Iraqi Journal of Veterinary Sciences, Vol. 38, Supplement II, 2024 (27-33)
Proceedings of the 2nd International Conference of Pharmaceutical and Applied Medical Sciences, Knowledge University

Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Molecular and phylogenetic analysis of *Babesia gibsoni* in dogs and its infested ticks in Nineveh province, Iraq

W.A. Alobaidii[1], S.Y. Albaroodi[1] and D.A. Almashhadany[2]

[1]Department of Microbiology, College of Veterinary Medicine, Mosul of University, Mosul, [2]Department of Medical Laboratory Science, College of Science, Knowledge University, Erbil, Iraq

Article information

Article history:
Received 11 May, 2024
Accepted 10 July, 2024
Published online 30 December, 2024

Keywords:
B. gibsoni
PCR
Dogs
Ticks
Mosul

Correspondence:
W.A. Alobaidii
wasenamkad@uomosul.edu.iq

Abstract

Through this study, 50 different dogs in sex, age, source clinical manifestation, and breeds. These animals were examined, and their apparent clinical signs were recorded; blood sample was collected from the cephalic veins of dogs, and the blood was transferred to anticoagulant EDTA tubes; 130 ticks were collected directly from animals. DNA was extracted from blood and ticks, and then DNA was detected using a polymerase chain reaction, which was applied to detect *B. gibsoni* using Gib599 and Gib1270 primers. The results Polymerase chain reaction, which can be used to detect the DNA of *B. gibsoni*, showed 19 positive blood samples and 80 ticks that gave positive to *B. gibsoni* DNA, a high prevalence of infection in animals that suffer from general weakness and enlargement of lymph nodes, the oldest animal showed high prevalence of infection with *B. gibsoni* when compare with youngest once, male dogs showed high prevalence than female, the native dogs recorded high prevalence than imported once, the largest breeds showed high prevalence than small breeds. 10 positive samples were selected. After that, the genetic sequencing was performed. A genetic sequence of the samples was detected by the findings of the sequencing of the small subunit ribosomal RNA gene of *B. gibsoni*. Only one isolate was found in the blood samples sent for sequencing. They have a serial number when they were registered in the gene bank. (OR944894.1) and another isolate from ticks (OR944895.1). in conclusions Dogs affected *B. gibsoni*, and there are more dominant parasites in dogs.in addition the role of ticks to transmit this parasite.

DOI: 10.33899/ijvs.2024.149751.3663,

Introduction

Dog blood protozoa can result in various clinical symptoms (1,2), including anemia and thrombocytopenia, even in cases where there is only a small number of parasites in the peripheral blood during acute infection (3,4). The issue is that the anemia's pathophysiology and the parasite's life cycle are poorly understood (5). When many parasites are present in blood smears, examining them is not too difficult (6). However, detecting parasites is far more difficult when their population is small. Thus, sensitive and repeatable techniques for parasite detection are required for basic and clinical veterinary research (4). Direct microscopy of blood smears stained with Giemsa stain is the method most frequently employed to discover parasites in the host (7). Furthermore, blood protozoa are stained with particular fluorescent dyes for deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), such as Hoechst 33342 and acridine orange (8). Nevertheless, these staining dyes are not parasite-specific; they also stain the host's cells. In the past, malaria was diagnosed using RNA and DNA probes specific to the disease (9). Although the sensitivity of the DNA probes is not as high as that of direct microscopy of blood smears, these probes enable the detection of small quantities of parasites (10). Individual parasite numbers and developmental stages are lost since these techniques

necessitate partial DNA or RNA purification (11). On the other hand, the precise staining of the parasites by these probes in blood smears or preserved tissues will significantly (11) aid in their microscopical detection (12).

Because there is a little study about *B. gibsoni* in dogs so this study aims to molecular and phylotegic study of this parasite.

Materials and methods

Ethical approval

This study obtained approval from the scientific board, College of Veterinary Medicine, Mosul University, Mosul, Iraq, the approval issue UM.VET.2023.076.

Sample collection

A blood sample was collected from the cephalic vein of 50 dogs (of different ages, sexes, sources, species, and health status). The blood was transferred to anticoagulant EDTA tubes (3).

