



## Survey and Molecular Study of *Babesia gibsoni* in Dogs of Baghdad Province, Iraq

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### A B S T R A C T

This study aimed to detect *Babesia gibsoni* (*B. gibsoni*) in dogs of different ages, sex and breeds in Baghdad province by microscopic and molecular investigations using polymerase chain reaction (PCR), sequencing, and phylogenetic analyses. The present study was investigated *B. gibsoni* in 310 blood samples of dogs for the period December 2018 to September 2019 in Baghdad province, Iraq. The molecular study was carried out by using universal primers of *Babesia spp.* (PIRO-A and PIRO-B) and specific primers of *B. gibsoni* (BAGIF and BAGIR) products size of 410 bp and 488 bp fragments of 18S rRNA gene respectively. The clinical signs revealed higher percentage and specific clinical signs of *B. gibsoni* as depression, anorexia, fever, pale mucus membrane, and ticks infestation, however icterus, and dead were low in which only occurred in two dogs out of infected dogs. The PCR assay and microscopic diagnosis revealed the infection rate of *B. gibsoni* 9 out of 310 (2.9%) in dogs. The sequence data analyses of nine DNA products were 98-100% similar to sequences of 18S rRNA gene of *B. gibsoni* data available in Gene bank. According to breed, age, and sex, the results revealed a significantly high-risk factor of infection in Husky dogs; *B. gibsoni* detected in females which was increased non-significantly than males; while the highest occurrence of disease was in young dogs aged three years or less in addition to the above, the infection rate of *B. gibsoni* was high in the spring season. In conclusion, this study was considered the first molecular record of *B. gibsoni* in Baghdad, Iraq documented no differences in diagnosis by blood smear and conventional PCR to amplify of 18S rRNA gene and partial sequencing of *B. gibsoni* with low-cost method and easily done.

**Keywords:** epidemiological, molecular, *Babesia gibsoni*, Iraq

### INTRODUCTION

Canine babesiosis is an important disease was distributed globally (1) which was recorded in Africa for the first time in 1896 as an intra-erythrocyte parasite of dog associated with clinical signs, while, it was firstly documented in United States in 1934. The common canine *Babesia spp.* is classified into two different species: the large *Babesia canis* (2.4-3×4-7 μm), that has a pear shape and small *Babesia gibsoni* (1-2×3-4 μm) that has an

annular shape (2), *Babesia gibsoni* (*B. gibsoni*) is a small parasite has a shape of single ring or pyriform bodies (3). The small *piroplasm* is an oval or annular shape that occupies more than 1/8 of the diameter of red blood cells (4). It is caused by canine babesiosis which is transmitted by *Haemaphysalis bispinosa* and *Haemaphysalis Longicornis* (2), the causative agent leads to anemia, fever, jaundice, thrombocytopenia and possible urinary system abnormalities (5), causing acute form disease which results in serious clinical problems, fever, regenerative anemia,

splenomegaly, and sometimes death (6). *B. gibsoni*, was endemic in Asia, North and East of both Africa and Europe and in North America (7). The epidemiology of canine babesiosis was recorded by many researchers; in Korea diagnosed *B. gibsoni* in 29 out of 117 dogs which were suspected of canine babesiosis (8). While in Romania, blood samples were studied from 49 symptomatic dogs and smears revealed 45/49 positive samples, whereas PCR results revealed 35/49 *B. canis* and 14/49 *B. gibsoni* (9). 12 dogs were positive by PCR detection for a *Babesia* spp., while specific PCR- species for *B. canis* and *B. gibsoni* recorded 5 (6.3%) *B. canis* and 7 (8.8%) *B. gibsoni* when 80 blood samples were studied in Japan (10). In Iran, the first molecular study on *B. gibsoni* recorded 5% infected dogs out of 60 anemic dogs (11). In Turkey, *B. gibsoni* was firstly detected in two dogs clinically, blood smear, and PCR methods (12).

On the other hand, the diagnosis of *Babesia* spp. in dogs depended mainly on microscopic examination of a stained blood smear which is considered having low sensitivity because it was represented fastidious method for blood parasites diagnosis, while PCR assay for diagnosis of apicomplexan parasites is considered confirmative technique and standard method for diagnosis blood parasites (13).

