Research Article

Infestation Rate and Molecular Detection of Hard Ticks in Pet Cats from Hilla City, Iraq

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Abstract | Ectoparasites such as hard ticks pose a significant health risk to domestic animals, including pet cats, as they can transmit a variety of pathogens and negatively affect animal well-being. This study aimed to assess the prevalence and distribution of hard ticks in pet cats in Iraq, considering the effects of age and sex. In the current study, a total of 70 pet cats were examined to assess the prevalence of hard tick infestation. Specimens were identified based on morphological characteristics according to a standard taxonomic key, and representative samples were deposited at the Iraq Natural History Research Center and Museum, Department of Vertebrates. Additionally, molecular identification using PCR techniques was performed to confirm the species. The results revealed that 34 cats (48.5%) were infested with a single species of hard tick, *Rhipicephalus* spp. Female cats showed a higher infestation rate (24 out of 47; 51.06%) compared to male cats (10 out of 23; 43.4%), although this difference was not statistically significant ($P>0.05$). In contrast, age showed a significant impact on infestation rates. Younger cats were more heavily infested than older cats, and statistical analysis confirmed a significant difference in infestation prevalence between age groups ($P<0.05$). Ticks were distributed across various body sites, with the highest concentrations found on the ears (79.4%), back (64.7%), and abdomen (61.7%). The lowest prevalence was observed on the neck (18.7%), tail, and perianal region (20.5%). The findings indicate that *Rhipicephalus* spp. is the predominant tick species infesting pet cats in the study area, with younger cats showing significantly higher infestation rates; ticks were most commonly found on the ears, back, and abdomen.

Keywords | Hard ticks, *Rhipicephalus*, Pet cats, Hilla city, Iraq

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INTRODUCTION

Understanding parasitic infections in pet cats is essential for both medical and veterinary fields, as these animals can serve as reservoirs for parasites that are transmissible to humans and other animals (Morelli *et al.*, 2021). Identifying tick species infesting pet cats is critical for developing effective prevention and control strategies that protect public and animal health (Mustafa, 2019). Both stray and pet cats are commonly infested with a variety of parasites (Beugnet *et al.*, 2014).

Ticks, as obligate blood-feeding ectoparasites, can cause a range of clinical issues, including skin disorders, irritation, allergic reactions, weight loss, and anemia (Jatav *et al.*, 2024). Moreover, they are capable of transmitting numerous bacterial, viral, and protozoal pathogens to both animals and humans (Rochlin and Toledo, 2020).

Despite the recognized importance of tick infestations in cats, there is a notable lack of comprehensive global data on their prevalence, associated disease burdens, and mortality rates. This data gap is particularly evident in Iraq, where systematic studies on tick infestations in pet cats are scarce. Understanding the epidemiology of these parasites is vital for formulating effective control and preventive measures.

Regional studies underscore the importance of this issue: a study in Turkey reported that approximately 40% of cats were infested with *Rhipicephalus* spp., with significant health impacts (Yildirim *et al*., 2019), while research in Egypt found a 35% infestation rate in domestic and stray cats, with PCR techniques revealing the presence of pathogens such as *Babesia* and *Ehrlichia* (El-Sherbini *et al*., 2021).

Given this context, the present study represents the first report on tick infestation in pet cats from Hilla City, Iraq. It aims to estimate the infestation rate and identify tick species using both morphological and molecular (PCR) methods, as visual inspection alone is often insufficient for accurate species classification.

MATERIALS AND METHODS

A total of 70 pet cats of various ages and sexes were collected from different locations in Hilla City, Iraq, through visits to veterinary clinics. The collection and handling of animals were conducted in accordance with the guidelines of the International Animal Ethics Committee or the Institutional Ethics Committee, as well as local laws and regulations (Ebani *et al*., 2020). Of the total cats examined, 47 were female and 23 were male.

Each cat was visually examined to determine its sex and age, with age estimated based on dentition. Cats were then grouped into two age categories: less than one year and more than one year. Ticks were also classified according to their location on the host's body.

Tick collection was performed using non-toothed forceps, and specimens were examined under a dissecting microscope for morphological identification (Fourie *et al*., 2014). All collected ticks were preserved in 70% ethanol and later subjected to DNA extraction and molecular identification using 16S rDNA-based PCR techniques (Ghafar *et al*., 2020).

Identification of Collected Ticks

Identification was accomplished using a stereomicroscope at 40x magnification and compared to features listed in the taxonomic key. Two specimens for each sample were placed at the Iraq Natural History Research Center and Museum's Department of Vertebrates.

