Annals of R.S.C.B., ISSN:1583-6258, Vol. 27, Issue 1, 2023, Pages. 772-783
Received 05-October-2023; Accepted 01 December 2023

Molecular Phylogenetic Analysis of *Theileria Annulata* in Hard Ticks of Cattle

Amal Hassan Abed [1]*, Monyer Abdulamier Abd [2]
[1, 2] Department of Veterinary Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq
Emails: amal.abd@qu.edu.iq [1], monyerr.abd@qu.edu.iq [2]

Abstract
The current study was aimed to detect the prevalence *T. annulata* in bovine ticks molecularly using the polymerase chain reaction (PCR) assay, with phylogenetic analysis of local strains to estimate their identity with the global NCBI-GenBank strains. A total of 109 cattle existed at different areas in Al-Qadisiyah province (Iraq) were subjected to clinical examination to detect and collection of ticks during December (2021) to August (2022). An overall 50 samples of tick were subjected for molecular examination using the conventional PCR assay. Targeting the *Cytochrom b* gene, a total of 26% positive samples for *T. annulata* were detected in study ticks using the conventional PCR assay. The genomic DNAs of 10 positive samples were subjected for phylogenetic analysis based on the primer of *Cytochrom b* gene. However, sequencing findings for *T. annulata* isolates were named as *T. annulata* voucher IQ1-10, and recorded in NCBI under an accession numbers of OR125071, OR125072, OR125073, OR125074, OR125075, OR125076, OR125077, OR125078, OR125079 and OR125080. Comparative analysis for *Cytochrom b*-nucleotides sequence of study samples with the numbers of *T. annulata* isolates existed in GenBank database was constructed, and the results showed nucleotide alignment similarities and substitution mutations in *Cytochrom b* gene. Comparative identity between the local *T. annulata* IQ-isolates and NCBI-BLAST *T. annulata* of *Cytochrom b* gene revealed a significant association with Tunisian isolates at 98% similarity and 0.0-0.2% total genetic changes. In conclusion, the molecular diagnosis of *T. annulata* in the prevalent tick species of the region has demonstrated the prevalence of bovine *Theileria* parasites in ticks, and the risk of cattle to contract theileriosis. Although only a limited number of ticks were studied, these preliminary data suggest that specific parasite genotypes may be selected during tick transmission from a carrier animal. Given its enormous burden on livestock farming in Iraq, further investigations are needed to better understand its epidemiology, vector transmission and potential clinical significance in cattle from neighboring countries.
Keywords: *T. annulata*, Bovine theileriosis, PCR, Sequencing, *Cytochrom b*, Iraq

Introduction
Theileria parasites are tick-transmitted, obligatory intracellular parasites which classified in Piroplasmida order under Apicomplexa phylum according to the scheme set by **Levine *et al.*** (**1980**). There are various developmental stages of different shapes and forms of *Theileria* that infected cattle, buffaloes, goats, sheep and camels. *Theileria* was recognized as like a ring form, slender spine-like form, an elongated structure or round measuring 3.75 μm in diameter. The nucleus located centrally surrounded by a cloud-like dispersed cytoplasm (**Hamed *et al.*, 2011**). *Theileria* has two forms the first form is (lymphocytic form) which presents in the lymph node

and called a Koch‘s blue bodies that represented the schizont of parasite. The schizont appears in two form macroschizont which consist of 8-12 nuclei and microschizont consists of 50-100 nuclei. While, the second form is erythrocytic form that presents in inside the erythrocytes and it takes several shapes like the ring, comma, rod and oval shape (**Mans *et al.*, 2015; Tallaf, 2017**). *Theileria* transmitted only by transstadial transmission from stage to stage because ticks are often present on animal in large numbers. Tick is infected when a larva or nymph is fed, and transferred to next developmental stage of tick. Also, via a vertical transmission was recorded (**Schnittger *et al.*, 2022**).

Transmission of *Theileria* is only happened by infected nymphal or adult ticks. The infected nymphor adult transmits infection during feeding when the sporozoites in its salivary glands have matured (**Marendy *et al.*, 2020**). Sporozoites enter lymphocytes and differentiate into schizonts inducing a lympho-proliferative disorder. The sporozoite entry process is not orientation-specific as has been observed with other apicomplexan parasites. In the lymphocytes, the schizonts differentiate into merozoites that invade the erythrocytes (**Tajeri *et al.*, 2022**). In *T. parva*, there is little or no multiplication in erythrocytes occurs exclusively in lymphocytes. In contrast, multiple rounds of asexual division have observed to occur in both the erythrocytes and lymphocytes from species like *T. annulata*. In the erythrocytes, merozoites develop into piroplasms, the stage of the parasite infective to ticks (**Tirloni *et al.*, 2015; Peckle *et al.*, 2022**). The sexual stage of development of *Theileria* spp. occurs in the gut of the tick. In the tick gut lumen gametogenesis and fertilization take place resulting in the production of a zygote. The zygote invades the gut cell and remains there throughout the tick moulting cycle and develops into a single motile kinete that escape the gut cells and invade the salivary glands. The parasites remain in the salivary gland until transmitted to another mammalian host following post-moult nymph or adult feeds. Feeding tick initiates rapid sporozoite development and infective sporozoites that release during the later stages of feeding (**Catalano *et al.*, 2015; Watts *et al.*, 2016**). Globally and locally, several studies have been focused on detection of *T. annulata* in animals (**A’aiz *et al.*, 2021; Alfatlawi *et al.*, 2021; Sray, 2021**); but few researchers have targeted the prevalence of theileriosis in ticks (**Abed and Hasso, 2019; Al-Fatlawi and Al-Fatlawi, 2019**). Therefore, the current study was aimed to detect the prevalence *T. annulata* in bovine ticks molecularly using the polymerase chain reaction (PCR) assay, with phylogenetic analysis of local strains to estimate their identity with the global NCBI-GenBank strains.

