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Morphological and Seroprevalence of Canine Babesiosis

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ABSTRACT
Background: Babesia species are parasitic microorganisms that invade the red blood cells of various mammals, causing Babesiosis. Babesia canis and Babesia gibsoni infect dogs and notably responsible for canine Babesiosis. This study first Seroprevalence of canine babesiosis in Baghdad, Iraq
Objectives: This study aimed to establish the incidence of Babesia spp. in dog populations within Baghdad City, Iraq, and to identify the epidemiological risk factors associated with canine Babesiosis
Methods: The study extended from November 2023 to May 2024, and involved 228 blood samples collected from numerous dog breeds across different regions of Baghdad. Blood samples were examined using Giemsa and Acridine Orange stains, ELISA, and immunochromatographic tests to detect the Babesia parasite. Statistical analysis of the prevalence rates affording the age, gender, breed of dogs, and their geographic districts.
Results: The study revealed a higher incidence of Babesia gibsoni 44% to Babesia canis35%, with total infection rates peaking in warmer months. Stray dogs exhibit the highest infection rate 33.3% of dog babesiosis in contrast to other breeds. exhibited the highest infection rates33.3%.
Conclusion: The current study provides important information on the epidemiology of canine babesiosis from Baghdad, Iraq.

Keywords: B. gibsoni, B. canis, dogs, Baghdad, Iraq

INTRODUCTION

Canine babesiosis is a vector-borne disease of a genus of Babesia belonging to Apicomplexa. This disease primarily affects canines and is transmitted through the bites of infected hard ticks (Ayoob et al., 2010). The notable species of canine babesiosis are B. canis and B.gibsoni exhibit distinct geographic distributions according to their tick vector (Depoix et al., 2002). More transmission in B. gbsoni occurs by direct contact with blood or via the placenta (Macintire et al.,2002) B. canis infections can induce secondary immunopathological effects, such as the formation of immune complexes and the generation of autoantibodies (Wężyk et al., 2023). Canine babesiosis is associated with anemia, dark urine, jaundice, and splenomegaly (Gboeloh et al., 2023). The prevalence of canine Babesiosis is recognized in different regions. For example, Baghdad city reported microscopically the infection rate at 65.68% (Fadhil & Kalef, 2012), other by PCR record 5.1% (Badawi & Yousif., 2020). will Mohsen & Abbas (2022) report 66.66% divided into 5.7% in stray dogs, 11.4% in police dogs, and 20% in pet dogs.
In Iran, demonstrated canine babesiosis at 6.6% (Hosseinzadeh et al., 2016), in Egypt recorded 12 for large Babesia species(Solano et al., 2016), other research in Nigeria documented 18% (Obeta et al., 2020; Gboeloh et al., 2023) and in India 6.01% infection rate for canine babesiosis (Kumar et al., 201

5), serological testing is a valuable diagnostic tool for detecting Babesia infections. A study deduced canin babesiosis serologically including Algiers found the infection rate 17.98% for B. canis (Kiouani et al., 2020).

In Romania, the study revealed a rate of 19.8% for B. canis (Imre et al., 2013), in Brazil a considerably positivity percentage of 41.98% was reported by ELISA (Lopes et al., 2020). immunochromatographic tests (ICT) used in Recent studies to estimate the efficacy of detecting serum antibodies for B. gibsoni in experimentally infected dogs (Castillo-León et al., 2021). Particularly the impact on public health, especially the health of dogs, caused by canine babesiosis, this study was designed to determine the prevalence of this disease serologically and identify the risk factors associated with its spread to develop strategies to control and prevent this parasite

.MATERIALS AND METHODS

Study area and sampling The study started from November 2023 to May 2024 in Baghdad City, Iraq. 228 blood samples were taken from different breeds of dogs examined for canine babesiosis. The blood samples were collected from each dog's cephalic antiseptically these samples were immediately divided into (3ml) anticoagulant gel tubes then centrifugation and kept at -20 ° C until serological tests were used. The distribution of breeds included: German Shepherd (29), Belgium Malinois (37), Stray (60), Husky (6), Terrier (16), and Pit Bull (8). The control group of dogs was selected as being clinically healthy, without tick exposure, and testing negative for ICT and ELISA tests.

Blood Smear Preparation

Direct Blood smears were prepared from the cephalic or ear vein. blood smears for Giemsa stain to detect intraerythrocytic parasites. Blood smears were fixed with methanol, stained for 30-40 minutes, washed, air-dried, and then examined under light microscopy (Harvey et al., 2011).

ELISA test

ELISA was utilized to detect anti-Babesia canis antibodies by using a Diagnostic kit (Demeditecs/Germany). A sample dilution of 1:100 µL was prepared, and the reaction was carried out according to the manufacturer's instructions. The plates were then read by an ELISA reader with an indicator of 405 nm. To interpret the results, the sample absorbance value was multiplied by 10 and divided by the cut-off value. Samples with intense yellowish coloration and an optical density equal to or greater than the cutoff index were positive for B. canis.

