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Case Study

A One-Month Survey on Infestation of *Ixodidae* (Acari: Ixodida) Ticks Collected from Dogs in the Robat Karim Region, Tehran Province, Iran

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ABSTRACT

This study aimed to investigate the infestation of *Ixodidae* ticks in herd and stray dogs in Robat Karim region of Tehran province, Iran. Ticks are among the most important external parasites in dogs that can cause various diseases through blood feeding. The growing population of stray dogs in the cities is one of the most important problems, especially in the outskirts of the cities, and the identification of the tick fauna in the area is very important. A total of 83 dogs (17 herd dogs and 66 stray dogs) were randomly sampled from 14 urban and rural points in the Robat Karim between September 1st and September 30th, 2023. After transferring the samples to the entomology laboratory, various species were identified. A total of 434 *Ixodidae* ticks belonging to 2 genera and 4 different species were identified from 72 infested dogs. The highest frequency was related to *Rhipicephalus sanguineus* (64.28%), *Rhipicephalus bursa* (17.28%), and the lowest frequency was related to Rhipicephalus turanicus (11.29%), *Hyalomma marginatum* (7.14%). Examining the age variables showed that there is a significant difference ($p \leq 0.05$) in the frequency of tick infestation in different age groups, with 44.23% of the total isolated ticks belonging to dogs aged 1-3 years. Such research, which deals with the identification and investigation of species diversity and the distribution of different species of ticks in a specific geographical area, will lead to better and more accurate decisions by the medical and veterinary professionals to control and prevent the spread of tick-borne diseases. Similar studies should be conducted in other regions of Iran to determine the level of tick infestation in dogs throughout Iran and the results of these studies can be used in strategic tick control programs.

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1. Introduction

The increasing number of dogs in the country and the increase in the presence of stray dogs in cities, as well as the l incomplete implementation of health regulations, has heightened the risk of transmitting common diseases from dogs to humans. The most important and common diseases in dogs are those caused by ectoparasite arthropods. Arthropods represent over 80% of all organisms on the planet and can act both as external parasites and as vectors for parasitic, viral, and bacterial infections (1). Ticks are among the most important arthropods in the world, causing high damages in the veterinary field of many countries each year. Therefore, after mosquitoes, ticks are are considered the second most important group of arthropods in the veterinary medicine, particularly in tropical countries (2, 3). *Ixodidae* ticks are the most important and common carriers of pathogens among ticks and are recognized as one of the most important external parasites affecting dogs in the world (4). *Ixodidae* ticks can cause a lot of damage to dogs, including blood loss, dermatitis, pain, and a variety of parasitic, bacterial, and viral infections such as tick-borne encephalitis virus, *Ehrlichia canis*, and *Babesia canis* (5, 6, 7). According to the surveys, there are about 700 million domestic dogs in the world, and 75% of this population are stray dogs (8). Today, due to the increasing presence of stray dogs in cities and their proximity to human communities, they are considered one of the biggest problems for public health (9).

Therefore, it is very important to determine the distribution and prevalence of ticks among all dogs, especially stray dogs (10, 11). As the capital of Iran, Tehran province is considered the most important and most populated region of Iran. With the growing population in Tehran, tend to settle in the surrounding areas, such as the Robat Karim region, which is close to Tehran. As the capital expands, the population of Robat Karim is also increasing. Therefore, investigating the risk factors of disease in this region is more important than ever.

2. Case Presentation

2.1. Study Area

Robat Karim region is located in the southwestern region of Tehran province, at a longitude of 51:4, latitude of 35:28 and an altitude of 1100 meters above the sea level. The area of this city is about 275 square kilometers, bordered by Ray and Islamshahr from the south, Shahriar to the north, Baharestan to the east, and Zarandieh to the west. The annual rainfall of this area is about 200 ml. The maximum temperature reaches 44 degrees Celsius ,while the minimum temperature drops to -20 degrees Celsius. Overall,the average air temperature in Robat Karim is 16 degrees Celsius. Due to its proximity to the capital of Iran, the population of this region is growing, with about 291,515 residents living in this region (Figure 1).