Materials And Methods

Ethical approval

This study was approved and carried out under the license of the Scientific Committee of the College of Veterinary Medicine (University of Al-Qadisiyah).

Samples

In Al-Qadisiyah province (Iraq), a total of 109 cattle were subjected to clinical examination to detect and collection of ticks during December (2021) to August (2022). The samples of ticks were removed manually by a rotating manner into plastic containers and transferred in ice-box to laboratory.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 27, Issue 1, 2023, Pages. 772-783
Received 05-October-2023; Accepted 01 December 2023

Molecular assay

According to manufacturer instructions of the AddPrep Genomic DNA Extraction Kit (AddBio, Korea), genomic DNAs for conventional PCR assay were extracted from the collected ticks, and evaluated for its concentration and purity by the Nanodrop System (Thermo Scientific, USA). Targeting *Cytochrom b* gene, one set of primers [F (5´- CAG GGC TTT AAC CTA CAA ATT AAC - 3´) and R (5´- CCC CTC CAC TAA GCG TCT TTC GAC AC -3´)] was served for preparation the Mastermix tubes at a final volume of (**Mhadhbi *et al*., 2015**). Electrophoresis of agarose-gel (1.5%) stained with Ethidium Bromide was performed at 100 volt and 80 AM for 1 hour. The results of PCR products were visualized under the UV transilluminator (Shandod Scientific, UK) to detect the positive ticks to *T. annulata* at an approximately product size of 1092 bp, and photographed using the digital camera (Nikon, Japan).

Phylogentic analysis

Ten positive DNAs were selected and sent by DHL for sequencing in the Macrogene Company (South Korea), and the data were received by the private email. Phylogenetic tree analysis was conducted using the Molecular Evolutionary Genetics Analysis (Mega 6.0) and Multiple Sequence Alignment Analysis based on the ClustalW alignment analysis; while, evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

Statistical analysis

One-Way ANOVA in the GrpahPad Prism Software was served for detection significant differences at P<0.05 (**Gharban *et al*., 2023**).

Results

Targeting the *Cytochrom b* gene, a total of 26% (13 /50) positive samples for T. annulata were detected in study ticks using the conventional PCR assay (**Figure 1**).

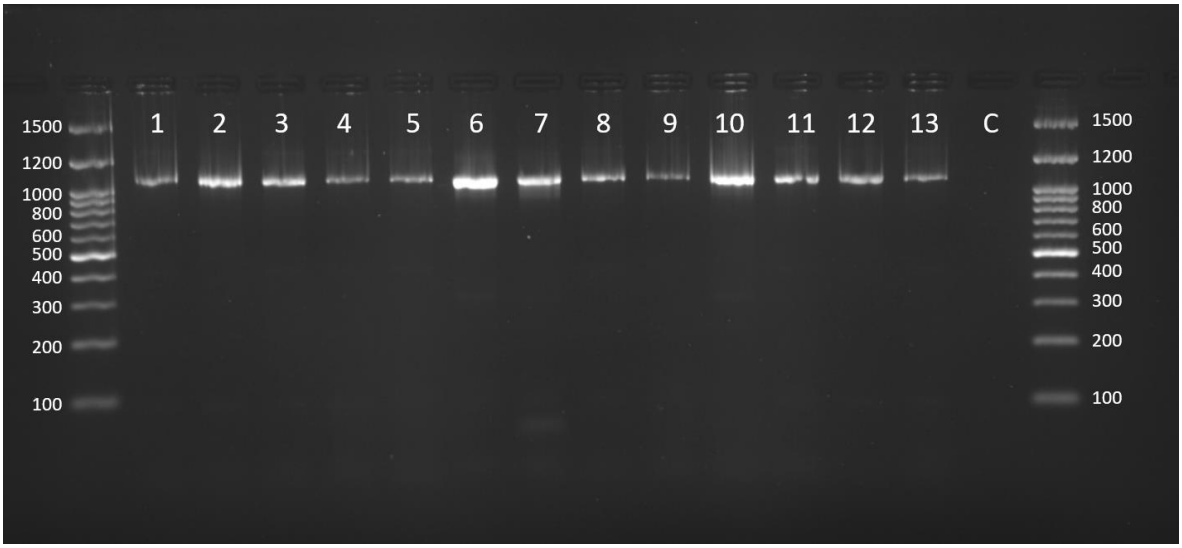

Figure (1): Gel electrophoresis image (1.5 % agarose) shows the amplicons of a partial region within *cytochrome b* gene (size= 1092 bp) for identification of *T. annulata*. Lanes (1-13) represent the positive amplicons while C is negative control

Annals of R.S.C.B., ISSN:1583-6258, Vol. 27, Issue 1, 2023, Pages. 772-783
Received 05-October-2023; Accepted 01 December 2023

The genomic DNAs of 10 positive samples were subjected for phylogenetic analysis based on the primer of *Cytochrom b* gene. However, sequencing findings for *T. annulata* isolates were named as *T. annulata* voucher IQ1-10, and recorded in NCBI under an accession numbers of OR125071, OR125072, OR125073, OR125074, OR125075, OR125076, OR125077, OR125078, OR125079 and OR125080. Comparative analysis for *Cytochrom b* -nucleotides sequence of study samples with the numbers of *T. annulata* isolates existed in GenBank database was constructed using the Clustal W Alignment of MEGA-6 software. The results showed that there were nucleotide alignment similarities (*****) and substitution mutations in *Cytochrom b* gene (Figure 2).

