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First molecular sequencing of *Babesia gibsoni* in ticks, Iraq

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ABSTRACT

Background: Tick is one of the most important ectoparasites distributed worldwide and plays an obvious role in the transmission of different infections to humans and animals as dogs.

Aim: This study conducted to molecular demonstration of *Babesia gibsoni* in ticks of stray dogs and phylogenetic analysis of study isolates to detect their identity to global isolates. Prevalence of ticks in dogs, identification of tick species, and their relationship to some risk factors were aimed, also.

Methods: A total of 97 stray dogs were inspected grossly to detect and collect ticks that existed in different body parts. After collection, all ticks were examined morphologically to identify their species, and then molecularly by the polymerase chain reaction (PCR) assay to detect *B. gibsoni* in different species of ticks. Local *B. gibsoni* isolates were sequenced, documented in the National Center For biotechnology information (NCBI) database, analyzed phylogenetically, and compared with the global GenBank-NCBI isolates.

Results: In the current study, ticks were detected in 43.3% of dogs, and were shown to be varied in number and distribution among different body parts of each dog. Concerning its distribution, ticks were observed significantly on the abdomen, ear, and perineal region. In relation to risk factors, ticks were increased significantly in dogs <6 months old in comparison to older dogs, males more than females; and in rural areas more than dogs of sub-urban and urban areas. Based on morphology, different tick species were seen including *Hylaomma anatolicum* (86.12%), *R. sanguineus* (11.99%), and *Rhipicephalus turanicus* (1.89%). Targeting the *18S rRNA* gene, PCR assay reported 3.79% positive ticks to *B. gibsoni* that were seen in *R. sanguineus* (13.16%) and *H. anatolicum* (2.56%). Based on phylogenetic analysis data of five local *B. gibsoni* isolates, this study demonstrated their close relations to the global NCBI-BLAST *B. gibsoni* Iraqi isolate (ID: MN385424.1).

Conclusion: This represents the first Iraqi study that demonstrated molecularly *B. gibsoni* in different species of ticks that infected stray dogs.

Keywords: Canine babesiosis, *Hylaomma anatolicum*, *Rhipicephalus sanguineus*, *Rhipicephalus turanicus*, Phylogeny.

Introduction

Babesia gibsoni is a tick-transmitted protozoan parasite, that belongs to Piroplasmida Order in the Aconoidasida Class under the Apicomplexa Phylum, which infects the domestic and wild canid species causing in a disease known as babesiosis (Baneth *et al*., 2019; Birkenheuer, 2021). Like other members, the life cycle of *B. gibsoni* requires two types of hosts; a tick and a canine host. Briefly, sporozoites enter the host's circulation with tick saliva during blood sucking to attach and penetrate erythrocytes by endocytosis. Once inside, sporozoites transform into trophozoites that develop into merozoites throughout the binary fission process (Baneth, 2018; Conesa *et al*., 2020; Martínez-García *et al*., 2021). Post ingestion of erythrocytes that contain gametocytes, ticks become infected and may remain infective for many generations by the transovarial and transstadial transmission. This process occurs due to the development of gametocytes into female and male gametes in the gut of female ticks to produce later the motile zygotes that multiply to vermicules and invade numerous organs including ovaries. Usually, sporogony resides in the salivary glands of different developmental stages (larval, nymphal, and/or adult) in female ticks that get an infection (Chauvin *et al*., 2009; Friedhoff, 2018; Ravindran *et al*., 2023).

Tick is an ectoparasite of widespread distribution, particularly in tropical and subtropical areas. Ticks have the ability to attach and feed the blood of different domestic and wild animals as well as humans to cause obvious health impacts, and to transmit several infectious viral, bacterial, and parasitic diseases (Mahlobo, 2018; Rajakaruna and Eremeeva, 2023). Scientifically, ticks are classified in the Parasitiformes order, Arachnida class, of the Chelicerata phylum (Proctor *et al*., 2015). Worldwide, different tick species belonging to *Boophilus*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, and *Rhipicephalus* genera can actively

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transmit several *Babesia* species (Aktas, 2014; Onyiche *et al*., 2021). Regarding *B. gibsoni*, *Rhipicephalus sanguineus*, *Haemaphysalis bispinosa*, *Haemaphysalis longicornis*, and *Dermacentor variablis* represent the most prevalent species in endemic areas (Karasová *et al*., 2022).

For diagnosis of the infected ticks, traditional diagnostic methods such as microscopy may yield inconclusive results, especially in absence the history of infection (Lager, 2020; Kahlig *et al*., 2021). The DNA-based methods such as polymerase chain reaction (PCR) and phylogeny can be applied as a highly sensitive, specific, and accurate diagnostic assay because of their capability to provide additional valuable information for genetic markers (Umesha and Manukumar, 2018; Ghosh *et al*., 2019).

