



Molecular Identification of *Theileria equi* and *Babesia caballi* from Ixodid Ticks Infesting Equids in Erbil Province, Northern of Iraq

KHALID JABAR AZIZ^{1*}, LOKMAN TAIB OMER AL-BARWARY²

¹Department of Animal Resources, College of Agricultural Engineering Sciences, Salahaddin University-Erbil;

²Department of Microbiology and Pathology, College of Veterinary Medicine, University of Duhok.

Abstract | The present study was conducted to detect of *Theileria equi* and *Babesia caballi* in Ixodid ticks collected from equids in Erbil province, Northern Iraq. A total of (98) adult ticks, belong to the five species from three ixodid genera (two species belong to the genus *Hyalomma* (*Hyalomma marginatum marginatum* and *H. anatolicum excavatum*), two species belong to the genus *Boophilus* (*Boophilus microplus* and *B. annulatus*) and one species belong to the genus *Rhipicephalus* (*Rhipicephalus turanicus*) were collected from 349 equids. Fifty DNA ticks (10 for each tick species) were chosen for molecular analyses to detect of *T. equi* and *B. caballi* using conventional and multiplex polymerase chain reaction technique (c-PCR, m-PCR) targeting 18S rRNA gene. Out of analyzed ticks, *Boophilus annulatus* was the most prevalent tick species (39.8%), whereas the lowest was *Hyalomma excavatum* (10.2%). The multiplex PCR finding revealed that five ticks species including (*Hyalomma marginatum marginatum*, *Hyalomma anatolicum excavatum*, *Boophilus microplus*, *Boophilus annulatus* and *Rhipicephalus turanicus*) were infected with *T. equi*, while only one species *Rhipicephalus turanicus* and *Boophilus annulatus* was found infected with *Babesia caballi* and both protozoa respectively.

Keywords | Ixodid, *Theileria equi* and *Babesia caballi*, PCR, Iraq

Received | July 17, 2020; **Accepted** | September 04, 2020; **Published** | November 15, 2020

***Correspondence** | Khalid Jabar Aziz, Department of Animal Resources, College of Agricultural Engineering Sciences, Salahaddin University-Erbil; **Email:** khalid.aziz1@su.edu.krd

Citation | Aziz, KJ, AL-Barwary LTO (2020). Molecular identification of *Theileria equi* and *Babesia caballi* from ixodid ticks infesting equids in Erbil province, northern of Iraq. Adv. Anim. Vet. Sci. 8(12): 1286-1293.

DOI | <http://dx.doi.org/10.17582/journal.aavs/2020/8.12.1286.1293>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

Copyright © 2020 Aziz and AL-Barwary. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Ixodid ticks are important ectoparasite due to their capacity to harbor and transmit a variety of zoonotic and veterinary important pathogens including (Protozoa, bacteria, virus and rickettsia) to domestic and wild animals in most region of the world (Ozdarendeli et al., 2008; Aziz and AL-Barwary, 2019a). Different diseases can be associated with this parasite and namely tick-borne disease (TBDs) (Otranto et al., 2014). In past twenty years, countless TBDs have been recognized in various countries (Kernif et al., 2016). Eight main species of ixodid ticks including (*Rhipicephalus turanicus*, *R. sanguines*, *Hyalomma anatolicum anatolicum*, *H. marginatum marginatum*, *H. anatolicum excavatum*, *Boophilus annulatus*, *H. turanicum* and *H. detritum detritum*) were previously recorded from

cattle, sheep and goats in Erbil region, Northern Iraq (Ameen, 2012). *Babesia caballi* and *Theileria equi* are the most important tick-borne protozoal pathogens causing equine piroplasmiasis (EP) infecting the equidae family (horses, mules, donkeys, ponies and zebras) in which it cause significant hygienic and economic losses in horses (Manna et al., 2018). *Babesia caballi* and *Theileria equi* have been classified taxonomically in the phylum Apicomplexa (protozoa) and in two different families, Babesiidae and Theileriidae, respectively (Taylor et al., 2007). Despite the fact that *Theileria equi* are more prevalent and more pathogenic than *Babesia caballi* in endemic areas, these two parasite always share the same tick vectors to infest equids (Katz, et al., 2000; Sigg et al., 2010). The protozoa are mainly transmitted by ticks of the Ixodidae family (Zangana and Naqid, 2011). Transmission through the

