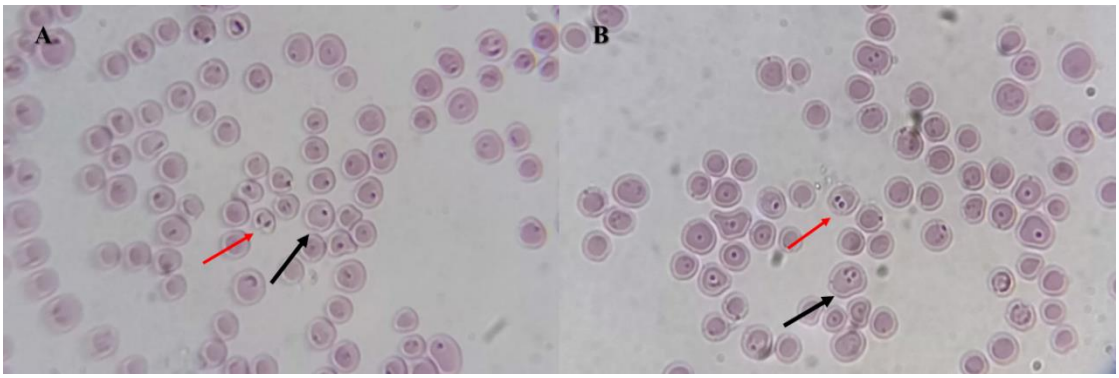

Figure 1: Morphological forms of *Theileria* spp. Within infected erythrocytes stained with Giemsa, examined under oil immersion lens (100X); A) Two pear-shaped merozoites (red arrowhead), One round-shaped merozoite (black arrowhead); B) Two round-shaped merozoites (red arrowhead), three round -shaped merozoites (black arrowhead).

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665

Table (2): Prevalence rates of *Theileria* spp. in cattle by microscopic and c-PCR examination.

Status	Total Tested Sample	Total Positive Samples	%	*P* value
Microscopy	236	51	21.6	0.001
c-PCR		106	44.9	

The prevalence of *Theileria* species infection in cattle is presented in Table 3. Out of the total of 106 positive samples, 89 (84%) were found to be infected with *Theileria annulata*, while 17 (16%) were infected with *Theileria sinensis*. A mixed infection was observed in 13 (12.3%) cattle. Statistical analysis revealed a significant association between *T. annulata* infection and the total prevalence ($p<0.001$).

Table (3): Prevalence rates of *Theileria* sp. infection in Cattle

***Theileria* Species**	**Cattle**	
	No. (%)	***p-value**
Single infection		
T. annulata	89 (84)	0.001
T. sinensis	17 (16)	
Mixed infection	13 (12.3)	
Total	106	

* p-value is determined using (p-value ≤ 0.05)

Multivariable Analysis of Molecular Prevalence of *Theileria* spp:

Table 4 revealed the prevalence of *Theileria* spp. Based on sex, age, breed, and the purpose of keeping cattle. In the current research, the prevalence rates of *Theileria* spp. in female cattle was higher (49.6%) compared to males (38.1%). The odds of infection were 1.6 times higher in females than in males (CI: 0.91–2.81), with a significant difference ($p<0.05$). Cattle aged 2–4 years had a prevalence rate of 53.8%, with odds of infection 1.8 times greater than cattle of other age groups (CI: 1.02–3.17), showing a significant difference ($p<0.001$). In contrast, cattle older than 4 years had the lowest prevalence (23.2%), while those under 2 years of age showed a prevalence of 49.4%. Imported breeds exhibited a higher prevalence of infection (49.2%) compared to local breeds (28.6%). The odds of infection were 2.42 times higher in imported breeds compared to local breeds (CI: 0.19–0.85), with a significant difference ($p<0.007$). However, no significant differences in the prevalence of *Theileria* spp. were observed between cattle kept for dairy production (49.3%) and those raised for beef production (38.8%).

Table (4): Sex, age, breed and purpose of keeping score -wise prevalence of *Theileria* sp. by c-PCR.