Until recently, limited studies have been conducted in Iraq to detect *B. gibsoni* in dogs (Otranto *et al*., 2019; Badawi and Yousif, 2020a,b). Therefore, this study conducted to molecular demonstration of *B. gibsoni* in ticks of stray dogs and phylogenetic analysis of study isolates to detect their identity to global isolates. Prevalence of ticks in dogs, identification of tick species, and their relationship to some risk factors were aimed, also.

Materials and Methods

Samples

Totally, 97 stray dogs were selected randomly from different rural, sub-urban, and urban areas in Basra province (Iraq) from November (2023) to April (2024). Initially, all study animals were subjected clinically to visual examination of the different parts of the body to detect and carefully manually collect of tick samples into labeled plastic tubes containing 70% Ethanol.

Morphology

All ticks were screened stereomicroscopically at the Iraqi Natural History Research Center and Museum (University of Baghdad, Baghdad, Iraq) to investigate their morphological characteristics following the taxonomic keys of related references (Estrada-Peña *et al*., 2004).

Molecular assay

AddPrep Genomic DNA Extraction Kit (AddBio, Korea) was served to extract the DNAs from all tick samples. Then, the obtained DNAs were tested spectrophotometrically by the Nnaodrop System (Thermo Scientific, UK) to estimate their concentration (ng/µl) and purity at an absorbance of 260/280. For DNA amplification, AddStart Taq Master Kit (AddBio, Korea) was utilized in addition to one set of primers (IMF: 5´-ACA ATT GGA GGG CAA GTC TG-3´) and (IMR: 5´-GGC AAA TGC TTT CGC AGT A-3´) was designed for the current study targeting the *18S rRNA* gene based on the sequence data of the National Center For Biotechnology Information (NCBI)-GenBank *B. gibsoni* Turkish isolate (ID: KJ513206.1) and using the Primer3Plus Software. In the Thermal Cycler System (MJ-Mini BioRad, USA), the prepared MasterMix tubes at a final volume of 20 µl were subjected to the steps of PCR reaction as follows: 1 cycle for initial denaturation (95°C/5 minutes), 35 cycles for denaturation (95°C/5 minutes), annealing (58°C/30 seconds) and extension (72°C/1 minute), and 1 cycle for final extension (72°C/5 minutes).

Electrophoresis of PCR products and Ladder marker (100–2,000 bp) in Agarose-Gel stained with 3 µl Ethidium Bromide was performed at 100 V and 80 Am for 90 minutes, and the resultants were visualized under the UV transilluminator (ATTA, Korea) to detect the positive samples at 375 bp.

Phylogeny

For documentation of local *B. gibsoni* isolates in the NCBI, five positive DNA samples were sent for sequencing in the Macrogen Company (Korea) using the Sanger method. The received data of study *B. gibsoni* isolates were reported in the NCBI database and analyzed phylogenetically by the MEGA-6 Software to detect their identity to the global NCBI-GenBank isolates.

Statistical analysis

GraphPad Prism Software *version 6.0.1* (GraphPad Inc, USA) was used to analyze the study data and indicate significant differences at $p<0.05$ using the *t*-test and odds ratio (Gharban, 2023).

Ethical approval

This study obtained a license from the Scientific Committee of the Department of Parasitology in the College of Veterinary Medicine (University of Basrah).

Results

The prevalence rate of ticks among a total of 97 stray dogs was 43.3% (Fig. 1). Also, the overall ticks collected from the study animals were 317 samples that varied significantly ($p<0.05$) in their numbers and distributions throughout the different body parts of each dog. Significantly ($p<0.0173$), 14 (33.33%) of

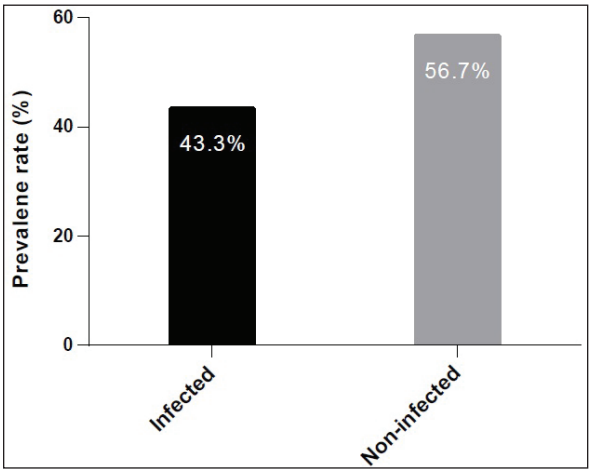

Fig. 1. Prevalence rate of ticks among totally 97 stray dogs.

Table 1. Number of ticks collected from the study dogs (total no: 42 dogs).

