Ann. For. Res. 65(1): 8232-8252, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

CONVENTIONAL AND MOLECULAR DIAGNOSTIC STUDY OF BABESIA SPECIES INFECTED CAMELS (CAMELUS DROMEDARIUS) IN AL-NAJAF AL-ASHRAF PROVINCE, IRAQ

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Abstract

The current study was carried out to determine Babesia species infections in Iraqi one-humped camels (Camelus dromedarius) based on microscopic and molecular methods. During the period from the beginning of December 2020 to the end of August 2021, 180 blood samples were randomly collected from camels of various ages and both sexes at the slaughter house of Al-Najaf Al-Ashraf province, Iraq. Giemsa stained blood smear examination revealed that 19/180 (10.55%) of camels positive for Babesia spp. infections. In relation to Babesia prevalence and the risk factors, the highest infection rate was at the age of > 1 year (adults) 18/133 (13.53%), females had the highest infection rate 5/27 (18.51%) compared to males was 14/153 (9.15%); the prevalence of Babesia spp. is distributed over the months of the study, except for January and February. However, the highest infection rate was recorded on August 6/20 (30%). Molecular study results based on nested PCR for the mitochondrial cytochrome c oxidase subunit I (coxI) gene showed that two species of Babesia, including Babesia bigemina with a high prevalence 21/100 (21%) and Babesia bovis 3/100 (3%) infected camels. For confirmation, DNA sequencing and phylogenetic tree relationship analysis (Mega 6.0) of local Babesia species and isolates related to the NCBI-Blast homology sequence indicated that the local Babesia bigemina camel blood isolates No.1- 5 showed closed genetic related into NCBI-BLAST Babesia bigemina China isolate (JQ518300.1) with genetic homology sequence identity (98.39%-99.60%) and local Babesia bovis camel blood isolates No. 1 and 2 showed closed genetic related into NCBI-BLAST Babesia bovis China isolate (JQ518301.1) with genetic homology sequence identity (99.13%-99.34%) then local Babesia species isolates were submitted into NCBI Genbank and identified by accession numbers (ON454068.1, ON454069.1, ON454070.1, ON454071.1 and OP094087.1) for B. bigemina (OP146607.1 and OP146608.1) for B. bovis.

Key words: camel, nested PCR, Babesia bigemina, Babesia bovis, Iraq.

Introduction

Camels are multipurpose animals of vital socioeconomic importance, it has been served millions of people since the old times especially in arid, semi-arid, mountainous and desert areas of the world (Karimi et al., 2015). Babesia, which are tick-transferred haemoprotozoan that infect mammals and birds, are well-known for their significant impact on domestic animal health and

associated economic expenses around the world. Babesia infections in wildlife can be lethal if they are linked to stressful management, which is becoming a growing public-health concern (Schnittger et al., 2012). Haemoparasitic infections are widespread and are characterized a partial or complete appetite loss, emaciation, paleness of mucous membranes, digestive disorders, diarrhea, rough hair coat, irregular heartbeats and lacrimation, while others had haemoglobinuria, coughing and exsistence of ticks on various body parts (Al-Obaidi et al., 2019).

Although their sensitivity and specificity are restricted, microscopic detection methods are still the cheapest and fastest procedures for identifying Babesia parasites (Mosqueda et al., 2012). With development of molecular methods such as DNA hybridization tests, polymerase chain reaction and its modifications, infection in the latent phase of the disease can be detected. assays based on nucleic acid are very sensitive, free from immunocompetency and can distinguish between morphologically similar parasites (Maharna et al., 2016).

There are some studies about Babesia spp. in camels that were conducted in Iraq by using blood smear microscopy and conventional PCR, Jasim et al. (2015) and Al-Saad et al. (2015) in Basrah province of southern Iraq, Al-Obaidi et al. (2019) in Nineveh governorate of Iraq recorded that camels were infected with Babesia caballi, while in Al-Diwaniyah province of Iraq, Al-Naily (2018) confirmed that Babesia bovis and Babesia bigemina infected camels. Salman et al. (2022) indicated that one-humped camels infected with Babesia bovis, B. bigemina and Babesia sp. using PCR assays in six Egyptian governorates.

The current study was carried out to investigate Babesia parasites in Iraqi camels (Camelus dromedarius) in Al-Najaf Al-Ashraf province by using blood smear examination and molecular diagnosis based on nested PCR for mitochondrial cytochrome c oxidase subunit I (coxI) gene followed by DNA sequencing and phylogenetic tree relationship analysis between the local Babesia spp. and global isolates.

Materials and Methods

Sample collection: A total of 180 blood samples of camel were collected randomly of different ages (47 Young ≤ 1 year and 133 Adult >1 year) and both sexes (153 males and 27 females) in weekly visitations to slaughter house of AL-Najaf province during the period from beginning of December 2020 to the end of August 2021. Five ml of blood sample was collected during the slaughtering of animal and kept in vacuum tubes with anticoagulant ethylene diamine tetra-acetic acid (EDTA). All samples were transferred in cooling conditions to the laboratory of College of Veterinary Medicine, University of Baghdad to conduct the blood smears tests to determine the infection with Babesia spp. (Garcia, 2009) after that, samples were freeze at (-20 C°) for DNA extraction and molecular diagnosis based on polymerase chain reaction (Romero-Salas et al., 2016). The data of animal's study including (No. of sample, sex, age, tick presence, etc.) were recorded

Parasitological examination: thick and thin blood smears were prepared and the dried thick and thin blood smears fixed by heating and absolute methyl alcohol for 3-5 minutes. Staining was performed using 10% Giemsa solution for 20-30 minutes. The stained thick and thin blood smears

were examined with the help of oil immersion lens (100X) to detect the presence of Babesia spp. (Garcia, 2009).

Blood DNA Extraction: Genomic DNA from camel blood samples was extracted by using the gSYAN DNA extraction kit (Frozen Blood) (Geneaid/USA). This was done according to the manufacturer's instructions. The extracted genomic DNA was checked by using a Nanodrop spectrophotometer (THERMO. USA), which checks and measures DNA purity by reading absorbance at (260/280 nm).