DNA Extraction of Ticks

Genomic DNA was extracted from 20 adult tick samples using the gSYNCTM Genomic DNA Purification Kit (Geneaid, Canada), with minor protocol adjustments. Each tick was incubated in a mixture of 300 µl comprising 200 µl of GST Buffer, 40 µl of Proteinase K solution, and 5 µl of RNase A solution at 60 °C for 2–3 hours, depending on the size of the specimen. After incubation, the ticks were mechanically ground using a sterile glass mortar and pestle. The homogenized material was centrifuged at 10,000 × g to remove insoluble debris.

The resulting supernatant was transferred to a new tube, followed by the addition of 200 µl of GSB Buffer. The mixture was vortexed vigorously for 10 seconds, then mixed with 200 µl of 100% ethanol. The solution was passed through a spin column by centrifugation at 13,000 rpm for 30 seconds.

The column was washed twice: first with 400 µl of W1 Buffer, and then with 600 µl of Wash Buffer, each followed by centrifugation at 14,000 rpm for 30 seconds. For elution, 100 µl of pre-warmed Elution Buffer was added to the center of the column membrane and incubated for 5 minutes at room temperature. DNA was finally eluted into sterile 1.5 ml Eppendorf tubes by centrifugation at 14,000 rpm.

Polymerase Chain Reactions

PCR was employed to confirm the identification of *Rhipicephalus* spp. using species-specific primers: forward primer 5′-TTGGGCAAGAAGACCCTATGAA-3′ and reverse primer 5′-CCGGTCTGAACTCAGATCAAGT-3′, which amplify a 495 base pair fragment (Faouzi *et al*., 2018). The PCR protocol began with an initial denaturation step at 95 °C for 5 minutes. This was followed by 10 amplification cycles, each consisting of denaturation at 92 °C for 1 minute, annealing at 72 °C for 1 minute and 30 seconds, and a final extension at 72 °C for 7 minutes.

Statistical Analysis

The Chi-square (X2) test was employed to compare the outcomes. According to Petrie and Watson (2006), differences were deemed statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

The incidence of hard tick (*Rhipicephalus*) infestation among pet cats in Hillah City was 34 out of 70 cats, representing a prevalence rate of 48.5%. Species identification was confirmed using polymerase chain reaction (PCR) analysis (Figure 1).

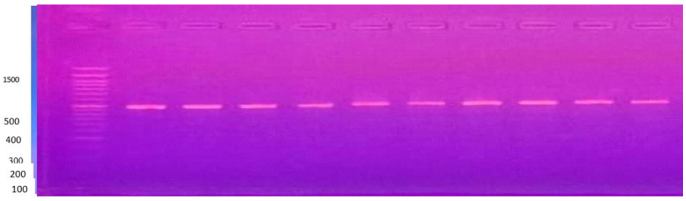

Figure 1: Gel electrophoresis of PCR products amplified using 16S rDNA primers, showing the DNA band of *Rhipicephalus turanicus* at 496 bp isolated from pet cats.

Rhipicephalus infestation Rate by Host Sex and Age

Examination of 70 pet cats revealed an overall *Rhipicephalus* infestation rate of 34 (48.5%) across both sexes. This prevalence varies considerably compared to other countries, such as 4.2% in Dubai (Schuster et al., 2009), 80.1% in cats from Egypt (Aboelhadid *et al*., 2018), 0.8% in the USA (Saleh *et al*., 2019), 1.79% in Ireland (Waal *et al*., 2020), 52.4% in Hungary (Capari *et al*., 2013), and 1.6% in Brazil (Mendes *et al*., 2011). Differences in infestation rates among nations may be influenced by the effectiveness of control programs, seasonal and environmental factors, as well as the molecular techniques employed for species identification, since morphological identification alone is often insufficient (Capari *et al*., 2013).

In this study, female cats exhibited a higher infestation rate (51.06%) compared to males (43.4%), although this difference was not statistically significant. These findings align with Kamaruddin *et al*. (2020), who reported no significant sex-based difference in infestation rates, suggesting that both males and females are equally affected by this parasite.

Table 1: Infection rate of *Rhipicephalus turanicus* according to host sex and age.

Sex / Age	Male			Female		
	No. of cats examined	No. of cats infected	Infection rate, %	No. of cats examined	No. of cats infected	Infection rate, %
≤ 1 year	9	6	66.6^{a}	18	14	73.6^{a}
>1 year	14	4	28.5^{b}	28	10	42.4^{b}
Total	23	10	43.4^{a}	47	24	51.06^{a}

Different superscript letters in column differ significantly at level of $p<0.05$.