Factor	**No. of examined cattle**	**c-PCR**			
		No. of positive sample	**Percentage (%)**	**OR (95%CI)**	***P value***
Gender					
Female	139	69	49.6	1.6 (0.91 to 2.81)	0.05
Male	97	37	38.1	0.63 (0.36 to 1.10)	
Age group					
<2	89	44	49.4	1.34 (0.76 to 2.35)	0.001
2-4	91	49	53.8	1.8 (1.02 to 3.17)	

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665

>4	56	13	23.2	0.28 (0.13 to 0.58)	
Breeds					
Local	49	14	28.6	0.41 (0.19 to 0.85)	0.007
Imported	187	92	49.2	2.42 (1.17 to 5.19)	
Purpose of keeping					
Dairy	138	68	49.3	1.53 (0.88 to 2.69)	0.071
Beef	98	38	38.8	0.65 (0.37 to 1.14)	
Total	236	106	44.9		

N=Number of positive samples, CI=Confidence interval, OR=Odd ratio

Association of Molecular Prevalence of *Theileria* spp with Management and Tick Vector Factors:

The current research showed that the prevalence of *Theileria* spp. was not statistically significant between cattle kept alone (45.2%) and those mixed with other animal species in stables (43.9%). Similarly, while grazing style did not show a statistically significant difference, cattle that grazed had a higher prevalence of infection (51.6%) compared to those kept in barns (42.4%), as presented in Table 5. Cattle with tick infestations had a prevalence rate of 52.9% and were 1.77 times more likely to be infected compared to those without tick infestations (CI: 1.02–3.09), with statistically significant differences ($p<0.021$). Additionally, cattle that did not receive acaricide treatment showed the highest prevalence (58.7%), with significantly increased odds of infection compared to those treated regularly (28%) or irregularly (50.4%) (CI: 0.99–4.08; $P<0.001$). Cattle housed on non-cemented floors had three times higher odds of infection compared to those housed on cemented floors (CI: 1.74–5.46), with significant differences ($P<0.001$). The prevalence was 57.6% in cattle housed on non-cemented floors, compared to 30.6% of those on cemented floors. Moreover, barns with ticks present exhibited a higher prevalence (50.4%) compared to barns without ticks (39.6%). The odds of infection were 1.65 times higher in barns with ticks (CI: 0.93–2.82; $p = 0.045$) (Table 5).

Table (5): Management and Tick Vector Factor Scores and Prevalence rates of *Theileria* spp. by c-PCR.

Factor	No. of examined cattle	c-PCR			
		No. of positive sample	Percentage (%)	OR (95%CI)	*p*
Animals in stable					
Only cattle	197	89	45.2	1.07 (0.51 to 2.28)	0.499
Mixed with other animals	39	17	43.9	0.94 (0.44 to 1.98)	
Management					
In grazing	64	33	51.6	1.44 (0.78 to 2.68)	0.135
In barn	172	73	42.4	0.69 (0.37 to 1.28)	
Tick infestation					
Present	102	54	52.9	1.77 (1.02 to 3.09)	0.021
Absent	134	52	38.8	0.56 (0.32 to 0.98)	
Acaricides application					

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665

No	46	27	58.7	2.0 (0.99 to 4.08)	
Regular	75	21	28	0.35 (0.18 to 0.65)	0.001
Irregular	115	58	50.4	1.55 (0.89 to 2.68)	
Floor					
Cemented	111	34	30.6	0.33 (0.18 to 0.58)	0.001
Non cemented	125	72	57.6	3.08 (1.74 to 5.46)	
Tick existence in barn					
Yes	127	64	50.4	1.65 (0.93 to 2.82)	0.045
No	109	42	39.6	0.62 (0.35 to 1.07)	
Total	236	106	44.9		

N=Number of positive samples, CI=Confidence interval, OR=Odd ratio

The results of the amplified (PCR) product using general or universal primers (Macrogen Inc., South Korea) revealed that the DNA band size for the initial reaction was 1098 bp, indicating a positive presence of *Theileria* spp. (Figure 2A). In contrast, the second reaction using specific primers for *T. annulata* displayed a DNA band size of 785 bp while specific primers for *T. sinensis* displayed a DNA band size of 887 bp (Figure 2C).