The sets of primers PIRO-A and PIRO-B were used to amplify a specific fragment of the 18S-rRNA gene in *Babesia* spp. (14) and this study was identified *B. gibsoni* in two out of 60 dogs by PCR and sequencings; and by using BAGI-F and BAGI-R specific primers of *B. gibsoni* for detection of *B. gibsoni* in dogs of Kerala in South India (15) as well as, previous primers were used for diagnosis *B. gibsoni* in dogs of South India by multiplex PCR (16).

To this date, there is no published data on the molecular detection of *B. gibsoni* in Iraq, the previous research only depended on blood smears diagnosis in addition to the above, using blood smear, the *B. gibsoni* was detected in 8 dogs in Nineveh, Iraq (17). In a study conducted to investigate the incidence of *B. gibsoni* among stray dogs in Baghdad province, Iraq using blood smear method, *B. gibsoni* was detected only in 48 out of 108 dogs sampled (18). This study aimed to detect *B. gibsoni* in dogs of different ages, both sexes and breeds in Baghdad Province by microscopic and molecular investigations using polymerase chain reaction (PCR), sequencing, and phylogenetic analyses.

## MATERIALS AND METHODS

### Animals and Clinical Examination

All procedures used in this study were reviewed and approved by the scientific committee at the University of Baghdad's College of Veterinary Medicine in compliance with animal welfare ethical standards.

This study included 310 dogs of different breeds (191 German Shepherds, 65 Malinois, 12 Huskies, 14 Cross breeds, and 28 other breeds), ages (171 dogs < 3 years and 139 > 3 years), and sexes (193 males and 117 females) that were introduced to Baghdad Veterinary Hospital, Baghdad, Iraq between December 2018, and September 2019. Body temperature, pulse, and respiratory rate were recorded, as well as any abnormal clinical signs and the case history for detecting previous tick infestations.

### Sampling and Microscopic Examination

Whole blood samples were collected in a 3-mL EDTA tube by direct needle insertion to the cephalic vein. The blood smear was then stained by a commercial Giemsa stain (Crescent diagnostic, Saudi Arabia). Then EDTA blood samples were preserved at -20 °C until molecular assessment.

### DNA Extraction

Genomic DNA was extracted from 310 whole blood samples collected. DNA extraction was performed using ReliaPrep™ gDNA Miniprep System (Promega, USA). The purity and concentration of the final DNA template was measured by NanoDrop spectrophotometer (Thermo Scientific, USA). The purity and concentration were considered pure when final elutes were between 1.7 to 1.8 at 260/280 nm and concentration between 46.7 to 99.1 ng/μL. Template DNA was loaded on the agarose (1%) with SYBR Safe stain (ABM, Canada) (19).

### Primers

Primers used in the study (Table 1) PIRO-A and PIRO-B primers for amplification of 410 bp region of 18S rRNA gene to determine *Babesia* spp., and BAGIF and BAGIR specific primers for amplification 488 bp region of 18S rRNA gene to detect *B. gibsoni*.

**Table 1.** Primers used for diagnosis *Babesia gibsoni* (18S rRNA gene)

Primers		Sequence (5'-3')	Product size	Reference
PIRO-A	F	AATACCCAATCCTGACACAGGG	410 bp	(14)
PIRO-B	R	TTAAATACGAATGCCCAAC		
BAGIF	F	TTGGCGGCGTTTATTAGTTC	488 bp	(15)
BAGIR	R	AAAGGGGAAAACCCAAAAG		

### Polymerase Chain Reaction (PCR)

PCR assay was conducted at the Laboratories of Internal and Preventive Veterinary Medicine Department, College of Veterinary Medicine, University of Baghdad, and PCR assay was done on 310 frozen blood samples by the following methods.

