Iraqi Journal of Veterinary Sciences

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Molecular identification of Theileria species in cattle in Mosul city

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Article information

Article history:
Received 26 June, 2023
Accepted 27 October, 2023
Available online 17 December, 2023

Keywords:
Theileria annulata
Theileria parva
Diagnosis
PCR

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Abstract

This study showed that total percentage of infection with *Theileria* species in 100 blood samples of cattle in Mosul City was 38% with no significant differences between male and female of cattle. Cattle older than 1 year old had a high percentage of *Thieleria* infection 40% compared to younger cattle 34.28%, with no significant variations in *Thieleria* infection and age groups of cattle. Theileria measurement in red blood cells ranged from 0.5 to 2 microns, while parasitemia levels ranged from 0.2 to 15%, with an average of 6.572%. The results of molecular diagnosis confirmed the detection *Theileria* genus, *Theileria annulata*, and *Theileria parva in blood samples of examined cattle* and PCR revealed that the amplification products were 237bp, 690bp, and 700bp, respectively. In this study, we recorded new strains of *Theileria* in cattle of the Nineveh governorate by using the BLAST program consisting of three strains of *T. annulata*, one strain of *Theileria ovis* and two strains of *Theileria parva which* differs from the normal isolates in many nucleotides in different worlds. The phylogenetic tree showed the relative relation among the *Theileria spp*, the results matching species between 8 and 11, 1 and 4, 6 and 10, 3 and 9, and 2 and 12; the species 7 were more closely linked to 11 and 8, the species five similar to 6 and 10.

DOI: 10.33899/ijvs.2023.141344.3112,

Introduction

Regarding meat and milk production, cattle and buffaloes are among the vital components of the livestock industry. Numerous infections that endanger these animals' health are exposed to them, including theileriosis (1-4). *Thieleria* is a genus of intracellular protozoan parasites in agricultural animals worldwide, particularly in the Middle East (5). Both domestic and wild cattle are affected by this substance. East Coast Fever (ECF) and Tropical Theileriosis (TT) are caused by the two most significant and pathogenic species, *T. parva* and *T. annulata* respectively (6). *Thielaria* can be transmitted trans-stadially by the tick vectors, mechanically through routine rearing practices, Rhipicephalus *appendiculatus*, a hard tick's vector for *Theileria parva*, and *Hyalomma* ticks, a vector for *Theileria annulata*. Both disseminate Theileria species (7,8). Pasture tick infestations can last up to two years depending on the weather. Skin injury and blood loss are the results. Meanwhile, the parasite prevents the animal from receiving nutrients by infecting the red blood cells of vertebrates, including pets (9). Theileriosis in cattle typically manifests as pale mucous membranes, depression, lethargy, lack of appetite, fever, widespread lymphadenopathy, anorexia, loss of condition, collapse, and, in some cases, death if the animal is made to move or run. Pregnant cows frequently experience miscarriages and stillbirths, or their milk supply may decrease while their somatic cell count rises in dairy cows (9). *Thielieria* and *Babesia* are closely related, However *Theileria* differs from them in that it has a leukocyte developmental stage profile before infecting erythrocytes (10). *Thieleria* infection decreases meat and milk production, causes several problems, and may increase mortality rates from an economic standpoint (11,12). For instance, 800 million dollars was expected to be lost annually in India's economy (9). The definitive diagnosis is reached by combining of appropriate clinical signs and lab examinations. *Theileria* parasites found in blood smears and a needle biopsy of a lymph node stained with the Giemsa stain are typically used to diagnose. Since most *Theileria* form piroplasm is

physically identical and schizonts are not always present in the superficial lymph nodes during illness cycles, type-specific detection is challenging. Sometimes used to detect *Theileria spp*., PCR assays plus a DNA probe are highly sensitive for detecting even low infection levels (13-15).

The study aimed to use microscopic and molecular diagnosis (Polymerase Chain Reaction, Sequencing, Phylogenic study) to know *Thieleria* species affecting cattle in order to control the disease due to the lack of studies on bovine *Theileria* parasite in Nineveh Governorate, as well as to find out, record the species of the genus, that microscopic differentiation is difficult between species, as well as with other species of genus *Babesia*.

Materials and methods

Ethical approve

This study has been approved by the scientific committee of department of Microbiology, Collage of Veterinary Medicine, University of Mosul at the first congress, dated 4/10/2021.

Blood samples collection

From cattle showing signs of theileriosis in various locations throughout the Nineveh Governorate, 100 blood samples were randomly selected from both sexes and different age groups. Blood samples were drawn from the jugular vein using sterile syringes and 70% ethyl alcohol to sterilize the region. The blood samples were kept in EDTA-containing tubes, and each sample's collection date, age, and sex were noted. The samples were then brought to the College of Veterinary Medicine/University of Mosul's Parasitological Laboratory for a laboratory analysis.

