



Ticks Prevalence and Possible Risk Factors Assessment on Domestic Dogs in Quetta District Balochistan, Pakistan



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TICKS and tick-borne diseases have always been a problem for animals and humans. This study aimed at the effect of risk factors based on univariable analysis affecting the number of ticks parasitized on domestic dogs. This research began in April and ended in July 2019. Most ticks recognized based on morphology were belonged to *Rhipicephalus sanguineus* (45.76%) followed by *Rhipicephalus* (Boophilus) *microplus* (32.85%), *Hyalomma dromedarii* (10.15%), *Haemaphysalis* spp. (7.01%), *Hyalomma anatolicum* (4.24%) respectively. Through the questionnaire, answers to various risk factors associated with tick infestation were discussed. It was revealed in the questionnaire results that most of the risk factors were recorded as non-significant ($p > 0.05$) except tick infestation on the host animal. The paper is extracted from the first author's M.Phil Thesis.

Keywords: Ticks, Risk Factors, Domestic, Dogs. N.B.

Introduction

Ticks are considered to be one of the arthropod vectors, which transmit diseases at the medical level as well as veterinary levels causing a detrimental impact on human beings in terms of their health-associated issues [1]. Hard ticks are the hematophagous ectoparasites almost all of the vertebrates worldwide. Their medical value is increasing day by day due to the transmission of viral, bacterial and protozoan infections which are known as Tick-Borne Diseases (TBDs) [2, 3].

They are mostly found to be attached with certain body parts of its host like head, neck, ear, abdominal region, perineal region or inside the parts of fore-limbs and hind-limbs [4]. Ticks, particularly belonging to the family Ixodidae that are being globally important as they direct attack on the skin and its tissues causing great damage to its host [5]. Ticks of the Ixodidae family infect a

large number of hosts and their population size is dependent upon temperature, humidity and host-searching ability [6]. The reproduction and life stages of ticks are dependent upon certain factors such as favorable environment and accessibility to its host. Moreover, some ticks are generally recognized for their adaptability to different types of climatic conditions and habitats, such adaptive features are responsible for their survival and successful reproduction [7, 8].

Ticks are regarded as the source of vector for pathogenic diseases of both humans and animals according to the previous study conducted in Pakistan [9]. In Pakistan, TBDs have a deleterious effect on both humans and animals including Crimean–Congo hemorrhagic fever (CCHF) [10], CCHF a fever caused by the biting of a tick-borne virus. Tick-borne diseases such as Theileriosis and babesiosis which are known to be the dreadful blood parasites and their occurrence

in water buffaloes and cattle have been reported in Pakistan [11].

Dogs are the most commonly owned companion pets throughout the world. They are considered to have a close relationship with humans or with their territory and are adapted to human habitation and may contribute to the physical, social and emotional well-being of their owners [12]. It is a possible fact that infested dogs carry ticks in the environment surrounding them and can transmit these to humans which contributes to the major public concerns and health issues [13]. The brown dog tick, *Rhipicephalus sanguineus* is the most prevalent tick species reported from Mediterranean countries, Latin America, Africa and most of the Asian countries [14, 15, 16, 17]. *Hyalomma anatolicum* has been reported from Iran and Pakistan [18]. *Haemaphysalis* are ixodid tick most common in temperate areas particularly in Asian countries [19].

The present study aimed to determine the tick species that are associated with the detrimental impact of tick-borne pathogens causing TBDs. Besides, risk factors associated with tick infestation along with seasonality were recorded and analyzed. Furthermore, the study motivates to understand the importance of implementation for effective tick eradication program and control strategies for domestic dogs.

Materials and Methods

Preliminary Studies

We used different online software such as Mendeley and Google Scholar to search relevant research articles published on the topic of prevalence, population, seasonal dynamics, tick infestation, and risk factor assessments. We focused our attention on those articles published recently between the year 2010 and 2019. We have studied about 200 research articles, of these, only 35 research articles are mentioned in this study.

Area search and samples collection

Quetta is the most populous district of Balochistan. It consists of 1,352 sq mi and surrounded by a series of mountains. Quetta is 5,510 feet high above sea level. Four different areas were chosen because dogs were readily available in every household. All house data were estimated nearest neighbor method. The tick's collection was continued for four months beginning in April-May and ending in June-July, 2019. The collection of samples was started

at 11 am and continued till 6 pm from selected localities. A total of 69 domestic dogs were clinically examined by expert veterinarians for the presence of ticks and their possible infestation. The dogs were handled during ticks collection in compliance with Pakistan's prevention of cruelty to animal act, 1890

The present 10-20 ticks or more per dogs were designated as highly infested, while below this range was characterized as low level infested animal respectively. A fine forceps was used to capture ticks from the attachment site and put them into a 50 mL falcon tube containing 70% ethanol.

Identification of ticks

This research is the first attempt on dog tick from this region therefore, co-authors of this research focused on the identification of adult ticks only, while manuscript on other stages is under preparation stage. Taxonomic identification was completed in two phases; In the first phase, the similar ticks were pooled into a separate tube using a stereoscopic compound microscope (Olympus CH-10, Japan). In the next phase, their permanent slides were made. Then, according to the morphological features such as basis capitula, small punctuation and based on scutum were identified using the available taxonomic keys [14, 20] under Lecia DM4000B microscope (Leica Microsystems GmbH, Wetzlar, Germany) furnished along with a digital camera (Lecia) at 40X magnification. The ticks were dehydrated after passing from different grades of alcohol (i.e., 20 %, 50 %, 75 %, and 100%) and prepared for Scanning Electron Microscope (SEM, Hitachi S3400-N, Type-II) in Centre of Excellence in Vaccinology and Biotechnology (CASVAB), Quetta.