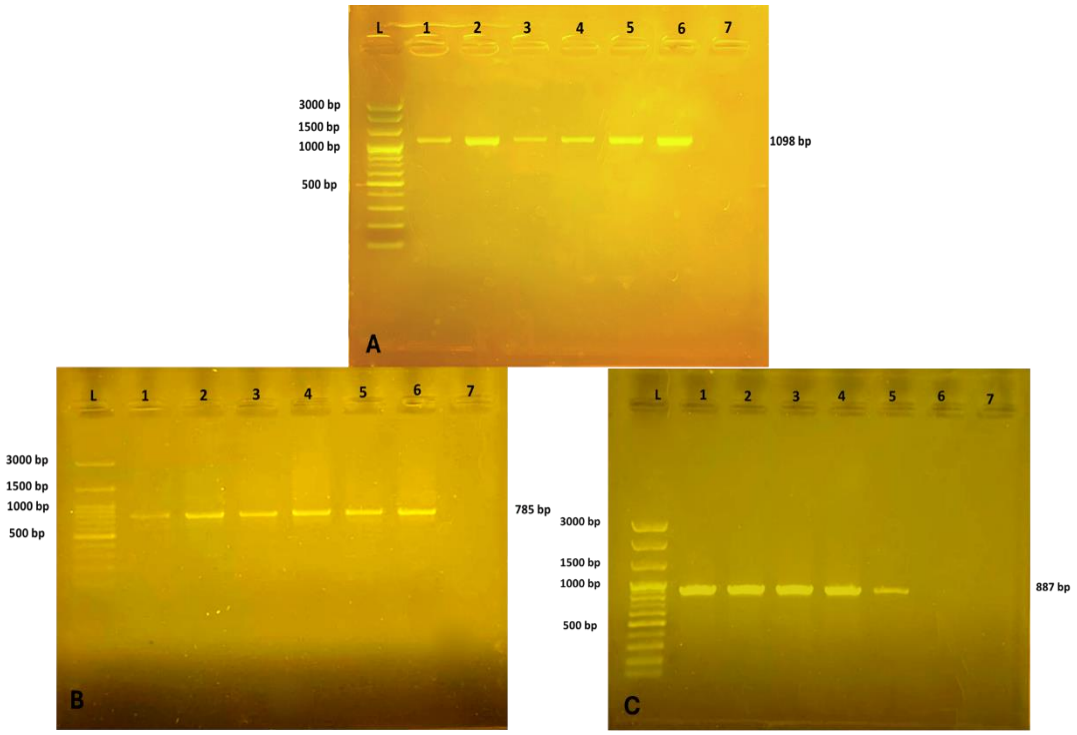

Figure 2: PCR gel electrophoresis results for *Theileria* detection. Lane M shows the 100 bp DNA ladder. (A) Lanes 1–6 show clear bands at approximately 1098 bp, indicating positive samples for *Theileria* spp.; lane 7 was used as a negative control. (B) Lanes 1–6 display bands at approximately 785 bp, confirming *T. annulata*; lane 7 was used as a negative control. (C) Lanes 1–5 show bands at approximately 887 bp, indicating positive results for *T. sinensis*; lane 7 was used as a negative control.

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665

The sequences of the DNA of the 18S rRNA gene have been made available at NCBI GenBank under the accession numbers: *T. annulata*: (PQ555159 and PQ555161); *T. sinensis*: (PQ555162 and PQ555223).

Phylogenetic trees of 18S rRNA sequence analyses based on a neighbor-joining program showed that *Theileria* spp. have a relation to *T. annulata* and *T. sinensis*. The two sequences blasted with previous GenBank registration and found that PQ555159 and PQ555161 showed 99.03% identity with HM628582/Iraq, MG569892/Turkey, KF429800/Iran, KT367878/ India, KT182871/China and OR364144/Egypt to those sequences previously reported *T. annulata* in NCBI GenBank (Figure 3).