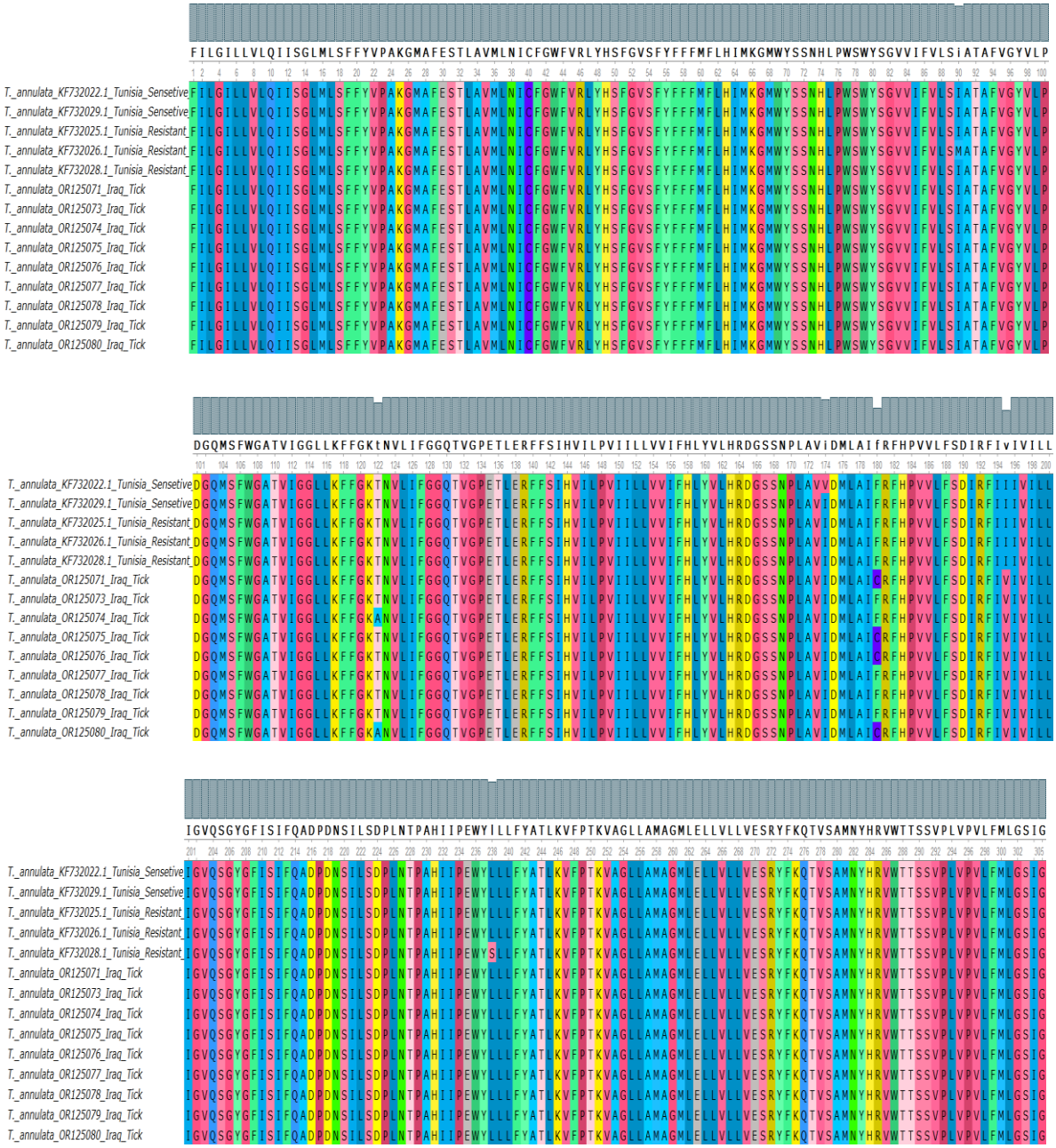

Figure (2): Multiple sequence alignment of the identified *T. annulata* targeting *Cytochrom b* gene. This highlights the similarity and differences between the identified sequences with four colors and compared with resistant and sensitive isolates from Tunisia

Annals of R.S.C.B., ISSN:1583-6258, Vol. 27, Issue 1, 2023, Pages. 772-783
Received 05-October-2023; Accepted 01 December 2023

Comparative identity between the local *T. annulata* IQ-isolates and NCBI-BLAST *T. annulata* of *Cytochrom b* gene revealed a significant association with Tunisian isolates at 98% similarity and 0.0-0.2% total genetic changes (Figure 3, Table 1).