tick vectors occurs by two main routes first; mechanical transmission when the mouthparts are contaminated, second; biological transmission when the protozoa develop or proliferate itself in the tick vector (Scoles and Ueti, 2015). The regional distribution of equine piroplasmiasis is greatly affected by the presence of ticks and international horse trade between endemic and sensitive areas (Friedhoff and Tenter, 1990; Bruning, 1996). It has been reported that in the areas where tick vectors are common, the outbreak of the disease usually associated with entry of the persistently carrier horses (Sumbria et al., 2015). Ticks belong to the family Ixodidae, called hard or Ixodid ticks (Acari: Arachnida), which are obligate blood sucking ectoparasites of various mammals, vertebrates, birds, amphibians and reptiles (Schmidt and Roberts, 1989). There is an obligatory relationship between piroplasms and their Ixodid tick vectors (Gou et al., 2013). Out of the 867 tick species currently known, there are 33 Ixodid tick species belong to six genera that have been reported as competent tick vectors for equine piroplasmiasis (Jongejan and Uilenberg, 2004). The Ixodid tick species include; *Amblyomma* spp., *Hyalomma* spp., *Rhipicephalus* spp., *Dermacentor* spp., *Ixodes* spp., and *Haemaphysalis* spp. these ticks have been reported as a risk factor to transmit *B. caballi* and *T. equi* to equids (Jongejan and Uilenberg, 2004). Twenty-seven tick species belong to these six genera, can be transmitted *T. equi* includes *Amblyomma* spp., *Dermacentor* spp., *Haemaphysalis* spp., *Hyalomma* spp., *Ixodes* spp., and *Rhipicephalus* spp. (Scoles and Ueti, 2015). Twenty-four tick species belong to the four genera can transmit *B. caballi* including *Dermacentor* spp., *Haemaphysalis* spp., *Hyalomma* spp., and *Rhipicephalus* spp. (Scoles and Ueti, 2015). Ixodid ticks are the final hosts as well as transporters for *T. equi* and *B. caballi*, due to the former parasites which undergo sexual-stage development in the tick vectors to complete their life cycle (Zapf and Schein, 1994). The most important program to prevent the spreading of the disease is controlling or removing the tick which is considered the main risk factors for EP (Kouam et al., 2010; Guidi et al., 2015).

Molecular tools have been considered as a perfect technique for detection of many species of *Babesia* and *Theileria* among different animals in the world. Seemingly, PCR techniques have higher sensitivity and specificity compared with other techniques including serological assays and Giemsa stained blood smears (Buling et al., 2007; Jefferies et al., 2007). Studies have been done to determine the prevalence of equine piroplasmiasis from different parts of Iraq using serological techniques and microscopic examination (Alsaad et al., 2012). However, there is a very few molecular epidemiological study on piroplasm parasites from infected animals in Erbil province (Aziz et al., 2019).

The status of EP in ixodid tick among equines has not been

evaluated in Erbil province, North of Iraq yet. Therefore, the current study aimed to use conventional and multiplex PCR techniques for the detection of *T. equi* and *B. caballi* parasite on tick's DNA samples collected from equines distributed in different districts of Erbil, Northern Iraq.

MATERIALS AND METHODS

STUDY AREA AND SAMPLING

This study was performed from April 2016 to March 2017 in Erbil province, North of Iraq. A total of 349 equids (Horse, Mule, Donkey and Pony) were inspected for tick infestations. Different parts of host body such as head, ears, neck, pectoral, per femoral region, tests, legs, back and buttock, inguinal and under tail areas, were inspected carefully for detection of any tick. A total of 98 ticks of both sexes (male, female) were collected in plastic vials containing 70% ethanol and stored at 4°C until morphological identification of ticks and molecular identification of EP from tick species by m-PCR.

IDENTIFICATION OF IXODID TICKS

The species and sex of the collected ticks was identified by studying the morphological characteristics microscopically according to the identification keys (Estrada-Peña et al., 2004). After appropriate identification, all identified ticks were kept individually in 70% ethanol before being treated for further analysis.

DNA extraction from ixodid ticks and PCR amplification DNA was purified from 50 Ixodid adult ticks (10 ticks) from each species after morphological identification which included: *Hyalomma marginatum*, *Hyalomma anatolicum excavatum*, *Rhipicephalus turanicus*, *Rh. (Boophilus) microplus*, *Rh. (Boophilus) annulatus*, using PrimePrep™ Genomic DNA Extraction kit (from Tissue) (GeNet Bio, Korea) according to the manufacturer's instructions.

In this study, two types of PCR reaction were applied: firstly, to identify the positive equine for all possible *Babesia* spp., and *Theileria* spp., the universal 'catch-all' primers (Bec-UF1 and Bec-UR) by c-PCR assay were used to amplify DNA fragments. Secondly, to differentiate between *B. caballi* and *T. equi* in all positive and negative samples in the first reaction, by using a single forward primer (Bec-UF2) and two reverse primers, one for identifying *B. caballi* and another for *T. equi*, which were Cab-R and Equi-R respectively by m-PCR assay (Table 1).