Polymerase chain reaction (PCR): The nested PCR technique was performed for the detection of Babesia species based on a highly specific variable region in the mitochondrial cytochrome c oxidase subunit I (cox1) gene according to (Romero-Salas et al., 2016). Five PCR and nested PCR primers for detection of Babesia species based on a conserved region in the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene were designed by the NCBI Genbank data base and primer3 plus. These primers were provided by Scientific Researcher Co. Ltd., Iraq, as shown in (Table 1):

Table (1): Primers that were used to diagnose Babesia species in blood samples of camels.

Primers		Sequence 5′-3′	Product size	Genbank code
PCR *Babesia bovis* cox1 gene	F	TGGGAAGTGGTACAGGTTGG	502bp	EU075182.1
	R	TGAGCCCAAACCAAACAACC		
Nested PCR *Babesia bovis* cox1 gene	F	GCAAGTGCAATGAGTGGTGC	219bp	EU075182.1
	R	TGGATCACCGGAATTTGAAGA		
PCR *Babesia bigemina* cox1 gene	F	GCAAGTGCAATGAGTGGAGC	440bp	AB499085.1
	R	GCACGAGTATCAGCCTCCAA		
Nested PCR *Babesia*	F	AGACAGATTAGGTCCAATAGCTTG	333bp	JQ518300.1
	R	TGAGCCCATACAAGACAACCA		

bigemina cox1 gene				
PCR *Babesia caballi* cox1 gene	F	GCAAGTGCAATGAGTGGAGC	629bp	AB499086.1
	R	ACATCAACTCCTGCATTTCCA		
Nested PCR *Babesia caballi* cox1 gene	F	TGAATCCAGTTGCTTGGTCA	526bp	AB499086.1
	R	ACTCCAGTTGTACCTCCAATAACA		
PCR *Babesia equi* Cox1 gene	F	TGATCTTACCGGCGTTTGGA	557bp	AB499091.1
	R	GACAAAATGCATTGGGGCGA		
Nested PCR *Babesia equi* Cox1 gene	F	AGCCATGCTATCAATTGGAATTCT	333bp	AB499091.1
	R	GCCCCAATTGACAGAACAAAGT		
PCR *Babesia canis* Cox1 gene	F	GCTAGTGCAATGAGTGGAGC	600bp	KC207822.1
	R	ACCAGTTGTTCCACCAATAACG		
Nested PCR *Babesia canis* Cox1 gene	F	AAAGTCTATTGGTCAAACAATGGAT	510bp	KC207822.1
	R	GCCATCATAACTATTCCAATGCTTCT		

Nested PCR master mix preparation

The GoTaqTM Green PCR Master Mix was used to prepare the first and Second round PCR master mix, which was done according to the company instructions, at a total volume of 25µL.

PCR Thermocycler Conditions

The conditions of the PCR and the nested PCR thermocycler using a conventional PCR thermocycler system are as follows: initial denaturation (95 °C / 5 min.) for 1 cycle, followed by denaturation (95 °C for 30 seconds), annealing (58 and 60 °C for 30 seconds), and extension (72 °C/ 1 min.) for 30 cycles; final extension (72 °C / 5 min.) for 1 cycle; and hold (4°C forever).

Nested PCR product analysis

The nested PCR products were analyzed by 1.5% agarose gel electrophoresis using 1X TBE, stained with ethidium bromide, and visualized using a UV Transilluminator.

DNA sequencing method

A DNA sequencing method was implemented to confirm the molecular detection of some positive Babesia species using nested PCR. In addition to studying the genetic relationship analysis between isolates of local Babesia species and isolates related to NCBI-Blast. The nested PCR products (5 of Babesia bigemina and 2 of B. bovis) for the cox1 gene were sent to Macrogen Company in Korea in an ice bag by DHL for DNA sequencing using the AB DNA sequencing system. DNA sequence analysis (phylogenetic tree analysis) was performed using Molecular Phylogenetic Analysis version 6.0 (Mega 6.0) and multiple sequence alignment analysis based on ClustalW Alignment Analysis. The evolutionary distances were computed using the Maximum Composite Probability method by the UPGMA phylogenetic tree method. Finally, identified Babesia species isolates were submitted to NCBI-Genbank to get a Genbank accession number.

Statistical analysis: The data for this study was analyzed using computer software, including Microsoft Office Excel and IBM/SPSS (version 26). To calculate the significant differences, the Chi-square test (x2) was applied at a $p \leq 0.05$ (Morgan et al., 2020).

Results and Discussion

Blood Smear: The study clarified that results of microscopical examination of Giemsa-stained blood smears collected from 180 camels with different ages and genders in Al-Najaf Al-Ashraf slaughter house were 19/180 (10.55%) of camels infected with Babesia species as shown in (Figure 1, Table 2).

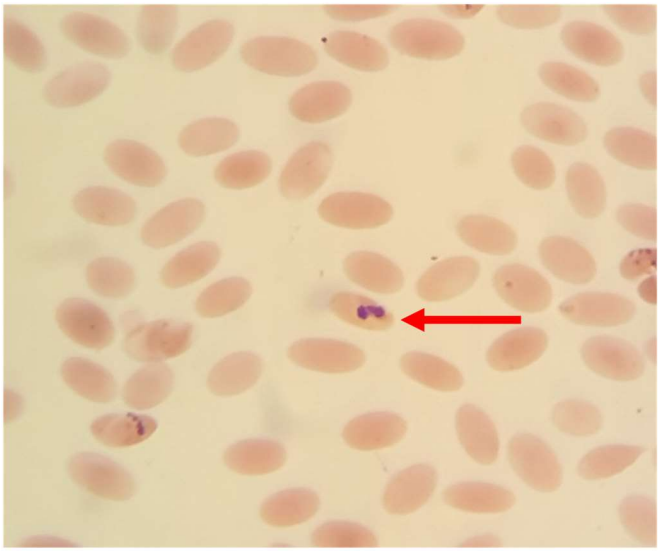

Figure (1): Thin blood smears stained with Giemsa stain show Babesia spp. inside the erythrocytes (100×).