DNA extraction

Extraction of DNA from blood samples and ticks using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH, Catalog number 51304)

PCR amplification

12.5 µl of PCR Master Mix, 1.0 µl of each forward and reverse primer, 5 µl of template DNA, 2.0 µl of Dimethyl sulfoxide (Genie®), and 5.5 µl of autoclaved triple-distilled water, instructions attempted PCR., described the PCR amplification procedure, which included an initial denaturation of 95 ºC for 5 min, followed by 35 cycles of denaturation (30 sec), annealing (30 sec), and extension (90 sec) at 95 ºC, 58 ºC, and 72 ºC, in that order. The final extension was run at 72 ºC for 5 minutes-Eppendorf® thermocycling. There was a Master Cycler Gradient. For PCR amplification, species-specific primers for the 18S rRNA gene, Gib599 forward and Gib1270 reverse (13), were employed, with an anticipated product size of 671 bp. Then, ten positive samples were chosen after numerous positive results were obtained. Each sample was then given a genetic sequencing procedure. In Marcrogen Laboratory, Korea, the Basic Local Alignment Search tool is used to find the degree of similarity between the genetic sequences in the database. It may be accessed on the National Center for Life Technologies NCBI website. After that, the MEGA 7 program generates a phylogenetic tree, and the Cluster Omega tool creates the multi-string alignment (Table 1).

Table 1: Showing forward and reverse primers used for the molecular diagnosis of *B. gibsoni* infection in dogs

Gene	Primer	Primer sequence 5' → 3	Expected product size
18S rRNA	Gib599 Forward Gib1270 Reverse	TCCGTTCCCACAACACCAGC TCCTCCTCATCATCCTCATTCG	671 bp

Results

To diagnose *B. gibsoni, a* polymerase chain reaction test was used, which showed the presence of 19 dogs infected with the parasite through the appearance of positive bands (671 bp) after the electrophoresis of the final products (Figure 1).

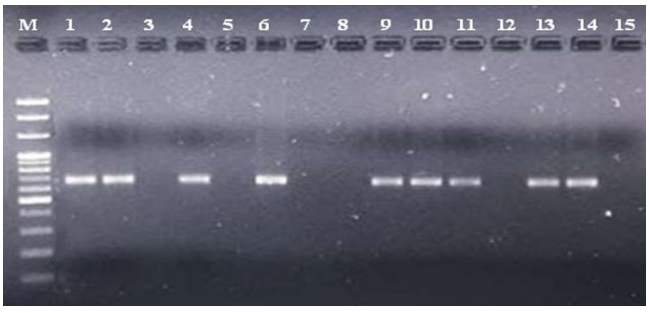

Figure 1: Gel electrophoresis image showing: Lane (M) DNA ladder; Lanes (3,5,7-8,12,15) negative samples to *B. gibsoni* DNA; Lanes (1-2,4,6,9-11,13-14) positive samples to *B. gibsoni* DNA in approximately band size 671 bp.

The PCR, which can be used to detect the DNA of *B. gibsoni*, showed a high prevalence of infection in animals that suffer from general weakness and enlargement of lymph nodes; the oldest animal showed a high prevalence of infection with *B.* gibsoni when compared with the youngest once with significant of variance, male dogs showed high prevalence than female with significant of variance, the native dogs recorded high prevalence than imported. Once with significant variance, the largest breeds showed a higher prevalence than small breeds with significant variance (Table 2).

Genetic sequencing of the *B. gibsoni* small subunit ribosomal RNA gene revealed the existence of a genetic sequence of the samples given; nevertheless, only one isolate was discovered among all the blood samples received for genetic sequencing. They were registered in the gene bank with a serial number (OR944894.1) and another isolate from ticks (OR944895.1). The results of the multiple sequence alignment of the ssRNA gene of *B. gibsoni* s showed the presence of fixed regions of the sequence of the nucleotide bases of the ssRNA gene and the presence of common, variable, and missing regions among the sequences. In contrast, the results showed a similarity between the local

isolates that carry the registry number of the Bank Genes (OR944894.1 and OR944895.1) and many global isolates registered in the gene bank. The highest similarity rate was 99.50%, and the lowest was 98.74%. The results of the study showed that the phylogenetic tree that was established, that the ssRNA gene of the two genotypes isolated during the study has a phylogenetic tree, while evolutionary with the recorded genetic species, as the proportion of the evolutionary relationship ranged from 98.74-99.50% (Table 3 and Figure 2).