Age appeared to be a significant factor in infestation rates, with younger cats showing a higher prevalence of tick infestation than older cats, as confirmed by statistical analysis (Table 1). The current study also observed significant differences in infestation rates between nymphal and adult ticks, with adults, particularly females, being more susceptible to infection. This may be attributed to their prolonged feeding periods and higher blood consumption, which increase the risk of acquiring and transmitting pathogens (Baker *et al*., 2024). Städel (2024) supports this by noting that the extended attachment of female ticks to their hosts increases their susceptibility to bloodborne diseases. In contrast, males tend to have a lower infection rate, possibly due to shorter feeding durations, although infection rates may rise during tick mating seasons (Ding *et al*., 2024).

Adult ticks generally exhibit higher infection rates than nymphs, likely because they feed longer on infected hosts, increasing their exposure to pathogens (Kalayou *et al*., 2024). The elevated infection rates in female ticks are particularly concerning for public health due to their greater potential for transmitting infections to humans and other animals. Consequently, tick control efforts should prioritize targeting adult female ticks.

Overall, multiple factors, including environmental conditions, seasonal variations, and host characteristics, influence tick infestation levels. These findings highlight the need for further research to better understand these dynamics and develop more effective control strategies.

Rhipicephalus Infestation by Body Site in Cats

This study examined the infestation patterns of *Rhipicephalus* ticks on domestic cats, focusing on the preferred attachment sites on the host's body. The data revealed a non-random distribution of ticks, with certain anatomical locations favored significantly over others. The ears and neck were the most commonly infested areas, consistent with previous reports of tick attachment in domestic animals. These sites feature thinner skin, richer blood supply, and are less accessible for grooming, making them ideal for tick attachment and feeding. The ears, in particular, provide a warm microclimate that protects ticks and enhances their feeding success.

Moderate infestation rates were also observed in the perineal and inguinal regions, areas that are less frequently groomed by cats and benefit from protective flesh folds combined with moisture, creating favorable conditions for tick survival. In contrast, limbs and dorsal parts of the body exhibited considerably lower infestation rates. These regions are more exposed and frequently groomed, reducing tick attachment and facilitating their removal through grooming behavior.

Kohler et al. (2018) noted that tick site selection is influenced by host traits such as hair density, skin thickness, and grooming habits. Understanding these attachment preferences is crucial for effective tick control strategies in cats. Focused inspection and treatment of known preferred sites can lead to timely tick removal, thereby reducing the risk of disease transmission. The concentration of ticks in specific areas underscores the importance of thorough examinations by veterinarians and pet owners, particularly around the ears, neck, and groin.

These findings also suggest that tick repellents could be applied more efficiently by targeting these strategic body regions rather than using uniform whole-body treatments. In this study, significant tick burdens were found on the ears (79.4%), back (64.7%), and abdomen (61.7%). Table 2 details the variation in tick concentrations across different body parts.

Table 2: Infection rate of *Rhipicephalus turanicus* according to attachment sites on the cat's body.

Attachment site	No. of infected cats	Infection rate, %
Head	15	44.1^{b}
Ears	27	79.4^{a}
Neck	5	18.7^{c}
Abdomen	21	61.7^{a}
Back	22	64.7^{a}
Legs and feet	8	23.5^{c}
Tail and perianal	7	20.5^{c}

Different superscript letters in column differ significantly at level of $p<0.05$.

Rhipicephalus ticks were especially prevalent on the ears, head, legs, and feet, consistent with Salant *et al.* (2013), who reported the highest *Rhipicephalus* infestations in these areas, and Geurden (2018), who identified the toes as common tick attachment sites.

CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrated that the infection rate of *Rhipicephalus* ticks was significantly higher in younger cats compared to older cats. Ticks were predominantly concentrated on specific body sites, such as the ears, back, and abdomen. Further epidemiological studies on the distribution of *Rhipicephalus* species in pet cats across various provinces of Iraq are needed. Additionally, a comprehensive examination of imported animals, particularly those in close contact with local pets, is essential to identify carrier animals that may harbor and transmit various diseases, especially parasitic infections.

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NOVELTY STATEMENTS

This study was conducted in Babylon province and provided data on ectoparasites infecting small animals. It showed that *Rhipicephalus spp*. is the predominant tick species infecting domestic cats, as it can transmit a variety of pathogens and negatively impact animal health.

AUTHOR'S CONTRIBUTIONS

Safaa Mohamed Karim conducted, supervised, and analyzed the study, while Ali Rabeea contributed to manuscript preparation. Ahmed Mosa participated in sample collection, laboratory testing, and epidemiological data collection. Fatima Hashim Abbas contributed to molecular analysis and carefully reviewed the manuscript. All authors approved the final version of the manuscript.

Conflict of Interest

The authors have declared no conflict of interest.

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