Table (2) Infection rate of Babesia spp. in camels by microscopic examination

No. of examined camel	No. of infected camel	Percent %
180	19	10.55

In giemsa stained blood films Babesia sp. merozoites pear shaped and arranged in pairs were observed near the margin of infected red blood cells (Swelum et al., 2014). This result is in agreement and close related to previous studies about Babesia species in camels, Jasim et al. (2015) who recorded (9.95%) in Basrah province south of Iraq, Faraj and AL-Amery (2018) whose recorded (17.5%) in camel of Al-Najaf province of Iraq, Mirahmadi et al. (2021) was (10%) in the southeastern regions of Iran, Al Malki et al. (2022) was (7.84%) in the Taif region of Saudi Arabia. This convergence and agreement are due to similarity in environmental conditions and the availability of tick vectors (Farhan and Hameed, 2017). Our result is in disagreement and lower than that recorded by Al-Naily (2018) was (53%) in Al-Qadisiyah province of Iraq. This variation in infection rates of Babesia by using Giemsa stained blood smear in camels is due to differences in the study area, sampling size, distribution of infected tick vectors and using of acaricides during tick infestation; adequate use of antiparasitic drugs, herd size, camel management systems in the study area, seasons of collecting samples and weather conditions of the year (Fesseha et al., 2022; Arwa and Kawan, 2022), add to that Camel resistance to various pathogenic diseases (El-Sayed et al.,2021).

Risk factors for Babesia spp. infections: Regarding the age of animals, the results of the present study showed that the highest infection rate was at the age of more than a year (adults) 18/133 (13.53%). While the lowest infection rate was at less than a year of age (young) 1/47 (2.12%), there were significant differences (P≤0.05) between the two age groups (Table 3).

Table (3) Infection rates of Babesia spp. in camels by microscopic examination to age of animals.

Age/ year	No. of examined animals	No. of infected animals (%)	P-value
Young ≤ 1 year	47	1 (2.12)	P<0.029
Adult >1 year	133	18 (13.53)	
Total	180	19 (10.55)	

*P≤0.05

This findings are in agreement with Farhan and Hameed (2017) recorded the highest infection rate of Babesia spp. in camels more than 3 years old (78.05 %) and AL-Amery et al. (2017) infection was higher in adult camels 7-8 years in Al-Najaf province, Al-Naily (2018) found high Babesia infections in camels for more than one year (57.33%), Faraj and AL-Amery (2018) revealed a high infection rate of Babesia in camels aged more than one year (28.33%), Kyari et al. (2021) recorded a significant difference of Babesia infection based on age of camels with higher prevalence rate

among adults, However, our results come incompatible with Karimi et al. (2015) and Azeem et al. (2019) clarified that differences were nonsignificant between age groups of infected camels with babesiosis, Al Malki et al. (2022) indicated that infection rate of Babesia was highly significant increase in younger camels than older in Saudi Arabia.

The high prevalence of Babesia in camels more than one year old may be due to heavy stress factors such as their use for transportation of goods and poor management, while low Babesia infections in young camels is due to the presence of maternal immunity of newborn animals, making them more resistant to infection and the low number of tick infestations in young animals (Farhan and Hameed, 2017; Al-mialy et al., 2018), In my opinion, adult camels often free grazing in open pastures, so they will be susceptible to infection with various parasites, especially ectoparasites of ticks that may be infected by Babesia.

The results of the study confirmed that the highest infection rate was in females (18.51%) compared to males was (9.15%), there were nonsignificant differences (P>0.05) between them, (Table 4).

Table (4) Infection rates of Babesia spp. in camels by microscopic examination to sex of animals.

Gender	No. of examined animals	No. of infected animals (%)	P-value
Males	153	14 (9.15)	P>0.144
Females	27	5 (18.51)	
Total	180	19 (10.55)	

P>0.05

Results of present study is compatible with El-Naga and Barghash (2016) showed that both sexes were at risk of Babesia infections, especially females, Farhan and Hameed (2017), Selmi et al. (2019) and Azeem et al. (2019) who reported that differences were nonsignificant between male and female of infected camels with babesiosis, Badawi and Yousif (2020) reported that Babesia was more common in females than males, but not significantly, Kyari et al. (2021) recorded that higher infection rate of Babesia among females of camel. Our results are different from AL-Amery et al. (2017) who reported high infection rate in males of camels, Faraj and AL-Amery (2018) illustrated that differences between male and female were significance that increased in males of camels, Al Malki et al. (2022) indicated that infection rate of Babesia was highly significant increase in males than females of studied camels in Saudi Arabia. There were non-significant differences in infection rates of Babesia between male and female camels. This means that both sexes are prone to infection due to the same environmental conditions, grazing in the same area and exposure to tick vectors (Farhan and Hameed, 2017), however; infection rates are slightly elevated in female camels. This is due to females' high exposure to stress factors such as gestation

and milk production compared to males. Also, females live longer than males because of the later slaughter for human consumption (Al-mialy et al., 2018).

The current study revealed that results of blood smear microscopy examination for Babesia spp. infections are distributed over the months of the study, except for January and February. However, the highest infection rate was recorded in August 6/20 (30%) while the lowest infection rates were in December, March and April 1/20 (5%), statistically the differences were significant (P≤0.05) among study months (Table 5).

Table (5) Infection rates of Babesia spp. in camels by microscopic examination distributed over the study months.

Months	No. of examined animals	No. of infected animals (%)	P-value
December-2020	20	1 (5.00)	P<0.018
January-2021	20	0 (0.00)	
February	20	0 (0.00)	
March	20	1 (5.00)	
April	20	1 (5.00)	
May	20	4 (20.00)	
June	20	2 (10.00)	
July	20	4 (20.00)	
August	20	6 (30.00)	
Total	180	19 (10.55)	

*P≤0.05

Results of current study is close with Al-Naily (2018) indicated that prevalence of blood parasites was increase in the October and decrease in January with significant differences among study months. Moreover, this finding come in disagreement of Farhan and Hameed (2017) discovered that infection rate of Babesia was high in rainy season (13.3%) whereas, dry season (6.2%).

These fluctuations in infection rates among study months return to the fact that there was a close relationship between the elevation in Babesia spp. infections and the seasonal activity of vector ticks and the environmental and climatic changes (Bakirci et al., 2012).

Molecular diagnosis of Babesia spp.