The total master mix reaction of PCR was 25 μL, according to instructions of (Promega, company, USA), total reaction containing 12.5 μL of the master mix, 0.5 μL of 10 pmol of each forward and reverse primers, 3 μL of DNA

template, and 8.5 µL of nuclease-free water. All reagents were procured by Promega, USA. The thermocycling protocol (Table 2) was according to Davitkov et al. (14) for PIRO-A and PIRO-B primers and according to Jain et al. (15) for BAGI F and BAGI R primers. The DNA products were loaded on 1.2% agarose gel with SYBR Safe stain (ABM, Canada), the positive band was compared with 100 bp commercial marker DNA ladder (Bio-Rad Laboratories, USA). Controls as non-template negative controls were included. Gels were photographed under UV light using a digital camera (Bio-Rad Laboratories, USA).

**Table 2.** PCR thermocycling conditions used for 18S rRNA gene for detection of *Babesia gibsoni*

Steps	PIRO-A and PIRO-B primers		
	Temperature (°C)	Time	No. Cycle
Initial denaturation	95	3 min	1
Denaturation	95	30 sec	40
Annealing	62	30 sec	
Extension	72	30 sec	
Final extension	72	7 min	1
Hold	4	10 min	1
Steps	BAGI F and BAGI R primers		
	Temperature (°C)	Time	No. Cycle
Initial denaturation	94	5 min	1
Denaturation	94	30 sec	35
Annealing	61	30 sec	
Extension	72	60 sec	
Final extension	72	5 min	1
Hold	4	10 min	1

### Partial Sequencing and Phylogenetic Analysis of 18S rRNA Gene

The PCR product was sent to Macrogen sequencing company (Korea) for partial sequence of 18S rRNA gene. The results of sequencing were compared with sequences available in the GenBank information using BLAST in NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST/>); and the phylogenetic tree of the 18S rRNA gene of *B. gibsoni* was done by Molecular Evolutionary Genetics Analysis (MEGA 6.0) program with multiple sequence alignment.

### Statistical Analysis

Statistical analysis was conducted by SPSS program (version 20) for analyzing data using the multiple logistic regression test (odds ratio test) for evaluating the risk factors.

**Table 3.** The clinical signs frequency of nine infected dogs by *Babesia gibsoni*

Specific Clinical signs	Infected/total	%	Nonspecific clinical signs	Infected/total	%
Depression	6/9	66%	Vomiting and diarrhea	3/9	33%
Anorexia	5/9	55%	Irregular heart rhythm	3/9	33%
Fever	5/9	55%	Emaciated	2/9	22%
Pale mucus membrane	4/9	44%	Heart beat weak	2/9	22%
Ticks' infestation	3/9	33%	Nervous manifestation	2/9	22%
Icterus and dead	2/9	22%	Vomiting and diarrhea	3/9	33%
Granulocytes (%)	6/9	66%			

## RESULTS AND DISCUSSION

### Clinical Signs and Microscopic Examination

The results of infected dogs with *Babesia gibsoni* infection revealed specific clinical signs of *Babesia gibsoni* as depression, anorexia, fever, pale mucus membrane, and 3 dogs showed ticks infestation, however death and icterus of 2 dogs (Figure 1). Whereas, other clinical signs as emaciated, nervous manifestation, vomiting and diarrhea, heart beat weak, and irregular heart rhythm were considered nonspecific signs of *Babesia gibsoni* (Table 3).

The detection of the presence *Babesia gibsoni* in the blood thin smear described as small *piroplasms* paired piriform or ring merozoites or single ring shape trophozoites intraerythrocytic parasites, blood parasitological smear observed 9 positive cases out of 310 total dogs (Figure 2).

The investigation of clinical signs associated with *B. gibsoni* infection revealed specific clinical signs as depression, anorexia, fever, pale mucus membrane, a history of ticks' infestation, and icterus developed only in the advanced stage of *B. gibsoni* infection. It was noticed that developing nonspecific clinical signs was due to disease complication or possible secondary infection. The latter was closely confirmed through which many authors recorded signs closed to present study as pale mucus membrane (11, 20), anorexia (12), fever (6, 20). In addition, *B. gibsoni* in Korea revealed signs of anorexia 48%, pale mucus membrane 48% and icterus 13% (8) while in Serbia anorexia 88%, pale mucus membrane 50% and icterus 28% was registered (14). In present study, these signs included anorexia 55%, pale mucus membrane 44% and icteric mucous membranes 22%. While Mahalingaiah et al. (21) identified the lowest percentage of developing icterus with a ratio of 7.5%. The vomiting and abnormal feces were 33% in the present study while other (14, 21) reported vomiting in 13.5% and 26%, and abnormal feces at 7.5% and 11.7% respectively. The different percentage between signs in these studies possibly due to variations in numbers of studied dogs and disease stages.