2.2. Data Collection

This study was conducted in 4 urban areas (Alard, Nasirshahr, Parand and Robat Karim) and 10 rural areas (Peyghambar, Anjemabad, Manjilabad, Hoseynabad-e Yangejeh, Shahrabad-e Ilat, Asgharabad, Laqeh Hesar Mehtar, Vahnabad and Hakimabad), from September 1st to September 30th, 2023 (Table 1). A total of 83 dogs (17 herd dogs and 66 stray dogs) were examined using random-cluster sampling. From these dogs, 434 ticks were detected accross 5 parts of the dog's body. In this method, the dog's body was divided into 5 parts: I. head, ears and neck; II. Dorsal; III. Abdomen, groin, axillary and inguinal; IV. Legs and feet and V. tail and perianal (12).

All sampling was done between 8:00 am and 12:00 pm. The age of the studied dogs was determined by asking the owner and assessing to the dental formula. Ticks were collected from the dogs' body using forceps slowly at a 45- degree angle and placed inside the numbered tubes containing 70% ethanol. These samples were sent to the Entomology Laboratory of Bu - Ali University ,Faculty of Agriculture, for further examination and clarification. They were transferred to Sinai Hamadan. A total of 279 *Ixodidae* ticks were identified using a stereomicroscope with a magnification of 40 to 80 times and compared with valid keys (13, 14).

2.3. Preparation of Slides and Clarification Of Ticks

The tick samples preserved in ethanol were washed to remove any adherent host tissue and then placed in glass vials. Blood contents of the ticks were drained from the abdominal area of the ticks using a syringe needle. The ticks were placed in 10% potassium hydroxide to dissolve unwanted chitin and debris, making them clear and clean for microscopic examination. Tick samples were washed several times with water and then dehydrated through successive dilutions of ethyl alcohol (70, 80, 90, 95 and 100). They were then clarified and cleared using xylene for 15 to 30 minutes.

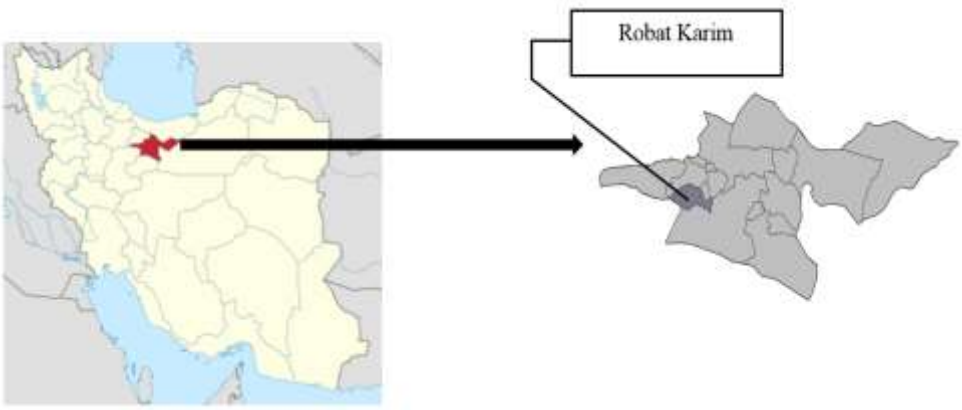

Figure 1. Map of Iran showing the location of Tehran province and Rabat Karim region.

Table 1. The names of urban and rural areas that have been studied.

Country	province	Region	Urban or Rural	The name of the sampling site	The number of dogs
				Alard	8
				Nasirshahr	7
			Urban	Parand	9
				Robat Karim	5
			Total	**4**	**29**
				Peyghambar	5
				Anjemabad	3
				Manjilabad	2
				Hoseynabad-e Yangejeh	3
				Shahrabad-e Ilat	1
				Asgharabad	3
				Laqeh	1
				Hesar Mehtar	6
				Vahnabad	2
Iran	Tehran	Robat Karim		Hakimabad	3
			Rural	Aliabad	10
				Keygavar	7
				Kazemabad	3
				Parandak	5
			Total	**14**	**54**

Tick samples were mounted on glass slides using Canadian balsam glue, covered with a coverslip, to dry at laboratory temperature and finally examined under a light microscope (15, 16) (Figure 2).

2.4. Statistical Analysis

The Chi-square test (2χ) was used to statistically analyze the obtained data and determine the relationship between the prevalence of infection by different species of ticks isolated with age, gender and place of isolation. Also, at first, the data collected from the isolation site was entered into Microsoft Excel 2016 software.

Final statistical analysis was performed using SPSS 2021 software, with a significance level of $p \leq 0.05$.