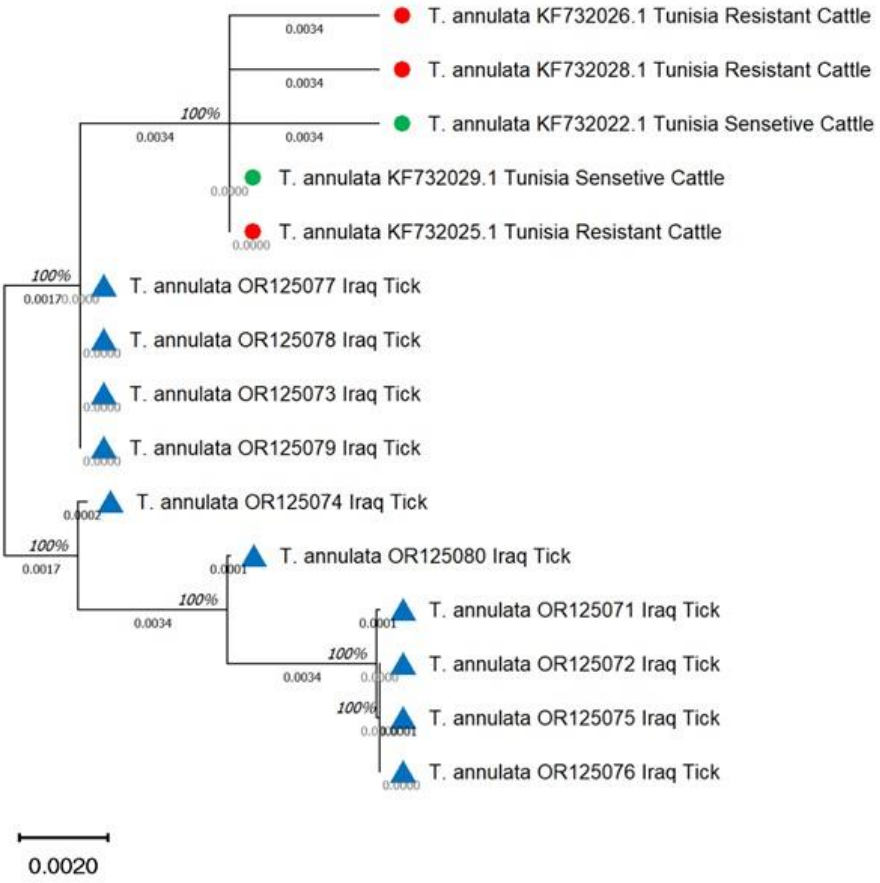

Figure (3): Evolutionary analysis of *T. annualata* in ticks targeting *Cytochrom b* gene. Iraqi isolates were referred with blue triangles while isolates from Tunisia referred with red circles (resistant isolates) and green circles in green isolates

Table (1): NCBI-BLAST homology Sequence identity (%) between the local and the global NCBI-BLAST *T. annulate* isolates

Local isolate		**NCBI-BLAST isolates**				
No.	**Access No.**	**Access No.**	**Country**	**%**	**Query cover**	**Host**
1	OR125071	KF732025	Tunisia	99.56	100	Cattle
2	OR125072	KF732025	Tunisia	99.56	100	Cattle
3	OR125073	KF732025	Tunisia	99.67	100	Cattle
4	OR125074	KF732025	Tunisia	99.56	100	Cattle
5	OR125075	KF732025	Tunisia	99.56	100	Cattle
6	OR125076	KF732025	Tunisia	99.67	100	Cattle
7	OR125077	KF732025	Tunisia	99.78	100	Cattle
8	OR125078	KF732025	Tunisia	99.67	100	Cattle
9	OR125079	KF732025	Tunisia	99.67	100	Cattle
10	OR125080	KF732025	Tunisia	99.45	100	Cattle

