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Hematological and Molecular Detection of *Theileria Annulata* from Thi-Qar Governorate and the Marshes Cattle Isolate

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ABSTRACT

This study aims to investigate *Theileria annulata* infection in cattle herds in Thi-qar governorate and the Marshes. The 115 blood samples were collected from cattle of different ages and sexes in the Dhi-qar governorate and the marshes in Iraq. Microscopic examination showed that the prevalence of *Theileria* in cattle was 43.4%, based on the microscopic examination of blood samples stained with Giemsa stain. Analysis of some blood parameters: RBCs, HGBPCV, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, WBCs, Lymph, and Granulocytes revealed significant differentiation due to *Theileria annulata* infection. Conventional PCR analysis of blood samples targeting the *tams-1* and *cox-1* genes revealed 69.56% positivity for *T. annulata*. The *cox-1* gene (1200 bp) and *tams-1* gene (441 bp) sequences were deposited in GenBank under accession numbers (LC852773.1, LC852774.1, LC852775.1) and (LC852776.1, LC852777.1, LC852778.1), respectively. Phylogenetic analysis showed that the *tams-1* gene of the isolates shared high homology and evolutionary relationships with strains from the Netherlands, Scotland, India, China, South Africa, and Japan, indicating genetic conservation across regions.

Key words: cbc, *cox-1* gene, marshes, pcr, tropical theileriosis.

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INTRODUCTION

Theileria is one of the most important blood parasites transmitted by hard ticks (Ixodidae) and poses a major threat to ruminants, especially cattle (Squarre *et al.*, 2020). The most critical *Theileria* species transmitted by the tick *Hyalomma anatolicum* is *Theileria annulata*, which causes tropical bovine theileriosis. This tick-borne protozoan disease is widespread and causes significant economic losses, leading to high morbidity and mortality rates (Ma *et al.*, 2020). Theileriosis is characterized by fever of 40-41.5°C, enlargement of superficial lymph nodes, ocular and nasal discharge, depression, leucopenia, anemia, and jaundice. Cattle recovering from acute infection serve as reservoirs of infection for ticks that feed on them (Zeb *et al.*, 2020).The life cycle of *Theileria annulata* begins with the vector tick feeding on cattle; the spores reach the bloodstream and then enter the white blood cells (monocytes or B lymphocytes); sporozoites then distinguished to macroschizonts induce uncontrolled divisions resembling cancer-like phenotype and production of a large number of microschizonts and merozoites by the merogony of macroschizonts which targeting the red blood cells to develop inside them into piroplasms, When the tick feeds on the infected cows, they become infected the cycle repeats (Elati *et al.*, 2024). Microscopic diagnosis using Giemsa stain to check for piroplasma stage in blood smear or Koch bodies in lymphocytes, especially in clinically acute theileriosis, but the infection may not appear in carrier animals (Talab and Falih, 2025).Molecular methods are highly sensitive and specific, and they are used to identify different species of *Theileria* in persistently infected cattle (Altay *et al.*, 2008). Molecular diagnosis using conventional PCR depending on several *Theileria annulata*-specific genes, *Theileria annulata* merozoite surface protein-1

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(*tams-1*), which encodes a major surface antigen of *T. annulata* merozoites and is extensively utilized in molecular diagnostics and epidemiological studies (Kundave *et al.*, 2021), and cytochrome c oxidase subunit 1 (*cox-1*) genes (Wang *et al.*, 2023), the *cox-1* gene serves as a valuable molecular marker for phylogenetic analyses in many organisms due to its conserved nature across various species (Yang *et al.*, 2025; Al-Fetly *et al.*, 2012).This current study aims to investigate *Theileria annulata* infection in cattle herds in Thi-qar governorate and the Marshes for the first time, as well as use the cytochrome oxidase subunit 1 (*cox-1*) gene in molecular diagnosis, in addition to the adoption of the *tams-1* gene as a specific gene for molecular diagnosis.

MATERIALS AND METHODS

This study was conducted for the first time in Thi-qar and Marshes Governorate in Iraq from January to October 2024, where 115 blood samples were obtained from cows of different ages and sexes. After clinical examination of the cattle, where signs of elevated rectal temperature, enlarged superficial lymph nodes, pale mucous membranes, and tick infestation were examined, blood samples (10 mL) were collected from the jugular vein into plastic tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) and divided into three parts. The first part (2 mL) was transferred to the laboratory to detect the presence of *Theileria* based on microscopical examination. The second part (4 mL) was used for hematological evaluation. The third part (4 mL) was stored at −80 °C and used for molecular detection. These three portions of the collected blood samples were subsequently processed as follows:

1. Microscopic examination: Thin blood films were stained with Giemsa stain at a concentration of 5% for 30 to 60 minutes and were viewed under a light microscope at 100x in oil immersion. Thin blood smears were utilized to determine the shape and measurement requirements for laboratory evaluation to diagnose the *Theileria* parasite in cattle (Alsulami *et al*., 2026).

2. Complete blood count (CBC): Some blood parameters were analyzed using a three-part haematological analyzer model (NP-36), including red blood cell count (RBCs), hemoglobin concentration (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width- coefficient of variation (RDW-CV), red blood cell distribution width-standard deviation (RDW-SD), total platelet count (PLT), white blood cells (WBCs), Lymphocytes (Lymph), Granulocytes (Gran).

3. Polymerase chain reaction: Genomic DNA from cattle blood, including 115 samples, was extracted using a DNA extraction kit (GeneiadBiotech, Taiwan) and performed according to the company's recommendations. Conventional PCR was used to detect *Theileria annulata* (Kawan, 2019) using specific primers provided by Macrogen Company (South Korea).