A phylogenetic tree of *T. sinensis* showed that the two sequence isolates (PQ555162 and PQ555223) observed in the present study clustered with HM538203/China, PP380183/ Thailand, MT271911/Malaysia and JQ037786/South Africa (Figure 3).

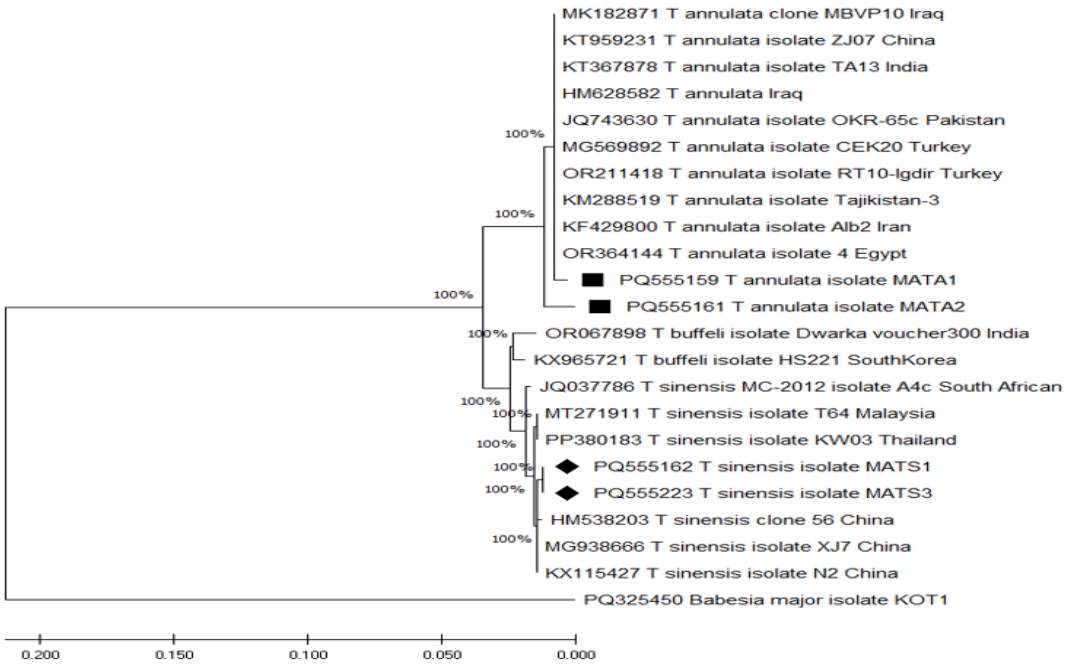

Figure 3: Phylogenetic tree showing *Thieleria* spp. some partial 18S sequences of the isolates. The sequences in this study are marked with a black square for *T. annulata* and black diamond for *T. sinensis*. The clade supports were indicated by the bootstrap values (from 1000 replicates) at each node. MEGA X software was employed to conduct the analysis and *Babesia major* (PQ325450) was the outgroup.

4. DISCUSSION

This study represents the first comprehensive investigation into the management practices and risk factors influencing bovine theileriosis in Erbil, Iraq. As one of the foremost tick-borne diseases, bovine theileriosis represents a serious threat to the health and productivity of cattle, especially in tropical and subtropical areas (Valente *et al.*, 2022). Study results showed a 21.6% prevalence based on examination of microscopically and 44.9% with PCR testing, indicating the advantage of sensitivity with molecular testing for sub-clinical infections. The findings are in line with earlier research, including that conducted by Aktas *et al.* (2006) in Turkey. PCR is more sensitive and specific than blood smear examination, allowing for the detection of low-level or subclinical Babesia infections that are often missed under microscopy due to low parasitemia or observer limitations (Almería *et al*. 2001).