In the current study, the nested PCR technique for detection of Babesia species in camel blood confirmed that two species, including Babesia bigemina (21%) and Babesia bovis (3%), infected camels. Statistical analysis revealed that the differences between two species were significant (P

≤ 0.05), while not recording positive cases of nested PCR for Babesia caballi, B. equi and B. canis (Table 6, Figures 2 and 3).

Table (6): Results of a nested PCR assay for Babesia species in camel blood

***Babesia* spp.**	**Total No.**	**Positives (%)**	**P-value**
Babesia bigemina	100	21 (21%)	P<0.000
Babesia bovis	100	3 (3%)	

* (P ≤ 0.05)

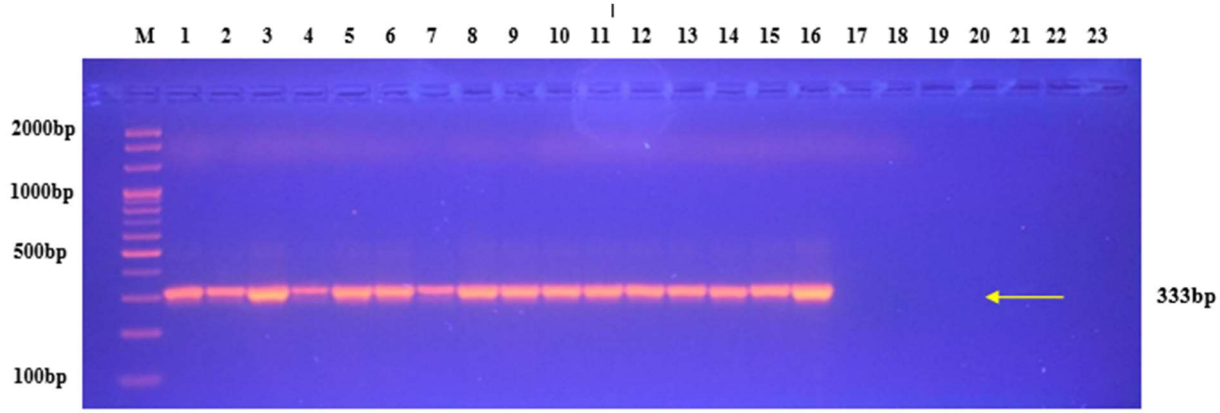

Figure (2): Agarose gel electrophoresis of nested PCR products that detected the mitochondrial cytochrome c oxidase subunit I (cox 1) gene in Babesia bigemina camel blood samples, M: Marker ladder (2000-100bp), and lanes (1-16) were showed only positive samples at (333bp) nested PCR product size.

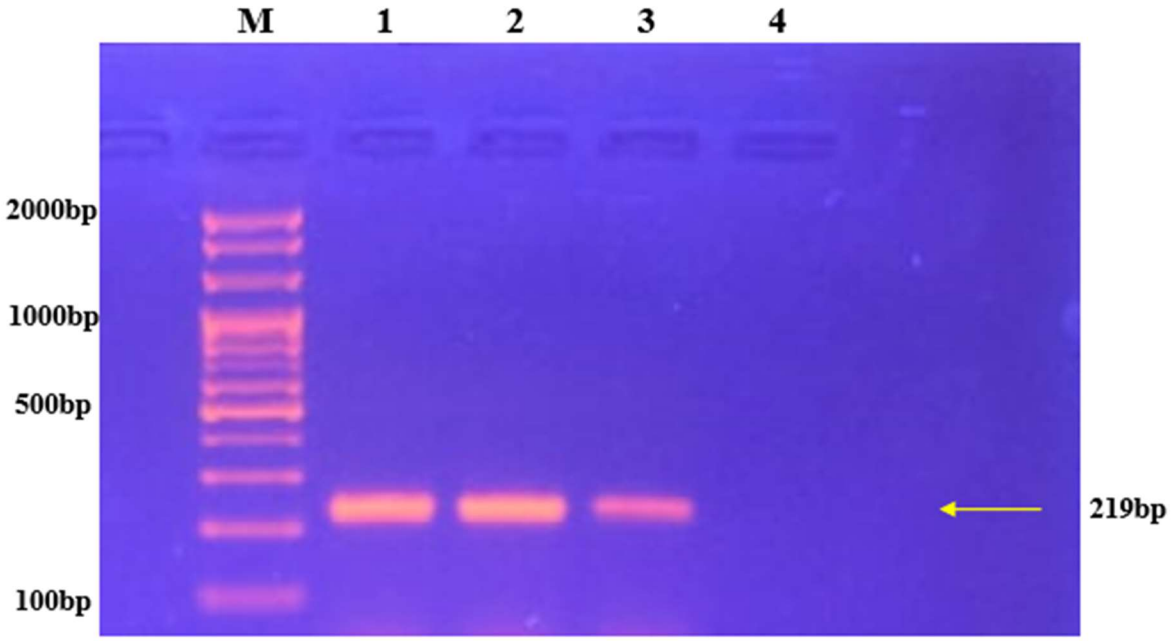

Figure (3): Agarose gel electrophoresis of nested PCR products that detected the mitochondrial cytochrome c oxidase subunit I (cox 1) gene in Babesia bovis camel blood samples, M: Marker ladder (2000-100bp), and lanes (1-3) were showed only positive samples at (219bp) nested PCR product size.

Results of the current study are in agreement with and close to those of Al-Naily (2018) recorded Babesia bigemina and Babesia bovis (12.22%) and (8.9%) respectively in camels by molecular methods in AL-Diwaniyah province, El-Sayed et al. (2021) detected that camel was infected with B. bovis (2.81%) by using nested PCR technique from two provinces of Egypt. Our results are differed from those of Sabbar and Aaiz (2016) indicated that Babesia bovis was in a high rate (47.91%) in cattle of Al-Qadisiyah province, Al-Abedi and Al-Amery (2020, 2021) detected that B. bovis was higher than B. bigemina in cattle and their ticks, Salman et al. (2022), who confirmed that Babesia bovis was at a rate (19.6%), which was higher than B. bigemina (14.9%) that infected one-humped camels using PCR assays in six Egyptian governorates, Moreover, our results are disagree with each of Qablan et al. (2012) in Jordan, Jasim et al. (2015) in Basrah province of Iraq, Bahrami et al. (2017) in Iran and Mirahmadi et al. (2021) in the southeastern regions of Iran, who reported B. caballi in camels by molecular methods with different percentages.