Sample	No. of ticks collected from each dog	No. of infected dogs	Total no.
Tick	3	1 (2.38%)	3
	4	8 (19.05%)	32
	5	3 (7.14%)	15
	6	7 (16.67%)	42
	9	14 (33.33%) *	126 *
	11	9 (21.43%)	99
p-value		0.0173 S	0.0484 S
Total No.		42	317

S: Significant increase * ($p < 0.05$).

study dogs were showed a higher rate of tick infestation (having a totally of 126 ticks) when compared to 9 (21.43%), 7 (16.67%), and 8 (19.05%) stray dogs having a totally 99, 42 and 32 ticks, respectively. Whilst, 1 (2.38%) and 3 (7.14%) of study dogs were showed significantly ($p<0.05$) a lower rate of tick infestation; 3 and 15 ticks, respectively (Table 1). Concerning the presence of ticks on different body parts, significant increases ($p<0.0282$) were observed in the abdomen (30.6%), ear (27.13%), and perineal region (23.34%); and decreased significantly ($p<0.05$) in the forelimb (1.58%), neck (4.1%), hindlimb (6.62%), and back (6.62%), (Table 2).

The prevalence rate of ticks was differed significantly ($p<0.05$) according to age, sex, and type of study areas (Table 3). Significantly, study dogs aged <6 months were showed a higher rate and risk of tick infestation (54.9% and 1.806, respectively) in comparison with the other age groups; 6-12 months (34.48% and 0.732, respectively), >12-24 months (20% and 0.448, respectively), and > 24 months (25% and 0.545, respectively). Significantly, male dogs showed an elevation in the prevalence rate of ticks (61.91%) and risk (1.62) than females (38.16% and 0.617, respectively), ($p < 0.0354$ and $p < 0.0002$, respectively). This study noticed that ticks were increased significantly ($p < 0.0234$) in dogs of rural areas (48.24%) more than those of sub-urban (11.11%) and urban (0%) areas. Subsequently, dogs from rural areas were seen at a significant ($p < 0.0001$) higher risk of infection (5.807) than those of sub-urban (0.238) and urban (0.238) areas.

Based on its morphology, all tick samples were screened stereomicroscopically, and the findings revealed the presence of 3 different tick species including *Hylaomma anatolicum* (86.12%), *R. sanguineus* (11.99%), and *Rhipicephalus turanicus* (1.89%) (Table 4; Fig. 2).

Molecularly, conventional PCR assay reported that the prevalence of *B. gibsoni* among study ticks was 3.79% (Fig. 3). Relation to tick species, a significant prevalence of *B. gibsoni* infections (p <0.0403) was seen in *R. sanguineus* (13.16%) than *H. anatolicum* (2.56%), and *R. turanicus* (0%) (Table 5).

Table 2. Distribution of ticks on different body parts of study dogs (total no: 317 ticks).

Body region	No. (%) of ticks
Ear	86 (27.13%) *
Neck	13 (4.1%)
Back	21 (6.62%)
Abdomen	97 (30.6%) *
Forelimb	5 (1.58%)
Hind limb	21 (6.62%)
Perineal region	74 (23.34%) *
p-value	0.0282 S

S: Significant increase * ($p < 0.05$).

For phylogeny, five positive local *B. gibsoni* isolates were sequenced, named, and recorded in the NCBI database (Fig. 4). Targeting the *18S rRNA* gene, multiple sequence alignment, phyologenetic tree analysis, and NCBI-BLAST homology demonstrated that the local study *B. gibsoni* isolates were closely related to the global NCBI-BLAST *B. gibsoni* Iraqi isolate (ID: MN385424.1) at a total similarity of 99.64%–99.66% and a total genetic changes/mutation of 0.007%–0.001% (Figs. 4–6; Table 6).

Discussion

Worldwide, ticks represent the second only to mosquitoes in transmitting several infections in both humans and animals. This study showed that 43.3% of stray dogs were infected with ticks. In Iraq, the prevalence rate of canine ticks was 30.95%–40.47% in Mosul province (Arsalan *et al*., 2006), 16.7% in central provinces (Mohammad, 2015), 68.75% in Basra province (Hatem, 2020), and 10.3% in Erbil province (Aziz and AL-barwary, 2019). In other countries, the prevalence rate of canine ticks was 51.53% in USA (Raghavan *et al*., 2007), 22.92% in

Table 3. Association of infected dogs to age, sex, and type of areas.

Factor	Group	Total no.	Positive		Risk	Odd ratio
			No.	%		
Age (month)	< 6	51	28	54.9 *	1.806 ****	2.779 ****
	6–12	29	10	34.48	0.732	0.592
	>12–24	5	1	20	0.448	0.311
	>24	12	3	25	0.545	0.389
p-value				0.0242 S	0.0001 S	0.0001 S
Sex	Female	76	29	38.16	0.617	0.38
	Male	21	13	61.91 *	1.62 ***	2.634 ****
p-value				0.0354 S	0.0002 S	0.0001 S
Area	Rural	85	41	48.24 *	5.807 ****	10.242 ****
	Sub-Urban	9	1	11.11	0.238	0.143
	Urban	3	0	0	0	0
p-value				0.0234 S	0.0001 S	0.0001 S

S: Significant increase * (p<0.05), **** (p<0.0001).