3. Results

3.1. Species diversity of *Ixodidae* ticks

Out of 83 examined dogs, 72 dogs were found to be infected with Ixodidae ticks. A total of 434 *Ixodidae* ticks, 2 genera of *Rhipicephalus* and *Hyalomma* and 4 species of *Rhipicephalus sanguineus*, *Rhipicephalus bursa*, *Rhipicephalus turanicus*, *Hyalomma marginatum* were detected. Among these, 279 (64.28%) of the identified Ixodidae ticks belonged to *Rhipicephalus sanguineus*,

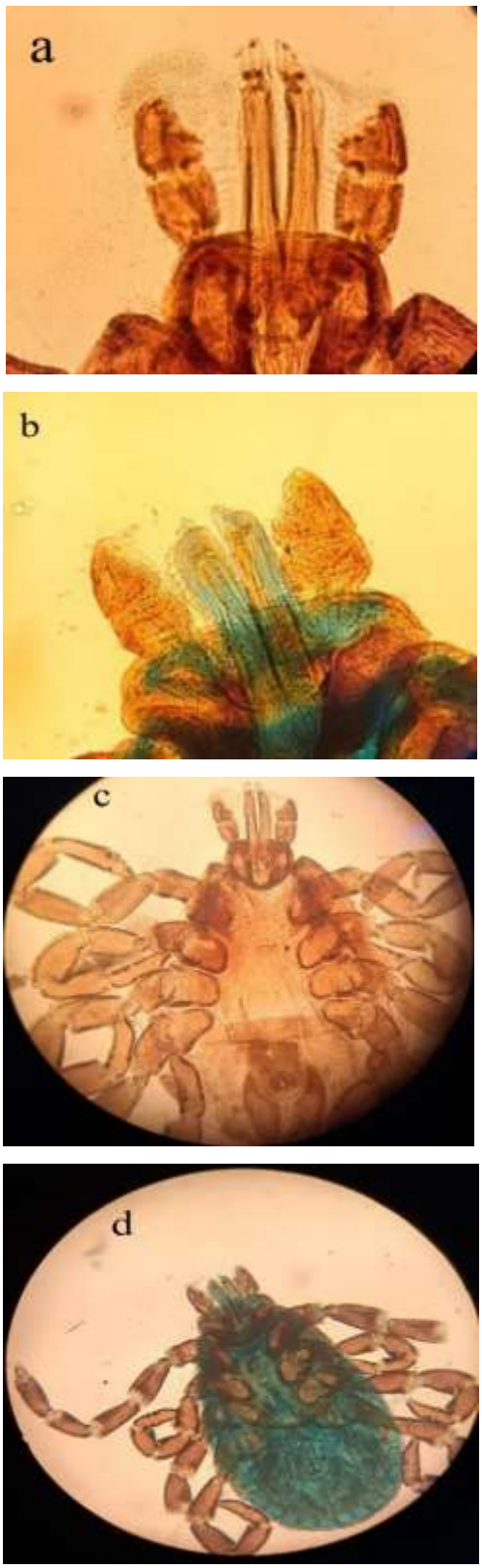

Figure 2. Tick samples isolated from dog, a. Oral appendages of *Hyalomma marginatum* b. Oral appendages of *Rhipicephalus sanguineus* c. *Hyalomma marginatum* d. *Rhipicephalus sanguineus*

which can be said to be the most common tick species in Robat Karim region in Tehran province. This was followed by 75 (17.28%) *Rhipicephalus bursa*, 49 (11.29%) *Rhipicephalus turanicus* and 31 (7.14%) *Hyalomma marginatum* of *Ixodidae* respectively (Table 2).

3.2. Geographical Distributions

The geographical distribution of ticks on dogs showed that *Rhipicephalus sanguineus* is the most common species, while *Hyalomma marginatum* is the least common species among all investigated areas in Rabat Karim region of Tehran province. In the region of Rabat Karim, the cities of Nasirshahr, Parand, Robat Karim and the villages of Anjemabad, Hoseynabad-e Yangejeh, Shahrabad-e Ilat, Vahnabad, Aliabad, Kazemabad, recorded the highest percentage of infection , with all the dogs examined in these areas were infected with Ixodidae ticks. Laqeh village was the only part that did not record any infestation with *Ixodidae* ticks. Out of a total of 434 *Ixodidae* ticks isolated from dogs in Robat Karim region, 153 ticks were in urban areas and 281 ticks were in rural areas, which indicates that tick infestation in dogs in rural areas is higher than in urban areas. The highest number of ticks in the urban area of Parand city with 50 (32.67%) , while Aliabad village had the highest count in rural areas , with 56 (19.92%) number of *Ixodidae* ticks recorded (Table 3).