This variation in worldwide prevalence of Babesia spp. was due to climate and ecological diversity, distribution of vectors, number of samples used, differences in targeted genes and primer design, type of PCR technique used and technician experience, in addition to the climatic conditions of the study region (Yıldırım et al., 2013; Faraj et al., 2019; Al-Obaidi et al., 2020). The source of Babesia bigemina and B. bovis infections in camels may be the result of free grazing of the animals. In addition to climatic conditions, tests used and vectors, especially ticks, are of importance in the transmission and management of disease in this study area (El-Naga and Barghash, 2016).

DNA Sequencing and Phylogenetic analysis

DNA Sequence results of Babesia bigemina:

The DNA sequencing method was carried out to identification genetic relationship analysis in mitochondrial cytochrome c oxidase subunit I (coxI) gene in local Babesia bigemina camel blood isolates and NCBI-Blast related country Babesia bigemina isolates (Figure 4). The phylogenetic tree genetic relationship analysis was showed that the local Babesia bigemina camel blood isolates (B. bigemina camel blood No.1- B. bigemina camel blood No.5) were showed closed genetic related into NCBI-BLAST Babesia bigemina China isolate (JQ518300.1) at total genetic changes (0.01%). as shown in (Figure 5).

The homology sequence identity between local Babesia bigemina camel blood isolates (B. bigemina camel blood No.1- B. bigemina camel blood No.5) and NCBI BLAST related Babesia bigemina China isolate (JQ518300.1) that originated from cattle and sheep (Gou et al., 2012) showed genetic homology sequence identity ranged from (98.39%-99.60%). As shown in (Table 7). Finally, the local Babesia bigemina camel blood isolates (B. bigemina camel blood No.1- B. bigemina camel blood No.5) were submitted into NCBI Genbank and identified by accession numbers (ON454068.1 into OP094087.1).

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ON454069.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTCCGGTTC
OP094087.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTCCGGTTC
ON454068.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGAATCAGTATTATATCAACATTTGTTCCGGTTC
ON454071.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTCCGGTTC
MW307310.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTTTGGTTC
MW307309.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTTTGGTTC
MW307308.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTTTGGTTC
AB499085.1      ATGTTCTTTGAGTCCTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTTTGGTTC
JQ518300.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTCYGGTTC
ON454070.1      ATGTTCTTTGAGTCTTCAAATTCAGGAGATCCAGTATTATATCAACATTTGTTCCGGTTC
                ************** **************  **********************  *****

ON454069.1      TTTGGTCATCCAGAGGTATCTATTTTAATATTACCTGCTTTTGGTGTGATAAGTGCTATA
OP094087.1      TTTGGTCATCCAGAGGTATCTATTTTAATATTACCTGCTTTTGGTGTGATAAGTGCTATA
ON454068.1      TTTGGTCATCCAGAGGTATATATTTTAATATTACCTGCTTTTGGTGTGATAAGTGCTATA
ON454071.1      TTTGGTCATCCAGAGGTATATATTTTAATATTACCTGCTTTTGGTGTGATAAGTGCTATA
MW307310.1      TTTGGTCATCCAGAGGTATATATTTTGATATTACCTGCTTTTGGTGTGATAAGTGCTATA
MW307309.1      TTTGGTCATCCAGAGGTATATATTTTGATATTACCTGCTTTTGGTGTGATAAGTGCTATA
MW307308.1      TTTGGTCATCCAGAGGTATATATTTTGATATTACCTGCTTTTGGTGTGATAAGTGCTATA
AB499085.1      TTTGGTCATCCAGAGGTATATATTTTGATATTACCTGCTTTTGGTGTGATAAGTGCTATA
JQ518300.1      TTTGGTCATCCAGAGGTATATATTTTAATATTACCTGCTTTTGGTGTGATAAGTGCTATA
ON454070.1      TTTGGTCATCCAGAGGTATATATTTTAATATTACCTGCTTTTGGTGTGATAAGTGCTATA
                ******************* ****** *********************************

ON454069.1      CTATCTTCTTATTGTACAAAAGAGCTATTAGGAAGTCAAACTATGATATTAGCTATGATT
OP094087.1      CTATCTTCTTATTGTACAAAAGAGTTATTAGGAAGTCAAACTATGATATTAGCTATGATT
ON454068.1      CTATCTTCTTATTGTACAAAAGAGCTACTTGGAAGTCAAACTATGATATTAGCTATGATT
ON454071.1      CTATCTTCTTATTGTACAAAAGAGCTATTTGGAAGTCAAACTATGATATTAGCTATGATT
MW307310.1      TTATCTTCTTATTGTACAAAAGAGCTATTTGGAAGTCAAACTATGATATTAGCTATGATT
MW307309.1      TTATCTTCTTATTGTACAAAAGAGCTATTTGGAAGTCAAACTATGATATTAGCTATGATT
MW307308.1      TTATCTTCTTATTGTACAAAAGAGCTATTTGGAAGTCAAACTATGATATTAGCTATGATT
AB499085.1      TTATCTTCTTATTGTACAAAAGAGCTATTTGGAAGTCAAACTATGATATTAGCTATGATT
JQ518300.1      CTATCTTCTTATTGTACAAAAGAGCTATTTGGAAGTCAAACTATGATATTAGCTATGATT
ON454070.1      CTATCTTCTTATTGTACAAAAGAGCTATTTGGAAGTCAAACTATGATATTAGCTATGATT
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Figure (4): Multiple sequence alignment analysis of mitochondrial cytochrome oxidase subunit I (coxI) gene in local Babesia bigemina Camel blood isolates and NCBI-Genbank Babesia bigemina country isolates. The multiple alignment analysis was constructed using ClustalW alignment tool in (MEGA 6.0 version). That showed the nucleotide alignment similarity as (*) and substitution mutations in mitochondrial cytochrome oxidase subunit I (coxI) gene between different Babesia bigemina isolates.