Primers and PCR reaction

The *tams-1* and *cox-1* gene primers used in the current study to detect *Theileria annulata* were amplified using forward and reverse primers, respectively. The amplified sequences of *tams-1* were 5'- CCAGGACCACCCTCAAGTTC - 3', 5'- GCATCTAGTTCCTTGGCGGA - 3', respectively, while the *cox-1* gene amplified 5'- TGGCTTGCTTATTGGTTTGGT - 3', 5'- CAACATCATAGTAGCTCCAA -3', respectively (Table 1).

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Table 1. Sequence of primers used in the study

Target gene	Sequence	Primer sequence 5'- 3' From (Microgen Korea)	Tm (°C)	GC%	Product size (bp)	Reference
tams-1	F	5'-CCAGGACCACCCTCAAGTTC- 3'	55 C	70%	441 bp	(Chauhan *et al.*, 2015)
	R	5'- GCATCTAGTTCCTTGGCGGA- 3'	55 C	55%		
cox-1	F	5'-TGGCTTGCTTATTGGTTTGGT- 3'	50 C	42.86%	1200 bp	(Gou et al., 2012)
	R	5'-CAACATCATAGTAGCTCCAA- 3'	50 C	42.86%		

Primer preparation

These primers were supplied by Macrogen Company in a lyophilized form. Each primer was dissolved in 230 µl of nuclease-free water to achieve a final stock concentration of 100 pmol/µl. A working solution was then prepared by diluting 10 µl of the stock solution (stored at -20 °C) in 90 µl of nuclease-free water, resulting in a final working concentration of 10 pmol/µl.

Preparation of PCR

The PCR reaction components were prepared by a mixture (13 µl) of Master Mix (Bioneer Corporation, South Korea), (1 µl) of each primer pair, (4 µl) DNA template, and (6 µl) of deionized water for a final volume of 25 µl (Table 2). Then, the PCR amplification program for *cox-1* gene was done through the use of an Applied Biosystem of PCR as follows: initial denaturation: 5 minutes at 95°C; denaturation: 30 sec. at 95°C; annealing as an important optimal condition: 30 sec. at 50°C, extension-1: 1 minute at 72°C, and extension-2: 10 minutes at 72°C, all (35) cycles except initial denaturation and extension-2(1) cycle. PCR amplification program for the *tams-1* gene was initial denaturation: 5 minutes at 95°C; denaturation: 30 sec. at 95°C; annealing: 30 sec. at 55°C, extension-1: 40 sec. at 72°C, and extension-2: 10 minutes at 72°C, all (35) cycles except initial denaturation and extension-2(1) cycle. The conventional PCR thermocycler system was done in steps. Electrophoresis using a 1.5% agarose gel stained with a safety green was performed to separate the bands at 100 volts and 80 mA for 1 hour. These PCR products were then evaluated using a UV imager (Mohanta *et al.*, 2023).

Table 2. The PCR reaction for the detection of *Theileria annulata*

Components	Concentration	Volume µl
Master Mix		13 µl
Primer forward	(10 pmol)	1 µl
Primer reverse	(10 pmol)	1 µl
DNA template	5-50 ng/µl	4 µl
Ionized water		6 µl
Final volume		25 µl

Sequencing of DNA and Phylogenetic Tree

The PCR product for three samples was amplified by PCR targeting the *tams-1* and *cox-1* genes, and the forward primers were submitted to the sequencing service (Macrogen, Korea) to perform Sanger sequencing. Then, the sequences were analyzed using BLAST (Basic Local Alignment Search Tool) at NCBI to investigate a similar sequence that had previously been registered in this genomic bank, and the phylogenetic tree was organized using the maximum composite likelihood and minimum evolution method by Molecular Evolutionary Genetics Analysis (MEGA) version 6.0. Software that eliminates the gaps and missing data in all positions (Tamura *et al.*, 2013).

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Analytical Statistics

The Statistical Packages of Social Sciences (SPSS) program (2019) was used to detect the effect of the different factors on study parameters. The least significant difference (LSD) or T-test was used to compare the means (ANOVA/ Two-way) in this study.

RESULTS AND DISCUSSION

Clinical signs observed on examination of infected animals included enlargement of superficial lymph nodes (especially the prescapular, submandibular, and anterior thigh lymph nodes), fever (40.3°C–41.5°C), pallor of mucous membranes, anemia, increased salivation, and lacrimation production, evidence of tick infestation, and weight loss (Figure 1), which was supported by previous studies in Iraq (Jameel and Majeed, 2013; Hassan *et al.*, 2024).

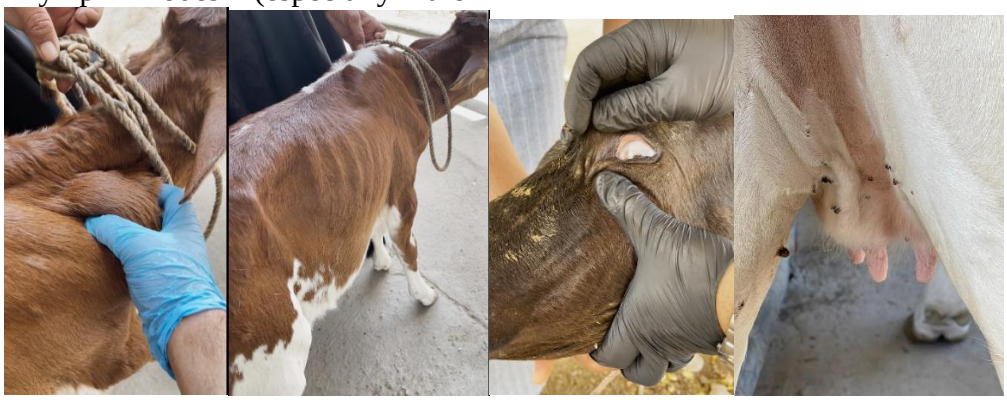

Figure 1. Clinical signs of bovine theileriosis in infected cattle: a severe emaciation, b enlargement of prescapular lymph node, c high infestation by ticks, d mucous membrane pallor.