Table 4. Classification of study ticks based on morphological characteristics.

Species	No.	Prevalence
H. anatolicum	273	86.12% *
R. sanguineus	38	11.99%
R. turanicus	6	1.89%
p-value	0.0103	

S: Significant increase * (p< 0.05).

Table 5. Distribution of *B. gibsoni* infections among different tick species.

Species	Total no.	Positive		*p*-value
		No.	%	
H. anatolicum	273	7	2.56	
R. sanguineus	38	5	13.16 *	0.0403 S
R. turanicus	6	0	0	
Total	317	12	3.79	-

S: Significant increase * (p < 0.05).

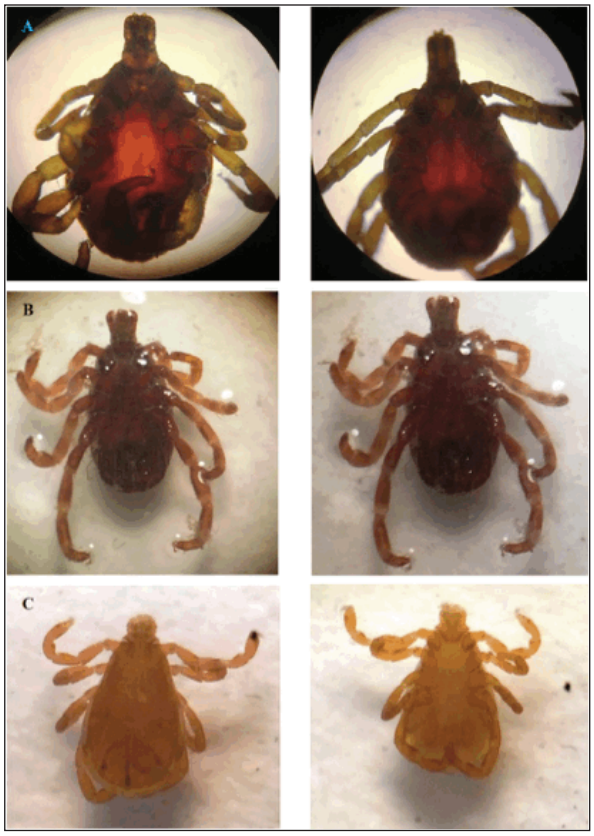

Fig. 2. Classification of study ticks based on its morphology. (A) *Hylaomma anatolicum*, (B) *R. sanguineus*, and (C) *R. turanicus*.

Great Britain (Smith *et al*., 2011), 46.39% in India (Sahu *et al*., 2013), 30% in UK (Abdullah *et al*., 2016), 67.9%–100% in Indonesia (Hadi *et al*., 2016), 45.7% in Italy (Maurelli *et al*., 2018), and 24.3% in Pakistan (Hussain *et al*., 2023). In general, many researchers have been concluded that higher rates of endoparasites and ectoparasites can be seen in stray than in pet dogs (Arsalan *et al*., 2006; Sahu *et al*., 2013), and this might because of the lack of systematic surveillance and preventive strategies in stray dogs. Léger *et al*. (2013) mentioned that alteration in abundance and spatial distribution of different tick species and their

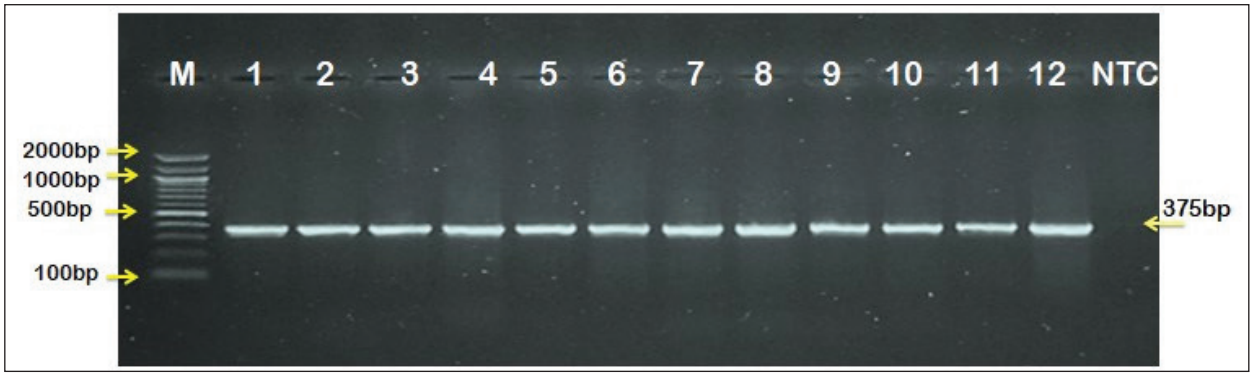

Fig. 3. Agarose-gel electrophoresis of PCR products targeting *18S rRNA* gene; in which, Lane (M): Ladder marker, Lanes (1-12): Positive samples to *B. gibsoni* infections at 375 bp, and Lane (NTC): Negative control (distilled water).