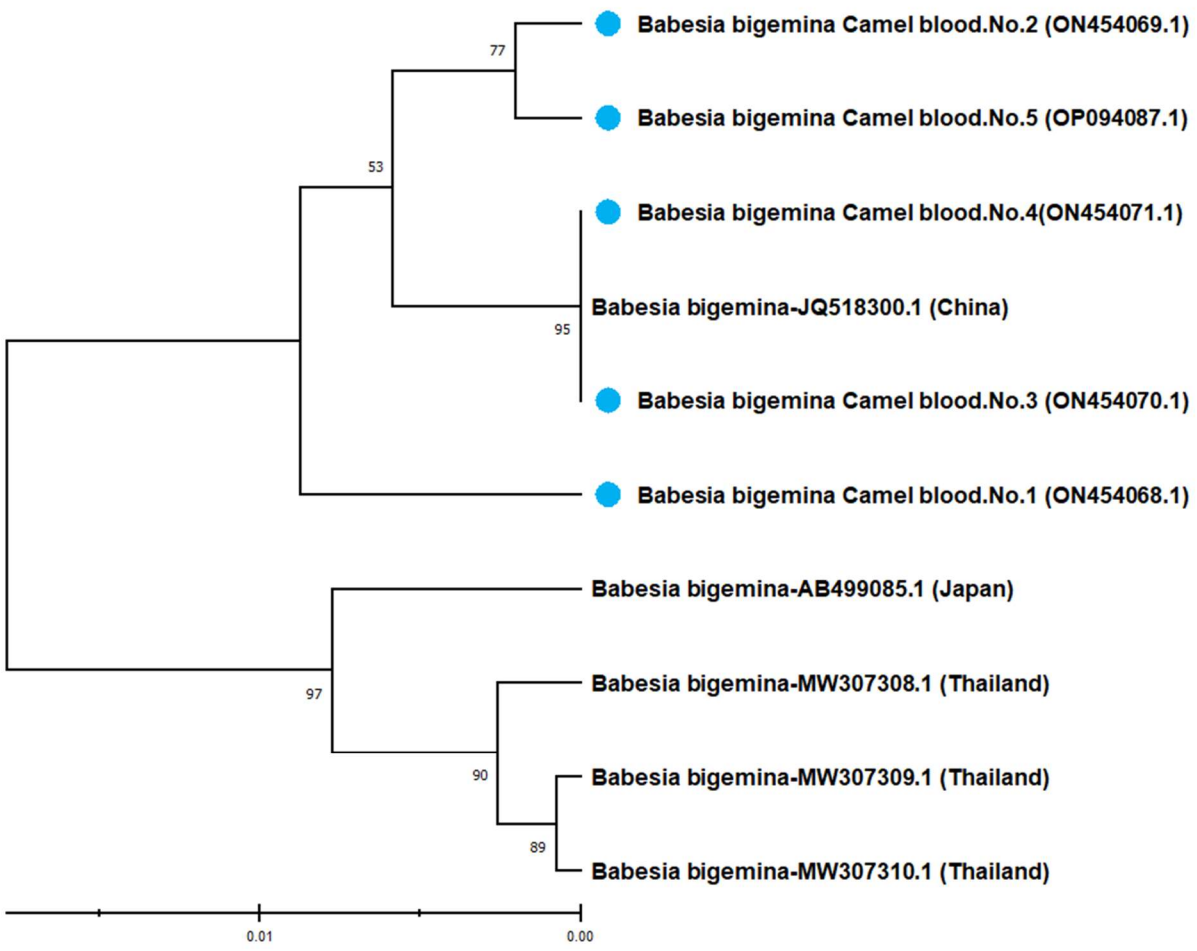

Figure (5): Phylogenetic tree analysis based on mitochondrial cytochrome oxidase subunit I (coxI) gene partial sequence in local Babesia bigemina Camel blood that used for genetic confirmative detection and genetic relationship identification. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local Babesia bigemina Camel blood isolates were showed closed related to NCBI-BLAST Babesia bigemina China isolate (JQ518300.1) at total genetic changes (0.01%).

Table (7) the NCBI-BLAST Homology Sequence identity (%) between local Babesia bigemina isolates and NCBI-BLAST submitted related China isolates:

***Babesia bigemina* isolate No.**	**Genbank Accession number**	**NCBI-BLAST Homology Sequence identity (%)**			
		Identical isolate	**Genbank Accession number**	**County**	**Identity (%)**

Camel blood.No.1	ON454068.1	*Babesia bigemina*	JQ518300.1	China	98.39%
Camel blood.No.2	ON454069.1	*Babesia bigemina*	JQ518300.1	China	98.80%
Camel blood.No.3	ON454070.1	*Babesia bigemina*	JQ518300.1	China	99.60%
Camel blood.No.4	ON454071.1	*Babesia bigemina*	JQ518300.1	China	99.59%
Camel blood.No.5	OP094087.1	*Babesia bigemina*	JQ518300.1	China	98.41%

DNA Sequence results of Babesia bovis:

Based on DNA sequencing data analysis there is a genetic relationship between mitochondrial cytochrome oxidase subunit I (coxI) gene in local Babesia bovis camel blood isolates and NCBI-Blast related country Babesia bovis isolates (Figure 6). The phylogenetic tree genetic relationship analysis was showed that the local Babesia bovis camel blood isolates (B. bovis camel blood No.1-B. bovis camel blood No.2) were showed closed genetic related into NCBI-BLAST Babesia bovis China isolate (JQ518301.1) at total genetic changes (1.5-0.5%). as showed in (Figure 7). The homology sequence identity between local Babesia bovis camel blood isolates (B. bovis camel blood No.1- B. bovis camel blood No.2) and NCBI BLAST related Babesia bovis China isolate (JQ518301.1) that originated from cattle and sheep (Gou et al., 2012) were showed genetic homology sequence identity ranged from (99.13%-99.34%). As shown in (Table 8). Finally, the local Babesia bovis camel blood isolates (B. bovis camel blood No.1- B. bovis camel blood No.2) were submitted into NCBI Genbank and identified by accession numbers (OP146607.1-OP146608.1).