This study showed that the prevalence of theileriosis in cattle was 43.4% by microscopic examination of blood samples stained with Giemsa stain (Table 3), which was higher than the prevalence reported by Salih *et al.* (2024), who suggested a prevalence of 24% in Fallujah city, Albakri, and his colleagues discovered that the infection rate in the city of Mosul was 38%, which was less than what we have reached (Albakri *et al.*, 2024), While this percentage was less than what the researcher reached, 66.88% for the Sumel area in the city of Dohuk in Iraq (Ismael, 2023), The result of Kawan was close to our result, as it recorded a percentage of 39.33% in the city of Baghdad (Kawan, 2019).*Theileria* species appeared in various shapes, such as comma, dot, rod-shaped, circular, signet ring-shaped, or pear-shaped, in addition to the deformation of red blood cells resulting from the effect of piroplasma or other shapes, as shown in Figures 2 and 3.

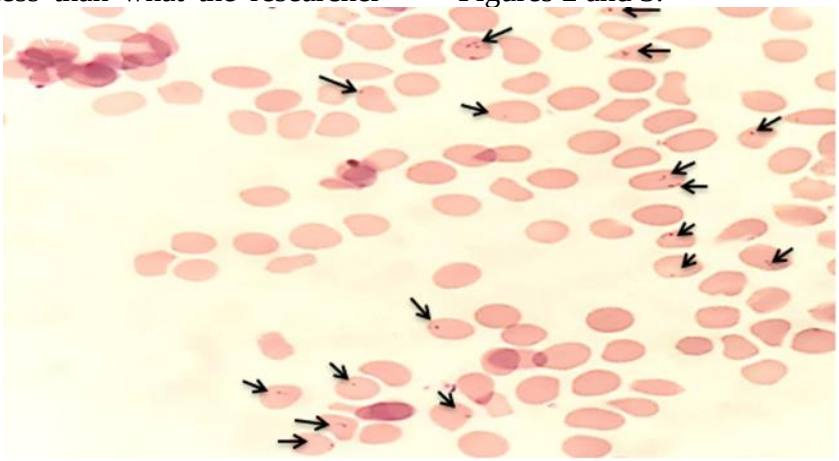

Figure 2. Numerous *Theileria* inside erythrocytes (black arrow) in thin blood smear of infected cattle stained by Giemsa stain (100x, oil immersion).

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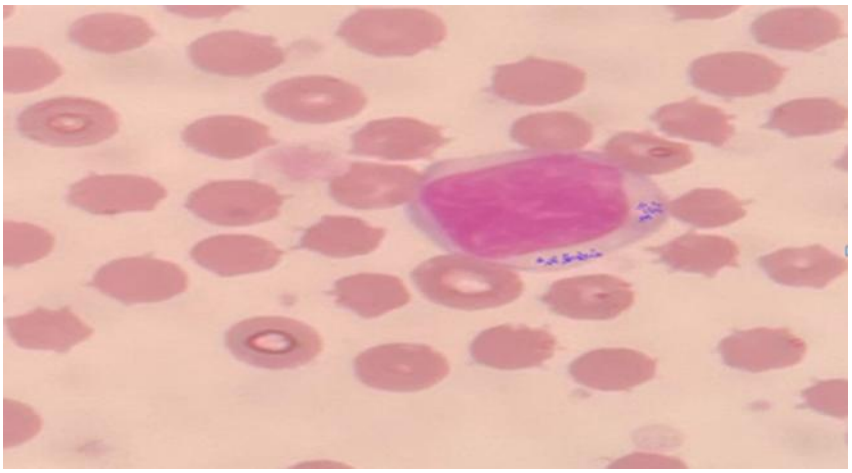

Figure 3. It shows the lymphocytic stage (Koch's blue body) (100x, oil immersion).

Polymerase chain reaction

In the PCR technique, the *cox-1* gene was used for the first time in the molecular diagnosis of *Theileria* in Iraq. The results for *cox-1* and *tams-1* genes from the tested blood samples showed that 69.56% were positive for *Theileria annulata* on agarose gel electrophoresis, as shown in Table 3, Figures 4 and 5. This prevalence was lower than the 73% reported in Fallujah city based on the *18S rRNA* gene (Salih *et al.*, 2024), Similarly, Hassan et al. (2012) found a comparable result (70%) in Sulaymaniyah, while Mohammad Al-Saeed *et al.* reported an infection rate of 68.9% in Iraqi Kurdistan (Mohammad Al-Saeed *et al.*, 2010), closely matching our findings. In contrast, Kawan (2019) detected a higher prevalence (88%) in Baghdad. A study analyzing the *cox-1* gene reported an 81.5% infection rate of *Theileria luwenshuni* in goats from Shandong Province, China (Wang *et al.*, 2023). Another study using the *cox III* gene for *Theileria annulata* detection in cattle revealed a much lower molecular prevalence (8.9%, 56/629) in clinically healthy cattle in Algeria (Kernif *et al.*, 2024).

Table 3. *Theileria* species infection rate in cattle by blood smears and PCR

Test	No. of examined	No. of infected	Percentage (%)
Blood smears	115	50	43.47%
PCR	115	80	69.56%