Table 2: Relationship of infection with *B. gibsoni* in dogs using molecular methods

Factors	Criteria	Number of animals positive for *B. gibsoni* using PCR (%)
Clinical signs	General weakness	3(15.7)
	Lethargy	2(10.5)
	Neurological symptoms,	0
	Loss of appetite	2(10.5)
	Paleness of the mucous membranes	3(15.7)
	Enlarged lymph nodes	3(15.7)
	Diarrhea	2(10.5)
	Hemoglobin urea	1(5.2)
	Presence of ticks	2(10.5)
	No clinical signs	2(10.5)
Age of dog	Less than 6 months	1(5.2) a
	6 months – 1 year	5(26.3) b
	1 year-2 years	13(68.4) b
Sex of dog	Male	13(68.4) a
	Female	6(31.5) b
Source of dog	Native	14(73.6) a
	Imported	5(26.3) b
Breed of dog	Terrier	1(5.2) a
	German shepherd	7(36.8) b
	Belgium Malenosis	4(21)c
	Sebrian huskey	3(15.7) c
	Pomeranian dog	0
	Pit bull	3(15.7) a
	Rottweiler	1(5.2) a
	Pekingese	0

Values significantly different at P<0.05 the different letters.

Table 3: There is a similarity between the local sequences of*B. gibsoni* and the same pathogen sequences in the GenBank using NCBI BLASTn

Accession No. of local sequences	Identified Pathogen	Query Cover %	Similarity Number %	Accession Number in the GenBank	Identification Country
OR944894.1 OR944895.1	*B. gibsoni*	100	99.50	MN385427.1	Iraq
		99	98.74	MN689648.1	China
		99	98.74	MN689646.1	China
		99	98.74	MN689645.1	Singapore
		99	98.74	KP666157.1	China
		99	98.74	OP445697	China
		99	98.74	KC461261.1	India

Discussion

The present study used a molecular technique to detect *Babesia spp*. infection in dogs, so this result corroborates with the results of Inokuma *et al.* (14). However, Das *et al.* (15) has reported a higher percentage of occurrence. The prevalence of tick populations, the time of year, the host's immune condition, and other management techniques may all impact these fluctuations in canine babesiosis. The results of the clinical examination of the animals included in the

study showed the presence of some animals suffering from general weakness, lethargy, neurological symptoms, loss of appetite, paleness of the mucous membranes, enlarged lymph nodes, diarrhea, hemoglobin urea, presence of ticks, while some of them were healthy, the severity of this clinical signs is more obvious in animals which infected with *Babesia spp* Since a larger number of the parasite in circulating in the blood can be noticed during the initial weeks after infection, light microscopy is advised in situations where there are clinical or epidemiological concerns of an acute phase of *Babesia spp.* infection (16-18). The clinical results in this investigation were consistent with those previously reported by Schäfer *et al.* (19).

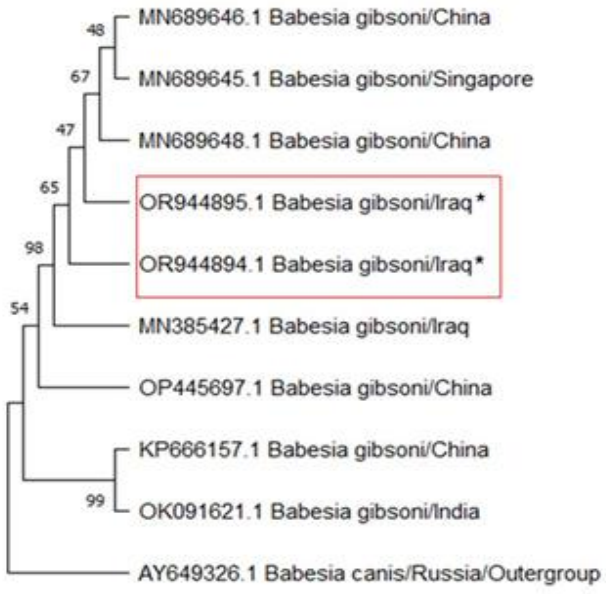

Figure 2: Phylogenic tree of *B. gibsoni* in Mosul city, Iraq (*). The phylogenic tree was built using bootstrap analysis with 1000 resamplings and the Maximum Likelihood technique based on the Tamura-Nei model in MEGA11 software. Concatenated partial *uvrC* gene partial DNA sequences were utilized as input data. The *Babesia canis* strain (AY649326.1) Russia was used as an outgroup.