The study found that several risk factors are associated with theileriosis prevalence. Age emerged as a significant determinant, with the highest prevalence of the disease observed in cattle aged 2–4 years. This could be due to greater exposure to tick infestation, exacerbated by physiological stressors of first lactation and reproductive cycles (Tuli *et al*., 2015). Younger calves were less at risk of infection as they had less exposure to pastures and tick infestation. These findings are analogous to those conducted by Bishop *et al.* (2008) that link grazing behavior to vector contact. Differences were also noted among the genders, where the prevalence of the disease was higher in females than males, even though the differences were not statistically significant. This

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665

increased susceptibility in females could be attributed to endocrine changes during pregnancy and lactation, along with prolonged physiological stress (Almería *et al.* (2001). More studies are needed to clarify the differences in the adaptive and innate immune response between genders in terms of *Theileria* infection.

Cattle breed was identified as the second risk factor in this study, which was more common in exotic and crossbred cattle than in native breeds. These differences could be due to the long-term adaptation of the local breed to local conditions, which provides an enzootic, stable habitat and tick resistance. These findings are in line with Zeb *et al.* (2020), who found that the local breed's resistance to disease stressors. The higher percentage of disease was among those with Tick infestation. Earlier studies, as shown by Aziz & Al-Barwary (2019)Highlighting the interaction between tick infestation and infection, it highlights the importance of effective tick control in controlling disease. Grazing practices also found to play a role in the prevalence of the disease; free-roaming cattle in tick-infested grazing fields had a higher risk of contracting the disease than cattle kept in barns, where exposure to tick vectors was decreased (Tuli *et al*., 2015).

Living conditions also proved a vital determinant, with cattle raised on non-cemented floors exhibiting far higher prevalence rates than those raised on cemented floors. Poor drainage and sanitation in non-cemented floors predispose the survival and proliferation of ticks, promoting infection. The same has been reported by Simuunza *et al.* (2011), crediting the contribution of environmental conditions towards the transmission of disease. Another of the critical factors that were determined was application of acaricides, with nonuniform or none acaricide application leading to significantly greater infection. This underlines the significance of frequent acaricide application as a pillar of disease avoidance, as also indicated by Selim *et al.* (2022). All these findings point towards the worth of integrated management measures in reducing disease prevalence.

Molecular characterization provided additional data on genetic diversity of *Thieleria* sp. in the region. Phylogenetic analysis revealed high genetic identity among T. annulata and T. sinensis strains from the region and neighboring countries such as Turkey, Iran, and China, suggesting regional patterns of transmission. The first detection of *T. sinensis* in Iraq adds valuable epidemiological information. The main strength of this study is its combined microscopic and molecular approach, enhancing diagnostic accuracy. However, its limitation is the restricted sampling to one governorate and a short time frame, which may not represent the entire country. Future studies across more regions and seasons are recommended.

CONCLUSION

The study emphasizes how host, environmental, and management factors interact to affect the epidemiology of bovine theileriosis. Age, breed, management status, tick infestation, and acaricide therapy were identified as significant risk factors for infection. The phylogenetic relatedness and genetic diversity of *Theileria* species necessitate integrated control programs. To minimize the effects of bovine theileriosis, a combination of vector control, better housing, extensive acaricide use, and focused observation is essential. Future research should focus on understanding host-pathogen interactions and developing sustainable countermeasures to address the multifaceted problems posed of this disease.

Acknowledgment:

We would like to gratefully acknowledge Salahaddin University-Erbil for their authorization and support throughout this study.

Author contribution

Both authors M. A. W., and K. J. A., contributed equally to the design of the research, the performance of experiments, data analysis, and the writing of the paper.

Ethics approval: Ethical Committee Number (CVM2024/1205UoD).