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AB499088.1      ATATGTACCAATGTTAAAATCTTCTGCTCTATT-TGAACCATCACTATCGTG-ATAGTTA
JX866785.1      ATATGTACCAATGTTAAGATCTTCTGCTCTATT-TGAACCATCACTATCGTG-ATAGTTA
EU075182.1      ATATGTACCAATGTTAAAATCTTCTACTCTATT-TGAACCATCACTATCGTG-ATAGTTA
JQ518301.1      TCCTTTATCAACTTCAATATCTTCACTTGGTGT-TGATTTTATAATATTTGG-ATTATTA
OP146608.1      -------TCAACTTCAATATCTTCACTTGGTGT-TGATTTTATAATATTTGG-ATTATTA
OP146607.1      -------TCAACTTCAATATCTTCACTTGGTGT-TGATTTTATAATATTTGG-ATTATTA
AK441127.1      AGTGTTTGGAATGTTAATATTTTCAGAAATATTACTATTTTTTGGTTTCATATGGGATTA
AK442018.1      AGTGTTTGGAATGTTAATATTTTCAGAAATATTACTATTTTTTGGTTTCATATGGGATTA
                         **  * ** ** ***        *   *        * *         ***

AB499088.1      ACTG-ATAAACATATAAATAAAACTCCTACA--CAAACGTGTAG----TGAATGTAGTCC
JX866785.1      ACTG-ATAAACATATAAATAAAACTCCTACA--CAAACGTGTAG----TGAATGTAGTCC
EU075182.1      ACTG-ATAAACATATAAATAAAACTCCTACA--CAAACGTGTAG----TGAATGTAGTCC
JQ518301.1      GCTT-CTGGAATTGCAAGTGCAATGAGTGGTGCTAATTTTGTTG----TTACTTTTGGAG
OP146608.1      GCTT-CTGGAATTGCAAGTGCAATGAGTGGTGCTAATTTTGTTG----TTACCTTTGGAG
OP146607.1      GCTT-CTGGAATTGCAAGTGCAATGAGTGGTGCTAATTTTGTTG----TTACTTTTGGAG
AK441127.1      TCTACATGCAAGATTGGGTAACATATATATTGATATGCCTTTAAATCTTGAACCTTATTT
AK442018.1      TCTACATGCAAGATTGGGTAACATATATATTGATATGCCTTTAAATCTTGAACCTTATTT
                 **   *  *        *   *    *      *    * *      * *   *

AB499088.1      AGTTATAATTAAGAAAGCAAGAGAAAACCAACT---GTCGTTTAATCCG--CA-TTGTAA
JX866785.1      AGTTATAATTAAGAAAGCAAGAGAAAACCAACT---GTCGTTTAATCCG--CA-TTGTAA
EU075182.1      AGTTATAATTAAGAAAGCAAGAGAAAACCAACT---GTCGTTTAATCCG--CA-TTGTAA
JQ518301.1      CTTTAAAGTCAATTGGACAAACTATAGACAGATTAAGTGTTTTAGCCTGGTCAATTGTAT
OP146608.1      CTTTAAAGTCAATTGGACAAACTATAGACAGATTAAGTGTTTTAGCCTGGTCATTTGTAT
OP146607.1      CTTTAAAGTCAATTGGACAAACTATAGACAGATTAAGTGTTTTAGCCTGGTCATTTGTAT
AK441127.1      AAATATAACTAGTTCTTTAAA-TATAACTAGTAGTGTAATATCAATACTAGTGTATAAAA
AK442018.1      AAATATAACTAGTTCTTTAAA-TATAACTAGTAGTGTAATATCAATACTAGTGTATAAAA
                   ** *   *       **   * *   *           * *          *  *

AB499088.1      AAATGTATATTCATCTCCTTGATAGGATAGGAATAATGA-ACCAATAAAGAATGCCGATG
JX866785.1      AAATGTATATTCATCTCCTTGATAAGATAGGAATAATGA-ACCAATAAAGAATGCTGATG
EU075182.1      AAATGTATATTCATCTCCTTGATAGGATAGAAATAATGA-ACCAATAAAGAATGCCGATG
JQ518301.1      TAACTGCTTTCTTGCTTTTAGTTTCTCTTCCTGTTGTAA-CTTCTGTTCTTTTAATGGTA
OP146608.1      TAACTGCTTTCTTGCTTTTAGTTTCTCTTCCTGTTGTAA-C--------------------
OP146607.1      TAACTCCTTTCTTGCTTTTAGTTACTCTTCCT-----------------------------
AK441127.1      TGACTGTTTCACATTTTTCTGATTTTGAAAAATGGTTGACATCGGCATTCTTTATTGGTT
AK442018.1      TGACTGTTTCACATTTTTCTGATTTTGAAAAATGGTTGACATCGGCATTCTTTATTGGTT
                  *    *       *    * *
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Figure (6): Multiple sequence alignment analysis of mitochondrial cytochrome oxidase subunit I (coxI) gene in local Babesia bovis blood isolates and NCBI-Genbank Babesia bovis country isolates. The multiple alignment analysis was constructed using ClustalW alignment tool in (MEGA 6.0 version). That showed the nucleotide alignment similarity as (*) and substitution mutations in mitochondrial cytochrome oxidase subunit I (coxI) gene between different Babesia bovis isolates.

