A one-month Survey on infestation of *Ixodidae* (Acari: Ixodida) ticks collected from dogs in the Robat Karim region, Tehran Province, Iran

Alireza Ghorbani[1], Erfan Darmanloo[2], Yagoob Garedaghi[3], Mohammad Jalali Niroumand[2]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

2. Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.

3. Department of Parasitology, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran.

Corresponding author: Dr. Yagoob Garedaghi.

Postal address: Iran, Tabriz, Islamic Azad University, Tabriz Medical Sciences, Department of Parasitology, Office of Dr. Yagoob Garedaghi University/organization **email address**: yagoob.garedaghi@gmail.com , y_garedaghi@iaut.ac.ir

ORCID ID: https://orcid.org/0000-0003-2976-2706.

Tel. number: 0989143110499

Abstract

The main objective of this study is to investigate the infestation of *Ixodidae* ticks in herd and stray dogs in Robat Karim region in Tehran province, Iran. Ticks are one of the most important external parasites in dogs that can cause various diseases by feeding on the blood of their hosts. Also, the increase in the number 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.

In total, a random sampling was conducted on 83 dogs (17 herd dogs and 66 stray dogs) from 14 urban and rural points in the Robat Karim region of Tehran Province, Iran, 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 from 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 variable showed that there is a significant difference ($p \leq 0.05$) in the frequency of tick infestation in different ages and showed that 44.23% of the total isolated ticks belonged 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 community to control and prevent the spread of diseases transmitted by ticks. Studies similar to our work should be done 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.

Key words: *Ixodidae* ticks, dogs, Robat Karim, Tehran, Iran.

1. Introduction

Today, due to the increasing number of dogs in the country and the increase in the presence of stray dogs in cities, as well as the lack of full implementation of health regulations, the risk of transmitting common diseases from dogs to humans is always of particular importance. The most important and common diseases in dogs are those caused by ectoparasite arthropods. More than 80% of all organisms on the planet are arthropods, these organisms can cause disease by themselves as an external parasite for other organisms and can also transmit many parasitic, viral and bacterial infections to the hosts have a role themselves (1). Ticks are one of the most important arthropods in the world, which cause high damages in the veterinary field of countries every year, and therefore, after mosquitoes, ticks are the second most important group of arthropods in the veterinary field for medicinal purposes in tropical countries (2, 3). *Ixodidae* ticks are the most important and common carriers of pathogens among ticks and are considered one of the most important external parasites in 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 increase in the 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. Also, with the increase in the population in Tehran, most of the new population who intend to enter the capital go to the surrounding areas of Tehran, such as the Robat Karim region, which is close to Tehran and the capital Iran is about to pass away and therefore the population of Robat Karim region is increasing. Therefore, investigating the risk factors of disease in this region is more important than before.

2. Material and method

2.1. Study area

Robat Karim region is located in the southwestern region of Tehran province with a longitude of 51:4, latitude of 35:28 and an altitude of 1100 meters from the sea. The area of this city is about 275 square kilometers, which is connected to Ray and Islamshahr cities from the south, Shahriar city from the north, Baharestan city from the east, and Zarandieh city from the west. The annual rainfall of this area is about 200 ml. The maximum temperature of this area is 44 degrees Celsius and the minimum temperature is -20 degrees Celsius, but in general the average air temperature of Robat Karim is 16 degrees Celsius. In terms of population, due to its proximity to the capital of Iran, the population of this region is growing, with about 291,515 people living in this region (**Figure 1).**