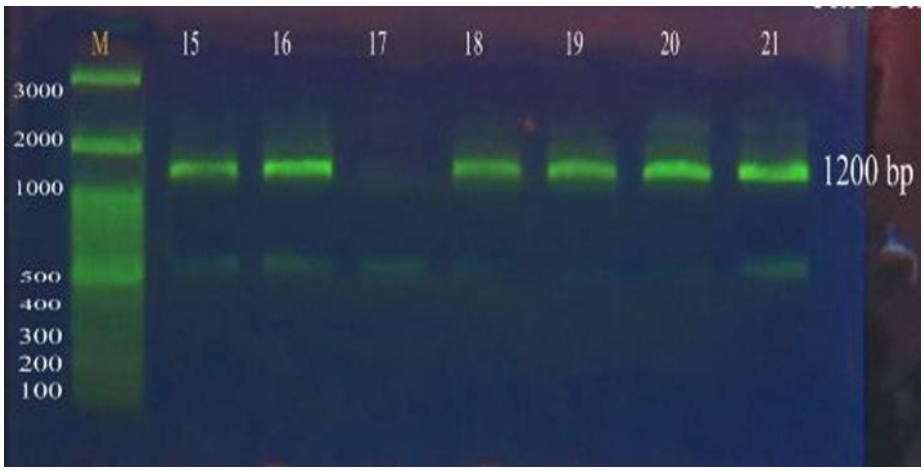

Figure 4. Gel electrophoresis shows PCR results using a 1.5% agarose gel stained with safety green. The bands were separated at 100 volts and 80 mA for 1 hour, displaying the *cox-1* gene (1200 bp) of *Theileria* species in blood samples of cattle. M represents the ladder (3000-100 bp). Lanes 15, 16, 18, 19, 20, and 21 were positive, while lane 17 was a negative sample

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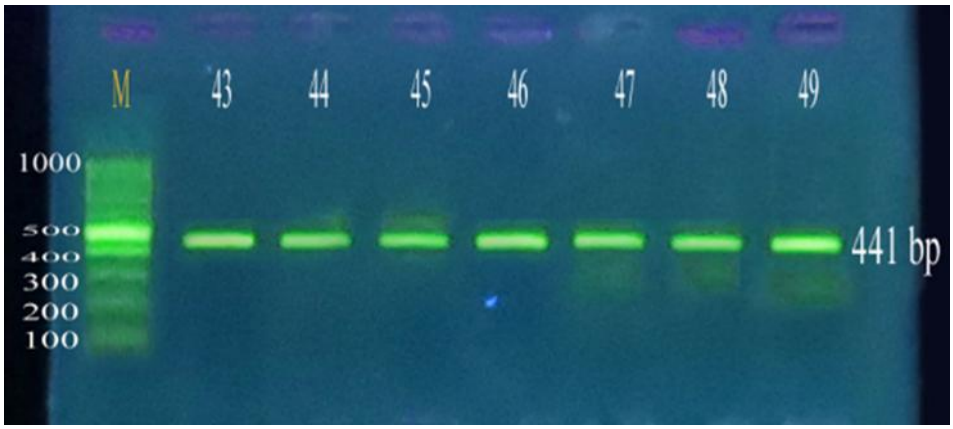

Figure 5. Gel electrophoresis shows PCR results using a 1.5% agarose gel stained with safety green. The bands were separated at 100 volts and 80 mA for 1 hour, displaying the *tams-1* gene (441 bp) of *Theileria annulata* in blood samples of cattle. M represents the ladder (1000-100 bp). Lanes 43–49 were positive samples

The results obtained in this study showed that the prevalence of theileriosis in cattle was 43.4% based on microscopic examination. Using the PCR technique, the results of the *cox-1* and *tams-1* genes from the tested blood samples revealed that 69.56% were positive for *Theileria annulata*. This molecular prevalence is higher compared to some previous studies conducted in different regions of Iraq. The high infection rates observed in this study can be attributed to several environmental and climatic factors, primarily the widespread presence of ticks, which are the main vectors responsible for the transmission of *Theileria*. Moreover, climatic conditions favorable for tick proliferation, such as elevated temperatures and extended periods of humidity, have played a significant role. The phenomenon of global warming and its associated climatic changes have directly contributed to an increase in tick populations and the spread of *Theileria*. Therefore, we believe that the prevailing environmental conditions in Iraq, particularly the changes associated with global warming, have significantly promoted the tick life cycle and enhanced the transmission dynamics of theileriosis. This has led to higher infection rates compared to other regions that are less affected by these environmental factors.

Hematological findings

In the present study, the comparison of some hematological parameters between infected and non-infected cattle showed that the differences were non-significant for all parameters, which revealed significant reduction in RBCs, Hb, PCV, MCV, MCHC and WBC, and Lymph ((P≤0.05); while showed a significant increase in MCH, RDW-CV, RDW-SD, PLT, and Granulocytes; compared with healthy ones (P≤0.05) (Table 4).

Table 4. Comparison between healthy and infected cattle groups in Hematological parameters

Hematological parameters	Mean ±SE		T-test
	Healthy cattle	Infected cattle	
RBCs (10^6/µl)	↑7.30 ±0.10 A	↓2.61 ±0.02 B	0.241 *
HGB (g/dl)	12.32 ±0.17 A	↓5.92 ±0.19 B	0.589 *
PCV (%)	35.84 ±1.14 A	↓19.40 ±1.81 B	4.948 *
MCV (fl)	51.32 ±0.99 A	↓41.98 ±1.15 B	3.503 *
MCH (pg)	12.90 ±0.21 B	↑←21.92 ±0.32 A	0.890 *
MCHC (g/dl)	35.27 ±1.10 A	↓28.39 ±0.35 B	2.671 *
RDW-CV (%)	15.00 ±0.71 B	↑←29.08 ±0.49 A	1.994 *
RDW-SD (fl)	36.20 ±0.86 B	↑←78.35 ±1.59 A	4.168 *
Platelet (10^3/µL)	209.00 ±4.19 B	↑←338.40 ±5.04 A	15.132 *
WBCs (10^3/µl)	10.51 ±0.21 A	↓5.91 ±0.07 B	0.514 *
Lymph (10^3/µl)	6.31 ±0.23 A	↓2.42 ±0.12 B	0.595 *
Gran(10^3/µl)	3.58 ±0.14 B	↑←7.34 ±0.32 A	0.814 *

Means having with the different letters in same row differed significantly * (P≤0.05).