A m-PCR was performed according to the method of Alhassan et al. (2005) and Qablan et al. (2013) with some modifications. Briefly, 25 µl of a mixture containing 2 µl of template DNA, 1 µl (10 pmol) of each of the reverse primers (EquiR) for *T. equi* and (CabR) for *B. caballi* 2

μl of a universal forward primer (UF2), 6.5 μl dH₂O and 12.5 μl of 2X PCR master-mix ready to use (GeNet Bio. Laboratory. Korea) with a final concentration of 0.5 mM of each dNTP in 10 mM Tris-HCl, pH 9.0, 4 mM MgCl₂, enzyme stabilizer, loading dye and 1 U Taq DNA polymerase. The mixtures were subjected to the following cycling conditions using a BioRad thermocycler: 96°C for 10 min, with 35 cycle of a denaturing step at 96°C for 1 min, an annealing step at 54°C for 1 min, and an extension step at 72°C for 1 min, followed by a final extension at 72°C for 10 min.

After amplification success, a 5 μl of the amplicons were run with gel electrophoresis in a 1.5% agarose for 45 min, then visualised by UV illuminator (Proxima 2500 Isogene Life science, Netherland). DNA positive controls for *T. equi* and *B. caballi* were confirmed by nucleotide sequencing and submission to gene bank with accession number MH047214 and MH047215 respectively. Further, DNA extracted from piroplasms-free ticks was used as negative control for PCR amplification.

DNA SEQUENCING AND PHYLOGENIC ANALYSIS

In this study a total of 9 PCR amplicons (8 for *T. equi* and 1 for *B. caballi*) that were positive and have strong bands by using m-PCR send to the commercial company for purified and sequencing (Macrogen Inc. South Korea).

The sequences similarity analyses with previous sequences published in GenBank were performed using the BLAST programme (<http://www.ncbi.nlm.nih.gov/BLAST>). BLAST analysis approved that all sequences were for *T. equi* and *B. caballi* 18S rRNA gene with 97-100 identities to previous published *T. equi* and *B. caballi*.

The four DNA samples that represent in Erbil province were applied in the phylogenetic analysis. Multiple sequence alignment was done employing MEGA7 version (<http://www.megasoftware.net>, July 2016). Sequences were aligned together with 18S rRNA gene of *T. equi* and *B. caballi* sequences, derived from GenBank, using the Muscle software (Edgar, 2004).

Three different algorithms: Maximum Likelihood, Neighbor-Joining were used to generate a phylogenetic tree and yielded topology-similar results (Tamura-Nei model) with 1000 Bootstrap replicates were performed to estimate the node reliability.

STATISTICAL ANALYSIS

Data were analyzed using SPSS from windows (Version 7). Chi-square and Z test were done to find significant differences between tick's species and sex. A value of $p < 0.05$ was considered as statistically significant.

PREVALENCE OF IXODID TICK INFESTING EQUINE IN ERBIL PROVINCE

The results show that five species from three ixodid genera (two species belong to the genus *Hyalomma* (*Hyalomma marginatum marginatum* and *H. anatolicum exacavatum*), two species belong to the genus *Boophilus* (*Boophilus microplus* and *B. annulatus*) and one species belong to the genus *Rhipocephalus* (*Rhipocephalus turanicus*) were observed on equids during the study. It was found that the tick species *B. annulatus* (39.8%) was the most frequently observed in equines, while *Hyalomma exacavatum* (10.2%) showed the minimum occurrence of tick species were observed. Whether as infestation rate of *Rhipocephalus turanicus*, *Boophilus microplus* and *Hyalomma marginatum* were 21.4%, 16.3% and 12.2% respectively Table 2.

MOLECULAR FINDINGS

For the first reaction finding of PCR amplified product using universal 'catch-all' primers (Bec-UF1 and Bec-UR) visualised that the DNA bands size was 876 bp and 913bp, meaning that the samples were positive for *Babesia* spp. and *Theileria* spp. respectively (Figure 1). While for the second PCR amplification reaction using single forward primer (Bec-UF2) and two reverse primers, one of them (Cab-R) was specific for *B. caballi* and another (Equi-R) for *T. equi*, visualized that the DNA bands size was 392 bp and 540 bp, indicating that the samples were positive for *T. equi* and *B. caballi*, respectively (Figure 2).

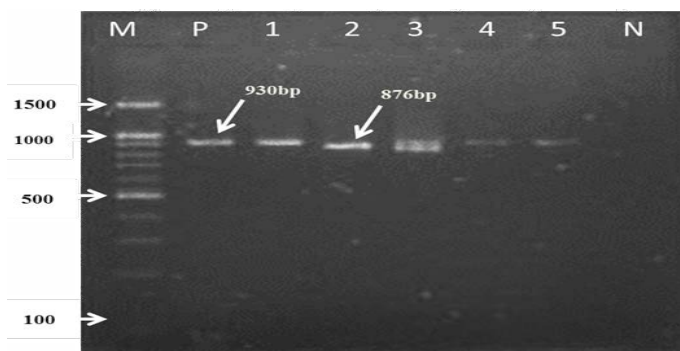


Figure 1: PCR detection of *B. caballi* and *T. equi* with a set of primer combinations (Bec-UF1 and Bec-UR): M= 100 bp DNA marker; Lanes 1-2=*B. caballi* in approximately band size 876bp; lanes 3-6= *T. equi* in approximately band size 930bp; Lane N) negative control.