Microscopic examination

Thin blood smears were prepared and stained with Giemsa stain at a concentration of 5% for 30 to 60 minutes and were viewed under a light microscope. Thin blood smears were utilized to determine the shape and measurement requirements for laboratory evaluation to diagnose the *Theileria* parasite in cattle (16). The following formula was used to get the parasitism percentage: Number of affected RBCs/numbers of calculated RBCs*100.

DNA extraction from blood

DNA was extracted from blood samples containing *Thailaria* using a DNA extraction kit (Qiagen). In order to rehydrate the DNA pellet, 100µ l of rehydration solution was added, and it was then stored at -20°C until the next test.

Polymerase chain reaction (PCR)

PCR was done to confirm the diagnosis of *Theileria spp*. by using the primers (Table 1).

The PCR reaction mixtures were created in 20 µl containers with 10 µl of Master mix (Promega 2X), 1 µl of each primer, 4 µl of DNA template, and 4 µl of PCR-grade water. The multiplication reaction was performed using the custom program, as stated in table 2, after the PCR was completed using a thermocycler (Optimum 96 G Germany).

Table 1: Types of primers and sequences of nucleotides of primers used for diagnosis of *Theileria genus*, *Theileria annulata and Theileria parva* by using PCR technique

Primer	Sequence	Target gene	Reference
Theileria genus 18s rRNA-F	GGTAATTCCAGCTCCAATAG	18s rRNA	17
Theileria genus 18s rRNA-R	ACCACCAAAATAGAACCAAAGTC		
Theileria.annulata-F	GTAACCTTTAAAAACGT	Major merozoite surface antigen DNA	13
Theileria annulata-R	GTTACGAACATGGGTTT		
Theileria parva-F	ATTTAAGGAACCTGACGTGACTGC	Bovine cytochrome b gene	18
Theileria parva-R	TAAGATGCCGACTATTAATGACACC		

Table 2: Steps of a conventional PCR program

Stage	ºC	Time
Initial denaturation	95	6 min.
Denaturation	95	45 sec.
Annealing	55	1.0 min.
Extension	72	1.0 min.
Final extension	72	5 min.

Electrophoresis separated the amplified products on 2% agarose gel in a 4 µl red safe. Each PCR result was placed into the agarose gel's well in a 4µl volume. The electrophoresis was performed using a power supply with 1X TBE buffer at 60 V for 45 minutes. The typical molecular marker was a 100 bp DNA marker (Biolaps), and 4µl of the gel was looked at using UV light (Gel Do cumintation).

Determination the nucleotide

Theileria's nitrogenous base sequences were performed by the Hitachi Genetic Analyzer 3130 (Japan) and matched with NCBI using the BLAST tool for 12 positive samples.

Results

Giemsa staining 100 blood smears of cattle, and 38 (38%) tested positive for *Theileria* species (Table 3). Males exhibit

significantly higher positive blood test results for *Theileria* than females (40 versus 35), with no discernible variations between the sexes (Table 4). *Theileria spp*. infection in the same litters did not significantly differ between males and females. Cattle older than one year old had a steady percentage of *Thieleria* infection 40% compared to younger cattle 34.28%, with no discernible variations in *Thieleria* infection and age groups of cattle (Table 5).

Table 3: Number of cattle investigated, quantity of cattle infected with *Theileria* species, and proportion of infection determined by Giemsa stain

Examined (n)	Infected (n)	% Infection
100	38	38

Table 4: Interdependence of infection with *Theileria* and sex of animals

Sex	Examined (n)	Infected [n(%)]
Female	40	14(35)a
Male	60	24(40)a
Total	100	38

Similar letters mean that there is no significant difference in the percentage of infection with *Theileria* and the sex of the cattle.

Table 5: Correspondence of infection with *Theileria* and age of animals

Age of animals	Examined (n)	Infected [n(%)]
Less one years	35	12(34.28)a
1-2 years	40	16(40)a
> 2years	25	10(40)a
Total	100	38

Similar letters mean that there is no significant difference in the percentage of infection with *Theileria* and the age of the cattle.

Blood smear microscopy analysis revealed the presence of intra-erythrocytic forms of *Theileria* species, including rod, round, oval, comma, ring- shaped, and organisms that resemble *Anaplasma* (Figure 1). Theileria's length in red blood cells ranged from 0.5 to 2 microns, while parasitemia levels ranged from 0.2 to 15%, with an average of 6.572%.

The bands of DNA extracted from positive blood samples for *Theileria* species in a 25 ng/µl concentration. The concentration of extracted DNA was 50- 100 ng with a purity of 1.7. The polymerase chain reaction results suggested that the DNA samples that were extracted and used in this reaction might be used to diagnose *Theileria* genus, *Theileria annulata*, and *Theileria parva*. PCR revealed that the amplification products were 237bp, 690bp, and 700bp, respectively (Figures 2-4). Ten *Theileria annulata* isolates and one *Theileria ovis* isolate's genetic sequences were documented. According to table 6, the International Genbank's website provided the sequence numbers (Table 6).

The six new isolates differ from the normal isolate in many nucleotides in different worlds, according to the sequencing results, and the new isolates of *Theileria* in cattle of the Nineveh governorate were reported using the BLAST tool consisting of three isolates of *Theileria annulata*, one isolate of *Theileria ovis* and two isolates of *Theileria parva* (Table 7).

