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

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### ABSTRACT

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

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

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

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

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

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

### Introduction

*Babesia gibsoni* is a tick-transmitted protozoan parasite, that belongs to Piroplasmida Order in the Aconoidasida Class under the Apicomplexa Phylum, which infects the domestic and wild canid species causing in a disease known as babesiosis (Baneth *et al.*, 2019; Birkenheuer, 2021). Like other members, the life cycle of *B. gibsoni* requires two types of hosts; a tick and a canine host. Briefly, sporozoites enter the host's circulation with tick saliva during blood sucking to attach and penetrate erythrocytes by endocytosis. Once inside, sporozoites transform into trophozoites that develop into merozoites throughout the binary fission process (Baneth, 2018; Conesa *et al.*, 2020; Martínez-García *et al.*, 2021). Post ingestion of erythrocytes that contain gametocytes, ticks become infected and may remain infective for many generations by the transovarial and transstadial transmission. This process occurs due to the development of gametocytes into female and male

gametes in the gut of female ticks to produce later the motile zygotes that multiply to vermicles and invade numerous organs including ovaries. Usually, sporogony resides in the salivary glands of different developmental stages (larval, nymphal, and/or adult) in female ticks that get an infection (Chauvin *et al.*, 2009; Friedhoff, 2018; Ravindran *et al.*, 2023).

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

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