Conflict of Interest:

The authors declare no conflicts of interest

REFERENCES

Aktas, M., Altay, K., & Dumanli, N. (2006). A molecular survey of bovine *Theileria* parasites among apparently healthy cattle and with a note on the distribution of ticks in eastern Turkey. *Veterinary parasitology, 138*(3-4), 179-185. **https://doi.org/10.1016/j.vetpar.2006.01.052**

Saeed, A. T., & AL-Badrani, M. A. (2014). Study of the parasites of the local chickens (*Gallus gallus domisticus*) in Duhok province, Kurdistan Region-Iraq. *Science Journal of University of Zakho, 2*(1), 18-23. **https://doi.org/10.25271/2014.2.1.110**

Albakri, H. S., Suleiman, E. G., & Al-Taee, A. F. (2024). Molecular identification of *Theileria* species in cattle in Mosul city. Iraqi *Journal* of Veterinary Sciences, 38(1), 183-189. **https://doi.org/10.33899/ijvs.2023.141344.3112**

Almería, S., Castella, J., Ferrer, D., Ortuno, A., Estrada-Peña, A., & Gutierrez, J. (2001). Bovine piroplasms in Minorca (Balearic Islands, Spain): a comparison of PCR-based and light microscopy detection. *Veterinary parasitology, 99*(3), 249-259. **https://doi.org/10.1016/s0304-4017(01)00464-2**

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665

Aulakh, G., & Singla, L. (2006). Clinico-haematobiochemical observations on bovines naturally infected with *Theileria annulata*. *Journal of Veterinary Parasitology, 20*(1), 49-52. **https://doi.org/10.1501/vetfak_0000001234**

Aziz, K. J., & Al-Barwary, L. T. O. (2019). Epidemiological study of equine piroplasmosis (*Theileria equi* and *Babesia caballi*) by microscopic examination and competitive-ELISA in Erbil Province North-Iraq. *Iranian journal of parasitology, 14*(3), 404. **https://doi.org/10.18502/ijpa.v14i3.1479**

Bishop, R., Musoke, A., Skilton, R., Morzaria, S., Gardner, M., & Nene, V. (2008). *Theileria*: life cycle stages associated with the ixodid tick vector. In: Cambridge University Press. **https://doi.org/10.1017/cbo9780511551802.015**

d'Oliveira, C., Van Der Weide, M., Habela, M.A., Jacquiet, P. and Jongejan, F. (1995). Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. *Journal* of Clinical Microbiology, 33(10), 2665-2669. **https://doi.org/10.1128/jcm.33.10.2665-2669.1995**

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research, 32*(5), 1792-1797. **https://doi.org/10.1093/nar/gkh340**

Gharbi, M., Darghouth, M. A., Elati, K., AL-Hosary, A. A., Ayadi, O., Salih, D. A., . . . Hassan, S. M. (2020). Current status of tropical theileriosis in Northern Africa: A review of recent epidemiological investigations and implications for control. *Transboundary and emerging diseases, 67*, 8-25. **https://doi.org/10.1111/tbed.13312**

Ismael, S., & Omer, L. (2021). Molecular identification of new circulating *Hyalomma asiaticum asiaticum* from sheep and goats in Duhok Governorate, Iraq. **https://doi.org/10.33899/ijvs.2020.126330.1298**

Kamani, J., Vieira, T. S. W. J., da Costa Vieira, R. F., Shekaro, A., Nahum-Biala, Y., Olubade, T., . . . Shand, M. (2023). Molecular detection of *Theileria annulata*, *Theileria mutans* and *Theileria velifera* but no evidence of Theileria parva infected or vaccinated cattle in Nigeria despite extensive transboundary migrations. *Veterinary Parasitology: Regional Studies and Reports, 41*, 100887. **https://doi.org/10.1016/j.vprsr.2023.100887**

Kirvar, E., Ilhan, T., Katzer, F., Hooshmand-Rad, P., Zweygarth, E., Gerstenberg, C., Phipps, P. and Brown, C.G.D. (2000). Detection of *Theileria annulata* in cattle and vector ticks by PCR using the Tams1 gene sequences. Parasitology, 120(3), 245-254. **https://doi.org/10.1017/S0031182099005466**