Rapid Immunochromatographic Test: Babesia gibsoni Ab was utilized in rapid ICT for qualitative detection. Twenty µL of the prepared specimen was mixed with a specific buffer and placed into the sample window. In positive cases, a colored line appears in the test area according to the manufacturer's instructions (https://m.globalsources.com/shanghai).

Statistical Analysis: The data from tables and figures were statistically analyzed using appropriate software. Descriptive statistics, means, standard deviations, and p-values were calculated for each parameter. Inferential statistics (t-tests or ANOVA, LSD) determined group differences with $p < 0.05$ and $p < 0.001$.

RESULTS

Blood smears diagnosed infections in dogs caused by B. gibsoni, B. canes, and mixed infections. Giemsa staining revealed small intraerythrocytic merozoites and annular bodies for B. gibsoni, while pyriform bodies indicated B. canis (Obeta et al., 2020). Microscopic examination showed various developmental stages of the parasite, including atypical trophozoites (**Figure 1,2**).

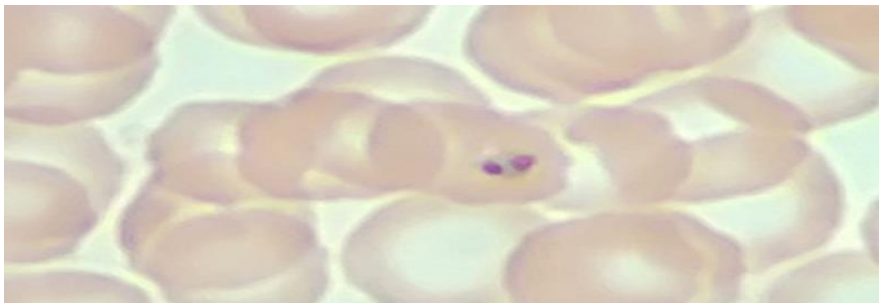


Figure 1: Giemsa-stained RBCs show the large intra-erythrocytic of B. canis, characterized by a clear cytoplasm and a round, basophilic nucleus, with 100X objective.

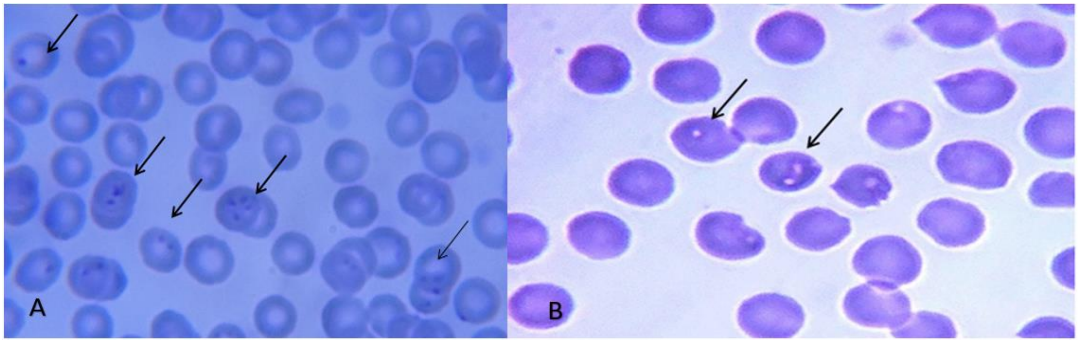
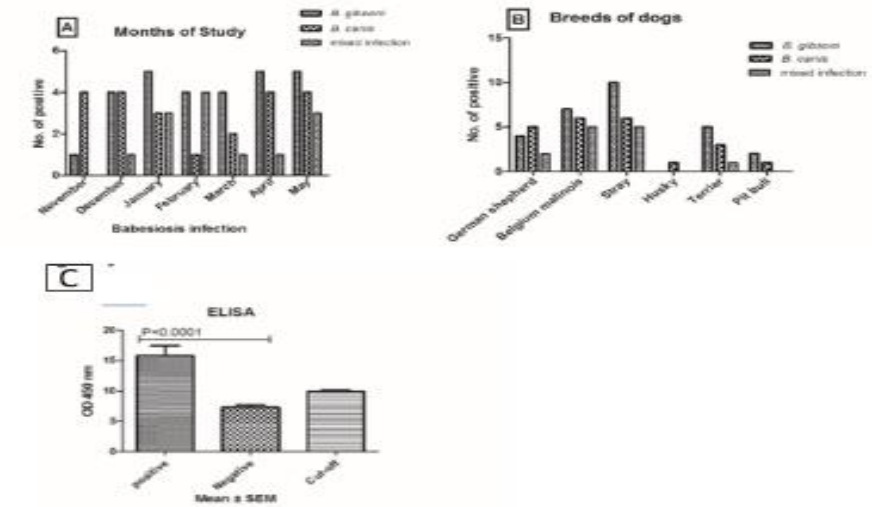


Figure 2: Giemsa-stained RBCs presents (A)show the small polymorphism of intracellular B. gibsoni. (B) mixed infection shows pyriform bodies with basophilic cytoplasm of B.canis and single-ring of B. gibsoni merozoites (black arrows). with a 100X objective.