**Figure 1.** Male Husky dog at three months of age infected with *Babesia gibsoni*. (A) icteric mucous membrane. (B) Yellowish serum in infected dogs

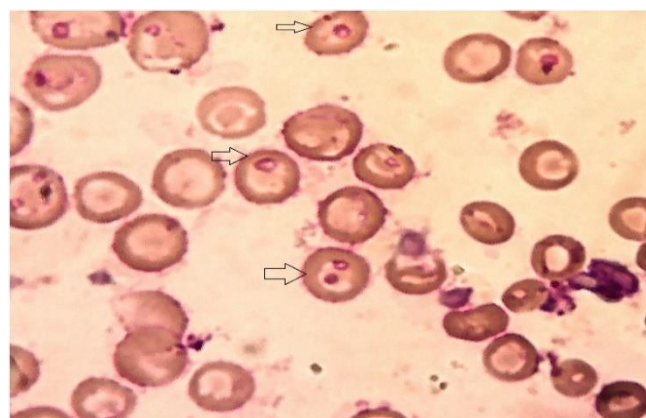
The microscopic diagnosis revealed that 9 out of 310 blood smears were positive for *B. gibsoni*. The microscopic diagnosis of *B. gibsoni* as small *Babesia* spp. was easier than other large *Babesia* spp., possibly because of the high distribution of *B. gibsoni* among erythrocytes in the blood smear however, expert in diagnosis is needed especially in low parasitemia (2). It is suggested to consider the blood smear test to be considered a gold test for detecting small *Babesia*.

### PCR Assay of *Babesia gibsoni*

The PCR assay and microscopic diagnosis revealed that the infection rate of *B. gibsoni* showed to have 9 out of 310 (2.9%) in dogs. All DNA samples were also examined with PIRO-A and PIRO-B universal primers for detecting of 410 bp of 18S rRNA gene of *Babesia* spp. (Figure 3). The DNA samples were investigated by BAGIF and BAGIR specific primers for amplification 488 bp region of 18S rRNA gene of *B. gibsoni* (Figure 4).

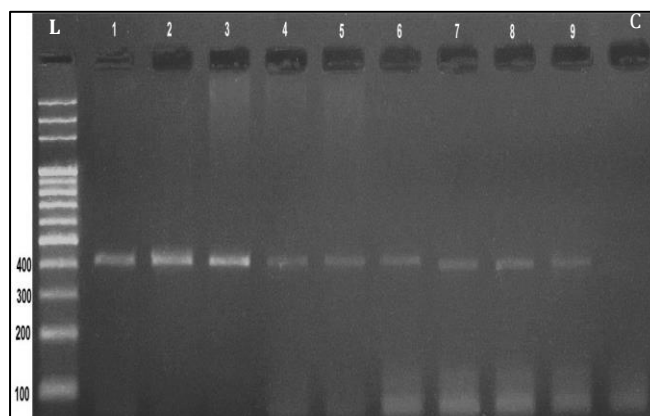
### Sequencing and Phylogenetic Analysis

The sequences data analyses of nine DNA products were 98-100% similar to sequences of 18S rRNA gene of *B. gibsoni* data available in Gene bank. The results of sequences were submitted to Gene bank according to accession numbers were (MN385424.1, MN385425.1, MN385426.1, MN385429.1, MN385422.1, MN385423.1, MN385430.1, MN385428.1, and MN385427.1). The phylogenetic tree analysis compared with 18S rRNA gene of *B. gibsoni* data were available in GenBank (Figure 5). All sequences data of study *B. gibsoni* isolates had homology 99% except MN385428.1 which was 98%, and MN385430.1 had 100% similarity to world isolates in India MN134517.1, China MG604346.1, Bangladesh LC008285.1, Japan:Yamaguchi LC012808.1, Turkey KJ513206.1, Saint Kitts and Nevis JX112784.1, Taiwan FJ769386.1, USA EU084677.1, Spain AY278443.1, Germany AF175301.1,