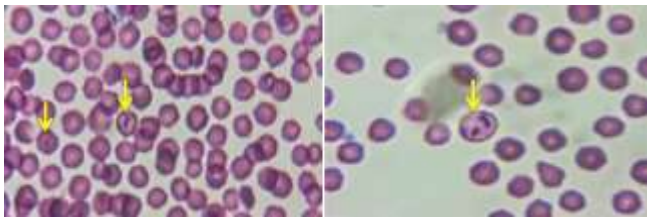

Figure 1: *Theileria spp*. were found in intra-erythrocytic (rod-shaped, circular, comma-shaped) in thin blood smears (Giemsa-stained 100X by using a digital camera).

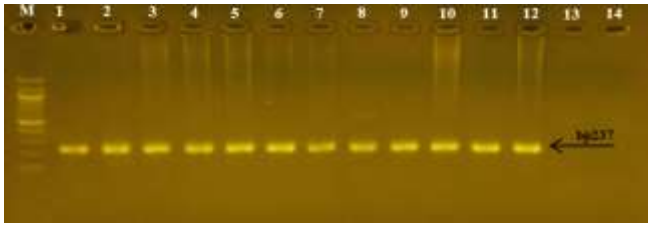

Figure 2: PCR reaction of *Theileria* genus with a reaction product of 237 bp.

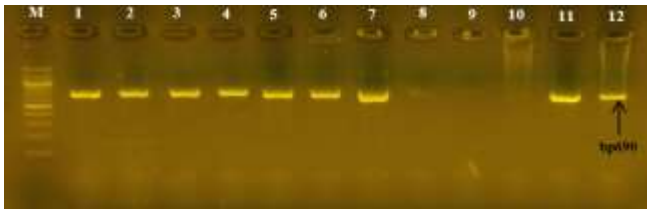

Figure 3: The PCR reaction of the parasite, *Theileria annulata*, with a reaction product of 690 bp.

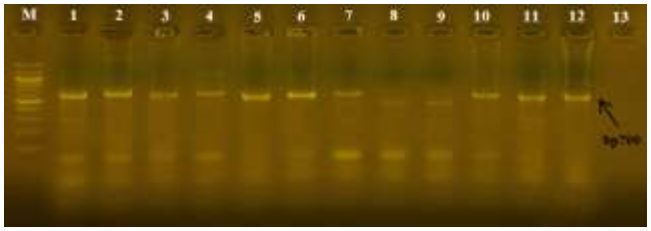

Figure 4: The PCR reaction of the, *Theileria prava*, with a reaction product of 700 bp.

Table 6: Determination of genetic diversity of *Theileria spp.* Parasite identified in bovine blood samples