The study investigated the presence of babesiosis in dogs, focusing on B. gibsoni and B. canis from November to May. B. gibsoni had a higher prevalence than B. canis, with low mixed infection rates. The infection peaked in January, April, and May, reaching 15.9% to 19% (**Figure 2A**). Among breeds, Stray dogs had the highest total infection rate at 33.35%, followed by Belgium Malinois (28.6%), and German Shepherds (17.5%). Pit Bulls and Huskies had lower rates, with Huskies showing no B. gibsoni or mixed infections (**Figure 2B**). Using ELISA, 29 samples tested positive for B. canis while 38 were negative. Twenty samples were close to the positive cut-off level of 9.82-10.35 (**Figure 2C**).

(Figure 2) Babesiosis occurrence in dogs. A: Infection rate of dog Babesiosis according to months of the study. **B:** Infection rate of Babesiosis related to dog breed. **C:**ELISA infection rate of B. canis in infected dogs during the study.

DISCUSSION



The prevalence of canine babesiosis demonstrates variability over the study duration, especially noted high prevalence in January, April, and May, this observation aligns with the findings of (Kiouani et al., 2020) in Algiers the recorded a high infection rate in April and May, but he records no infection rate in Winter (December, January, February) disagree with him, while (Soulsby., 2005; Oguche et al.,2020) found that B. canis infections are more common in winter due to increased activity of the brown dog tick, Rhipicephalus sanguineus, during rainy seasons, leading to higher rates in December, January, and February .B. gibsoni showed a higher prevalence than B. canis throughout the months, with an overall infection rate of 44%. This is attributed to additional transmission routes for B. gibsoni, including blood transmission from fights and placental transmission (Karasová et al.,2022).

The research revealed notable variations in the prevalence of Babesia infections across various dog breeds. The German Shepherds exhibited a moderate prevalence of babesiosis with a total of 17.5% revers to the prevalence of (Hornok et al. 2006) who found that antibodies to B. canis were significantly higher among German shepherds. The higher rates observed in Stray dogs and Belgian Malinois may suggest greater exposure to environmental risk factors or higher susceptibility to infection(Al-Rubaii.,2008). It is noteworthy to find that B. gibsoni infected the majority of the stray dogs, which is indicative of their living in groups, and fighting among each other besides the maternal transmutation of parasite and lack of regular veterinary care compatible with *(*Fadhil.,2012) in Baghdad found %56.56 Babesia spp and (Oguche et al.,2020*)*in Nigeria recorded 21.66% in local breeds. The data on Huskies and Pit Bulls indicated a lower prevalence of babesiosis, It may be due to the small size of the samples taken and, the spread of parasites and vectors (Sabbar.,2016). Our finding with an ELISA infection rate of 33.3%. This aligns with the infection rate of (Corassa et al., 2020), 41.98% in Brazil, (Pantchev et al., 2015), and 16.2% in Bulgaria. (Veneziano et al., 2018)14.0% In Southern Italy, and (Kiouani et al., 2020) in Algiers reported 17.98% positivity for B. canis antibodies. Global differences in Babesiosis prevalence are influenced by infection rates, climate and ecological variations, vector distribution, and socioeconomic factors (Al-Obaidi et al.,2020). The elevated infection rate could result from chronic infections, where antibody levels can be detected for up to 420 days' post-infection (Fukumoto et al., 2004). The results of the study highlight sensitive diagnostic methods like ELISA to successfully identify infected animals for treatment and preventive measures (Al-Gharban& Dhahir 2015*)* canin babesiosis higher in female dogs because of their immunodepression, caused by pregnancy and lactation risk Factors which can facilitate the disease's progression (Abd Al-Rhman 2009; Al-Taie& Abdulla 2011).

These observed differences may be explained by different proportions of infected tick vector populations in addition to other risk factors such as quantity of samples, canine interaction, handling techniques, and medical histories. (Hasso & AL-Nashy.,2002; Azhar et al., 2023).

CONCLUSIONS

The results of this study provide a valued understanding of the spread of canine babesiosis infection in Baghdad City. The general infection rate was 44% for Babesia gibsoni and 35% for Babesia canis. Microscopic identification and serological analysis were used to evaluate the existence of anti-B. gibsoni and B. canis antibodies in numerous groups of dogs. The results presented high prevalence rates, transmission passageways in the infected dogs. This research underscores the potential public health implications of canine babesiosis, particularly in urban areas with high dog populations and suitable environmental conditions for parasite transmission.

Ethical Considerations

The study was assigned Protocol No. P.G. 12640 according to all necessary licenses and guidelines and suggested procedures approved by the Iraqi Ethics Committee of the College of Veterinary Medicine, Baghdad University, to collection and usage of dogs' blood for this study

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Authors' contributions

Dalia Ahmed Kalef: Conceptualization, Data Curation, Investigation, Methodology, Formal Analysis, Writing - Original Draft Preparation.

Zahra Akram Saleh: Data collection, recording and arranging of the samples, Investigation, writing the first draft

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