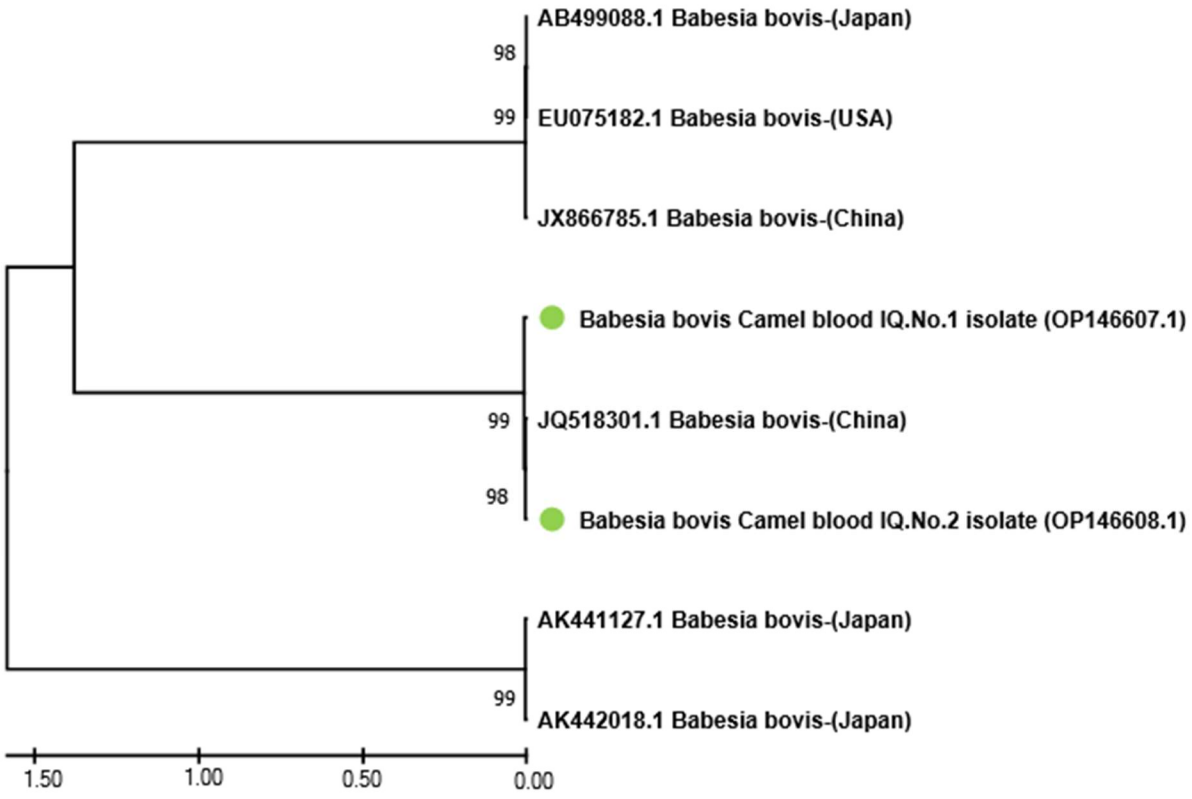

Figure (7): Phylogenetic tree analysis based on mitochondrial cytochrome oxidase subunit I (coxI) gene partial sequence in local Babesia bovis blood isolates that used for genetic confirmative detection and genetic relationship identification. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local Babesia bovis blood isolates were showed closed related to NCBI-BLAST Babesia bovis China isolate (JQ518301.1) at total genetic changes (1.5-0.5%).

Table (8) the NCBI-BLAST Homology Sequence identity (%) between local Babesia bovis isolates and NCBI-BLAST submitted related China isolates:

***Babesia bovis* isolate No.**	**Genbank Accession number**	**NCBI-BLAST Homology Sequence identity (%)**			
		Identical isolate	**Genbank Accession number**	**County**	**Identity (%)**
B. bovis blood No.1	OP146607.1	*Babesia bovis*	JQ518301.1	China	99.34%
B. bovis blood No.2	OP146608.1	*Babesia bovis*	JQ518301.1	China	99.13%

The current study involved DNA sequencing of mitochondrial cytochrome oxidase I (COX1) gene was performed for confirmative molecular detection of Babesia species that previously

detected by Nested PCR technique. The precise DNA sequence analysis was detected by matching the local mitochondrial cytochrome oxidase subunit 1 (COX1) sequence of Babesia species isolates with NCBI-Genbank global Babesia species isolates.

The mitochondrial DNA offers several advantages compared with nuclear DNA, including rapid evolution, limited exposure to recombination, a lack of introns and a high copy number. The mitochondrial cytochrome oxidase subunit 1 (Cox1) has high inter-specific divergence and low intra-specific divergence. Mitochondrial cytochrome oxidase subunit 1 (COX1) had a high average discrimination accuracy; this parameter's value was 100% at the genus level and 97% at the species level (Gou et al., 2012).

The mitochondrial genomes have a linear form and range from 5767 to 5946 bp in length; they include three protein-encoding genes, cytochrome c oxidase subunits I and III (cox1 and cox3), cytochrome b (cob), six large subunit rRNA genes (LSU), and two terminal inverted repeats (TIR) on both ends (Wang et al. 2020).

The present study showed that mitochondrial cytochrome oxidase subunit I (COX1) sequences consist of hypervariable regions surrounded by highly conserved regions, allowing for amplification of a wide range of Babesia spp. But, differences in the 18S rRNA gene sequences of distantly related clades make designing assays that amplified all Babesia species whilst excluding amplification from other eukaryotes difficult (Qurollo et al., 2017). The existence of additional variable sites in the Cox gene allowed improved interspecies discrimination between Babesia species (Tian et al., 2013)

The analysis of mitochondrial cytochrome I (COX1) can routinely provide species identification. Because the mitochondrial cytochrome c oxidase I (COX1) gene has a large number of copies, it has been used as a target in various studies, such as DNA barcoding methodology and DNA based amplification (Tu et al., 2021). The COI gene is suitable for genetic and phylogeographic studies because of its conserved sequence among conspecifics and because it has a rapid mutation rate. Consequently, it is a good target for identifying closely related species (Rach et al., 2008).

Our findings of homology sequence that confirmed identity between local Babesia bigemina from camel blood isolates and NCBI BLAST related Babesia bigemina from China (JQ518300.1), Thailand (MW307308.1) and Japan (AB499085.1) isolates, moreover identity between local Babesia bovis from camel blood isolates and NCBI BLAST related Babesia bovis from China (JQ518301.1), Japan (AB499088.1) and USA (EU075182.1) isolates. This indicates that our isolate species and the isolates of these countries may have descended from one ancestor, and there are factors that play a role in the spread of this ancestor around the world, such as the transportation of animals and importation, exportation and smuggling processes. In addition, this could suggest that these isolates may have descended from a specific species associated with different parts of the world other than these countries or Iraq (Hajeel and Abd Alfatlawi, 2019). In accordance with these results, this means that ticks have a significant role in the transmission of Babesia among livestock herds, in particular cattle and camels, between neighboring areas and countries (Al-Abedi, 2020).

Conclusion:

Camels were infected with Babesia bigemina and B. bovis at an Al-Najaf Al-Ashraf slaughterhouse and the prevalence of Babesia is closely related to the seasonal activity of vector ticks, which were prevalent in some months of the year in both sexes and significantly increasing with the age of camels. DNA sequencing and phylogenetic tree relationship analysis confirmed that the local Babesia bigemina and B. bovis camel blood isolates were closely related to the China isolate.

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