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The significant decrease in RBCs, Hb, and PCV in the present study confirmed the previous results obtained by Al-Emarah *et al.* (2012), who found a decrease in red blood cells, PCV, and Hb in cows infected with *Theileria annulata* compared to healthy cows in the north of Basra Governorate. These results were identical to those recorded in cattle by Minnat *et al*. in the Diyala province, who explained this decline due to the destruction of red blood cells by the parasite as it multiplies inside them and causes their destruction (hemolysis) (Minnat *et al.*, 2016). These results agreed with the results of Ismael and Al-Samarai in Baghdad (Ismael and Al-Samarai, 2019). PCV, RBC, and Hb showed a significant decrease, which is consistent with Al-Shammari et al. in cattle in the Babylon governorate (Al-Shammari *et al.*, 2024), Al-Fetly in Al-Diwaniya province (Al-Fetly, 2012), and Kim *et al*., (Kim *et al.*, 2024). The significant reduction in MCV was consistent with Ismael and Al-Samarai (2019) and Elsayed *et al*. (2024). The significant decrease in MCHC, which agreed with Abdul-Husin in *Theilaria* infection in cattle in Babylon (Abdul-Husin *et al.*, 2016), Al-Emarah *et al*. (2012), and Aziz *et al.* (2022). Yousef *et al*. (2020) showed normocytic normochromic anemia in mild and moderate anemic *theileria*-infected groups relative to the control group.The significant decline in WBCs observed in this study is consistent with the findings of Abdul-Husin *et al.* (2016) and Durrani *et al.* (2010) Similarly, the decrease in lymphocyte count aligns with the results reported by Durrani *et al.* (2010) and Elsayed *et al*. (2024); These studies suggest that the terminal stages of leukopenia may result from extensive lymphocyte destruction. *Theileria* parasites infect lymphocytes, multiply within them, and subsequently destroy or impair their function. This leads to the leakage of lymphocytes into various organs, reducing their numbers in peripheral circulation. Such redistribution can significantly affect lymphocyte counts in the bloodstream.The significant increase in MCH, RDW-CV, RDW-SD, PLT, and Granulocytes in infected cattle compared with healthy ones ($P \leq 0.05$); these results were identical to those recorded by Elsayed *et al.* (2024). The nature of the morphological changes in red blood cells resulting from infection with the *Theileria annulata* parasite is irregular external protrusions through changes in membrane phospholipids that may develop due to the continuation of the parasitic infection into hemolysis that stimulated bone marrow to produce new immature cells (reticulocytes) that are larger than mature cells, which leads to increased diversity in cell sizes (Elati *et al.*, 2024) and consequently increases RDW-CV and RDW-SD. The presence of this parasite causes multiple petechial hemorrhages, tissue damage in organs such as the liver, kidneys, and lungs, and lesions in the lining of blood vessels. This parasite stimulates platelet production to help clot and close the damaged vessels due to extensive bleeding or inflammation (Ma *et al.*, (2020). The hematological analysis conducted in this study revealed significant alterations in infected cattle compared to healthy ones. The observed reductions in RBCs, Hb, PCV, MCV, MCHC, WBCs, and lymphocytes, along with the increases in MCH, RDW-CV, RDW-SD, platelets, and granulocytes, align with previous findings reported in various regions of Iraq. These changes reflect the pathological impact of *Theileria annulata* infection, particularly the parasite-induced hemolysis and damage to blood components. Given the widespread presence of ticks and the favorable climatic conditions for their proliferation in Iraq, such hematological disturbances are expected. The continuous exposure of cattle to infection cycles contributes significantly to the severity of these changes, highlighting the urgent need for integrated control programs against theileriosis in the region.

Sequencing and phylogenetic tree construction of *tams-1* and *cox-1* genes

The *cox-1* gene at 1200 bp was registered in GenBank at NCBI with accession numbers (LC852773.1, LC852774.1, LC852775.1), in addition to recording the *Theileria annulata* merozoite surface protein-1 (*tams-1*) gene at 441bp with accession numbers (LC852776.1, LC852777.1, LC852778.1).Representative comparisons based on the *cox-1* gene revealed varying degrees of identity between the Iraqi

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isolates (LC852773.1, LC852774.1, LC852775.1) and strains from geographically diverse regions. The Iraqi isolates exhibited 100% identity among themselves. When compared with the Chinese isolates, the Iraqi sequences showed 99.49% identity with isolate JQ518298.1 (China 1) and 99.19% identity with isolate JQ518297.1 (China 2). Similarly, comparison with the South African isolates revealed 99.66% identity with KC961516.1 (South Africa 1) and a lower identity of 90.98% with KC961513.1 (South Africa 2), as shown in Table 5 and Figure 6.

Table 5. Comparative analysis of the *cox-1* gene for *Theileria* species

	Accession	Country	Source	Compatibility
1	ID: LC852773.1	Iraq 1	*Theileria_annulata*	100%
2	ID: LC852774.1	Iraq 2	*Theileria_annulata*	100%
3	ID: LC852775.1	Iraq 3	*Theileria_annulata*	100%
4	ID: JQ518298.1	China 1	*Theileria_annulata*	99.49%
5	ID: JQ518297.1	China 2	*Theileria_annulata*	99.19%
6	ID: KC961516.1	South Africa 1	*Theileria_annulata*	99.66%
7	ID: KC961513.1	South Africa 2	*Theileria_annulata*	90.98%