Discussion

In the present study, conventional PCR assay was applied to detect *T. annulata* infection in study ticks that confirmed morphologically and molecularly as *H. anatolicum*. Worldwide, different methods have been used to study infection rates of *Theileria* species in salivary glands of ticks (**Tajeri *et al*., 2016; Abdigoudarzi, 2013**). In general, histological and histochemical methods for detecting the infection have the disadvantage that it is not possible to determine with certainty the parasite species infecting the tick (**Mallesh *et al*., 2017**). Methyl green pyronin (MGP) is one of the most and simple histological methods which used traditionally to detect infection rates of tick-borne pathogens in ticks (**Lempereur *et al*., 2017**). However, it can only be performed on freshly collected ticks and does not allow differentiation between the closely related *Theileria* species such as *T. lestoquardi* and *T. equi* which can be transmitted by the same tick but do not infect cattle (**Kirvar *et al*., 2000; Tallaf, 2017**). This has been overcome by using PCR targeting the *T. annulata*-specific genes. One major advantage of the described PCR tests over traditional diagnostic methods is that they distinguish *T. annulata* from other *Theileria* and *Babesia* species in bovine blood and *T. annulata* from *T. lestoquardi* and *T. equi* in the tick. Thus this work has gone some way to address the concerns expressed by other workers on accurate parasite diagnosis in *Hyalomma* spp. (**Salih *et al*., 2015; Kumar *et al*., 2022**). The level of sensitivity, specificity and cross-reactivity in PCR tests is undoubtedly due to a variety of factors such as sequences of primers, amplification conditions, DNA extraction methods, storage of samples and DNA. Standardization of such parameters has been recommended for *Plasmodium* spp. PCR tests (**Costa *et al*., 2021; Yadav *et al*., 2021**). **Kirvar *et al.*** (**2000**) showed that the PCR methods have been shown to work sensitively and specifically under laboratory conditions using commercial kits to extract DNA which can remove chemicals present in samples that may inhibit the PCR. It would, however, be useful for wide-scale epidemiological surveys if simpler and cheaper DNA extraction methods could be tested and developed to detect *T. annulata* in bovine blood and in ticks. Data regarding prevalence of *Theileria* infected hard ticks is important to devise necessary control program for ticks and theileria infection. In this study, *T. annulata* were detected in 13% of study ticks (*H. anatolicum*). In a previous study in the Sudan, *H. anatolicum* was shown to have high natural infection rates with *Theileria*, ranging from 38 % to 86% (**Walker and McKellar, 1983**). Infection rates of *H. anatolicum* with *T. annulata* were subsequently estimated to be 96 % in ticks fed experimentally on infected calves (**Bakheit 1998**), and 80% in the ticks collected from the field (**El Imam, 1999**). These studies attributed the high infection rate in ticks to the animal husbandry system used, which consist of small enclosures, in which, susceptible crossbred cattle are maintained, and this creates an ideal microhabitat for *H. anatolicum* ticks. **Salih *et al.*** (**2005**) detected the infection rate of *T. annulata* in *H. anatolicum* (49.6%) indicated that cattle are subjected to a high challenge with *T. annulata* sporozoites and that ticks feeding as nymphs have a high chance of becoming infected with the parasite. **Khan *et al.*** (**2004**) detected that the high incidence of theileriosis in the districts under investigation may be the indication of high infestation of *Hyalomma* species. **Tavassoli *et al.*** (**2011**) revealed that 39.9% of *H. anatolicum*, 3.5% of *H. asiaticum*, and 18.2% *H. excavatum*, were infected with *T. annulata*. **Ali *et al.*** (**2013**) found that *T. annulata* was only detected in *H. annatolicum* and *H. dromedari* but not in *H. marginatum*. These results suggest that *H. anatolicum* may play a major role in transmission of *T. annulata* infection in Iran. These findings were similar with

that shown by other studies In Ethiopia and Sudan as *H. anatolicum* is the principal vector of *T. annulata*, the main cause of tropical theileriosis; therefore, surveillance of tropical theileriosis should be considered in the area in which *H. anatolicum* circulates (**Mossaad *et al*., 2021; Kaba, 2022**).

In the current study, phylogenetic analysis of positive *T. annulata* local isolates, based on the *cytochrome b* gene, showed that there was a significant identity with the NCBI-BLAST *Theileria annulata* Tunisian isolates. In many countries, field veterinarians reported unusual failures in the treatment of infected cases with ticks; indeed, these cases result in either a fatal outcome or a survival of the cattle with however a persistent high level of parasiteamia and a small number of degenerative forms of *T. annulata* (**Dryden, 2009; Coles and Dryden, 2014; Padula, 2016**). *In vivo* and *in vitro*, several researchers have confirmed the emergence of resistant and treatment failure for a number of parasitic infections such as *T. annulata* (**Mhadhbi *et al*., 2010**), *Plasmodium falciparum* (**Nam *et al*., 2011**), and T*oxoplasma gondii* (**Alday *et al*., 2017**). The analysis of these resistant parasites has associated the resistance to mutations in the *cytochrome b* ubiquinone binding site (**Huang *et al*., 2021; Hacılarlıoglu *et al*., 2023**). Different previous (**Hacilarlioglu *et al*., 2012; Sharifiyazdi *et al*., 2012; Mhadhbi *et al*., 2015**) and recent (**Fotoukkiaii *et al*., 2020; Flampouri, 2021; Zein *et al*., 2022**) studies have observed an association between the resistance to acaricides and the mutation in the *cytochrome b* catalytic site, the oxidation quinol site, namely coenzyme Q_0. These studies have revealed the single mutations in the *cytochrome b* gene in the samples of *T annulata* isolated from clinical cases of treatment failure. Indeed, the analysis of *cytochrome b* gene sequences showed mutations in the functional drug binding regions Q_0 (**Hacılarlıoglu *et al*., 2023**). In the Iranian study, the mutations identified were detected in eight *T. annulata* isolates and have been reported to result in the substitution of two amino acids (**Gholami *et al*., 2016**). The recent study conducted in Iraq reveals thirteen non-synonymous mutations in codons 11, 22, 33, 63, 103, 128, 130, 129, 172, 178, and 190; while, three silent mutations were found in codons 124, 128, and 275 (**Albayati *et al*., 2023**). In last study, the authors have been postulated that a rise in the prevalence of *T. annulata*-caused bovine theileriosis could lead to a greater prevalence of mutations in the aforementioned areas or other functional regions of the protein, rendering the medication ineffective. However, the transforming *T. annulata* into a drug-resistant strain may be responsible for the severe clinical signs that occur in the infected animals. This transformation requires exchanging the hydrophobic amino acids leucine and proline with the hydrophilic serine. This decrease in hydrophobicity may explain why acaricides has a lower affinity for *cytochrome b* (**Kessl *et al*., 2005; Fotie, 2014**). An in silico model confirms that these mutations prevent the medication from binding by altering the protein's shape and shrinking the binding pocket (**Walker *et al*., 1998**). The high rate of spontaneous mutations in the mitochondrial genome has been attributed to a variety of factors, including poor proofreading in the nucleus (**Weir *et al*., 2011**), multiple copies of the *cytochrome b* gene in mitochondria (**Sivakumar *et al*., 2019**), and the production of hydroxyl radicals in the mitochondrial respiration chain (**Raha and Robinson, 2000**). Perhaps the resistance is a result of selection pressure brought about by the extensive use of acaricides over more than three decades.