Kozhabaev, M., Kuzerbaeva, A., Baizhanov, K., Tulemetova, S., & Nurkhodzhaev, N. (2023). Study of the dynamics of distribution, seasonality, and degree of infection with bovine theileriosis in the territory of Turkestan Region. **https://doi.org/10.47278/journal.ijvs/2022.203**

Lakew, B. T., Eastwood, S., & Walkden-Brown, S. W. (2023). Epidemiology and Transmission of *Theileria orientalis* in Australasia. *Pathogens, 12*(10). doi:10.3390/pathogens12101187 **https://doi.org/10.3390/pathogens12101187**

Larcombe, S. D., Capewell, P., Jensen, K., Weir, W., Kinnaird, J., Glass, E. J., & Shiels, B. R. (2022). Susceptibility to disease (tropical theileriosis) is associated with differential expression of host genes that possess motifs recognised by a pathogen DNA binding protein. *PloS one, 17*(1), e0262051. **https://doi.org/10.1371/journal.pone.0262051**

Li, Y., Galon, E. M., Guo, Q., Rizk, M. A., Moumouni, P. F. A., Liu, M., . . . Xuan, X. (2020). Molecular detection and identification of *Babesia* spp., *Theileria* spp., and *Anaplasma* spp. in sheep from border regions, northwestern China. *Frontiers in Veterinary Science, 7*, 630. **https://doi.org/10.3389/fvets.2020.00630**

Liu, A., Guan, G., Liu, Z., Liu, J., Leblanc, N., Li, Y., . . . Ren, Q. (2010). Detecting and differentiating *Theileria sergenti* and *Theileria sinensis* in cattle and yaks by PCR based on major piroplasm surface protein (MPSP). *Experimental Parasitology, 126*(4), 476-481. **https://doi.org/10.1016/j.exppara.2010.05.024**

Liu, Z., Hou, J., Bakheit, M. A., Salih, D. A., Luo, J., Yin, H., . . . Seitzer, U. (2008). Development of loop-mediated isothermal amplification (LAMP) assay for rapid diagnosis of ovine theileriosis in China. *Parasitology research, 103*, 1407-1412. **https://doi.org/10.1007/s00436-008-1149-3**

Ma, Q., Liu, J., Li, Z., Xiang, Q., Wang, J., Liu, A., . . . Luo, J. (2020). Clinical and pathological studies on cattle experimentally infected with *Theileria annulata* in China. *Pathogens, 9*(9), 727. **https://doi.org/10.3390/pathogens9090727**

McFadden, A., Rawdon, T., Meyer, J., Makin, J., Morley, C., Clough, R., . . . Geysen, D. (2011). An outbreak of haemolytic anaemia associated with infection of *Theileria orientalis* in naive cattle. *New Zealand Veterinary Journal, 59*(2), 79-85. **https://doi.org/10.1080/00480169.2011.552857**

Norouzi, M., Dayer, M. S., & Ghaffarifar, F. (2023). Molecular detection and characterisation of *Theileria* in hard ticks of small ruminants in Zarrin Dasht County, Southern Iran. *Veterinary Medicine and Science, 9*(1), 372-379. **https://doi.org/10.1002/vms3.1027/v1/review3**

Oakes, V. J., Yabsley, M. J., Schwartz, D., LeRoith, T., Bissett, C., Broaddus, C., . . . Brookhart, M. (2019). *Theileria orientalis* Ikeda genotype in cattle, Virginia,

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665

USA. *Emerging Infectious Diseases, 25*(9), 1653. **https://doi.org/10.3201/eid2509.190088**

Omer, O. H., El-Malik, K., Magzoub, M., Mahmoud, O., Haroun, E., Hawas, A., & Omar, H. (2003). Biochemical profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. *Veterinary research communications, 27*, 15-25.