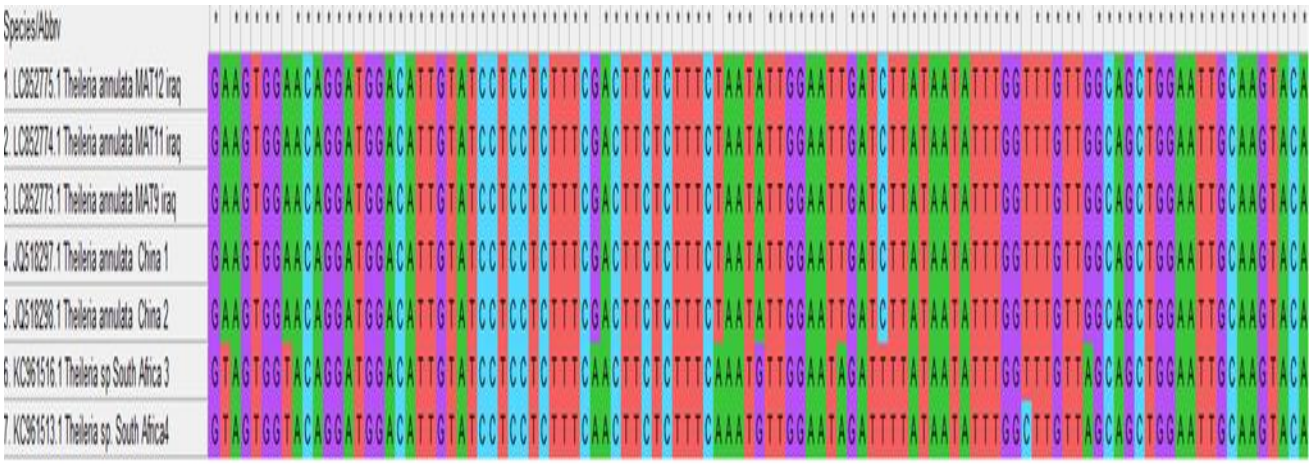

Figure 6. DNA sequencing of *Theileria. annulata* by PCR using the *cox-1* gene

The evolutionary divergence analysis of the *cox-1* gene among *Theileria annulata* isolates demonstrated minimal genetic variation among the Iraqi isolates (LC852775.1, LC852774.1, and LC852773.1), with a calculated genetic distance of 0.0000, indicating complete genetic identity. When comparing the Iraqi isolates to the Chinese strains, slight genetic divergences were observed: 0.0051 with the Chinese isolate (JQ518297.1) and 0.0081 with the second Chinese isolate (JQ518298.1), suggesting a high level of genetic conservation between Iraqi and Chinese strains. In contrast, markedly higher divergence values were recorded when compared to *Theileria sp.* isolates from South Africa: 0.1050 with isolate (KC961516.1) and 0.1052 with isolate (KC961513.1), reflecting significant genetic differentiation between geographically distinct populations (Table 6).These results are consistent with the phylogenetic tree (Figure 7), where the Iraqi isolates cluster closely with the Chinese strains, while the South African isolates form a separate and distant branch. The findings emphasize the genetic homogeneity of *Theileria annulata* populations in Iraq and their close relationship with strains from similar ecological zones, likely due to shared evolutionary pressures and host adaptations. Meanwhile, the pronounced divergence from South African isolates highlights the influence of geographic isolation in driving parasite genetic diversity. The observed conservation of the *cox-1* gene across these populations supports its value as a reliable and stable marker for phylogenetic and evolutionary studies of *Theileria annulata*.

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Table 6. Evolutionary divergence analysis of *Theileria annulata* using *cox-1* gene isolates

	1	2	3	4	5	6	7
1. LC852775.1 *Theileria annulata* MAT12 Iraq							
2. LC852774.1 *Theileria annulata* MAT11 Iraq	0.0000000000						
3. LC852773.1 *Theileria annulata* MAT9 Iraq	0.0000000000	0.0000000000					
4. JQ518297.1 *Theileria annulata* china1	0.0050807172	0.0050807172	0.0050807172				
5. JQ518298.1 *Theileria annulata* China 2	0.0081495565	0.0081495565	0.0081495565	0.0043091014			
6. KC961516.1 *Theileria sp.* South Africa	0.1049592372	0.1049592372	0.1049592372	0.1049960526	0.1049960526		
7. KC961513.1 *Theileria sp.* South Africa	0.1052033480	0.1052033480	0.1052033480	0.1052240098	0.1052240098	0.0248490213	

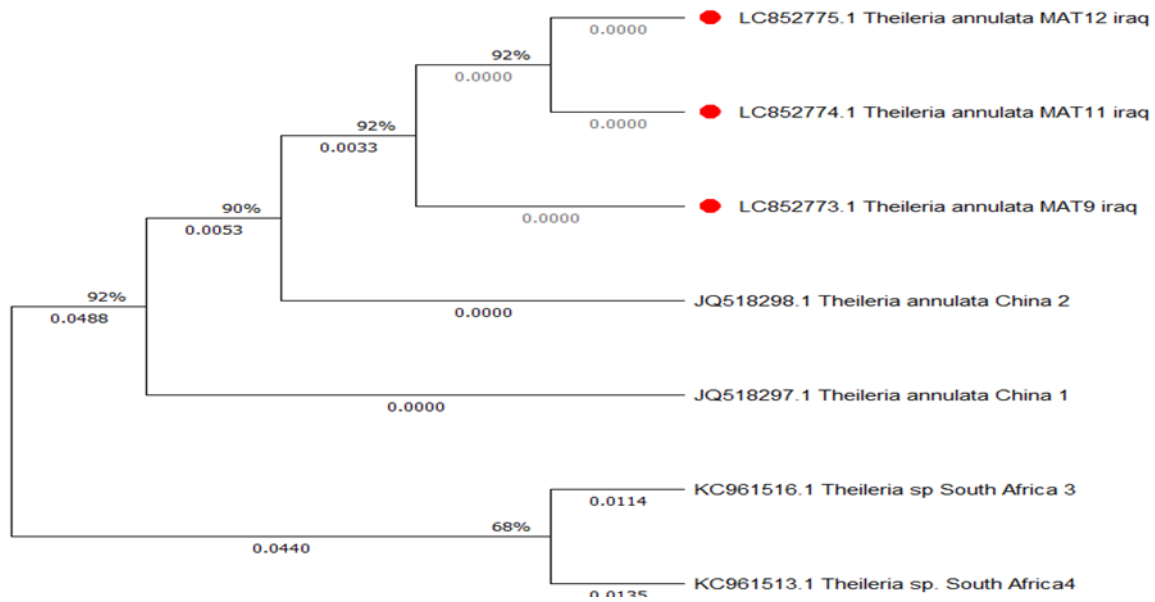

Figure 7. Phylogenetic tree of *Theileria annulata* using the *cox-1* gene. The isolates used in the current study are labeled with red circles. MEGA software version 6.0 was used to create the analysis using the neighbor-joining method.