Conclusion

This study concluded that the molecular diagnosis of *T. annulata* in the prevalent tick species of the region has demonstrated the prevalence of bovine *Theileria* parasites in ticks, and the risk of cattle to contract theileriosis. Although only a limited number of ticks were studied, these preliminary data suggest that specific parasite genotypes may be selected during tick transmission from a carrier animal. Given its enormous burden on livestock farming in Iraq, further investigations are needed to better understand its epidemiology, vector transmission and potential clinical significance in cattle from neighboring countries.

Authors Contribution

AHA: Collection of ticks and molecular examination. MAA: Phylogenetic analysis of local strains and statistical analysis of obtained data. Both authors contributed equally and approved the final copy of the manuscript.

Conflict of interest

No.

Funding

No external funds (private fund)

References

[1] A'aiz, N. N., Ayyez, H. N., and Neamah, A. J. (2021). Molecular assay proves the presence of Theileria annulata infection in camels in Al-Diwaniyah Province, Iraq. *Iranian Journal of Parasitology*, *16*(2), 289.

[2] Abdigoudarzi, M. (2013). Detection of naturally infected vector ticks (Acari: Ixodidae) by different species of Babesia and Theileria agents from three different enzootic parts of Iran. *Journal of arthropod-borne diseases*, *7*(2), 164.

[3] Abed, M. J., and Hasso, S. A. (2019). Molecular detection of Babesia bigemina in ticks infesting water buffaloes (Bubalus bubalis) in Iraq. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, *18*(1).

[4] Albayati, H. H., Al Khafaji, A. M., and Al-karagoly, H. (2023). Microscopical and phylogenetic analysis of Theileria annulata in Iraqi local breed cattle: https://doi. org/10.12982/VIS. 2023.032. *Veterinary Integrative Sciences*, *21*(2), 457-472.

[5] Alday, P. H., Bruzual, I., Nilsen, A., Pou, S., Winter, R., Ben Mamoun, C., and Doggett, J. S. (2017). Genetic evidence for cytochrome b Qi site inhibition by 4 (1 H)-quinolone-3-diarylethers and antimycin in Toxoplasma gondii. *Antimicrobial agents and chemotherapy*, *61*(2), 10-1128.

[6] Alfatlawi, M. A., Jasim, A. A., Jarad, N. E., and Khlaif, S. F. (2021). Clinical and molecular identification of ruling Theileria annulata strains in cattle calves in Al-Diwaniyah province, Iraq. *Iraqi Journal of Veterinary Sciences*, *35*(1), 115-119.

[7] Al-Fatlawi, M. S. H., and Al-Fatlawi, M. A. A. (2019). Molecular and phylogenetic study of Theileria SPP isolated from ticks in AL-Diwaniyah city, Iraq. *The Iraqi Journal of Agricultural Science*, *50*(1), 475-479.

[8] Ali, Z., Maqbool, A., Muhammad, K., Khan, M., and Younis, M. (2013). Prevalence of Theileria annulata infected hard ticks of cattle and buffalo in Punjab, Pakistan. *DNA*, *862*, 846.

[9] Bakheit, M.A. (1998). Susceptibility of Kenana cattle to tropical theileriosis in the Sudan. M.V.Sc. thesis, University of Khartoum.

[10] Catalano, D., Biasibetti, E., Lynen, G., Di Giulio, G., De Meneghi, D., Tomassone, L., and Capucchio, M. T. (2015). "Ormilo disease" a disorder of zebu cattle in Tanzania: bovine cerebral theileriosis or new protozoan disease?. *Tropical Animal Health and Production*, *47*, 895-901.

[11] Coles, T. B., and Dryden, M. W. (2014). Insecticide/acaricide resistance in fleas and ticks infesting dogs and cats. *Parasites and vectors*, *7*(1), 1-10.

[12] Costa, A. D. T., Aguiar, A. C. C., Silva, A. M., and Pereira, D. B. (2021). Point-of-care strategies applied to malaria diagnosis. In *Current Topics and Emerging Issues in Malaria Elimination*. IntechOpen.

[13] Dryden, M. W. (2009). Flea and tick control in the 21st century: challenges and opportunities. *Veterinary dermatology*, *20*(5-6), 435-440.

[14] El Imam, AH (1999). Ecological and epidemiological studies on ticks and tick-borne diseases in Kosti Province, Sudan. MVSc. thesis, University of Khartoum, Sudan.

[15] Flampouri, E. (2021). Agrochemicals inhibiting mitochondrial respiration: Their effects on oxidative stress. In *Toxicology* (pp. 3-10). Academic Press.

[16] Fotie, J. (2014). The potential of peptides and depsipeptides from terrestrial and marine organisms in the fight against human protozoan diseases. *Bioactive Natural Products: Chemistry and Biology*, 279-320.

[17] Fotoukkiaii, S. M., Tan, Z., Xue, W., Wybouw, N., and Van Leeuwen, T. (2020). Identification and characterization of new mutations in mitochondrial cytochrome b that confer resistance to bifenazate and acequinocyl in the spider mite Tetranychus urticae. *Pest Management Science*, *76*(3), 1154-1163.