>PP788635.1 Babesia gibsoni isolate IQ.No.1 small subunit ribosomal RNA gene, partial sequence
ATAGCGTATATTAAACTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCGTTGCCCGACTG
GCTACTTGCCTTGTCTGGTTTCGCTTTCGGGGTTTTCCCCTTTTCACTTTGAGAAAATTAGAGT
GTTTCAAGCAGACTTGTGTCTTGATTACTTCAGCATGGAATAATAAAGTAGGACTTTGGTTCTA
TTTTGTTGGTTTGTGAACCTTAGTAATGGTTAATAGGAACGGTTGGGGGCATTCGTATTTAACT
GTCAGAGGTGAAATTCTTAAATTTGTTAGAG

>PP788636.1 Babesia gibsoni isolate IQ.No.2 small subunit ribosomal RNA gene, partial sequence
ATAGCGTATATTAAACTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCGTTGCCCGACTCGGCTAC
TTGCCTTGTCTGGTTTCGCTTTCGGGGTTTTCCCCTTTTCACTTTGAGAAAATTAGAGTGTTTCAAGCAG
ACTTGTGTCTTGATTACTTCAGCATGGAATAATAAAGTAGGACTTTGGTTCTATTTTGTTGGTTTGTGAA
CCTTAGTAATGGTTAATAGGAACGGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAAATT
TGTTAGAG

>PP788637.1 Babesia gibsoni isolate IQ.No.3 small subunit ribosomal RNA gene, partial sequence
ATAGCGTATATTAAACTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCGTTGCCCGACTCGGCTAC
TTGCCTTGTCTGGTTTCGCTTTCGGGGTTTTCCCCTTTTCACTTTGAGAAAATTAGAGTGTTTCAAGCAG
ACTTGTGTCTTGATTACTTCAGCATGGAATAATAAAGTAGGACTTTGGTTCTATTTTGTTGGTTTGTGAA
CCTTAGTAATGGTTAATAGGAACGGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAAATT
TGTTAGAG

>PP788638.1 Babesia gibsoni isolate IQ.No.4 small subunit ribosomal RNA gene, partial sequence
ATAGCGTATATTAAACTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCGTTGCCCGACTCGGCTAC
TTGCCTTGTCTGGTTTCGCTTTCGGGGTTTTCCCCTTTTCACTTTGAGAAAATTAGAGTGTTTCAAGCAG
ACTTGTGTCTTGATTACTTCAGCATGGAATAATAAAGTAGGACTTTGGTTCTATTTTGTTGGTTTGTGAA
CCTTAGTAATGGTTAATAGGAACGGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAAATT
TGTTAGAG

>PP788639.1 Babesia gibsoni isolate IQ.No.5 small subunit ribosomal RNA gene, partial sequence
ATAGCGTATATTAAACTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCGTTGCCCGACTCGGCTAC
TTGCCTTGTCTGGTTTCGCTTTCGGGGTTTTCCCCTTTTCACTTTGAGAAAATTAGAGTGTTTCAAGCAG
ACTTGTGTCTTGATTACTTCAGCATGGAATAATAAAGTAGGACTTTGGTTCTATTTTGTTGGTTTGTGAA
CCTTAGTAATGGTTAATAGGAACGGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAAATT
TGTTAGAG

Fig. 4. Sequence data of local study *B. gibsoni* isolates in the GenBank-NCBI.