Comparative analysis of the *tams-1* gene demonstrated variable levels of sequence identity between the Iraqi isolates and those from diverse geographical origins. The Iraqi isolates (LC852776.1, LC852777.1, and LC852778.1) showed complete (100%) identity among themselves. In comparison with isolates from other countries, the Iraqi sequences shared 99.01% identity with KX981029.1 from Scotland (UK), 98.77% with AF214917.1 from the Netherlands, and 98.52% with MK034700.1 from India. Furthermore, alignment with the Chinese isolate EU563912.1 indicated 97.04% identity, whereas the Japanese isolate LC467542.1 (*Theileria spp.*) exhibited the lowest sequence identity at 96.31%, as shown in Table 7 and Figures 8.

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Table 7. Comparative analysis of the *tams-1* gene for *Theileria* species

	Accession	Country	Source	Compatibility
1	ID: LC852776.1	Iraq 1	*Theileria_annulata*	100%
2	ID: LC852777.1	Iraq 2	*Theileria_annulata*	100%
3	ID: LC852778.1	Iraq 3	*Theileria_annulata*	100%
4	ID: KX981029.1	Scotland UK	*Theileria_annulata*	99.01%
5	ID: AF214917.1	Netherlands	*Theileria_annulata*	98.77%
6	ID: MK034700.1	India	*Theileria_annulata*	98.52%
7	ID: EU563912.1	China	*Theileria_annulata*	97.04%
8	ID: LC467542.1	Japan	*Theileria_sp.*	96.31%

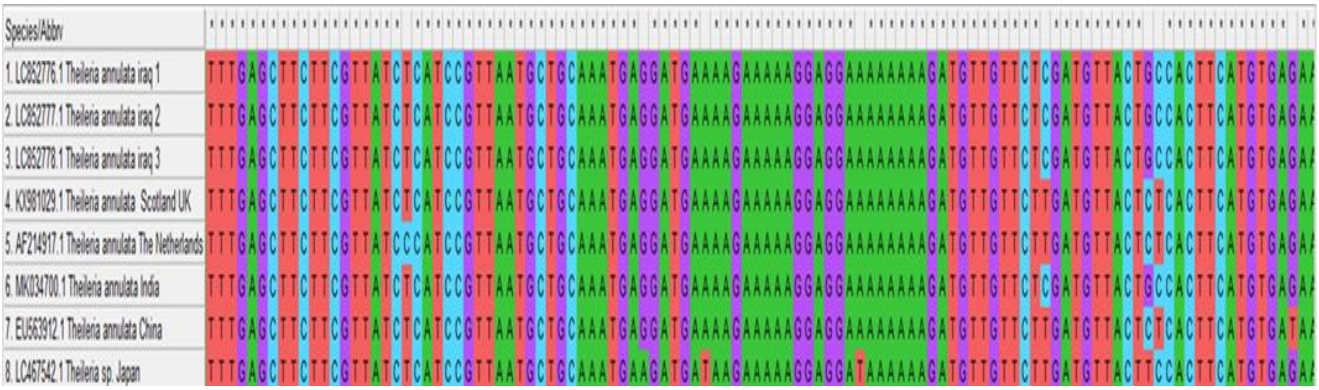

Figure 8. DNA sequencing of *T. annulata* by PCR using the *tams-1* gene

The evolutionary divergence analysis of the *tams-1* gene among *Theileria annulata* isolates revealed minimal genetic variation among the three Iraqi isolates (LC852776.1, LC852777.1, and LC852778.1), with a genetic distance of 0.0000, confirming complete genetic identity among them. When comparing the Iraqi isolates with global isolates, the smallest genetic divergence was observed with the Scottish isolate (KX981029.1) at a distance of 0.0099, followed by the Netherlands isolate (AF214917.1) at 0.0124, and the Indian isolate (MK034700.1) at 0.0149. Moderate divergence was noted with the Chinese isolate (EU563912.1) at 0.0302, whereas the highest divergence was recorded with the Japanese isolate (LC467542.1) at 0.0380, as shown in Table 8 and Figure 9.These findings suggest that the *tams-1* gene among the Iraqi isolates is highly conserved, which is consistent with previous studies reporting low genetic variability among *Theileria annulata* populations across different regions. The low levels of divergence observed in this study may reflect the parasite's evolutionary strategy to maintain genetic stability, possibly due to adaptation to similar environmental conditions and host factors in these regions. Thus, the evolutionary divergence analysis supports the hypothesis that *Theileria annulata* strains circulating in Iraq are genetically homogeneous and closely related to strains from Europe and Asia, with slight variations likely attributable to localized evolutionary pressures or host–parasite interactions.

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Table 8. Evolutionary divergence analysis of *Theileria annulata* using *tams-1* gene isolates

	1	2	3	4	5	6	7
1. LC852776.1 *Theileria annulata* Iraq 1							
2. LC852777.1 *Theileria annulata* Iraq 2	**0.0000000000**						
3. LC852778.1 *Theileria annulata* Iraq 3	**0.0000000000**	**0.0000000000**					
4. KX981029.1 *Theileria annulata* Scotland, UK	**0.0099118024**	**0.0099118024**	**0.0099118024**				
5. AF214917.1 *Theileria annulata* The Netherlands	**0.0124002514**	**0.0124002514**	**0.0124002514**	**0.0025326390**			
6. MK034700.1 *Theileria annulata* India	**0.0149581270**	**0.0149581270**	**0.0149581270**	**0.0323317476**	**0.0321792408**		
7. EU563912.1 *Theileria annulata* China	**0.0302211225**	**0.0302211225**	**0.0302211225**	**0.0127748840**	**0.0127153955**	**0.0414704289**	
8. LC467542.1 *Theileria sp.* Japan	**0.0380439837**	**0.0380439837**	**0.0380439837**	**0.0912945524**	**0.0915897982**	**0.0919624589**	**0.0976019420**