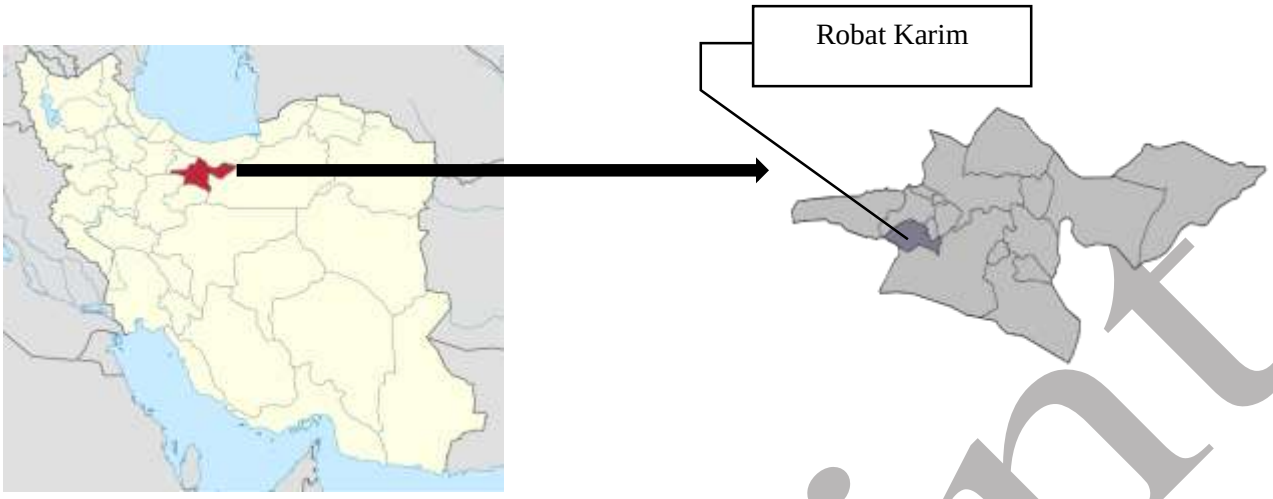

Figure 1. Map of Iran showing the location of Tehran province and Rabat Karim region.

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)**. 83 dogs (17 herd dogs and 66 stray dogs) were examined by random-cluster sampling, of these dogs, 434 ticks were detected in the 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 according to the dental formula. The ticks were collected from the dogs' body using forceps slowly and at an angle of 45 degrees and placed inside the numbered tubes containing 70% ethanol and sent to the entomology laboratory of Bu - Ali University Faculty of Agriculture for further examination and clarification of the samples. They were transferred to Sinai Hamadan. A number 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. The blood contents of the ticks were drained from the abdominal area of the ticks using a syringe needle. Ticks were placed in 10% potassium hydroxide to dissolve unwanted chitin and debris and make them clear and clean for microscopic examination. Tick samples were washed several times with water and then dehydrated with successive dilutions of ethyl alcohol (70, 80, 90, 95 and 100), then clarified and cleared using xylene for 15 to 30 minutes. Tick samples were mounted on glass slides using Canadian balsam glue and covered with a coverslip, to dry at laboratory temperature and finally examined under a light microscope (15, 16) **(Figure 2).**