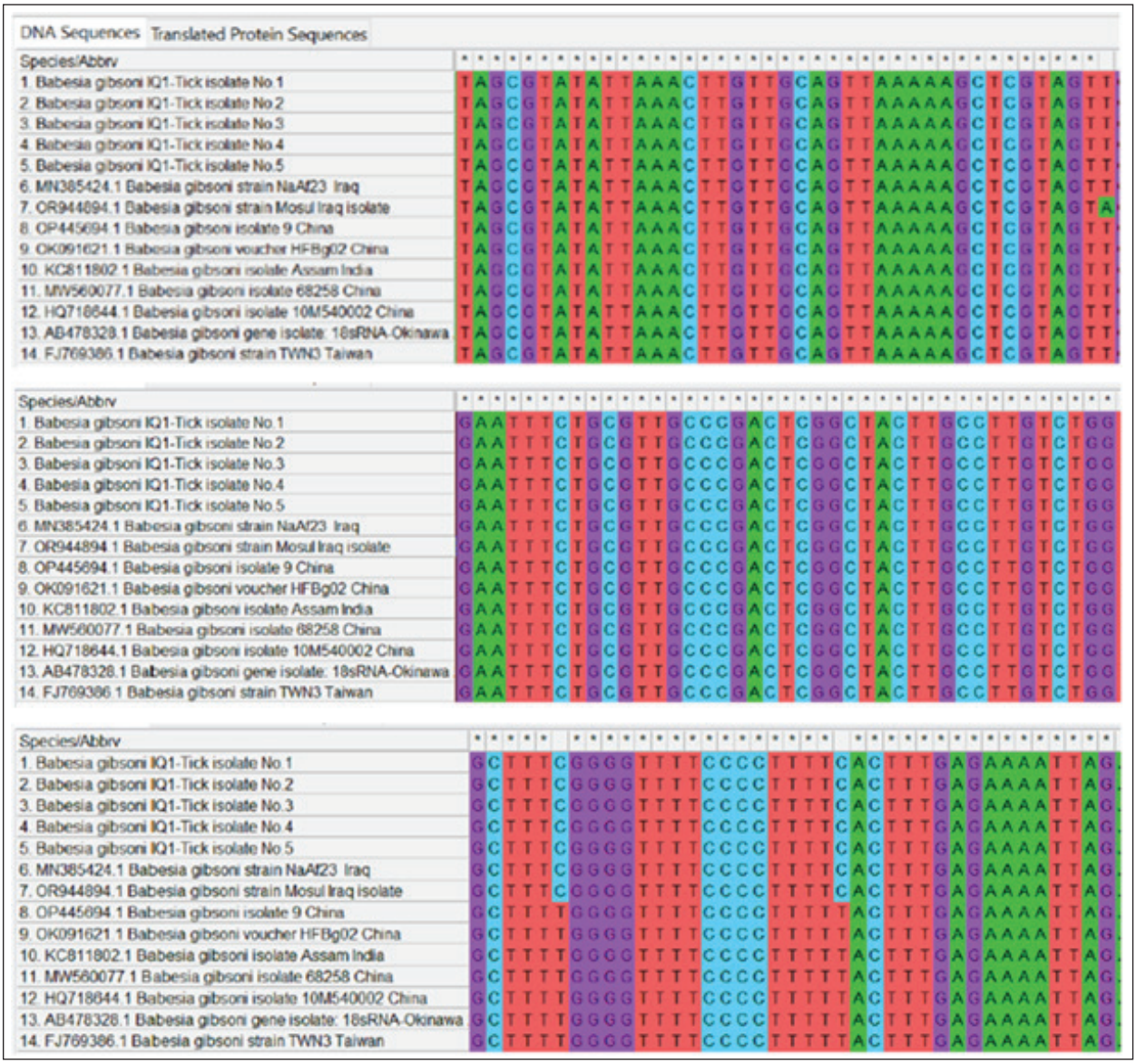

Fig. 5. Multiple sequence alignment analysis of local study and GenBank-NCBI *B. gibsoni* isolates in ticks targeting the *18S rRNA* gene.

associated pathogens may occur due to globalization of human activities, habitat modification, and climate changes.

The number of existing ticks on different body parts was shown in the current study as in agreement with that recorded by other studies that saw the presence of at least one tick on each infested dog (Smith *et al*., 2011; Maurelli *et al*., 2018). However, the low number of obtained ticks from study animals might be affected by the season in which the ticks were collected (autumn and winter). The presence of ticks on different body parts of study dogs might be because these are the most exposed sites to attachment ticks (Claerebout *et al*., 2013; Ramos *et al*., 2020). Hadi *et al*. (2016) mentioned that tick predilections in dogs distributed on the back (35%), head, ears, and neck (29%), legs and interdigital spaces (14.5%), as well as abdomen and tail (10.9%). Maurelli *et al*. (2018) reported that ticks were located insignificantly on different parts of the body including the head (37.4%), neck (28.8%), muzzle (15.5%), and back (15.3%).

In the current study, significantly higher levels of tick infestation were identified in dogs of <6 months age old, males more than females, and dogs in rural than in other study areas. In multiple logistic regressions, Raghavan *et al*. (2007) mentioned that younger, male, and sexually intact dogs have an elevated risk of tick infection. Smith *et al*. (2011) revealed no significant