Name of gene	Genetic diversity	nucleotides	GenBank number
Theileria annulata clone Gxyl small subunit ribosomal RNA gene, partial sequence.	TTCTGCTGCATTGCTTGTGTCCCTCTGGGGTCTGNGCATGTGGC TTTTTTCGGACGGAGTTTCTTTGTCTGAATGTTTACTTTGAGAA AATTAGAGTGCTCAAAGCAGGCTTTCGCCTTGAATAGTTTAGC ATGGAATAATAAAGTAGGACTTTGGTTCTATTTTGGTGGTA	430	MK089831.1
Theileria annulata isolates IQ-Camel NO.10 small subunit ribosomal RNA gene,partial sequence	CTGCTGCATTGCTTGTGTCCCTCTGGGNNNNNNNNATGTGGCT TTTTTCGGACGGAGTTTCTTTGTCTGAATGTTTACTTTGAGAAA ATTAGAGTGCTCAAAGCAGGCTTTCGCCTTGAATAGTTTAGCA TGGAATAATAAAGTAGGACTTTGGTTCTATTTTGGTGGTANAA TAATGGAATAGGACTTTGNTTCTATTTTGGTG	461	MT491139.1
Theileria annulata isolate IQ-camel No.7 small ribosomal RNA gene - partial sequence	TTCTGCTGCATTGCTTGTGTCCCTCTGGGGTCTGTGCATGTGGC TTTTTTCGGACGGAGTTTCTTTGTCTGAATGTTTACTTTGAGAA AATTAGAGTGCTCAAAGCAGGCTTTCGCCTTGAATAGTTTAGC ATGGAATAATAAAGTAGGACTTTGGTTCTATTTTGG TGGTA	455	MT491136.1
Theileria annulata isolates IQ-camel No.5 small subunit ribosomal RNA gene- partial sequence	TTGNNTTTCTGCTGCATTGCTTGTGTCCCTCTGGGGTCTGTGCA TGTGGCTTTTTTCGGACGGAGTTTCTTTGTCTGAATGTTTACTT TGAGAAAATTAGAGTGCTCANNNNNGGNTTNCNCCTNNNANA NTTNANNNNGNAATAATAAAGNAGGACTTTGGTTCTNTTTTGG TGGTANN	461	MT491134.1
Theileria annulata isolates IQ-camel No.4 small subunit ribosomal RNA gen -partial sequence	TTGNNTTTCTGCTGCATTGCTTGTGTCCCTCTGGGGTCTGTGCA TGTGGCTTTTTTCGGACGGAGTTTCTTTGTCTGAATGTTTACTT TGAGAAAATTAGAGTGCTCAAAGCAGGCTTTCGCCTTGAATAG TTTAGCATGGAATAATAAAGTAGGACTTTGGTTCTATTTTGGT GGTA	439	MT491133.1
Theileria annulata isolates IQ-camel No.2 small subunit ribosomal RNA gene-partial sequence	GCTCGTANTTGNATTTCTGCTGCATTGCTTGTGTCCCTCTGGGG TCTGNGCATGTGGCTTTTTTCGGACGGAGTTTCTTTGTCTGAAT GTTTACTTTGAGAAAATTAGAGTGCTCAAAGCAGGCTTTCGCC TTGAATAGTTTAGCATGGAATAATAAAGTAGGACTTTGGTTCT ATTTTGGTGNN	468	MT491131.1
Theileria annulata small subunit ribosomal RNA gene -partial sequence	GCTCGNAGTTGNNTTTCTGCTGCATTGCTTTTGCTCCTTTACGA GNCTTTGCATTGTGGCTTATTTCGGACTTTGTTTTACAATGTCC GGATGTTTACTTTGAGAAAATTAGAGTGCTCAAAGCAGGCTTT CGCCTTGAATAGTTTAGCATGGAATAATAAAGTAGGACTTTGG TTCTATTTTGGTGGTANN	1679	MT26171.1
Theileria annulata small subunit ribosomal RNA gene -partial sequence	GCTCGTANTTGNATTTCTGCTGCATTGCTTGTGTCCCTCTGGGG TCTGNGCATGTGGCTTTTTTCGGACGGAGTTTCTTTGTCTGAAT GTTTACTTTGAGAAAATTAGAGTGCTCAAAGCAGGCTTTCGCC TTGAATAGTTTAGCATGGAATAATAAAGTAGGACTTTGGTTCT ATTTTGGTGNNNNN	1679	MT341858.1
Theileria ovis 18S ribosomal RNA gene,complete sequence	GCTCGNAGTTGNNTTTCTGCTGCATTGCTTTTGCTCCTTTACGA GNCTTTGCATTGTGGCTTATTTCGGACTTTGTTTTACAATGTCC GGATGTTTACTTTGAGAAAATTAGAGTGCTCAAAGCAGGCTTT CGCCTTGAATAGTTTAGCATGGAATAATAAAGTAGGACTTTGG TTCTATTTTGGTGGTANNN	1764	MT260171.1
Theileria annulata isolate T178 small subunit ribosomal RNA gene - partial sequence	TTGNATTTCTGCTGCATTGCTTGTGTCCCTCTGGGGTCTGTGCA TGTGGCTTTTTTCGGACGGAGTTTCTTTGTCTGAATGTTTACTT TGAGAAAATTAGAGTGCTCAAAGCAGGCTTTCGCCTTGAATAG TTTAGCATGGAATAATAAAGTAGGACTTTGGTTCTATTTTGGT GGTANN	1056	MT31816.2
Theileria annulata isolate T179 small subunit ribosomal RNA gene - partial sequence	GCTCGTANTTGNATTTCTGCTGCATTGCTTGTGTCCCTCTGGGG TCTGTGCATGTGGCTTTTTTCGGACGGAGTTTCTTTGTCTGAAT GTTTACTTTGAGAAAATTAGAGTGCTCAAAGCAGGCTTTCGCC TTGAATAGTTTAGCATGGAATAATAAAGTAGGACTTTGGTTCT ATTTTGGTGNN	1056	MT318159.1

Table 7: Thieleria new isolates from cattle blood samples diagnosed by (NCBI) according to the BLAST program

No. of *Thieleria* isolate in NCBI	Name of isolate
LC714837.1	*Theileria annulata* EM1 gene for 18S ribosomal RNA, partial sequence
LC714839.1	*Theileria annulata* EM3 gene for 18S ribosomal RNA, partial sequence
LC714842.1	*Theileria ovis* EM6 gene for 18S ribosomal RNA, partial= sequence
LC714838.1	*Theileria annulata* EM2 gene for hypothetical protein, partial sequence GenBank
LC714841.1	*Theileria parva* EM5 gene for 18S ribosomal RNA, partial sequence:
LC211084.1	*Theilaria parva* Ahlam-E-H gene for SSUrRNA, partial sequence

The phylogenetic tree showed the relative relation among the twelve *Theileria isolates* from cattle, the results matching species between *Theileria annulata* (8 and 11), *Theileria annulata* (1 and 4), *Theileria annulata* (6 and 10), *Theileria annulata* (3 and 9), and *Theileria annulata* 2 and *Theileria ovis* 12, the isolate Theileria annulata 7 were more closely linked to isolate *Theileria annulata* 11 and 8,the isolate *Theileria annulata* five similar to isolate *Theileria annulata* (6 and 10) (Figure 5).