[18] Gharban, H. A., Al-Ghuraibawi, H. N., Al-Rubaye, Z. A., Jahlol, H. A., Al-Zergany, A. A., and Al-Abedi, G. J. (2023). Most Clinically Detected Viral Diseases in Field Animals of Wasit Province, Iraq. *Annals of the Romanian Society for Cell Biology*, *27*(01), 154-168.

[19] Gholami, S., Laktarashi, B., Shiadeh, M. M., and Spotin, A. (2016). Genetic variability, phylogenetic evaluation and first global report of Theileria luwenshuni, T. buffeli, and T. ovis in sheepdogs in Iran. *Parasitology research*, *115*, 2125-2130.

[20] Hacilarlioglu, S., Bilgic, H. B., Bakirci, S., Weir, W., Tait, A., Unlu, A. H., and Karagenç, T. (2012). Are mutations in the cytochrome b gene of Theileria annulata responsible for resistance to the theilericidal drug, Buparvaquone. In *Joint conference on emerging and re-emerging epidemics affecting global health. Orvieto, Italy*.

[21] Hacilarlioglu, S., Bilgic, H. B., Bakırcı, S., Tait, A., Weir, W., Shiels, B., and Karagenc, T. (2023). Selection of genotypes harbouring mutations in the cytochrome b gene of Theileria annulata is associated with resistance to buparvaquone. *Plos one*, *18*(1), e0279925.

[22] Hamed, M. I., Zaitoun, A. M., El-Allawy, T. A., and Mourad, M. I. (2011). Investigation of Theileria camelensis in camels infested by Hyalomma dromedarii ticks in Upper Egypt. *Journal of Advanced Veterinary Research*, *1*(1), 4-7.

[23] Huang, L. S., Lümmen, P., and Berry, E. A. (2021). Crystallographic investigation of the ubiquinone binding site of respiratory Complex II and its inhibitors. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1869*(9), 140679.

[24] Kaba, T. (2022). Geographical distribution of ixodid ticks and tick-borne pathogens of domestic animals in Ethiopia: a systematic review. *Parasites and vectors*, *15*(1), 108.

[25] Kessl, J. J., Ha, K. H., Merritt, A. K., Lange, B. B., Hill, P., Meunier, B., and Trumpower, B. L. (2005). Cytochrome b mutations that modify the ubiquinol-binding pocket of the cytochrome bc1 complex and confer anti-malarial drug resistance in Saccharomyces cerevisiae. *Journal of Biological Chemistry*, *280*(17), 17142-17148.

[26] Khan, M. Q., Zahoor, A., Jahangir, M., and Mirza, M. A. (2004). Prevalence of blood parasites in cattle and buffaloes. *Pak. Vet. J*, *24*(4), 193-194.

[27] Kirvar, E., Ilhan, T., Katzer, F., Hooshmand-Rad, P., Zweygarth, E., Gerstenberg, C., 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.

[28] Kumar, B., Maharana, B. R., Thakre, B., Brahmbhatt, N. N., and Joseph, J. P. (2022). 18S rRNA gene-based piroplasmid PCR: an assay for rapid and precise molecular screening of Theileria and Babesia species in animals. *Acta Parasitologica*, *67*(4), 1697-1707.

[29] Lempereur, L., Beck, R., Fonseca, I., Marques, C., Duarte, A., Santos, M., and Zintl, A. (2017). Guidelines for the detection of Babesia and Theileria parasites. *Vector-Borne and Zoonotic Diseases*, *17*(1), 51-65.

[30] Levine, N. D., Corliss, J. O., Cox, F. E. G., Deroux, G., Grain, J., Honigberg, B. M., and Wallace, F. G. (1980). A Newly Revised Classification of the Protozoa* The committee on systematics evolution of the society of protozoologists. *The Journal of protozoology*, *27*(1), 37-58.

[31] Mallesh, P., Manchukonda, U. K., and Murthy, G. S. S. (2017). Studies on the salivary glands of Argas persicus. *The Pharma Innovation*, *6*(7, Part C), 180.

[32] Mans, B. J., Pienaar, R., and Latif, A. A. (2015). A review of Theileria diagnostics and epidemiology. *International Journal for Parasitology: Parasites and Wildlife*, *4*(1), 104-118.

[33] Marendy, D., Baker, K., Emery, D., Rolls, P., and Stutchbury, R. (2020). Haemaphysalis longicornis: the life-cycle on dogs and cattle, with confirmation of its vector status for Theileria orientalis in Australia. *Veterinary Parasitology*, *277*, 100022.

[34] Mhadhbi, M., Naouach, A., Boumiza, A., Chaabani, M. F., BenAbderazzak, S., and Darghouth, M. A. (2010). In vivo evidence for the resistance of Theileria annulata to buparvaquone. *Veterinary Parasitology*, *169*(3-4), 241-247.

[35] Mhadhbi, M., Chaouch, M., Ajroud, K., Darghouth, M. A., and BenAbderrazak, S. (2015). Sequence polymorphism of cytochrome b gene in Theileria annulata Tunisian isolates and its association with buparvaquone treatment failure. *PloS one*, *10*(6), e0129678.

[36] Mossaad, E., Gaithuma, A., Mohamed, Y. O., Suganuma, K., Umemiya-Shirafuji, R., Ohari, Y., and Xuan, X. (2021). Molecular characterization of ticks and tick-borne pathogens in cattle from Khartoum state and east Darfur state, Sudan. *Pathogens*, *10*(5), 580.