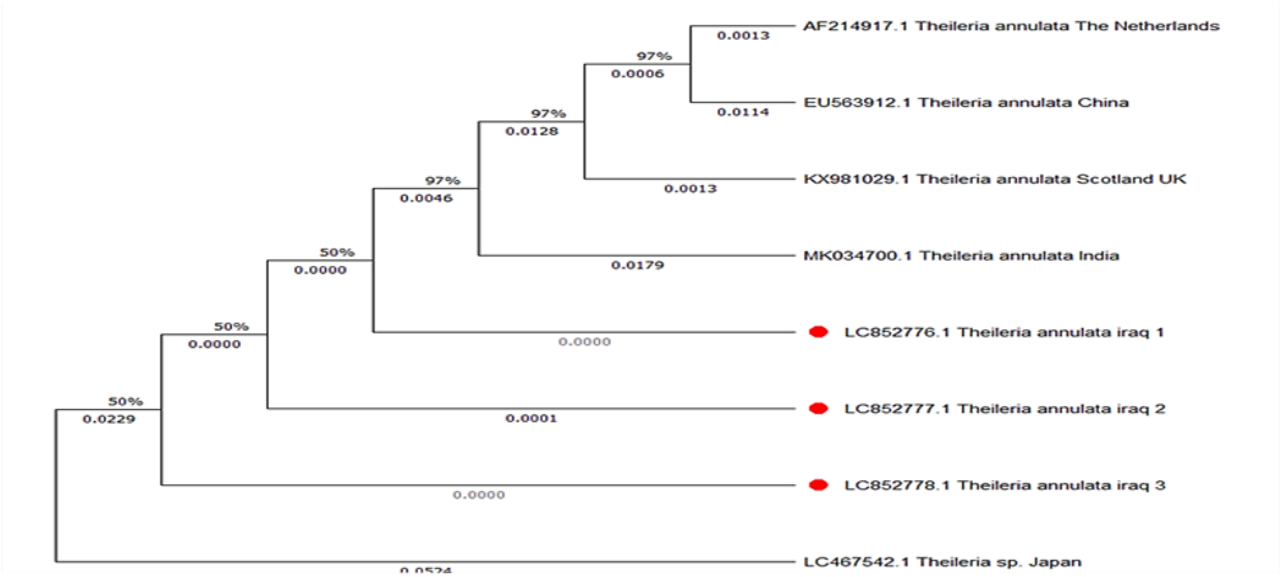

Figure 9. Phylogenetic tree of *Theileria annulata* using the *tams-1* gene. The isolates used in the current study are labeled with red circles. MEGA software version 6.0 was used to create the analysis using the neighbor-joining method.

The findings indicate a strong evolutionary conservation of the *cox-1* and *tams-1* genes among *Theileria annulata* populations from diverse geographic regions, as reflected by the consistently high sequence identity. Nevertheless, the absence of closely related Iraqi or Arab isolates, along with the distinct genetic profiles of the newly characterized Iraqi strains, suggests the presence of subtle genetic variation within *T. annulata* populations. This observed divergence underscores ongoing evolutionary dynamics and highlights the potential influence of regional factors on the genetic structure of the parasite. Furthermore, the overall conservation of these genes supports their utility as reliable molecular markers for diagnostic applications and epidemiological investigations.

CONCLUSION

In conclusion, *Theileria annulata* infection is highly prevalent in Iraqi cattle and causes significant hematological alterations, such as severe anemia. Utilizing PCR with *cox-1* and *tams-1* genes provides a highly sensitive diagnostic approach, revealing strong genetic conservation among local isolates while

highlighting the impact of environmental factors on disease spread.

CONFLICT OF INTEREST

The authors declare no conflicts of interest..

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الكشف الدموي والجزيئي عن الثيليريا الحلقية المعزولة من أبقار محافظة ذي قار والأهوار

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المستخلص

هدفت الدراسة إلى الكشف عن ظهور إصابات بالطفيلي *Theileria annulata* في قطعان الأبقار بمحافظة ذي قار والأهوار. تم جمع 115 عينة دم من أبقار مختلفة الأعمار والأجناس. أظهرت نتائج الفحص المجهري لعينات الدم المصطبغة بصبغة جيمسا أن نسبة انتشار *Theileria annulata* بلغت 43.4%. كما أظهرت تحاليل بعض المؤشرات الدموية (RBCs، HGB، PCV، MCV، MCH، MCHC، RDW، PLT، WBCs، Lymph، Granulocytes) وجود فروق معنوية مرتبطة بالإصابة. وقد أظهرت نتائج تفاعل البوليميراز المتسلسل التقليدي للمورثين *cox-1* عند 1200 و *tams-1* عند 441 ، عن نسبة إصابة بلغت 69.56%, وسجل المورثين في قاعدة بيانات بنك الجينات في المركز الوطني لمعلومات التقانة الحيوية تحت أرقام الوصول (LC852773.1، LC852774.1، LC852775.1) لمورث *cox-1*، و(LC852776.1، LC852777.1، LC852778.1) لمورث *tams-1*. أظهر التحليل الوراثي تشابهًا عاليًا بين العزلات العراقية وعزلات من هولندا واسكتلندا والهند والصين وجنوب افريقيا واليابان، مما يشير إلى حفظ موروث كبير للمورثين *cox-1* و *tams-1* بين سلالات *Theileria annulata* المختلفة جغرافيًا، ويؤكد أهميته كمؤشر تشخيصي.

الكلمات المفتاحية: الأهوار، تعداد الدم الكامل، تفاعل البوليميراز المتسلسل، داء الثيلريات الاستوائية، المورث *cox-1*.