DETECTION RATE OF *T. EQUI* AND *B. CABALLI* AND BOTH PROTOZOA IN IXODID TICKS USING M-PCR

Out of 50 examined adult ticks 10 (20%) were positive for equine piroplasmosis (Table 3). DNA of *T. equi* was detected in 8 (16%) adult ticks of species *H. marginatum marginatum* (n=2), *H. anatolicum exacavatum* (n=1), *Rhipocephalus turanicus* (n=1), *Boophilus microplus* (n=1) and

Table 1: The Oligonucleotide primers used to amplify the hemoparasites 18S rRNA genes of piroplasms. (Alhassan et al., 2005).

Primers	Sequences 5'-3'	Target gene	Expected size (bp)
Bec-UF1	5'-GTTGATCCTGCCAGTAGTCA-3'	"Catch-all" (<i>Babesia</i> spp. and <i>Theileria</i> spp.)	876 <i>Babesia</i> spp.
Bec-UR	5'-CGGTATCTGATCGTCTTCGA-3'		913 <i>Theileria</i> spp.
Bec-UF2	5'-TCGAAGACGATCAGATACCGTCG-3'	Specific primers for <i>B. caballi</i> and <i>T. equi</i>	-----
Cab-R	5'-CTCGTTCATGATTAGAAATTGCT-3'		540
Equi-R	5'-TGCCTTAAACTTCCTTGCGAT-3'		392

Bec-UF1 and Bec-UF2: catch-all forward primers; Bec-UR: catch-all reverse primers; Cab-R: *B. caballi*-specific reverse primer, Equi-R: *T. equi*-specific reverse primer.

Table 2: Prevalence and ratio of Ixodid tick according to sex infesting equine in Erbil province.

Specimens of adult ticks	Male no. (%)	Female no. (%)	Total no. (%)	♂/♀	P value
<i>Hyalomma marginatum</i>	7	5	12 (12.2%)	1.45:1	0.315
<i>Hyalomma excavatum</i>	9*	1	10 (10.2%)	9.37:1	0.103
<i>Boophilus microplus</i>	2	14*	16 (16.3%)	0.14:1	0.942
<i>Boophilus annulatus</i>	21	18	39 (39.8%)	1.21:1	0.001**
<i>Rhipocephalus turanicus</i>	9	12	21(21.4%)	0.78:1	0.86
Total	48	50	98	0.96:1	

*: (p>00.5); **: (p>00.1).

B. annulatus (n=3). DNA of *B. caballi* was detected in 1 (2%) adult ticks of species *Rhipocephalus turanicus* (n=1). DNA of both protozoa were detected in 1 (2%) adult ticks of species *B. annulatus* (n=1). This finding that *T. equi* was more common detected in ticks compared with *B. caballi* and both protozoa.

Table 3: Detection of *T. equi* and *B. caballi* and both protozoa in Ixodid ticks using m-PCR.

Tick species	No. of tested ticks	No. of positive ticks	Type of protozoa		
			T. equi	B. caballi	Both protozoa
HM	10	2	2	0	0
HE	10	1	1	0	0
RT	10	2	1	1	0
BM	10	1	1	0	0
BA	10	4	3	0	1
Total	50	10 (20%)	8 (16%)	1 (2%)	1 (2%)

No.: Number of samples; HM: *Hyalomma marginatum*; HE: *Hyaloma excavatum*; RT: *Rhipocephalus turanicus*; BM: *Boophilus microplus*; BA: *Boophilus annulatus*.

PHYLOGENIC ANALYSIS

T. equi and *B. caballi* sequences for the hypervariable V4 region of the 18SrRNA gene were obtained from four and five samples respectively. The sequences were deposited in GenBank with some subsequently employed for the phylogenetic analysis.

These sequences when analysed by BLASTn shared

identify ranging from 98-100% with *T. equi* and *B. caballi* sequences previously detected. The sequences were clustered in two different groups, B and C for *T. equi* while for *B. caballi* they clustered to group B.

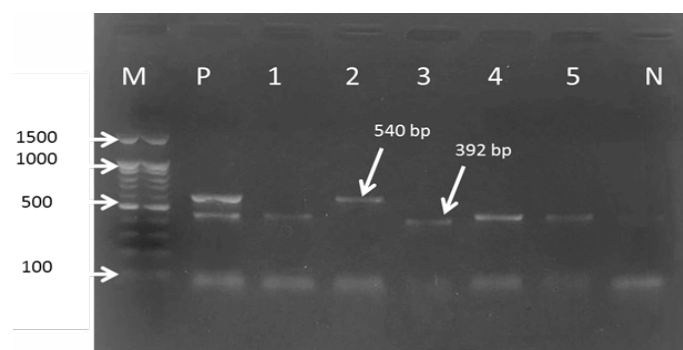


Figure 2: Gel electrophoresis image showing: lane M) 100bp DNA ladder; Lane P) positive control for *T. equi* and *B. caballi*; Lane1-4) multiplex PCR detected *T. equi* and *B. caballi* in approximately band size 392 and 540 bp respectively; Lane N) negative control.