4. Discussion

In this study, which was the first specialized investigation on *Ixodidae* ticks in the Robat Karim region of Tehran province, 2 different genera of *Ixodidae* ticks , *Rhipicephalus* and *Hyalomma*, were identified, along with 4 species *Rhipicephalus sanguineus*, *Rhipicephalus bursa*, *Rhipicephalus turanicus* and *Hyalomma marginatum*. The findings indicated that the predominant tick species in the Robat Karim region is *Rhipicephalus sanguineus*, accounting for 64.28% of the total ticks identified. This prevalence is approximately equal to the research conducted in Argentina with a prevalence rate of 73% and Thailand with a prevalence rate of 74.20% (17, 18).

Additionally, studies conducted in Iran, in Ilam province (27.50%) and the Gilanegharb region in Kermanshah province (35.36%), also identified *Rhipicephalus sanguineus* as the dominant tick species (19, 20). The number of ticks found on male dogs compared to female dogs shows a significant difference, with 284 (65.44%) ticks found on male dogs and 150

Table 2. The prevalence of *Ixodidae* ticks has been investigated.

Variable			N. (%)
Total number of dogs			83(100%)
Infected dogs			72(86.77%)
Non Infected dogs			11(13.23)
The number of ticks			434(100%)
Genus	*Rhipicephalus*	*Rhipicephalus sanguineus*	279(64.28%)
		Rhipicephalus bursa	75(17.28%)
		Rhipicephalus turanicus	49(11.29%)
	Hyalomma	*Hyalomma marginatum*	31(7.14%)

Table 3. Prevalence of different species of ticks isolated according to the study areas.

Area type	Name of the area	The number of dogs	Positive case	Negative case	The number of ticks	*Rhipicephalus sanguineus*	*Rhipicephalus bursa*	*Rhipicephalus turanicus*	*Hyalomma marginatum*
Urban	Alard	8(27.58%)	6(75%)	2(25%)	42(27.45%)	36(85.71%)	3(7.14%)	3(7.14%)	0(0%)
	Nasirshahr	7(24.13%)	7(100%)	0(0%)	37(24.18%)	25(67.56%)	1(2.70%)	1(2.70%)	10(27.02%)
	Parand	9(31.03%)	9(100%)	0(0%)	50(32.67%)	32(64%)	7(14%)	0(0%)	11(22%)
	Robat Karim	5(17.24%)	5(100%)	0(0%)	24(15.68%)	13(54.16%)	6(25%)	5(20.83%)	0(0%)
Total	-	**29(100%)**	**27(93.10%)**	**2(6.89%)**	**153(100%)**	**106(69.28%)**	**17(11.11%)**	**9(5.88%)**	**21(13.20%)**
Rural	Peyghambar	5(9.25%)	4(80%)	1(20%)	19(6.76%)	11(57.89%)	1(5.26%)	7(36.84)	0(0%)
	Anjemabad	3(5.55%)	3(100%)	0(0%)	22(7.82%)	8(36.36%)	6(27.27%)	8(36.36%)	0(0%)
	Manjilabad	2(3.70%)	1(50%)	1(50%)	5(1.77%)	5(100%)	0(0%)	0(0%)	0(0%)
	Hoseynabad-e Yangejeh	3(5.55%)	3(100%)	0(0%)	15(5.33%)	10(66.66%)	5(33.33%)	0(0%)	0(0%)
	Shahrabad-e Ilat	1(1.85%)	1(100%)	0(0%)	10(3.55%)	0(0%)	0(0%)	0(0%)	10(100%)
	Asgharabad	3(5.55%)	1(33.33%)	2(66.66%)	3(1.06%)	0(0%)	0(0%)	3(100%)	0(0%)
	Laqeh	1(1.85%)	0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Hesar Mehtar	6(11.11%)	5(83.33%)	1(16.66%)	25(8.89%)	19(76%)	0(0%)	6(24%)	0(0%)
	Vahnabad	2(3.70%)	2(100%)	0(0%)	10(3.55%)	5(50%)	5(50%)	0(0%)	0(0%)
	Hakimabad	3(5.55%)	2(66.66%)	1(33.33%)	32(11.38%)	21(65.62%)	11(34.37%)	0(0%)	0(0%)
	Aliabad	10(18.51%)	10(100%)	0(0%)	56(19.92%)	37(66.07%)	12(21.42%)	7(12.50%)	0(0%)
	Keygavar	7(12.96%)	6(85.71%)	1(14.28%)	26(9.25%)	16(61.53%)	8(30.76%)	2(7.69%)	0(0%)
	Kazemabad	3(5.55%)	3(100%)	0(0%)	18(6.40%)	13(72.22%)	0(0%)	5(27.77%)	0(0%)
	Parandak	5(9.25%)	4(80%)	1(20%)	40(14.23%)	28(70%)	10(25%)	2(5%)	0(0%)
Total	-	**54(100%)**	**45(83.33%)**	**9(16.66%)**	**281(100%)**	**173(61.56%)**	**58(20.64%)**	**40(14.23%)**	**10(3.55%)**