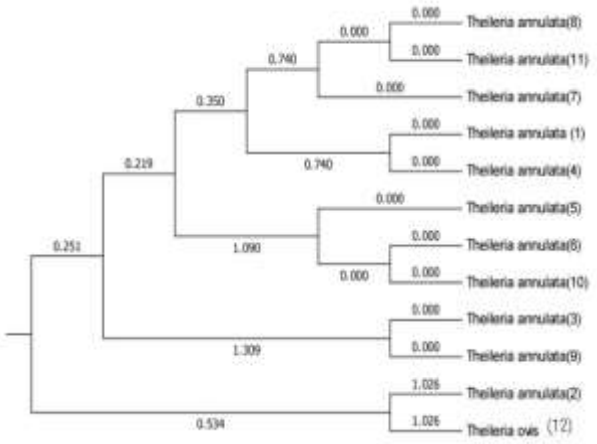

Figure 5: Phylogenetic tree analysis from *Theileria spp.*

Discussion

Although the clinical indicators and blood smear examination are frequently accurate in detecting parasitic infection, the disease cannot be accurately diagnosed with these techniques due to their insufficient sensitivity. Using Giemsa-stained blood smears, the current investigation found that 38% of the investigated animals were positive for the *Theileria* species. This result was in accordance with Kundave *et al.* (19), who discovered that the percentage of *Theileria* infection by utilizing Giemsa-stained thin blood smear was 30.98%, while Yaghfoori and Gh (8), El-Dakhly *et al.* (20), Rad *et al.* (21) and Tookhy *et al.* (22) recorded lower percentages of infection by Giemsa staining method, namely 22%,12.93%,9.31%, and 3.75%. Another study stated that 4% and 37% of cattle in the Herat region had *Theileria spp*. (23). Higher infection rates may be connected to early acute stage microscopic examination making it simple to find both intracellular piroplasms and intralymphatic Koch's blue bodies (21,24).

In our study, the proportion of *Theileria* infection appeared to be high prevalence in cattle aged 1-2 years and cattle aged two years was 40%. This result was consistent with findings from studies (20,25), which found that *T. annulata* infection was more common in adult cattle, while Yaghfoori and Gh (8), and Durrani (26) were concerned that the high occurrence of *Theileria* was seen in animals under a year old, these findings conflicted with them. The physiological aspects, such as oestrus, were disclosed by Durrani (26) and Morzaria (27). Additionally, the antibodies to *Theileria* that protect the calves against *Theileria* infection may be the reason for the low incidence of disease in calves under one year old (28) due to pregnancy and lactation's temporary reduction of immunity and the increase in the percentage of infection in adult cattle.

A more significant percentage of *Theileria* species infection was identified in the current study in female cattle than in male cattle, which was in keeping with Ayadi *et al.* (14), Inci *et al.* (29) and Kamani *et al.* (30). According toAl-Saeed *et al.* (13), females have higher hormonal swings and a relatively weaker immune system, which enhances the likelihood of disease. Additionally, because female cattle are kept for various functions, including reproduction and milk production, and because they spend much time in the meadows, Kamani *et al.* (30) found that tick-borne diseases in female cattle occur at a high incidence.

Theileria annulata and *Theileria parva* were diagnosed using primers developed based on Al-Saeed *et al.* (13), Odongo *et al.* (17), and Al-Hosary *et al.* (18) which yielded a base pair of 237, 690, and 700. This study's findings in diagnosing the genus *Theileria*, *Theileria annulata,* and *Theileria parva* coincided with the investigations of Lempercur *et al.* (5), Yaghfoori and Gh(8), d'Oliveira *et al.* (12), Silatsa *et al.* (31), Khatoon *et al.* (32) and Al-Shabbani and Alfatlawi (33). PCR technique is one of the most sensitive and specific methods for determining the pathogens (34-36) and using microscopic analysis of blood smears stained with Giemsa, is not sufficient for detecting species of *Theileria* that are challenging to discriminate and define the species of *Theileria* (37-39).

Phylogenetic research helps establish the basis for genetic differences and evolutionary relationships between

parasite species. Genetic diversity in parasites is crucial for establishing control strategies like medication treatment and immunization and parasite species (40). Each species might have a unique nucleotide, because the environment impacts it. As a result, the species can adapt to shifting environmental factors. The sequencing results demonstrated the discovery of new isolates, detected for the first time and differ from typical isolates in many nucleotides. The organisms adjust to this situation by mutating to build new genes for proteins, which helps them endure the harsh conditions and prepare for development. The nucleotides change may be caused by environmental causes. When an organism is isolated from its environment, it often responds to the conditions. In these cases, the organism may undergo mutations that affect one or more nucleotides, depending on the triple sequence of the protein molecule, or that affect amino acids with more than one code.