The tree of *T. equi* shows two major clades representing the genotype (B and C) Figure 3. Two sequences (MH368310 and MH368318) detected in ticks were clustered with sequences previously detected from Iran (KF704750), Egypt (MF192854), Jordan (KX227638), Brazil (KJ573337), Cuba (KY111760) and Turkey (MG569905) belong to the clade C, and one sequences (MH368342) were positioned near to sequences previously detected from Turkey (MG569901), Trindida (KU289006), Sudan (AB515315), Turkey (KU921663), Brazil (MG052902), South Africa

(EU642508), Jordan (KX227637), India (LC042536) and Iran (KJ418423) belong to the clade B.



Figure 3: Rooted phylogenetic tree of *Theileria equi* as interfered from partial sequences of 18S rRNA gene. Black diamond indicated sequences obtained from the present study; others represent sequences from references.

Tree of *Babesia caballi* shows one clades representing the known genotype of *Babesia caballi* genotype B Figure 4, one sequence (MH368304) were positioned near to the sequences previously detected from Jordan (JN596979), Mongolia (JQ288735), South Africa (EU642513), (EU888904), (Z15104) and (EU888901), Jordan (JQ417262) and Malaysia (KU879026) and (KU879023) belong to the clade B.

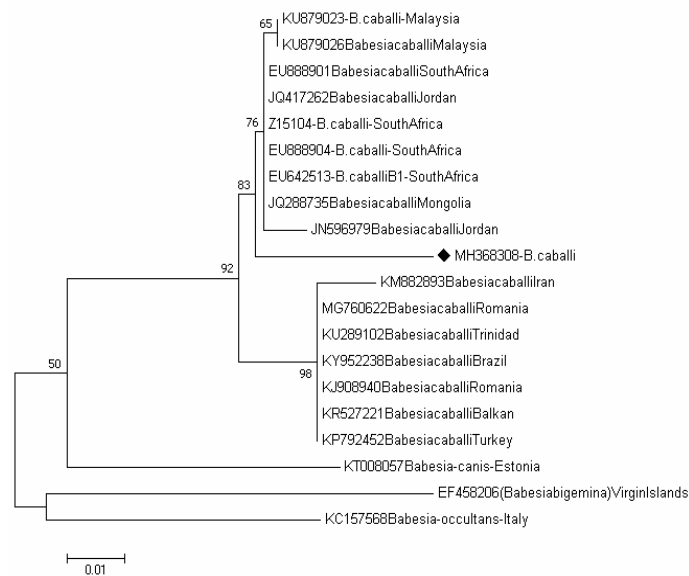


Figure 4: Rooted phylogenetic tree of *Babesia caballi* as interfered from partial sequences of 18S rRNA gene. Black diamond indicated sequences obtained from the present study; others represent sequences from references.

Ticks and tick-borne diseases have a great economical and vital importance throughout the world, and they affect animals health through transmitting of different diseases including: protozoan, bacterial, rickettsial, and viral agents (Ozdarendeli et al., 2008). Equine piroplasmosis which are important tick-borne diseases infect a great variety of equids (Jongejan and Uilenberg, 2004).

Several studies have conducted about tick-borne diseases in equids and other animals using different methods in some regions of Iraq (Estrada-Peña et al., 2004; Alsaad et al., 2010; Aziz et al., 2019). The scarcity of literature related to equids showing any previous molecular study targeting tick population responsible for transmission of theileriosis and babesiosis in Erbil state has not performed any comparison on the prevalence of ixodid tick vectors in the region.

In this study, five ixodid species were identified morphologically on equids during the study, However, according to the (Estrada-Peña et al., 2004). The comparison between these results with other previous studies carried out in different parts of Iraq and other neighboring countries indicated that the main three genera; *Hyalomma*, *Rhipocephalus* and *Boophilus* were observed (Omer et al., 2007; Hasson, 2012; Biglari et al., 2018; Aziz and AL-Barwary, 2019b)

Among Five tick species examined in terms of *Theileria equi* and *Babesia caballi* species, all of them were positive for *Theileria equi* while just one species *Rhipocephalus turanicus* and *Boophilus. annulatus* was positive for *Babesia caballi* and both protozoa respectively.

A previous study conducted in Jordan reported that the prevalence rate of infections was 18.8% for *T. equi* and 7.3% for *B. caballi* in horses used PCR technique (Qablan et al., 2013). Another study demonstrated that the infection rate of Equine piroplasmosis was 4.93% in Turkey; 2.96% and 1.97% for *T. equi* and *B. caballi* respectively (Kizilarslan et al., 2015).