Discussion

The present study used a molecular technique to detect *Babesia spp.* infection in dogs, so this result corroborates with the results of Inokuma *et al.* (14). However, Das *et al.* (15) has reported a higher percentage of occurrence. The prevalence of tick populations, the time of year, the host's immune condition, and other management techniques may all impact these fluctuations in canine babesiosis. The results of the clinical examination of the animals included in the study showed the presence of some animals suffering from general weakness, lethargy, neurological symptoms, loss of appetite, paleness of the mucous membranes, enlarged lymph nodes, diarrhea, hemoglobin urea, presence of ticks, while some of them were healthy, the severity of this clinical signs is more obvious in animals which infected with *Babesia spp* Since a larger number of the parasite in circulating in the blood can be noticed during the initial weeks after infection, light microscopy is advised in situations where there are clinical or epidemiological concerns of an acute phase of *Babesia spp.* infection (16-18). The clinical results in this investigation were consistent with those previously reported by Schäfer *et al.* (19).

Dogs that were infected in this investigation ranged in age from one to two years, which is more prevalent than the other age groups. The younger animals recorded a lower prevalence of infection with blood parasites; the prevalence of hemoprotozoan infections has been noted by several authors to be highest in older dogs and younger dogs. Breed predisposition is present in larger breeds, which recorded a high infection prevalence compared to small breeds. However, the low prevalence of the disease decreased according to statistical analysis. No significant association was noted between age alteration in our study. Other studies showed that Blood parasites were more common in German shepherds than mongrels. This can result from the owner's desire for German shepherds and insufficient immunogenic response (20,21).

According to the study, male dogs had a higher frequency of blood parasites than female canines in terms of sex. This result was in line with observations made by Liu *et al.* (22) and Obeta *et al.* (23), who noted that male dogs had a higher prevalence of infection than female dogs. However, the results of our study differed from those of Amritpal *et al.* (13), who discovered that the predominance of male dogs is much higher than that of female dogs. This may be explained by the stronger preference for male dogs over female dogs (24). Based on the data collected for this study, it can be inferred that there is no statistically significant variation in the prevalence of the disease between male and female canines based on the sex of the host. These findings don't align with Irwin *et al.* (25). More samples were found by PCR in the current investigation, suggesting greater sensitivity and specificity of the test, which is consistent with the results Ionita *et al.* (26) and Porchet *et al.* (27). The current study indicated that the age group of >1-2 years had a greater incidence of canine babesiosis 23%, which is consistent with data from Bulusu *et al.* (16) and Laha *et al.* (28). This conclusion may be because young dogs' immune systems are less developed than those of adults. Numerous authors have documented cases of babesiosis in dogs of various ages. Thus, it may be concluded that the host's immune system and the transmitting vector determine whether a person contracts Babesia, rather than age being the determining factor. According to the results of Guo *et al.* (29), most affected canines in this study were male 57.5% instead of female. Nevertheless, some Bulusu *et al.* (16),

Amuta *et al.* (30) and Selvaraj *et al.* (31) have suggested no substantial correlation between the host's breed and the incidence of babesiosis. The results of the relationship between the infection of *B. gibsoni* and the clinical manifestation in dogs showed high prevalence in animals that suffer from general weakness and enlargement in lymph nodes, while the animals which appear neurological symptoms not record any infection with *B. gibsoni*, Hassanen *et al.* (32) recorded of the 75 dogs that were tested, 26.67% (20/75) had clinical symptoms consistent with a canine babesiosis infection, including fever, anorexia, dullness, weakness, crimson urine, and pale mucous membranes. The relationship of age of dogs with infection *B. gibsoni* showed a high prevalence in old dogs compared with young ones; several studies showed high the findings were consistent with those of Selvaraj *et al.* (31), showing that the condition was more common in older canines than in younger ones. Similar studies with a high illness incidence in elderly animals are shown in the current study.