Conclusion

PCR technique is one of the most sensitive and specific methods for determining the *Theileria* and using microscopic analysis of blood smears stained with Giemsa, is not sufficient for detecting species of *Theileria* that are challenging to discriminate and define the species of *Theileria*. In this study new isolates of *Theileria* in cattle of the Nineveh governorate were reported by using the BLAST tool consisting of three isolates of *Theileria annulata*, one isolate of *Theileria ovis* and two isolates of *Theileria parva*

Acknowledgments

The authors like to thank department of microbiology, college of Veterinary Medicine, University of Mosul for their effort and support given to the current study.

Conflict of interest

The authors confirm no conflicts of interest in the publication of this paper.

Reference

1. Wisam S, Sadam DH, Karam MH, Al-Obaidi QT. Serological detection of the latent infection of Brucellosis in calves in Mosul city, Iraq. Iraqi J Vet Sci. 2022;36(I):7-10. DOI: 10.33899/ijvs.2022.134936.2421
2. Ali WH, Dahl OM. Comparison of California mastitis test and Draminski mastitis detector as on-farm methods for monitoring udder health in lactating buffalo. Iraqi J Vet Sci. 2022;36(I):55-60. DOI: 10.33899/ijvs.2022.135362.2469
3. Al-Alo ZK, Mohammed JA. A cross sectional study on the seroprevalence of bovine Brucellosis in Al-Najaf province in Iraq. Iraqi J Vet Sci. 2021;35(4):617-620. DOI: 10.33899/ijvs.2021.127143.1471
4. Ridhae KM, Hussein SA. Serological and molecular investigations of Brucellosis in dairy cows at certain areas of Al-Sulaymaniyah governorate- Iraq. Iraqi J Vet Sci. 2021;35(4):657-662. DOI: 10.33899/ijvs.2021.127688.1520
5. Lempercur L, Beck R, Fonseca I, Marques C, Duarte A, Santos M, Zuquete S, Gomes J, Walder G, Domingos A, Antunes S, Baneth G, Holman P, Zintl A. Guidelines for the detection of Babesia and Theileria. Vector Borne Zoonotic Dis. 2017;17(1):51-65. DOI: 10.1089/vbz.2016.1955
6. Brown CD. Dynamics and impact of tick-borne diseases of cattle. Trop Anim Health Prod. 1997;29:15-35. DOI: 10.1007/bf02632905
7. Nayel M, El-Dakhly KM, Aboulaila M, Elsify A, Hassan H, Ibrahim E, Salama A, Yanai T. The use of different diagnostic tool for Babesia and Theileria in parasites in cattle in Menofia, Egypt. Parasitol Res. 2012;111:1019-1024. DOI: 10.1007/s00436-012-2926-6
8. Yaghfoori S, Gh R. Molecular detection of *Theileria annulata* among dairy cattle and vector ticks in the Herat area, Afghanistan. Arch Razi Inst. 2021;76(1):79. DOI: 10.22092/ari.2019.128212.1407
9. Wamuyu L, Obanda V, Kariuki D, Gakuya F, Makanda M, Otienda M, Ommeh S. Molecular detection and characterization of Theileria infecting wildebeest (*Connochaetes. taurinus*) in the Massai Mara national reserve, Kenya. Pathogens. 2015;4:626-638. DOI: 10.3390/pathogens4030626
10. Hunfeld K, Hildebrandt A, Gray J. Babesiosis: Recent insights into an ancient disease. Int J Parasitol. 2008;38:1219-1237. DOI: 10.1016/j.ijpara.2008.03.001
11. Singh NK, Singh H, Bhat JA, Rath ST. Prevalence of *Theileria annulata* infection in *Hyalomma anatolicum anatolicum* collected from crossbred cattle of Ludhiana Punjab. J Parasitol Dis. 2015;39(1):57-61. DOI: 10.1007/s12639-013-0279-4
12. d'Oliveira C, Van Der Weide M, Habela MA, Jacquiet P, Jongejan F. Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. J Clin Microbiol. 1995;13:2665-2669. DOI: 10.1128/jcm.33.10.2665-2669.1995
13. Al-Saeed AM, Omer LT, Abdo J, Habibi G, Salih DA, Seitzer U, Ahmed J. Epidemiological studies on tropical theileriosis (*Theileria annulata* infection of cattle) in Kurdistan region, Iraq. Parasitol Res. 2010;106:403-407. DOI: 10.1007/s00436-009-1675-7
14. Ayadi O, Rjeibi MR, Elfegoun MC, Gharbi M. Prevalence and risk factors of tropical theileriosis, and sequencing of *Teileria annulata*, the causative pathogen in Setif region (Algeria) before and after tick season. Rev Elev Med Vet Pays Trop. 2016;69(4):161-6. DOI: 10.19182/remvt.31201
15. Altay K, Aydin MF, Dumanli N, Aktas M. Molecular detection of the Theileria and Babesia infections in cattle. Vet Parasitol. 2008;158(4):295-301. DOI: 10.1016/j.vetpar.2008.09.025
16. Sibeko KP, Oosthuizen MC, Collins NE, Geysen D, Rambritch NE, Latif AA, Groeneveld HT, Potgieter FT, Coetzer JA. Development and evaluation of a real-time polymerase chain reaction test for the detection of *Theileria parva* infections in Cape buffalo (*Syncerus caffer*) and cattle. Vet Parasitol. 2008;155(1-2):37-48. DOI: 10.1016/j.vetpar.2008.03.033
17. Odongo DO, Sunter JD, Kiara HK, Skilton RA, Bishop RP. A nested PCR assay exhibits enhanced sensitivity for detection of *Theileria parva* infections in bovine blood samples from carrier animals. Parasitol Res. 2010;106:357-65. DOI: 10.1007/s00436-009-160-2
18. Al-Hosary AT, Ahmed J, Nordgrahn A, Merza M. Assessment of the first commercial ELISA kit for the diagnosis of *Theileria annulate*. J Parasitol Res. 2015;2015:1-4. DOI: 10.1155/2015/787812
19. Kundave VR, Patel AK, Patel PV, Hasnani JJ, Joshi CG. Detection of theileriosis in cattle and buffaloes by polymerase chain reaction. J Parasit Dis. 2015;39:508-13. DOI: 10.1007/s12639-013-0386-2
20. El-Dakhly KM, Arafa W, Ghanem SS, Abdel-Fatah OR, Wahba AA. Microscopic and molecular detection of *Theileria annulata* infection of cattle in Egypt. J Adv Parasitol. 2018;5(2):29-34. DOI: 10.17582/journal.jap/2018/ 5.2.29.34
21. Rad NH, Ghaemi P, Shayan Eckert B Shirazi NS. Detection of native carrier infected with *Theileria annulata* by semi nested PCR and smear method in Golestan province of Iran. World App Sci J. 2011;12(3):317-323. [available at]
22. Tookhy NA, Fazly MJ, Shalshes S, Mohmand NA. Prevalence study of bovine theileriosis in Herat province, Afghanistan. J Agric Vet Sci. 2018;11:7-9. [available at]