(34.56%) on female dogs. However, overall infestation rates for external parasites are 85.71% in male dogs and 88.88% in female dogs. Regarding age-related variables, out of the total of 434 *Ixodidae* ticks,

192 (44.23%) were related to dogs aged 1-3 years, while the lowest number, 53 ticks(12.21%) belonged to the dogs under 1 year. The intensity of infestation in stray dogs compared to owned dogs does not show a significant difference: 82.35% of owned dogs and 87.87% of stray dogs were infested with *Ixodidae* ticks. On average, 8.5 ticks per dog were identified on owned dogs, while stray dogs had 5.43 ticks per dog, showing a lower number

compared to a study conducted by Yi Yan and colleagues in 2023, where Malaysia reported an infestation rate of 8.13 ticks per dog, and the Philippines reported 25.75 ticks per dog for stray dogs (21, 22) (Table 4). Some studies, reported by different researchers, have also isolated dog ixodid ticks from the skin of other animals, such as cats and ruminants (23-26).

The analysis showed that specific body parts significantly affect the presence of ticks in dogs. Of the total ticks collected, 211 (48.61%) ticks were found in the Abdomen, axillary, groin, and inguinal region, 70 (16.12%) in the Legs and feet region, 67 (15.43%) in the

dorsal region, 51 (11.75%) There were 35(8.06%) in Tail and perianal area and 35(8.06%) in Head, ears and neck area, which shows that Abdomen, axillary, Groin, inguinal area is the most heavily infected area in the body of dogs in Robat Karim area with *Ixodidae* ticks. (Table 5).

Examining the infected areas of the dogs' body with different types of ticks found shows that the highest number of *Rhipicephalus sanguineus* and *Rhipicephalus bursa* were in Abdomen, axillary, groin, inguinal region, while the highest number of *Rhipicephalus turanicus* and *Hyalomma marginatum* were in dorsal region (Figure 3).

This study provides valuable insights into *Ixodidae* tick infestation and associated risk factors in herding and stray dogs. Adapting preventive strategies and interventions based on sensitive and vulnerable body parts can more effectively protect dogs against ticks and reduce health risks. As in this study, variables such as gender and age have been examined in detail, which helps implememt controlling and preventive plans in the region. Our study showed that *Rhipicephalus sanguineus*, as a tick of tropical lineage, is the dominant tick in Robat Karim area of Tehran province.

However, this study did not assess seasonal prevalence due to limitations, nor did it evaluate the pathogens transmitted by *Ixodidae* ticks. Such studies conducted on larger scales and accross larger regions of Iran can help to identify the tick fauna specific to each region and control the biological problems in that region. Studies similar to our work should be done in other regions of Iran to determine the national level of tick infestation in dogs and the results of these studies can be used in strategic tick control programs.

Table 4. Prevalence of different species of ticks isolated according to the study areas.