The current investigation discovered that several dog breeds were positive, including Great Dane, Doberman, Spitz, Terrier, Irish setter, Pug, and non-descript dogs. Amritpal *et al.* (13) observed that, among 68 dogs suspected of having babesiosis, there was a statistically nonsignificant difference in the incidence of *B. gibsoni* among different breeds and nondescript dogs. Breeding management is another reason for the difference in the prevalence of infection with *B. gibsoni* according to breed. Large breeds of dogs are already present in gardens outside of homes, which results in contact with different arthropods that transmit the Babesia infection, while small breeds are already present in the home, which decreases direct contact with arthropods. In relation to the host's breed, the findings showed that PCR and blood smear analysis found a statistically nonsignificant variation in the prevalence of *B. gibsoni* across the different breeds and nondescript dogs. Hornok *et al.* (33) discovered 21 female and 47 male dogs, seven males, and two female dogs contracting *B. gibsoni* infections, while Fisher's exact test revealed no statistically significant difference (34). Nine of the 68 canines in the current investigation, which used blood smear and/or PCR, were positive for *B. gibsoni* infection. Other researchers Deepa *et al.* (35) showed that every dog that tested positive for blood smears tested positive for PCR. Low parasitemia was thought to be the cause of the two samples' erroneous negative results from blood smear tests. The current investigation confirms that PCR analysis was more effective in diagnosing infection than blood smear inspection. Laha *et al.* (28) discovered that PCR molecular analysis was more accurate in diagnosing *B. gibsoni* infection than standard blood smear examination. The presence of a genetic sequence for the samples of dogs sent in from the city of Mosul was revealed by the findings of the genetic sequencing of the *Babesia gebsoni* 18S rRNA gene. Out of all the samples that were sent for genetic sequencing, it was discovered that there were just two isolates (OR944894.1 and OR944895.1). This variation in the similarity rate indicates the genetic diversity of the parasite, which is the result of genetic changes and mutations in it (36). The results of conducting the genetic sequence of the 18S rRNA gene of *B. gebsoni*, comparing it to the genetic sequence of some international strains registered in GenBank, showed that the highest percentage of similarity to the local isolate was 98.47% with the strain under the genetic name MN689648.1, which was isolated from China, Singapore, and India. This relationship is conclusive evidence of a high genetic correlation between these species (37). At the same time, the isolated strains in the study in terms of their location in the phylogenetic tree (38). This divergence and convergence between local and global strains indicates the common evolutionary origin of the strains from a genetic standpoint (39).

Conclusions

Dogs affected *B. gibsoni*, and there are more dominant parasites in dogs in Nineveh province.

Acknowledgment

The authors would like to thank the College of Veterinary Medicine at the University of Mosul for providing financial support for this work and the veterinary teaching hospital for their valuable assistance.

Conflict of interest

The authors declare that there is no conflict of interest in the manuscript.

References

1. Alseady HH, Al-Dabbagh SM. Isolation and molecular identification of cutaneous leishmaniasis in humans and dogs in middle Euphrates, Iraq. Iraqi J Vet Sci. 2024;38(2):427-35. DOI: 10.33899/ijvs.2023.143821.3259
2. Al-Malachi HB, Al-Farwachi MI. A comparison between different laboratory methods and stains for detection of microfilaremic dogs. Iraqi J Vet Sci. 2023;37(1):171-5. DOI: 10.33899/ijvs.2022.133610.2267
3. Alobaidii WA. The serological diagnosis of canine Leishmaniasis was made using ELISA in Nineveh province. Iraqi J Vet Sci. 2019;33(2):11-114. DOI: 10.33899/IJVS.2019.163194
4. Alobaidii WA, Almashhadany DA. Molecular detection of Leishmania in cutaneous scrapping and blood samples of dogs in Mosul city. Iraqi J Vet Sci. 2023;37(I):15-19. DOI: 10.33899/IJVS.2023.1386030.2815
5. Alobaidii WA, Ali SA. Molecular detection of *Dirofilaria immitis* in dogs. Indian Vet J. 2024;101(1):7-11. DOI: 10.62757/iva.2024.101.1.7-11
6. Kubo S, Tateno M, Ichikawa Y, Endo Y. A molecular epidemiological survey of Babesia, Hepatozoon, Ehrlichia and Anaplasma infections of dogs in Japan. J Vet Med Sci. 2015;77(10):1275-9. DOI: 10.1292/jvms.15-0079
7. Mandal M, Banerjee PS, Kumar S, Garg R, Ram H, Raina OK. Development of recombinant BgP12based enzyme linked