23. Muslih NJ, Zangana IK, Arsalan SH. Incidence of various clinical diseases. in sheep and goats in north Iraq (Mosul). Int J Anim Sci. 1988;3:157-163. [available at]
24. Ananda KJ, D'Souza PE, Puttalakshmamma GC. Prevalence of Haemoprotozoan diseases in crossbred cattle in Banglore north. Vet World. 2009;2(1):15. DOI: 10.5455/vetworld.2009.15-16
25. Razmi G, Yaghfoori S. Molecular surveillance of *Theileria ovis*, *Theileria lestoquardi* and *Theileria annulata* infection in sheep and ixodid ticks in Iran. Ondersteport J Vet Res. 2013;80:01-05. DOI: 10.4102/ojvr.v80i1.635
26. Durrani AZ. Epidemiology, serodiagnosis and chemoprophylaxis of theileriosis in cattle [Ph.D. dissertation]. Pakistan: University of Veterinary and Animal Sciences, Lahore; 2003.
27. Morzaria SP, Musoke AJ, Latif AA. Recognition of *Theileria parva* antigens by field sera from Rusinga Island Kenya. Kenya Vet. 1988;12(2):88-90. [available at]
28. Parveen A, Alkhaibari AM, Asif M, Almohammed HI, Naqvi Z, Khan A, Aktas M, Ozubek S, Farooq M, Iqbal F. Molecular epidemiology of *Theileria annulata* in cattle from two districts in Punjab (Pakistan). Anim. 2021;11(12):3443. DOI: 10.3390/ani11123443
29. Inci A, Ica A, Yildirim A, Vatansever Z, Çakmak A, Albasan H, Cam Y, Atasever A, Duezlue O. Epidemiology of tropical theileriosis in the Cappadocia region. Turk J Vet Anim Sci. 2008;32(1):57-64. [available at]
30. Kamani J, Sannusi A, Egwu OK, Dogo GI, Tanko TJ, Kemza S, Tafarki AE, Gbise DS. Prevalence and significance of haemoparasitic infections of cattle in north- central, Nigeria. Vet World. 2010;3(9):445-448. DOI: 10.5455/vetworld.2010.445-448
31. Silatsa BA, Simo G, Githaka N, Kamga R, Oumarou F, Keambou Tiambo C, Machuka E, Domelevo JB, Odongo D, Bishop R, Kuiate JR. First detection of *Theileria parva* in cattle from Cameroon in the absence of the main tick vector *Rhipicephalus appendiculatus*. Transbound Emerg Dis. 2020;67:68-78. DOI: 10.1111/tbed.13425
32. Khatoon S, Kolte SW, Kurkure NV, Chopde NA, Jahan A. Detection of tropical bovine theileriosis by polymerase chain reaction in cattle. J Parasit Dis. 2015;39:53-6. DOI: 10.1007/s12639-013-0270-0
33. Al-Shabbani AH, Alfatlawi MA. Morphological and genotypical identification of *Hyalomma anatolicum* isolated from cattle in Al-Daghara city, Al-Qadisiyah province, Iraq. Iraqi J Vet Sci. 2023;37(30):683-687. DOI: 10.33899/IJVS.2023.137021.2630
34. Abdelmonem GJ, Amer AM, Hussein EA, Aboezz ZR, Habashi AR, Sharawi SS. Assessment of multiplex PCR for detection of FMDV, BVDV, BTV, and possible coinfection with *Pasteurella multocida* in cattle. Iraqi J Vet Sci. 2022;36(4):1053-9. DOI: 10.33899/ijvs.2022.132983.2158
35. Sayhood MH, Mohammed AL, Abdulhameed MF, Jori MM. Classical and molecular identification of *Staphylococcus aureus* isolated from infestation cattle wounds with myiasis in Basrah governorate, Iraq. Iraqi J Vet Sci. 2022;36(3):641-646. DOI: 10.33899/ijvs.2021.131196.1930
36. Bogema DR, Deutscher AT, Fell S, Collins D, Eamens GJ, Jenkins C. Development and validation of a quantitative PCR assay using multiplexed hydrolysis probes for detection and quantification of *Theileria orientalis* isolates and differentiation of clinically relevant subtypes. J Clin Microbiol. 2015;53:941–950. DOI: 10.1128/JCM.03387-14
37. Pulford DJ, Gias E, Bueno IM, McFadden. Developing high throughput quantitative PCR assays for diagnosing Ikeda and other *Theileria orientalis* types common to New Zealand in bovine blood samples. N Z Vet J. 2016;64(1):29-37. DOI: 10.1080/00480169.2015.1089798
38. Perera PK, Gasser RB, Pulford DJ, Stevenson MA, Firestone SM, McFadden AM, Jabbar A. Comparison of the performance of three PCR assays for the detection and differentiation of *Theileria orientalis* genotypes. Parasit Vectors. 2015;31(8):192. DOI: 10.1186/s13071-015-0812-7
39. Bilgiç HB, Karagenç T, Simuunza M, Shiels B, Tait A, Eren H, Weir W. Development of a multiplex PCR assay for simultaneous detection of *Theileria annulata*, *Babesia bovis* and *Anaplasma marginale* in cattle. Exp Parasitol. 2013;133(2):222-9. DOI: 10.1016/j.exppara.2012.11.005
40. Alanazi AD, Alouffi AS, Alshahrani MY, Alyousif MS, Abdullah HH, Allam AM, Elsawy BS, Abdel-Shafy S, Alsulami MN, Khan A, Iqbal F. A report on tick burden and molecular detection of tick-borne pathogens in cattle blood samples collected from four regions in Saudi Arabia. Ticks Tick Borne Dis. 2021;12(3):101652. DOI: 10.1016/j.ttbdis.2021.101652