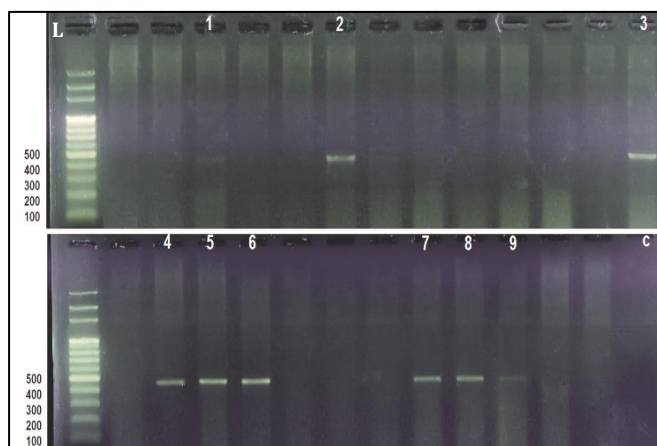


**Figure 2.** Blood thin smear of female Malinois dog at four months of age infected with *Babesia gibsoni* inside its erythrocytes (intracellular) have small ring-form trophozoites which was stained by Giemsa stain and examined under light microscope (100×)

and Australia AY102164.1. Our isolate MN385428.1 was also located in single clade and different to other isolates of present study by one transversion and three transitions, whereas total transitions were significantly decreased with 13 transitions compared to total transversions 18 out of 31 total substitutions, as well as T>C considered the main type of transversion (Table 4).



**Figure 3.** Agarose gel (1.2%) electrophoresis with SYBR Safe stain shows amplification of 410 bp fragment of 18S rRNA gene (PIRO A and PIRO B primers). Lane L: DNA ladder (100 bp); Lane C: non-template negative control; Lanes 1-9 positive cases of *Babesia gibsoni*



**Figure 4.** Agarose gel (1.2%) electrophoresis SYBR Safe stain shows amplification of 488 bp fragment of 18S rRNA gene (BAGIF and BAGIR primers). Lane L: DNA ladder (100 bp); Lane C: non-template control; lanes 1-9: positive cases of *Babesia gibsoni*; and Lanes without band: negative cases