immunosorbent assays for serodiagnosis of *B. gibsoni* infection in dogs. Vet Immunol Immunopathol. 2016;169:27–33. DOI: 10.1016/j.vetimm.2015.10.013
8. Mahalingaiah MC, Asoor M, Thimmaiah RP, Narayanaswamy HD, Mukartal SY, Elattuvalappil AM, Chikkahonnaiah N, Gupta S, Singh S. Prevalence of canine babesiosis in different breeds of dogs in and around Bengaluru. Adv Anim Vet Sci. 2017;5(3):140-144. DOI: 10.14737/journal.aavs/2017/5.3.140.144
9. Das MK, Baidya S, Mahato A, Pandit S, Ghosh JD, Chadhuri S, Das M, Incidence of canine babesiosis in and around Kolkata, west Bengal, India. Explor Anim Med Res. 2015;5(1):102-107. [available at]
10. Xu D, Zhang J, Shi Z, Song C, Zheng X, Zhang Y, Hao Y, Dong H, Wei L, El-Mahallawy HS, Kelly P. Molecular detection of vector-borne agents in dogs from ten provinces of China. Parasit Vectors. 2015;8:1-7. DOI: 10.1186/s13071-015-1120-y
11. Ukwueze CS, Akpa PO, Odo RI, Aronu CJ, Anene BM. The beneficial effects of resveratrol supplementation on parasitemia, oxidative stress and serum biochemical parameters in *Trypanosoma brucei* infected dogs. Iraqi J Vet Sci. 2022;36(3):753-60. DOI: 10.33899/ijvs.2022.131843.2009
12. Singh MN, Raina OK, Sankar M, Rialch A, Tigga MN, Kumar GR, Banerjee PS. Molecular detection and genetic diversity of *B. gibsoni* in dogs in India. Infect Genet Evol. 2016;41:100–106. DOI: 10.1016/j.meegid.2016.03.025
13. Amritpal Singh AS, Harkirat Singh HS, Singh NK, Singh ND, Rath SS. Babesiosis in northwestern India: Molecular detection and assessment of risk factors. BioMed Res Int. 2014;2014:1°. DOI: 10.1155/2014/741785
14. Inokuma H, Yoshizaki Y, Matsumoto K, Okuda M, Onishi T, Nakagome K, Kosugi R, Hirakawa M. Molecular survey of Babesia infection in dogs in Okinawa, Japan. Vet Parasitolog. 2004;121(3-4):341-6. DOI: 10.1016/j.vetpar.2004.03.012
15. Das MK, Baidya S, Mahato A, Pandit S, Ghosh JD, Chadhuri S, Das M, Incidence of canine babesiosis in and around Kolkata, west Bengal, India. Explor Anim Med Res. 2015;5(1):102-107. [available at]
16. Bulusu S, Rajkumar K, Thanislass J, Vijayalakshmi P, Prabavathy A. Molecular diagnosis of *B. gibsoni* infection. Int J Livest Res. 2018;8(5):98-103. DOI: 10.5455/ijlr.20180214070823
17. Guo WP, Xie GC, Li D, Su M, Jian R, Du LY. Molecular detection and genetic characteristics of *B. gibsoni* in dogs in Shaanxi Province, China. Parasit Vectors. 2020;13:1-4. DOI: 10.1186/s13071-020-04232-w
18. Akhtardanesh BA, Saberi ME, Nurollahifard SR, Aghazamani MA. Molecular detection of *Babesia spp.* in tick-infested dogs in southeastern Iran. J Dis Glob Health. 2016;8(2):72-7. [available at]
19. Schäfer I, Helm CS, von Samson-Himmelstjerna G, Krücken J, Kottmann T, Holtdirk A, Kohn B, Hendrickx G, Marsboom C, Müller E. Molecular detection of *Babesia spp.* in dogs in Germany (2007–2020) and identification of potential risk factors for infection. Parasit Vectors. 2023;16(1):396. DOI: 10.1186/s13071-023-06005-7
20. Chandra BS, Rajkumar K, Das SS, Vijayalakshmi P, Abiramy Prabavathy A. Prevalence of *B. gibsoni* infection in dogs of Puducherry region. Int J Curr Microbiol App Sci. 2018;3:1275-8. DOI: 10.20546/ijcmas.2018.703.151
21. Deepa CK, Varghese A, Kumar KG, Nandini A, Kumar GS, Hembram PK, Dinesh CN, Juliet S, Vergis J, Sindhu OK, Ravindran R. Evaluation of recombinant *B. gibsoni* thrombospondin-related adhesive protein (BgTRAP) for the sero-diagnosis of canine babesiosis. Exp Parasitol. 2023;254:108621. DOI: 10.1016/j.exppara.2023.108621
22. Liu M, Ruttayaporn N, Saechan V, Jirapattharasate C, Vudriko P, Moumouni PF, Cao S, Inpankaew T, Ybañez AP, Suzuki H, Xuan X. Molecular survey of canine vector-borne diseases in stray dogs in Thailand. Parasitol Int. 2016;65(4):357-61. DOI: 10.1016/j.parint.2016.04.011