Ota, N., Mizuno, D., Kuboki, N., Igarashi, I., Nakamura, Y., Yamashina, H., . . . Hata, H. (2009). Epidemiological survey of *Theileria orientalis* infection in grazing cattle in the eastern part of Hokkaido, Japan. *Journal of Veterinary Medical Science, 71*(7), 937-944. **https://doi.org/10.1292/jvms.71.937**

Payne, R., Murray, D., Harding, S., Baird, D., & Soutar, D. (2009). GenStat for windows introduction. *VSN International, Hemel Hempstead, 204*.

Qin, G., Li, Y., Liu, J., Liu, Z., Yang, J., Zhang, L., . . . Yin, H. (2016). Molecular detection and characterization of *Theileria infection* in cattle and yaks from Tibet Plateau Region, China. *Parasitology research, 115*, 2647-2652. **https://doi.org/10.1007/s00436-016-5011-8**

Riaz, M., & Tasawar, Z. (2017). Identification of Theileria species (*Theileria ovis* and *Theileria lestoquardi*) by PCR in apparently healthy small ruminants in and around Multan, Southern Punjab, Pakistan.

Schnittger, L., Ganzinelli, S., Bhoora, R., Omondi, D., Nijhof, A. M., & Florin-Christensen, M. (2022). The Piroplasmida *Babesia*, *Cytauxzoon*, and *Theileria* in farm and companion animals: species compilation, molecular phylogeny, and evolutionary insights. *Parasitology research, 121*(5), 1207-1245. **https://doi.org/10.1007/s00436-022-07424-8**

Selim, A., Weir, W., & Khater, H. (2022). Prevalence and risk factors associated with tropical theileriosis in Egyptian dairy cattle. *Veterinary world, 15*(4), 919. **https://doi.org/10.14202/vetworld.2022.919-92**

Shahedi, A., Habibi, G., Fathi, S., Abdigoudarzi, M., Afshari, A., Bozorgi, S., & Chegini, M. R. (2022). Molecular identification of *Theileria* spp. in ruminants and ticks from southern littoral of Caspian Sea, Iran. *Tropical Animal Health and Production, 54*(3), 157. **https://doi.org/10.1007/s11250-022-03156-0**

Simuunza, M., Weir, W., Courcier, E., Tait, A., & Shiels, B. (2011). Epidemiological analysis of tick-borne diseases in Zambia. *Veterinary parasitology, 175*(3-4), 331-342. **https://doi.org/10.1016/j.vetpar.2010.09.027**

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution, 38*(7), 3022-3027. **https://doi.org/10.1093/molbev/msab120**

Tuli, A., Singla, L. D., Sharma, A., Bal, M. S., Filia, G., & Kaur, P. (2015). Molecular epidemiology, risk factors and hematochemical alterations induced by *Theileria annulata* in bovines of Punjab (India). *Acta parasitologica, 60*(3), 378-390. **https://doi.org/10.1515/ap-2015-0053**

Valente, D., Gomes, J., Coelho, A. C., & Carolino, I. (2022). Genetic resistance of bovines to theileriosis. *Animals, 12*(21), 2903. **https://doi.org/10.3390/ani12212903**

Zajac, A. M., Conboy, G. A., Little, S. E., & Reichard, M. V. (2021). *Veterinary clinical parasitology*: John Wiley & Sons.

Zangana, I.K., & Naqid, I.A. (2011). Prevalence of piroplasmosis (Theileriosis and Babesiosis) among goats in Duhok Governorate. Al-Anbar J Vet Sci, 4(2), 50-7.

Zangana, I.K., Ali, B.A., & Naqid, I.A. (2013). Distribution of ectoparasites infested sheep and goats in Duhok province, north Iraq. Bas. J. Vet. Res, 12(1), 54-64.

Zeb, J., Shams, S., Din, I. U., Ayaz, S., Khan, A., Nasreen, N., . . . Senbill, H. (2020). Molecular epidemiology and associated risk factors of *Anaplasma marginale* and *Theileria annulata* in cattle from North-western Pakistan. *Veterinary parasitology, 279*, 109044. **https://doi.org/10.1016/j.vetpar.2020.109044**

DOI: https://doi.org/10.25271/sjuoz.2026.14.1.1665