Variable					Tick species			
Sex	Total	N. Infected dogs (%)	N. Non Infected dogs (%)	N. The number of ticks (%)	*Rhipicephalus sanguineus*	*Rhipicephalus bursa*	*Rhipicephalus turanicus*	*Hyalomma marginatum*
Male	56	48(85.71%)	8(14.29%)	284(65.44%)	201(70.77%)	33(11.61%)	26(9.15%)	24(8.45%)
Female	27	24(88.88%)	3(11.12%)	150(34.56%)	78(52%)	42(28%)	23(15.33%)	7(4.66%)
Total	83	72(86.77%)	11(13.23)	434(100%)	279(64.28%)	75(17.28%)	49(11.29%)	31(7.14%)
Age grope								
<1	14	13(92.85%)	1(7.14%)	53(12.21%)	27(50.94%)	15(28.30%)	10(18.86%)	1(1.88%)
1-3	35	30(85.71%)	5(14.28%)	192(44.23%)	136(70.83%)	25(13.02%)	19(9.89%)	12(6.25%)
3-6	26	22(84.61%)	4(15.38%)	126(29.03%)	70(55.55%)	32(25.39%)	16(12.69%)	8(6.34%)
>6	8	7(87.50%)	1(12.50%)	63(14.51%)	46(73.01%)	3(4.76%)	4(6.34%)	10(15.87%)
Total	83	72(86.74%)	11(13.25)	434(100%)	279(64.28%)	75(17.28%)	49(11.29%)	31(7.14%)
Type of dogs								
Herd dogs	17	14(82.35%)	3(17.64%)	119(27.41%)	70(58.82%)	30(25.21%)	9(7.56%)	10(8.40%)
Stray dogs	66	58(87.87%)	8(12.12%)	315(72.58%)	209(66.34%)	45(14.28%)	40(12.69%)	21(6.66%)
Total	83	72(86.77%)	11(13.25)	434(100%)	279(64.28%)	75(17.28%)	49(11.29%)	31(7.14%)

Table 5. Prevalence of different species of ticks isolated according to the study areas.

Tick species	Place of isolation of ticks					Total
	Abdomen, axillary, Groin , inguinal	Dorsal	Tail and perianal	Legs and feet	Head, ears and neck	
Rhipicephalus sanguineus	159(56.98%)	37(13.26%)	31(11.11%)	39(13.97%)	13(4.65%)	279(64.28%)
Rhipicephalus bursa	26(34.66%)	0(0%)	12(16%)	17(22.66%)	20(26.66%)	75(17.28%)
Rhipicephalus turanicus	15(30.61%)	17(34.69%)	8(16.32%)	7(14.28%)	2(4.08%)	49(11.29%)
Hyalomma marginatum	11(35.48%)	13(41.93%)	0(0%)	7(22.58%)	0(0%)	31(7.14%)
Total	211(48.61%)	67(15.43%)	51(11.75%)	70(16.12%)	35(8.06%)	434(100%)

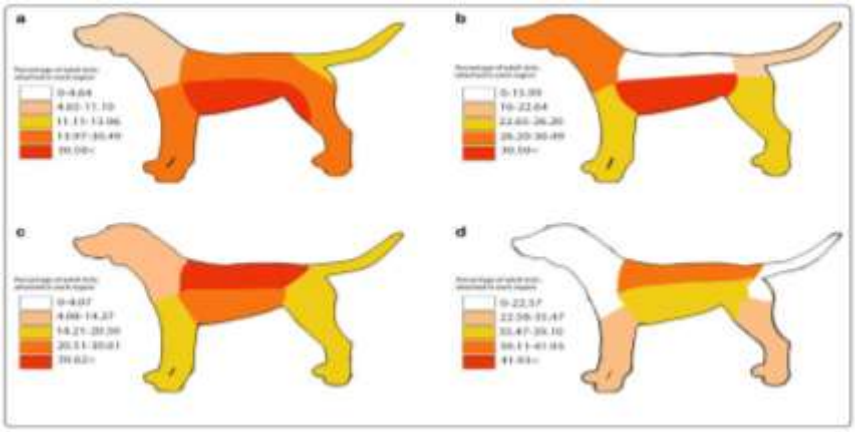

Figure 3. Distribution of attachment sites of adult ticks on dogs. A) *Rhipicephalus sanguineus*. B) *Rhipicephalus bursa*. C) *Rhipicephalus turanicus*. D) *Hyalomma marginatum*.

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Authors' Contribution

Study concept and design: G.A, G.Y.
Acquisition of data: G.A, D.E.
Analysis and interpretation of data: G.A, G.Y, D.E.
Drafting of the manuscript: G.A, G. Y.
Critical revision of the manuscript for important: G.Y.
Intellectual content: G.A, D.E.
Sampling : G.A, D.E, J, M.
Statistical analysis: G.A, D.E, J. M.

Ethics

All principles of medical ethics have been observed in this study.

Conflict of Interest

The authors declare no competing interests.

Funding

Not applicable.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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