In the present study, the highest infection rate of Equine Piroplasmosis was found in *Boophilus annulatus* as compared to other identified ticks. *H. anatolicum* was established in the previous study as the main vector responsible for transmission of EP among the population equine (Kumar et al., 2007). This may be due to the climatic change in last decade and the little number of ticks which observed in horses during this survey due to the great interest in periodic hygiene of horses as a continuous horses cleaning.

The data found that the infected rate of *Theileria equi* in

Ixodid ticks by m-PCR was statistically more significant than that of *B. caballi* ($P < 0.05$). These findings are agreed with a report by others whom study horses and donkeys from Iraq and neighbouring countries using different techniques (Alsaad et al., 2012; Kizilarlan et al., 2015). This situation is likely to be as result of the effectively elimination of *B. caballi* by active treatment and host immune system, in contrast to the long-life persistence of *T. equi* (Laus et al., 2015).

The 18S rRNA gene is widely used for molecular detection and phylogenic analysis of piroplasms in equids (Hall et al., 2013). Here in the molecular detection and phylogenic analysis of *T. equi* and *B. caballi* sequence amplified among ticks from Erbil province were assessed and appear to be the first study of its kind from this area.

The present study has provided first data on the genetic diversity of piroplasms in equids population from Iraq based on 18S rRNA gene sequences. Until today, five genotypes of *T. equi* and three genotypes of *B. caballi* lineages were established in equine populations from different countries (Bhoora et al., 2010). However by finding a new sequence from various geographical area, further increase in the genetic diversity of *T. equi* and *B. caballi* has been predictable (Qablan et al., 2013). This variation in distribution of piroplasms 18S rRNA gene sequences may be related to the horse and mule business movement from other countries especially neighbor countries that primarily included turkey, Iran and Jordan.

The *T. equi* isolates in the current study clustering within genotypes (B and C), this may revealed an overlapping occurrence of different genotypes within the same population of equids and tick vectors, or may represent a novel variant would require further confirmation (Qablan et al., 2013; Salim et al., 2013).

The *B. caballi* isolates obtained in our study clustered within the genotype B which corresponds to *B. caballi* was previously isolated from Jordan (JN596979), Mongolia (JQ288735), South Africa (EU642513), (EU888904), (Z15104) and (EU888901), Jordan (JQ417262), Malaysia (KU879026) and (KU879023).

In results, the high prevalence rate of *T. equi* was found in ticks in Erbil province, although this considerable diversity in 18SrRNA was found in *T. equi*, may be related to the history horse business movement between neighboring and our country by illegal modes. The importation of horses into Kurdistan region of Iraq has more involved in the last decade.

CONCLUSIONS AND RECOMMENDATIONS

It has been concluded that five tick species were prevalent from equids in Erbil province, the infected rate of *Theileria equi* in ticks by conventional PCR was statistically more higher than *B. caballi*. The molecular diagnostic tool in the detection of *T. equi* and *B. caballi* from ticks has been used for the first time in Erbil province in this study. In order to provide better insight of epizootiological situation of tick prevalence and transmission cycle of equine piroplasmosis in this region of our country.

ACKNOWLEDGMENT

We want to thank Dr. Nazhad H. Qader and Dr. Yunis A. Ahmad and the farmers for their assistance with the sample collection. We would also like to thanks Mr. Muhsin J. Abdulwahid (Scientific Research Center/ Salahaddin University) for their help to facilities using laboratory of molecular biology.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

- Alhassan A, Pumidonming W, Okamura M, Hirata H, Battsetseg B, Fujisaki K, Igarashi I (2005). Development of a single-round and multiplex PCR method for the simultaneous detection of *Babesia caballi* and *Babesia equi* in horse blood. *Vet. Parasitol.*, 129(1-2): 43-49. <https://doi.org/10.1016/j.vetpar.2004.12.018>
- Alsaad KM, Hassan SD, Al-Obaidi QT (2012). Detection of *Babesia equi* and *Babesia caballi* antibodies in horses and donkeys in Mosul, Iraq. *Res. Opin. Anim. Vet. Sci.*, 2(4): 291-294. http://www.roavs.com/pdf-files/Issue_4_2012/291-294.pdf
- Alsaad KM, Alsaad EA, Al-Derawie HA (2010). Clinical and diagnostic study of equine babesiosis in drought horses in some areas of Basrah Province. *Res. J. Anim. Sci.*, 4(1): 16-22. <https://doi.org/10.3923/rjnasci.2010.16.22>
- Ameen KA (2012). Occurrence of hard tick and its possible role in the transmission of pathogenic agents. Thesis submitted to University of Mosul, Iraq.
- Aziz KJ, AL-barwary LT (2019b). Prevalence rate of ixodid ticks in equids and some nearby farm animals in erbil governorate, north of Iraq. *Bas. J. Vet. Res.*, 18(1): 337-359.
- Aziz KJ, AL-barwary LT, Mohammed ZA, Naqid IA (2019). Molecular identification and phylogenetic analysis of theileria equi and babesia caballi infections in equids from Erbil Province, North of Iraq. *Adv. Anim. Vet.*