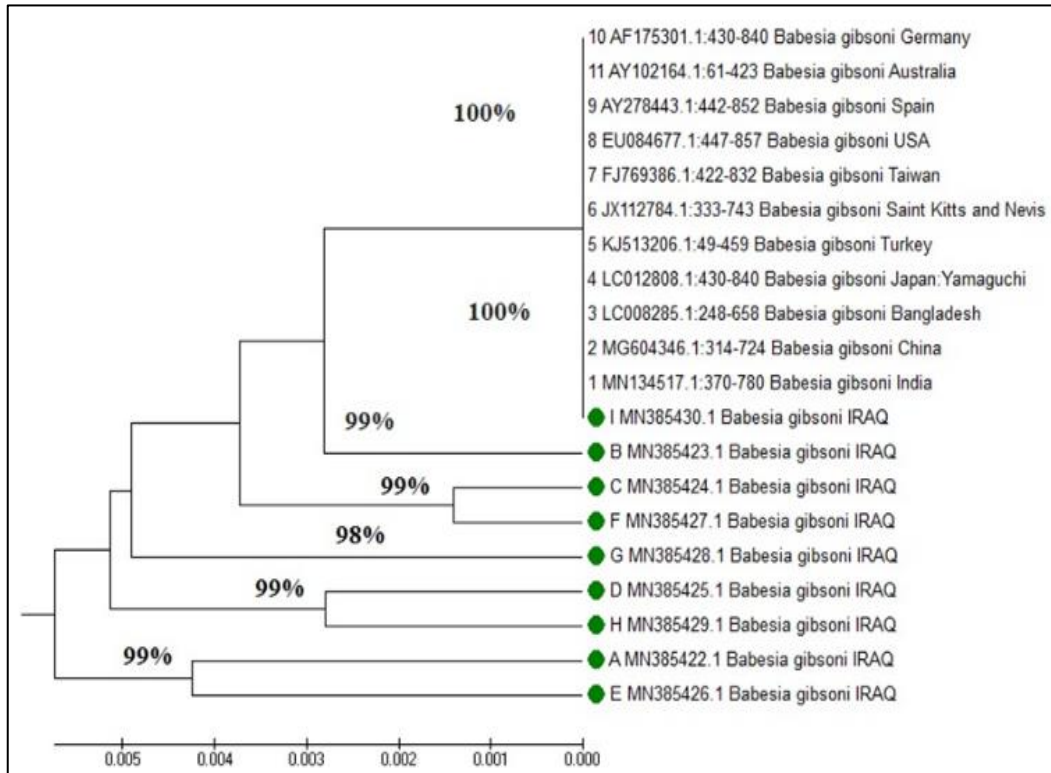


Figure 5. Phylogenetic tree of *Babesia gibsoni* (18S rRNA gene) made by (MEGA 6.0)

Table 4. The Frequency substitution of *Babesia gibsoni* nucleotide sequencing

Numbers	A>G	A>T	C>G	C>T	C>A	G>C	G>A	T>C
MN385422.1	-	1	1	-	-	-	-	2
MN385423.1	-	-	-	-	1	-	1	1
MN385424.1	1	1	-	-	-	-	1	2
MN385425.1	-	1	1	1	1	-	-	1
MN385426.1	-	-	-	-	-	1	-	2
MN385427.1	-	-	-	-	-	-	1	2
MN385428.1	1	-	1	-	-	-	-	2
MN385429.1	-	1	1	-	1	-	-	-
MN385430.1	-	-	-	-	1	-	-	-
<b>Total</b>	2	4	4	1	4	1	3	12
	Total transitions 13				Total transversions 18			

PCR assay revealed nine positive samples to different primers (PIRO-A and PIRO-B) and (BAGIF and BAGIR) according to the thermo-cycle protocol of the present study, the results of PCR resembled the microscopic diagnosis. This result of the present study disagreed with other studies which recorded higher PCR detection when compared to microscopic observations (3, 9, 15, 16, 20, 22, 23). The blood smear should be examined accurately for diagnosis the *B. gibsoni*, so, it is recommended using blood smear for identifying *B. gibsoni* as a confirmative method.

Molecular characterization and phylogenetic analysis of *B. gibsoni* depended on 18S rRNA partial sequence which revealed that the isolates were very close to groups of many other countries, the present isolate MN385430.1 shared the other countries in sister clade, and this *B. gibsoni* isolate was also in 4 months age Malinois dog in Baghdad; the clinical signs of this dog were described by severely emaciated, depression, weakness, and diarrhea. However, Asia isolates of a neighboring tree were revealed 99% similarity to other isolates of the present study except for

MN385428.1 isolate which showed 98% similarity. The above isolate was located in the single clade returned to crossbreed stray dogs less than one year in age. Moreover, the molecular research related of Asian isolates of the conducted phylogenetic tree of the present study that was explained by infection rate of *B. gibsoni* 23% in Taiwan FJ769386.1 (24), *B. gibsoni* was 9% in dogs of Turkey KJ513206.1(25), in Japan 2.4% infection rate LC012808.1(26), while Terao et al. (27) study under accession number LC008285.1 recorded isolate of stray dogs in Bangladesh with 30% infection rate. All these studies had high homology with the present study.

The nucleotides substitutions of the present study were recognized only in one substitution of MN385430.1 when compared to other partial of sequences of 18S rRNA gene of other isolates. This case that returned to the same Malinois dog had sister clade with world isolates. It could be possibly explained by the fact that this dog was exported to Iraq and MN385430.1 isolate was not local *Babesia gibsoni* strain of Iraq. In the phylogenetic tree of *B. gibsoni* of this study, crossbreed infected dog isolates varied than other isolates, through which it is suggested that these dogs were infected by a specific strain of *B. gibsoni* that developed specific nucleotides substitutions consider local strain, and the differences of sequences and genotyping of crossbreed stray dogs need for more investigation. The numbers of nucleotides substitutions between 1 to 5 nucleotides among all *B. gibsoni* isolates; and the averages of transitions and transversions were 1.44 and 2, respectively. Although these numbers of nucleotide polymorphism are predicted, they may have occurred due to climate changes, natural diversity of *Babesia gibsoni* strains in Iraq, or other disease-related factors.