23. Obeta SS, Ibrahim B, Lawal IA, Natala JA, Ogo NI, Balogun EO. Prevalence of canine babesiosis and their risk factors among asymptomatic dogs in the federal capital territory, Abuja, Nigeria. Parasit Epidemiol Control. 2020;11:e00186. DOI: 10.1016/j.parepi.2020.e00186
24. Alobaidii WA, Allhaibi RA, Alkhero A. Detection of blood protozoa in pet dogs in Nineveh province. Assiut Vet Med J. 2019;65(162):1-8. DOI: 10.21608/avmj.2019.169005
25. Irwin PJ. Canine babesiosis: From molecular taxonomy to control. Parasit Vectors. 2009;2(1):S4. DOI: 10.1186/1756-3305-2-s1-s4
26. Ionita M, Mitrea IL, Pfister K, Hamel D, Buzatu CM, Silaghi C. Canine babesiosis in Romania due to *Babesia canis* and *Babesia vogeli*: A molecular approach. Parasitol Res. 2012;110:1659-64. DOI: 10.1007/s00436-011-2683-y
27. Porchet MJ, Sager H, Muggli L, Oppliger A, Müller N, Frey C, Gottstein B. A descriptive epidemiological study on canine babesiosis in the Lake Geneva region. Schweiz Arch Tierh. 2007;149(10):457-65. DOI: 10.1024/0036-7281.149.10.457
28. Laha R, Bhattacharjee K, Sarmah PC, Das M, Goswami A, Sarma D, Sen A. Babesia infection in naturally exposed pet dogs from a north-eastern state (Assam) of India: Detection by microscopy and polymerase chain reaction. J Parasit Dis. 2014;38:389-93. DOI: 10.1007/s12639-013-0261-1
29. Guo WP, Xie GC, Li D, Su M, Jian R, Du LY. Molecular detection and genetic characteristics of *B. gibsoni* in dogs in Shaanxi province, China. Parasit Vectors. 2020;13:1-4. DOI: 10.1186/s13071-020-04232-w
30. Amuta EU, Atu BO, Houmsou RS, Ayashar JG. *Rhipicephalus sanguineus* infestation and *Babesia canis* infection among domestic dogs in Makurdi; Benue. State-Nigeria; Int J Acad Res. 2010;2(3):170–172. [available at]
31. Selvaraj P, Kumar KS, Vairamuthu S, Prathaban S, Srinivasan SR. *B. gibsoni*-an emerging challenge in canine pediatric practice in Chennai. Tamilnadu J Vet Anim Sci. 2010;6(3):122-4. [available at]
32. Hassanen EA. Clinical, morphological and molecular characterization of canine babesiosis and its compatible tick vector in naturally infected dogs, in Egypt. Zagazig Vet J. 2020;48(3):242-53. DOI: 10.21608/zvjz.2020.24982.1103
33. Hornok S, Edelhofer R, Farkas R. Seroprevalence of canine babesiosis in Hungary suggesting breed predisposition. Parasitol Res. 2006;99:638-42. DOI: 10.1007/s00436-006-0218-8
34. Habibi G, Imani A, Afshari A, Bozorgi S. Detection and molecular characterization of *Babesia canis vogeli* and *Theileria annulata* in free-ranging dogs and ticks from Shahriar county, Tehran province, Iran. Iranian J Parasitol. 2020;15(3):321. DOI: 10.18502/ijpa.v15i3.4196
35. Deepa CK, Varghese A, Bora CA, Kumar KG, John L, Asaf M, Chulliparambil S, Ravindran R. Phylogenetic analysis of *B. gibsoni* isolates of south India using apical membrane antigen, 50 kDa surface antigen, and 70 kDa heat shock protein genes. Exp Parasitol. 2023;246:108461. DOI: 10.1016/j.exppara.2023.108461
36. Rashid ZM, Aziz SA, Ali OJ, Kakarash NK, Marif HF. Coprological detection of Toxocariosis in domicile and stray dogs and cats in Sulaimani province, Iraq. Iraqi J Vet Sci. 2022;36(4):1047-51. DOI: 10.33899/IJVS.2022.132976.2157
37. Tayyub M, Ashraf K, Lateef M, Anjum AA, Ali MA, Ahmad N, Nawaz M, Nazir MM. Genetic diversity of canine Babesia species prevalent in pet dogs of Punjab, Pakistan. Anim. 2019;9(7):439. DOI:10.3390/ani9070439
38. Phuyal S, Jha VC, Subedi M. Prevalence of blood parasites in dogs of Kathmandu Valley. Nepal Vet J. 2017;34:107-12. DOI: 10.3126/nvj.v34i0.22909
39. Karasová M, Tóthová C, Grelová S, Fialkovičová M. The etiology, incidence, pathogenesis, diagnostics, and treatment of canine babesiosis caused by *B. gibsoni* infection. Anim. 2022;12(6):739. DOI: 10.3390/ani12060739