- Sci., 7(12): 10601066. <https://doi.org/10.17582/journal.aavs/2019/7.12.1060.1066>
- Aziz KJ, AL-Barwary TO (2019a). Epidemiological Study of Equine Piroplasmosis (*Theileria equi* and *Babesia caballi*) by Microscopic Examination and Competitive-ELISA in Erbil Province North-Iraq. Iran J. Parasitol., 14(3): 404-412. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6815869/pdf/IJPA-14-404.pdf>
 - Bhoora R, Quan M, Zweygarth E, Guthrie AJ, Prinsloo SA, Collins NE (2010). Sequence heterogeneity in the gene encoding the rhoptry-associated protein-1 (RAP-1) of *Babesia caballi* isolates from South Africa. Vet. Parasitol., 169(3-4): 279-288. <https://doi.org/10.1016/j.vetpar.2010.01.009>
 - Biglari P, Bakhshi H, Chinikar S, Belqeisizadeh H, Ghaffari M, Javaherizadeh S, Faghihi F, Telmadarraiy Z (2018). Hyalomma anatolicum as the main infesting tick in an important livestock rearing region, central area of Iran. Iran. J. Public Health, 47(5): 742. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6005964/pdf/IJPH-47-742.pdf>
 - Brüning A (1996). Equine piroplasmosis an update on diagnosis, treatment and prevention. Br. Vet. J., 152(2): 139-151. [https://doi.org/10.1016/S0007-1935\(96\)80070-4](https://doi.org/10.1016/S0007-1935(96)80070-4)
 - Buling A, Criado-Fornelio A, Asenzo G, Benitez D, Barba-Carretero JC, Florin-Christensen M (2007). A quantitative PCR assay for the detection and quantification of *Babesia bovis* and *B. bigemina*. Vet. Parasitol., 20;147(1-2): 16-25. <https://doi.org/10.1016/j.vetpar.2007.03.031>
 - Edgar RC (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput, Nucl. Agric. Res., 32(5): 1792-1797. <https://doi.org/10.1093/nar/gkh340>
 - Estrada-Peña A, Bouattour A, Camicas JL, Walker AR (2004). Ticks of domestic animals in the Mediterranean region. Vol. 131.
 - Friedhoff KT, Tenter AM, Müller I (1990). Haemoparasites of equines: impact on international trade of horses. Rev. Sci. Tech. Int. Off. Epizoot., 9(4): 1187-1194. <https://doi.org/10.20506/rst.9.4.535>
 - Gou H, Guan G, Liu A, Ma M, Chen Z, Liu Z, Ren Q, Li Y, Yang J, Yin H, Luo J (2013). Coevolutionary analyses of the relationships between piroplasmids and their hard tick hosts. Eco. Evol., 3(9): 2985-2993.
 - Guidi E, Pradier S, Lebert I, Leblond A (2015). Piroplasmosis in an endemic area: analysis of the risk factors and their implications in the control of Theileriosis and Babesiosis in horses. Parasitol. Res., 1;114(1): 71-83. <https://doi.org/10.1007/s00436-014-4161-9>
 - Hall CM, Busch JD, Scoles GA, Palma-Cagle KA, Ueti MW, Kappmeyer LS, Wagner DM (2013). Genetic characterization of *Theileria equi* infecting horses in North America: evidence for a limited source of US introductions. Parasit. Vect., 6(1): 35. <https://doi.org/10.1186/1756-3305-6-35>
 - Hasson RH (2012). Tick distribution and infestation among sheep and cattle in Baghdad's south suburb Raad Hammodi Hasson. Kufa J. Vet. Med. Sci., 3(1): 77-90.
 - Jefferies R, Ryan UM, Irwin PJ (2007). PCR-RFLP for the detection and differentiation of the canine piroplasm species and its use with filter paper-based technologies. Vet. Parasitol., 15,144(1-2): 20-27. <https://doi.org/10.1016/j.vetpar.2006.09.022>
 - Jongejan F, Uilenberg G (2004). The global importance of ticks. Parasitol. Cambridge, 1, 129: S3.
 - Katz J, Dewald R, Nicholson J (2000). Procedurally similar competitive immunoassay systems for the serodiagnosis of *Babesia equi*, *Babesia caballi*, *Trypanosoma equiperdum*, and *Burkholderia mallei* infection in horses. J. Vet. Diag. Invest., 12(1): 46-50.
 - Kernif T, Leulmi H, Raoult D, Parola P (2016). Emerging tick-borne bacterial pathogens. Emerg. Infect., 20: 295-310.