[37] Nam, T. G., McNamara, C. W., Bopp, S., Dharia, N. V., Meister, S., Bonamy, G. M., and Winzeler, E. A. (2011). A chemical genomic analysis of decoquinate, a Plasmodium falciparum cytochrome b inhibitor. *ACS chemical biology*, *6*(11), 1214-1222.

[38] Padula, A. M. (2016). Tick paralysis of animals in Australia. *Clinical Toxinology: Clinical Toxinology*, 1-20.

[39] Peckle, M., Santos, H., Pires, M., Silva, C., Costa, R., Vitari, G., and Massard, C. (2022). Dynamics of Theileria equi Infection in Rhipicephalus (Boophilus) microplus during the Parasitic Phase in a Chronically Infected Horse. *Pathogens*, *11*(5), 525.

[40] Raha, S., and Robinson, B. H. (2000). Mitochondria, oxygen free radicals, disease and ageing. *Trends in biochemical sciences*, *25*(10), 502-508.

[41] Salih, D. A., Sharieff, O. E., Lazarus, A. G., Hassan, S. M., and El Hussein, A. M. (2005). Natural infection rates and transmission of Theileria annulata by Hyalomma anatolicum anatolicum ticks in the Sudan. *Onderstepoort Journal of Veterinary Research*, *72*(4), 303-307.

[42] Salih, D. A., El Hussein, A. M., and Singla, L. D. (2015). Diagnostic approaches for tick-borne haemoparasitic diseases in livestock. *Journal of Veterinary Medicine and Animal Health*, *7*(2), 45-56.

[43] Schnittger, L., Ganzinelli, S., Bhoora, R., Omondi, D., Nijhof, A. M., and 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.

[44] Sharifiyazdi, H., Namazi, F., Oryan, A., Shahriari, R., and Razavi, M. (2012). Point mutations in the Theileria annulata cytochrome b gene is associated with buparvaquone treatment failure. *Veterinary parasitology*, *187*(3-4), 431-435.

[45] Sivakumar, T., Fujita, S., Tuvshintulga, B., Kothalawala, H., Silva, S.S.P., Yokoyama, N., (2019). Discovery of a new Theileria sp. closely related to Theileria annulata in cattle from Sri Lanka. Sci. Rep. 9(1), 1-10.

[46] Sray, A. H. (2021). First Serological Detection of Theileria Annulatain Buffaloes in Iraq. *Annals of the Romanian Society for Cell Biology*, *25*(7), 1262-1273.

[47] Tajeri, S., Razmi, G., and Haghparast, A. (2016). Establishment of an artificial tick feeding system to study Theileria lestoquardi infection. *PloS one*, *11*(12), e0169053.

[48] Tajeri, S., Momeux, L., Saintpierre, B., Mfarrej, S., Chapple, A., Mourier, T., and Langsley, G. (2022). Theileria annulata histone deacetylase 1 (TaHDAC1) initiates schizont to merozoite stage conversion. *Scientific Reports*, *12*(1), 12710.

[49] Tallaf, A. K. (2017). *Detection of Theileriasis in cattle by using polymerase chain reaction in AL-Muthanna Province* (Doctoral dissertation, MSc Thesis in Veterinary Medicine, Baghdad University, Iraq).

[50] Tavassoli, M., Tabatabaei, M., Nejad, B. E., Tabatabaei, M. H., Najafabadi, A., and Pourseyed, S. H. (2011). Detection of Theileria annulata by the PCR-RFLP in ticks (Acari, Ixodidae) collected from cattle in West and North-West Iran. *Acta Parasitologica*, *56*, 8-13.

[51] Tirloni, L., Islam, M. S., Kim, T. K., Diedrich, J. K., Yates, J. R., Pinto, A. F., and Da Silva Vaz, I. (2015). Saliva from nymph and adult females of Haemaphysalis longicornis: a proteomic study. *Parasites and vectors*, *8*(1), 1-23.

[52] Walker, A. R., and McKellar, S. B. (1983). The maturation of Theileria annulata in Hyalomma anatolicum anatolicum stimulated by incubation or feeding to produce sporozoites. *Veterinary Parasitology*, *13*(1), 13-21.

[53] Walker, D. J., Wakefield, A. E., Dohn, M. N., Miller, R. F., Baughman, R. P., Hossler, P. A., and Meshnick, S. R. (1998). Sequence polymorphisms in the Pneumocystis carinii cytochrome b gene and their association with atovaquone prophylaxis failure. *The Journal of infectious diseases*, *178*(6), 1767-1775.

[54] Watts, J. G., Playford, M. C., and Hickey, K. L. (2016). Theileria orientalis: a review. *New Zealand veterinary journal*, *64*(1), 3-9.

[55] Weir, W., Karagenç, T., Gharbi, M., Simuunza, M., Aypak, S., Aysul, N., and Tait, A. (2011). Population diversity and multiplicity of infection in Theileria annulata. *International Journal for Parasitology*, *41*(2), 193-203.

[56] Yadav, N. I. D. H. I., and Upadhyay, R. (2021). Tick saliva toxins, host immune responses and its biological effects. *Int J Pharm Pharm Sci*, *13*(8), 9-19.

[57] Zein, H., Afifi, A. M., Ali, F. S., Shaurub, E. S., and Ahmed, M. (2022). Target-site insensitivity to some acaricides in a field population of Tetranychus urticae Koch (Acari: Tetranychidae) from Egypt. *Persian Journal of Acarology*, *11*(2), 323-337.