### Distribution of *Babesia gibsoni* According to Breed, Age, Sex, and Months

Results of *Babesia gibsoni* infection rate distributed based on the breed, age, and sex of the dogs are presented in Table 5. High significant infection appeared in Husky dogs (16.7%), non-significant in crossbreed (7.14%) compared to German Shepherd (2.09%) and other breeds (0%) dogs (Terrier, Labrador retriever, Pekingese, Lolo fox, Boo, Sheepdog, Hawshar and Cocker spaniel). *B. gibsoni* was detected at 3.41% (4/117) in females which was numerically but not significantly higher than that recorded in males (2.59%, 5/193). The highest occurrence of disease was in young dogs < 3 years in age when compared to dogs more than 3 years.

Although, the results of distributing *B. gibsoni* infection showed no significant values among the months, March and April had the highest numbers (Table 6).

Epidemiological approaches of *B. gibsoni* in dogs, the infection rate of *B. gibsoni* in dogs was 2.9% which was the nearest to what Davitkov et al. (14) found in selected symptomatic dogs of 3.3% percentage in deference to the present study where randomly dogs were selected. Another study in North American (28) identified *Babesia spp.* in 269 out of 9345 total dogs (2.9%); and 186 out of 9345 dogs (2%) infected by *B. gibsoni*. The results showed that highly infection may occur in dogs younger than 3 years, female dogs, Husky then crossbreed stray dogs. Many authors reported the higher rate of infection in the young dogs (21, 23); a significant increase in dogs younger than five years was noted by other authors (11, 14, 29).

**Table 5.** Distribution of infection rate of *Babesia gibsoni* according to breed, age, and sex of dogs

	Infected/total	%	Odds ratio	CI 95%
<b>Breed</b>				
German Shepherd	4/191 B	2.09		
Malinois	2/65	7.17		
Husky	2/12 A	16.7	7.95	1.32-47.9
Crossbreeds	1/14	7.14		
Other breeds <sup>1</sup>	0/28	0		
<b>Age</b>				
≤3 years	7/171	4.09	2.84	0.54-13.9
≥3 years	2/139	1.44		
<b>Sex</b>				
Male	5/193	2.59		
Female	4/117	3.42	1.32	0.34-5.01

<sup>1</sup>Terrier, Labrador retriever, Pekingese, Lolo fox, Boo, Sheepdog, Hawshar and Cocker spaniel

The reason for the high infection of young dogs with *B. gibsoni* in this study may be due to immunocompromised (21); and frequent vaccinated of young dogs against other diseases as vaccinations program in Iraq can also be played a role to immune system stress. The sex epidemic pattern in the present study showed a non-significant increase of infection percentage in female dogs which was disagreed to

other research studies (8, 14, 21, 30). These studies recorded increase of infection in male dogs by *B. gibsoni*, however the lack of relationship between infection and sex documented in other studies (3, 23). The increase of infection rate in some studies occurred due to increase chance of ticks' exposure; while another study revealed increase of the infection rate of indoor dogs may indicate

indoor dogs are easily exposing to ticks (8). It is suggested that pregnancy and lactating period in females can be considered roles in immunity depression, the correlation between sex and infection may also be needed for further and specific studies.

Several research studies worked on the association between *B. gibsoni* infection in dogs and their breeds and many studies recorded that the infection rate is higher in

pure breed than that mixed breeds or stray dogs (14). Another study found that the infection rate was significant in some breed as terriers (20) while others reported the non-significant values between breed (23). It was revealed in this study a high incidence infection rate to Husky than other dogs in Iraq due to that Husky dog intolerant to the Iraq environment but more susceptible to many diseases.