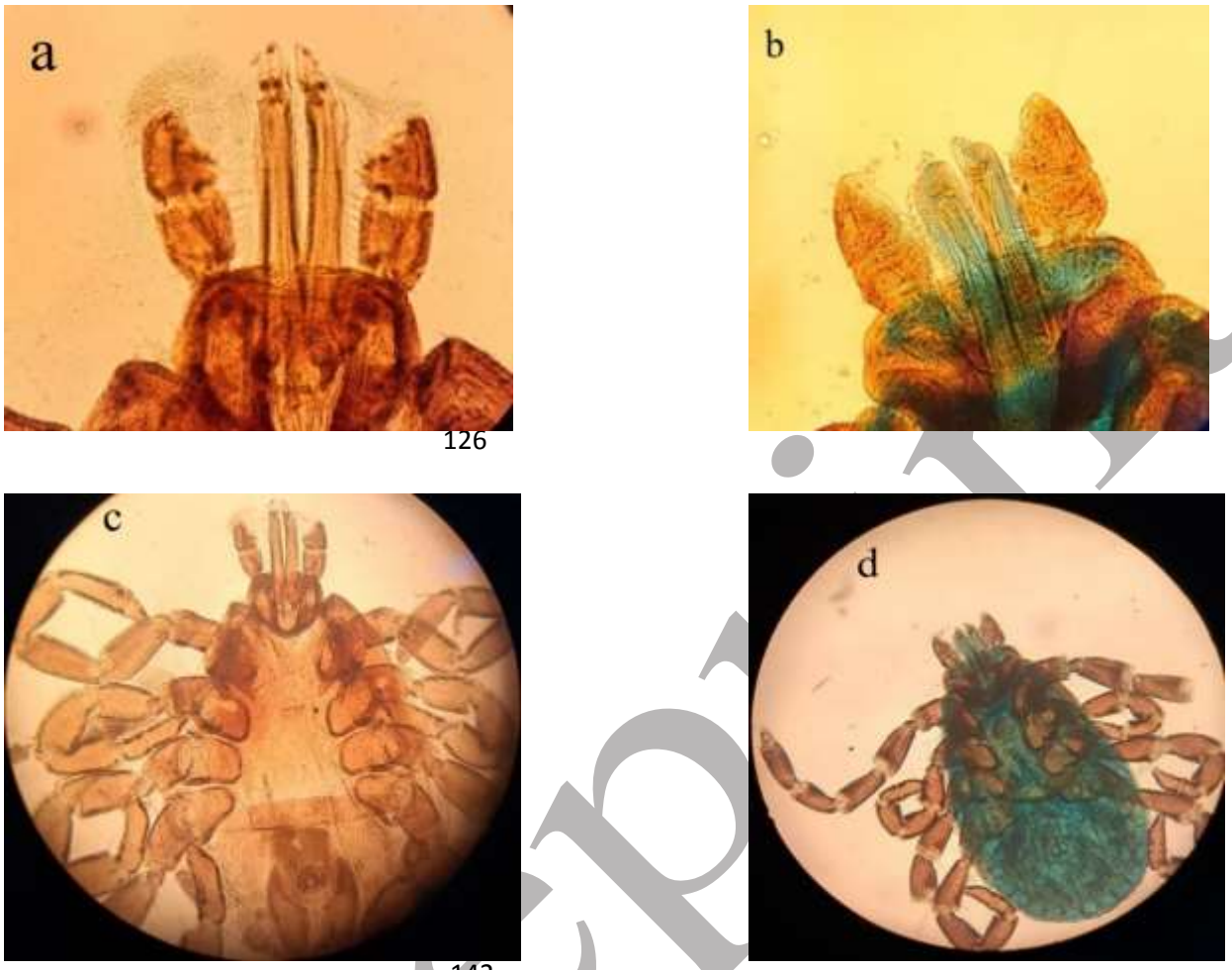

Figure 2. Tick samples isolated from dog, a. Oral appendages of *Hyalomma marginatum* b. Oral appendages of *Rhipicephalus sanguineus* c. *Hyalomma marginatum* d. *Rhipicephalus sanguineus*

2.4. Statistical analysis

Chi-square test (2χ) was used to statistically analyze the obtained data and obtain the relationship between the prevalence of infection of different species of ticks isolated with age, gender and place of isolation. Also, at first, the data collected at the isolation site was entered into Microsoft Excel 2016 software, and then SPSS 2021 software was used for the final analysis, where the significance level of $p \leq 0.05$ was considered.

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
Iran	Tehran	Robat Karim	Urban	Alard	8
				Nasirshahr	7
				Parand	9
				Robat Karim	5
			Total	**4**	**29**
			Rural	Peyghambar	5
				Anjemabad	3
				Manjilabad	2
				Hoseynabad-e Yangejeh	3
				Shahrabad-e Ilat	1
				Asgharabad	3
				Laqeh	1
				Hesar Mehtar	6
				Vahnabad	2
				Hakimabad	3
				Aliabad	10
				Keygavar	7
				Kazemabad	3
				Parandak	5
			Total	**14**	**54**

3. Result

3.1. Species diversity of *Ixodidae* ticks

Out of 83 examined dogs, 72 dogs infected with Ixodidae ticks were identified, and 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. 279 (64.28%) of the identified Ixodidae ticks belonged to *Rhipicephalus sanguineus*, which can be said to be and most common tick species in Robat Karim region in Tehran province. Then 75 (17.28%) *Rhipicephalus bursa*, 49 (11.29%) *Rhipicephalus turanicus* and 31 (7.14%) *Hyalomma marginatum* of *Ixodidae* respectively **(Table 2)**.

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%)

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186 **3.2. Geographical distributions**

187 The geographical distribution of ticks on dogs showed that *Rhipicephalus sanguineus* is the most common species,
188 and *Hyalomma marginatum* is the less common species among all investigated areas in Rabat Karim region of
189 Tehran province. In the region of Rabat Karim, the cities of Nasirshahr, Parand, Robat Karim and the villages of
190 Anjemabad, Hoseynabad-e Yangejeh, Shahrabad-e Ilat, Vahnabad, Aliabad, Kazemabad recorded the highest
191 percentage of infection and all the dogs examined in these areas were infected with Ixodidae ticks, have been
192 infected Laqeh village was the only part that did not record any infestation with *Ixodidae* ticks. Out of a total of
193 434 *Ixodidae* ticks isolated from dogs in Robat Karim region, 153 ticks were in urban areas and 281 ticks were in
194 rural areas, which indicates that tick infestation in dogs in rural areas is higher than in urban areas. Be the most
195 isolated ticks in the urban area of Parand city with 50 (32.67%) number of *Ixodidae* ticks and Aliabad village with
196 56 (19.92%) number of *Ixodidae* ticks recorded the highest number of ticks on dogs **(Table 3)**.