التحديد الجزيئي لأنواع الثايليريا في الأبقار في مدينة الموصل

هيثم صديق البكري وإيمان غانم سليمان و أحلام فتحي الطائي

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الخلاصة

أظهرت نتيجة هذه الدراسة أن النسبة الكلية للإصابة بأنواع الثيليريا في ١٠٠ عينة دم من الأبقار في مدينة الموصل كانت ٣٨٪ مع عدم وجود فروق معنوية بين الذكور والإناث. الأبقار التي يزيد عمرها عن سنة واحدة لديها نسبة اعلى من الاصابة بالثيليريا ٤٠٪ مقارنة الأصغر سنًا (٣٤,٢٨ ٪، مع عدم وجود اختلافات كبيرة في الاصابة بالثيليريا والفئات العمرية للأبقار. تراوحت قياسات الثيليريا في خلايا الدم الحمراء بين ٠,٥-٢ ميكرون، بينما تراوحت مستويات التطفل في الدم بين ٠,٢-١٥٪ بمعدل ٦,٥٧٢٪. أكدت نتائج التشخيص الجزيئي تشخيص كل جنس الثيليريا والثيليريا الحلقية والثيليريا بارفا في عينات دم الأبقار المفحوصة إذ بلغت نتائج التضخيم باستخدام تقنية تفاعل البلمرة المتسلسل ٢٣٧ زوجا قاعديا و ٦٩٠ زوجا قاعديا و ٧٠٠ زوجا قاعديا على التوالي. في هذه الدراسة سجلت سلالات جديدة من الثيليريا في أبقار محافظة نينوى باستخدام برنامج تقصي الترتب الموضعي الأساسي وهي (ثلاث سلالات من الثيليريا الحلقية وسلالة واحدة من ثيليريا الأغنام وسلالتان من ثيليريا بارفا والتي تختلف عن العزلات الطبيعية الأخرى في العديد من النيوكليوتيدات في مختلف دول العالم. أظهرت شجرة النشوء والتطور العلاقة النسبية بين أنواع الثيليرية المشخصة إذ ظهر تطابق بين ٨ و ١١ ، ١ و٤ ، ٦ و١٠ ، ٣ و ٩ ، ٢ و ١٢ ؛ كانت الأنواع ٧ أكثر ارتباطًا بالأنواع ١١ و ٨ ، والأنواع ٥ تشبه ٦ و ١٠.