التحليل الجزيئي والتطوري لبابيزيا جيبسوني في الكلاب والقراد في محافظة نينوى بالعراق

وسن أمجد العبيدي[1]، صفوان يوسف البارودي[1] و ضاري عليوي المشهداني[2]

[1]فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، [2]قسم علوم المختبرات الطبية، كلية العلوم، جامعة المعرفة، أربيل، العراق

الخلاصة

من خلال هذه الدراسة تم فحص ٥٠ كلبًا متباينا في الجنس والعمر والمظهر السريري والسلالات. تم فحص هذه الحيوانات وتسجيل العلامات السريرية الظاهرة عليها، وتم جمع عينة الدم من الوريد الرأسي للكلاب، ونقل الدم إلى أنابيب مضادة للتخثر، بالإضافة إلى جمع ١٣٠ قرادة مباشرة من الحيوانات، تم استخلاص الحمض النووي من الدم والقراد ثم تم تطبيق الكشف عن الحمض النووي باستخدام تفاعل البوليميراز المتسلسل للكشف عن البابيزيا جيبسوني باستخدام البادئات المتخصصة. أظهرت نتائج تفاعل البوليميراز المتسلسل للكشف عن الحمض النووي للطفيلي وجود ١٩ عينة دم إيجابية و٨٠ عينة إيجابية للحمض النووي للطفيلي في القراد، وارتفاع نسبة الإصابة بالحيوانات التي تعاني من ضعف عام وتضخم في الغدد الليمفاوية، أظهر الحيوان الأكبر سنا انتشارا عاليا للإصابة بالمقارنة مع الأصغر منها، وأظهرت الكلاب الذكور انتشارا أعلى من الإناث، وسجلت الكلاب المحلية انتشارا أعلى من المستوردة، وأظهرت السلالات الأكبر انتشارا أعلى من السلالات الصغيرة. تم اختيار ١٠ عينات إيجابية. وبعد ذلك تم إجراء التسلسل الجيني تم الكشف عن التسلسل الجيني للعينات من خلال نتائج تسلسل الوحدة الفرعية الصغيرة لجين الريبوسوم لجزية الرنا لطفيلي البابيزيا ومن بين عينات الدم التي تم إرسالها للتسلسل تم العثور على عزلة واحدة فقط. لديهم رقم تسلسلي عندما تم تسجيلهم في بنك الجينات (OR944894.1)وعزلة أخرى من القراد (OR944895.1). نستنتج من خلال هذه الدراسة ان للطفيلي دور كبير على صحة الكلاب فضلا عن دور القراد في انتقاله.