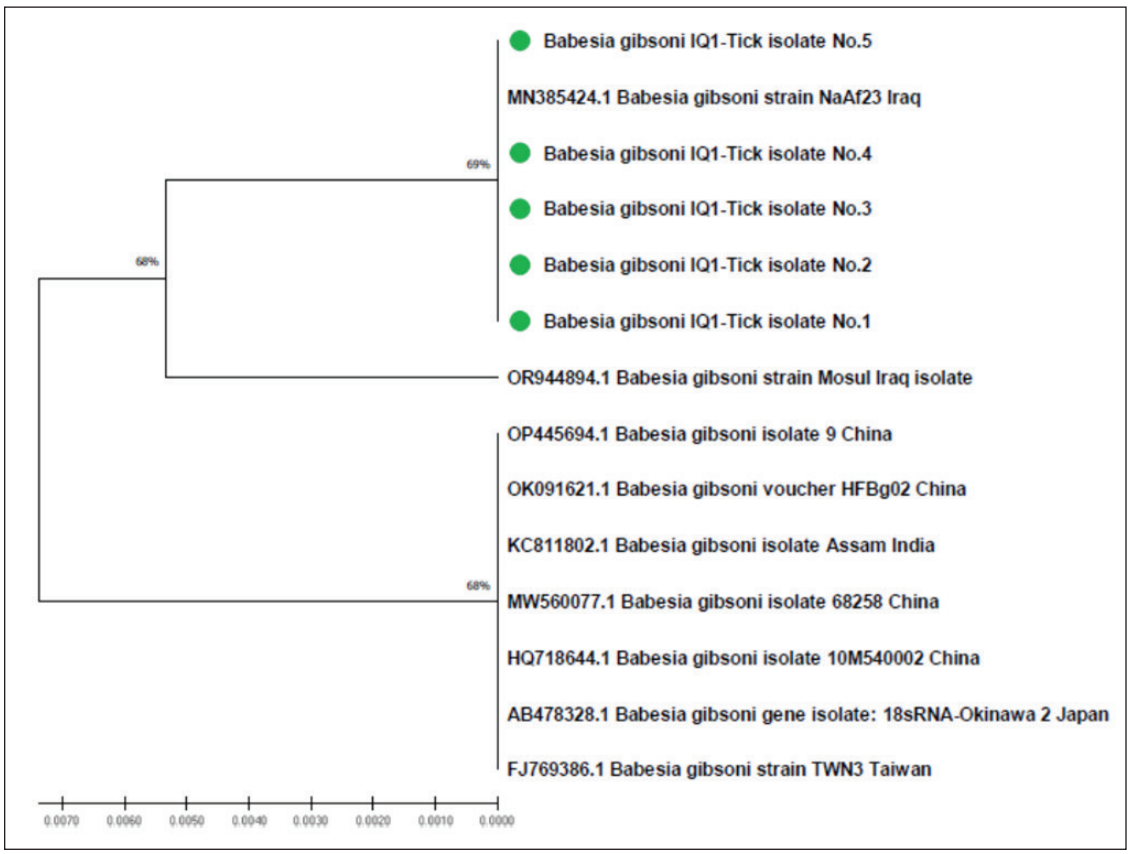

Fig. 6. Phylogenetic tree analysis of local study and GenBank-NCBI *B. gibsoni* isolates in ticks targeting the *18S rRNA* gene.

Table 6. Homology Sequence identity for local and NCBI-BLAST *B. gibsoni* isolates.

Local isolate				NCBI isolate			
Name	**Access no.**	**Host**	**Species**	**Host**	**Country**	**Access no.**	**Identity**
IQ.No.1	PP788635.1	Tick	*B. gibsoni*	Dog	Iraq	MN385424.1	94.81%
IQ.No.2	PP788636.1	Tick	*B. gibsoni*	Dog	Iraq	MN385424.1	94.74%
IQ.No.3	PP788637.1	Tick	*B. gibsoni*	Dog	Iraq	MN385424.1	94.68%
IQ.No.4	PP788638.1	Tick	*B. gibsoni*	Dog	Iraq	MN385424.1	94.70%
IQ.No.5	PP788639.1	Tick	*B. gibsoni*	Dog	Iraq	MN385424.1	94.69%

association between infection and risk factors such as age, sex, and body size. Sahu *et al*. (2013) summarized that the prevalence rate of ticks was higher in dogs of <1 year of age (53.41%) than > 1 year (45.21%), and in males (53.97%) than females (38.31%). Hadi *et al*. (2016) recorded that purebred male dogs were more infested than the crossbred, local, and female dogs. In comparison to results to Maurelli *et al*. (2018), it found that ticks existed significantly in females (18.6%) more than males (6.6%). However, the effect of age on tick infestation might be due to the development of resistance and effective scratching activity in adult dogs than in younger age groups; while for sex variables, hormonal factors and activity might be playing some roles to predispose male dogs more to tick infestations. In this study, *H. anatolicum* was the almost prevalent tick species than *R. sanguineus* and *R. turanicus*. In fact, various studies demonstrated that different species of ticks can parasitize dogs (Latrofa *et al*., 2017; Saleh *et al*., 2019, 2021). In Iraq, earlier studies in dogs have been detected two types of tick species in both Mosul province (Northern part) and Basra province (Southern part) including *R. sanguieus* and *R. turanicus* (Arsalan *et al*., 2006; Awad *et al*., 2006). Smith *et al*. (2011) detected three species belonging to *Ixodes* spp. are *I. ricinus* (72.1%), *I. hexagonus* (21.7%), and *I. canisuga*