**Table 6.** *Babesia gibsoni* infection in dogs among months of the year

Months	Infected/total	%	Infected/total
December 2018	0/25	0	Winter 0/75
January 2019	0/25	0	
February 2019	0/25	0	
March 2019	2/25	8.00*	Spring 7/149*
April 2019	2/29	6.89*	
May 2019	2/46	4.34	
June 2019	1/49	2.04	
July 2019	1/36	2.78	Summer 2/86
August 2019	1/25	4.00	
September 2019	0/25	0	

\*Confidence interval 95%= 0.41-9.94, Odd ratio= 2.02

The present results supported a non-significant increase in the infection rate through warm months or spring season especially in March and April. Although the infection-related widespread to ticks vectors, the ticks manifestation noted in 33% of infected dogs and there was different researches explained ticks with *Babesia* infection in dogs as 73% (21) and 55% (14). *Babesia* infected dogs of some reports were noted ticks free at the time of samples collection which may be presented other routes of transmission as transplacental or directly by the wounds (9). Another study included a history of dog fights with *Babesia gibsoni* (28) and the incidence of disease among months depended on climate conditions, control management as a used insecticide and ticks seasons activity especially warm or humid periods through the year (3, 14 and 15) and dogs were infected by ticks in Iraq which showed the lowest rate 7.69% in winter (31).

In conclusion, the molecular study of 18S rRNA gene in *B. gibsoni* was recorded for the first time in Iraq and estimated some nucleotides polymorphisms and substitutions in Iraq isolates. The above study detected no differences between microscopic and PCR assay; PCR assay used only as a confirmative technique and for phylogenetic analysis and the study also concluded the Husky dogs to be more susceptible breed to infection by *B. gibsoni* in Iraq.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## مسح وبائي و دراسة جزيئية للكثيريات الجيبسونية في الكلاب في محافظة بغداد ، العراق

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### الخلاصة

هدف هذه الدراسة تثبيت طفيلي الكثيريات الجيبسونية في الكلاب في كلا الجنسين و لمختلف الاعمار و الفصائل من خلال الكشف المجهرى و الجزيئى باستخدام فحص تفاعل البلمرة المتسلسل و الكشف عن تعاقب القواعد النيتروجينية و النمط الجينى. تم اخذ 310 من عينات الدم للكلاب خلال فترة شهر كانون الاول للعام 2018 حتى شهر ايلول 2019 في محافظة بغداد/ العراق. استخدمت الدراسة الجزيئية نوعين من البراميرات احدهم خاص لجنس الكثيريات(PIRO-A and PIRO-B) و الثاني خاص للكثيريات الجيبسونية(BAGIR and BAGIF) لتضخيم منطقة حجمها 411 و 488 على التوالي الموجودة في الجين (18S rRNA). طورت الكلاب المصابة نسبة عالية من العلامات السريرية الخاصة للإصابة بداء الكثيريات في الكلاب مثل الاكتئاب و فقدان الشهية و الحمى و شحوب الاغشية المخاطية و الإصابة بالقراد بينما كان الموت و اصفرار الاغشية المخاطية فقط في كلبين من مجموع الكلاب المصابة. توصل اختيار تفاعل البلمرة و الفحص المجهرى الى نسبة الإصابة في الكثيريات الجيبسونية حوالي 9 كلاب من 310 (2.9%). تحليل نتائج تعاقب القواعد النيتروجينية اظهر تشابه بنسبة 98% الى 100% مع تعاقب جين (18S rRNA) للكثيريات الجيبسونية المتوفر في مصرف الجينات العالمى. حسب نتائج تأثير السلالة و العمر و الجنس اظهرت النتائج وجود عامل خطر كبير للإصابة في كلاب الاسكيمو و من ثم السلالات المضربة، وكذلك عند الإناث مقارنة بالذكور، في حين كانت أعلى نسبة حدوث للمرض في الكلاب الفتية التي تساوي أو تقل عن ثلاث سنوات في العمر. تم تسجيل معدل الإصابة في الكثيريات الجيبسونية في كل شهر و كانت النسبة الاعلى في الربيع. في الختام، اعتبرت هذه الدراسة أول دراسة جزيئية للكثيريات الجيبسونية في بغداد / العراق ، و قد تم تأكيد الإصابة بتفاعل البلمرة المتسلسل لتضخيم الجزيئى لجين (18S rRNA) و التسلسل الجزيئى للكثيريات الجيبسونية.

الكلمات المفتاحية: وبائيا ، جزيئيا ، الكثيريات الجيبسونية، العراق