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199 **Table 3 –** Prevalence of different species of ticks isolated according to the study areas

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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%)**

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 named *Rhipicephalus* and *Hyalomma*, along with 4 species *Rhipicephalus sanguineus*, *Rhipicephalus bursa*, *Rhipicephalus turanicus* and *Hyalomma marginatum* were discovered. The findings indicated that the predominant tick species in the Robat Karim region of Tehran province is *Rhipicephalus sanguineus*, accounting for 64.28%. 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 (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 a total of 434 *Ixodidae* ticks, 192 (44.23%) were related to the age group of 1-3 years, and the lowest number of ticks separated belonged to the age group less than 1 year, comprising 53 (12.21%) ticks. The intensity of infestation in stray dogs compared to owned dogs does not show a significant difference, as 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).

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%)

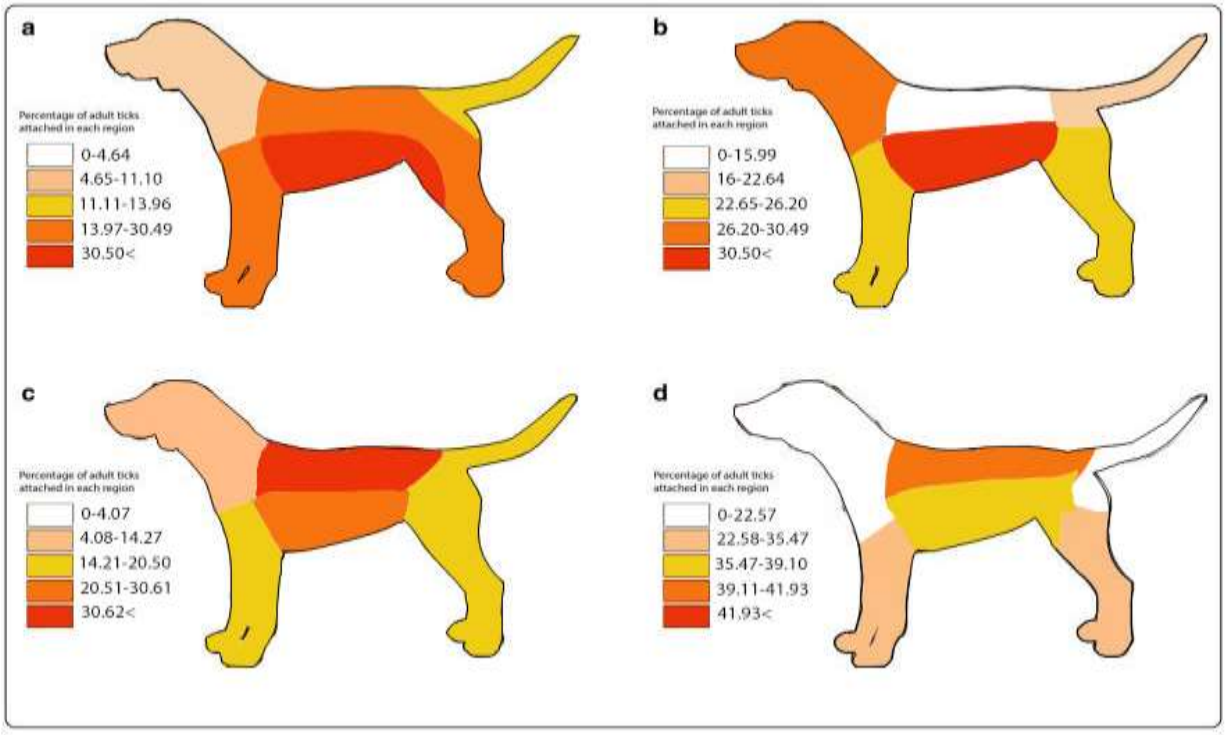

Figure 3. Distribution of attachment sites of adult ticks on dogs. a *Rhipicephalus sanguineus*. b *Rhipicephalus bursa*. c *Rhipicephalus turanicus*. d *Hyalomma marginatum*

The analysis showed that body parts significantly affect the presence of ticks in dogs. Of the total number of ticks obtained, 211 (48.61%) ticks were found in the Abdomen, axillary, groin, 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 infected area in the body of dogs of Robat Karim area with *Ixodidae* ticks. (**Table 5**).

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%)

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, but the highest number of *Rhipicephalus turanicus* and *Hyalomma marginatum* was in dorsal region. (**Figure 3**).

5. Conclusions

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 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 control 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, in this study, the ratio of prevalence to different seasons was not measured due to limitations, and in addition, the pathogens transmitted by *Ixodidae* ticks were not evaluated. Such studies on larger scales and larger regions of Iran can help to identify the tick fauna of 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 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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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 and 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 and J, M

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Ethics:

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

Conflict of Interest:

The authors declare no competing interests.

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