(5.6%). Livanova *et al*. (2018) identified 11 tick species, most commonly *Dermacentor reticulates* with a prevalence of 40.7%. Maurelli *et al*. (2018) observed that the tick samples collected from study dogs were belonged to four genera and 14 species, with a higher prevalence of *Rhipicephalus* spp. (10.8%), in particular *R. sanguineus* (63.6%), compared to *Dermacentor* spp. (0.6%), *Haemophysalis* spp. (0.2%), and *Ixodes* spp. (0.2%). Ramos *et al*. (2020) inspected 139 dogs from nine species, and collected tick species from three species including *Amblyomma sculptum*, *R. sanguineus,* and *R. microplus* with a dominance of *A. sculptum*. A number of authors reported that the brown dog tick (*R. sanguineus*) is the most common tick species in dogs, particularly in urban areas (Galaviz-Silva *et al*., 2013; Ojeda-Chi *et al*., 2019; Beristain-Ruiz *et al*., 2022). In Iraq, Obaid *et al*. (2023) showed significantly that the higher prevalence of *Hyalomma* spp. in different provinces was in Duhok (88.6%), Maysan (83.9%), Baghdad (83.47%), Erbil (78.3%), Basra (78.11%), Dhi-Qar (75.85%), Al-Qadisiyah (74.42%), Najaf (73.33%), and Karbala (70.42%). Other recent studies have confirmed morphologically and molecularly that different species of *Ixodid* ticks collected from ruminants were belong to the genus of *Hyalomma* (Karawan *et al*., 2021; Aziz, 2022; Makawi and Hadi, 2023). Therefore, a significant incidence of *H. anatolicum* in rural areas might explain its high existence in study dogs.

Hard ticks of *Hyalomma* genus have been demonstrated as a vector for *Babesia* species in most endemic parts of Asia and Africa (Kumar *et al*., 2020; Karshima *et al*., 2022). In Iraq, despite the extensive work to elucidate patterns of *Babesia* spp. and abundance in various animals (AL-Shabbani and Faraj, 2023; Hossein, 2023; Al-Ani and Yousif, 2024; Al-Shammari *et al*., 2024), relatively two studies have systematically evaluated *Babesia* spp. in dogs (Otranto *et al*., 2019), as well as *B. canis* and *B. gibsoni* in dogs only (Badawi and Yousif, 2020a,b). In the present work, we showed molecularly that 3.79% of study ticks were having *B. gibsoni* distributed in *R. sanguineus* and *H. anatolicum* but not in *R. turanicus*. Globally, different *Babesia* species was demonstrated in various tick species for example *B. microti* (0%–0.9%), *B. canis* (0%–66.7%), *B. venatorum* (0%–0.4%), *B. crassa* (0%–4%), *B. vogeli* (0%–0.9%), and *B. divergens* (0%–0.4%) in Russia (43); and *B. venatorum* (84.3%), *B. vulpes* (10%), *B. divergens* / *B. capreoli* (2.9%), and *B. microti* (1.4%) in UK (Abdullah *et al*., 2018). Phylogenetically, analyzing of local *B. gibsoni* isolates revealed a close relation to the global NCBI-BLAST *B. gibsoni* strains isolated from Iraqi dogs by Badawi and Yousif (2020a,b). This indicates that local *B. gibsoni* isolates might be circulated between ticks and stray and domestic dogs in Iraq. The *18S rRNA* gene, used in this study, is widely applied in Asia, Australia, America, and Europe to establish phylogenetic relationships as well as to differentiate the genotypes or subspecies of canine babesiosis (Schäfer *et al*., 2023; Yin *et al*., 2023; Zygner *et al*., 2023).

Conclusion

To the best of our knowledge, this represents the first study in Iraq demonstrated molecularly and phylogenetically the presence of *B. gibsoni* in different tick species including *H. anatolicum*, *R. sanguineus,* and *R. turanicus*. Subsequently, we first indicate the high prevalence of *H. anatolicum* in stray dogs suggesting its role in the transmission of canine babesiosis. Current concerns over the potential impacts of climate change and the increased movement between countries of stray animals on the distribution of ticks highlight the need for an accurate understanding of existing prevalence patterns. Furthermore, epidemiological screening, surveillance, and monitoring of parasitic infection could be carried out broadly with the help of appropriate diagnostic assays such as molecular and phylogenetic techniques. In Iraq, there was limited available data to identify various parasites in ticks and the exact role of each tick species in the transmission of infection among different types of animals.

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Conflict of interest

The authors have no conflict of interest to disclose.

Authors' contributions

GYAA: Designation of the work, collection of tick samples their related data as well as information concerning stray dogs, and statistical analysis. IME: Molecular assaying of ticks and detection of *B. gibsoni* in different tick species. NKT: Phylogentic analysis of study local *B. gibsoni* isolates. All authors contributed equally in writing, reading, and approving the final copy of the manuscript.

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Data availability

All data supporting the findings of this study are available within the manuscript.

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