 - Kizilarslan F, Yildirim A, Duzlu O, Inci A, Onder Z, Ciloglu A (2015). Molecular detection and characterization of *Theileria equi* and *Babesia caballi* in horses (*Equus ferus caballus*) in Turkey. J. Equi. Vet. Sci., 35(10): 830-835.
 - Kouam MK, Kantzoura V, Gajadhar AA, Theis JH, Papadopoulos E, Theodoropoulos G (2010). Seroprevalence of equine piroplasms and host-related factors associated with infection in Greece. Vet. Parasitol., 169(3-4): 273-278.
 - Kumar S, Malhotra DV, Sangwan AK, Goel P, Kumar A, Kumar S (2007). Infectivity rate and transmission potential of *Hyalomma anatolicum anatolicum* ticks for *Babesia equi* infection. Vet. Parasitol., 31, 144(3-4): 338-343. <https://doi.org/10.1016/j.vetpar.2006.10.009>
 - Laus F, Spaterna A, Faillace V, Veronesi F, Ravagnan S, Beribé F, Tesi B (2015). Clinical investigation on *Theileria equi* and *Babesia caballi* infections in Italian donkeys. BMC Vet. Res., 11(1): 100. <https://doi.org/10.1186/s12917-015-0411-z>
 - Manna G, Cersini A, Nardini R, Del Pino LE, Antognetti V, Zini M, Conti R, Lorenzetti R, Veneziano V, Autorino GL, Scicluna MT (2018). Genetic diversity of *Theileria equi* and *Babesia caballi* infecting horses of Central-Southern Italy and preliminary results of its correlation with clinical and serological status. Ticks Tick-borne Dis., 1, 9(5): 1212-1220. <https://doi.org/10.1016/j.ttbdis.2018.05.005>
 - Omer LT, Kadir MA, Seitzer U, Ahmed JS (2007). A survey of ticks (Acari: Ixodidae) on cattle, sheep and goats in the Dohuk Governorate, Iraq. Parasitol. Res., 1, 101(2): 179-181. <https://doi.org/10.1007/s00436-007-0690-9>
 - Otranto D, Dantas-Torres F, Giannelli A, Latrofa MS, Cascio A, Cazzin S, Ravagnan S, Montarsi F, Zanzani SA, Manfredi MT, Capelli G (2014). Ticks infesting humans in Italy and associated pathogens. Parasite Vectors, 1, 7(1): 328. <https://doi.org/10.1186/1756-3305-7-328>
 - Ozdarendeli A, Aydin KE, Tonbak S, Aktas M, Altay K, Koksall I, Bolat Y, Dumanli N, Kalkan A (2008). Genetic analysis of the M RNA segment of Crimean-Congo hemorrhagic fever virus strains in Turkey. Arch. Virol., 1, 153(1): 37-44.
 - Qablan MA, Obornik M, Petrzekova KJ, Sloboda M, Shudiefat MF, Horin P, Modry D (2013). Infections by *Babesia caballi* and *Theileria equi* in Jordanian equids: epidemiology and genetic diversity. Parasitology, 140(9): 1096-1103.
 - Salim B, Bakheit MA, Sugimoto C (2013). Rapid detection and identification of *Theileria equi* and *Babesia caballi* by high-resolution melting (HRM) analysis. Parasitol. Res., 112(11): 3883-3886. <https://doi.org/10.1007/s00436-013-3581-2>
 - Schmidt GD, Roberts LS (1989). Foundation of parasitology. 4th ed. St. Louis Mirror: Mosby College Publishing. pp. 660-669.
 - Scoles GA, Ueti MW (2015). Vector ecology of equine piroplasmosis. Ann. Rev. Entomol., 60: 561-580. <https://doi.org/10.1146/annurev-ento-010814-021110>
 - Sigg L, Gerber V, Gottstein B, Doherr MG, Frey CF (2010). Seroprevalence of *Babesia caballi* and *Theileria equi* in the Swiss horse population. Parasitol. Int., 1, 59(3): 313-317. <https://doi.org/10.1016/j.parint.2010.02.005>
 - Sumbria D, Singla LD, Sharma A, Bal MS, Kumar S (2015).

- Multiplex PCR for detection of *Trypanosoma evansi* and *Theileria equi* in equids of Punjab, India. *Vet. Parasitol.*, 211(3-4): 293-239. <https://doi.org/10.1016/j.vetpar.2015.05.018>
- Taylor AM, Coop RL, Wall RL (2007). *Veterinary parasitology*. 3rd ed. Oxford: UK: Blackwell. pp. 295-297.
 - Zangana IK, Naqid IA (2011). Prevalence of piroplasmosis (Theileriosis and Babesiosis) among goats in Duhok Governorate. *Al-Anbar J. Vet. Sci.*, 4(2): 50-57.
 - Zapf F, Schein E (1994). The development of *Babesia* (*Theileria*) *equi* (Laveran, 1901) in the gut and the haemolymph of the vector ticks, *Hyalomma* species. *Parasitol. Res.*, 1, 80(4): 297-302. <https://doi.org/10.1007/BF02351869>