Male and female hard ticks were separated on after identification of each genera based on their scutum on the anterior dorsum. Sample size and the percentage of infestation were calculated using the formula,

$$\text{Prevalance} = \frac{(\text{Number of animal found positive})}{(\text{total number of animal sampled})}$$

Statistical Analysis

The monthly prevalence of tick species was estimated on the Chi-square table. Student t-test on alpha value < 0.05 if the result was statistically significant, applied to calculate the sex ratio using the Paleontological Statistics Software Package

for educating purpose and analysis of data 3.26. All calculated values were reconfirmed through online statistical software. Possible risk factors were assessed using Win Epi-info® 7.0 statistical software based on Mantel-Haenszel analysis. The graph was designed using Graph Pad Prism®8.3.0 (San Diego, CA, USA). ArcGIS® 10.8 was used to illustrate ticks collection.

Results

A total of 63 domestic dogs were examined included in this study (Fig.1) and only 42

were infested with ticks. We have identified five species of ticks i.e., *Haemaphysalis spp.*, *Hyalomma anatolicum*, *Hyalomma dromedarii*, *Rhipicephalus (Boophilus) microplus* and *Rhipicephalus sanguineus* (Fig.2). A total of 542 ticks were collected from making 7.74 % ticks attachment on a single host. Table 1 describes the differences between the sex of the species of the tick. Almost all of the collected samples had the same ratio of sex (1,2) except *Rhipicephalus sanguineus* (1,3) and *Rhipicephalus (Boophilus) microplus* (1,5).

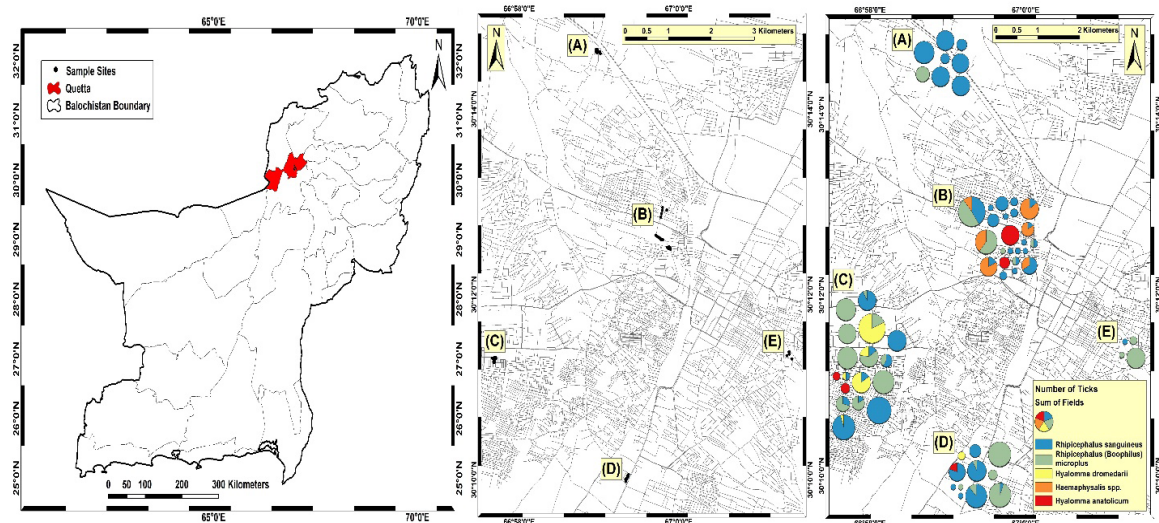


Fig.1. Map showing geographic distribution of tick parasitizing in the Quetta district.

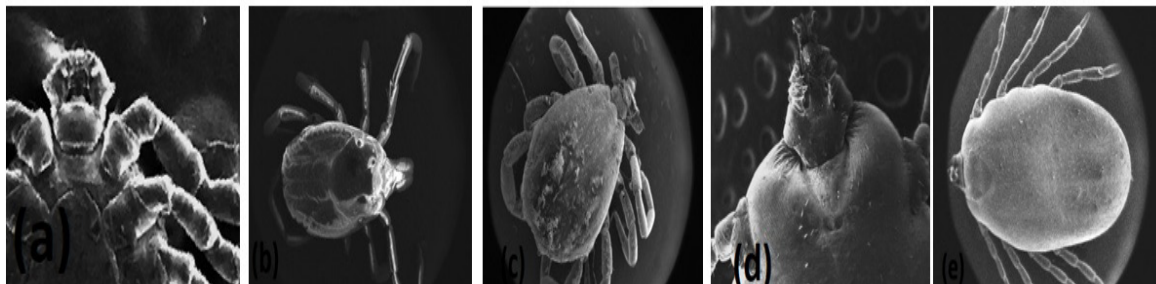


Fig.2. Electron micrograph of (a) *Haemaphysalis spp.* (b) *Hyalomma anatolicum* (c) *Hyalomma dromedarii* (d) *Rhipicephalus (Boophilus) microplus* (e) *Rhipicephalus sanguineus*.

TABLE 1. Sex ratio of tick's population parasitizing on dogs in District Quetta.

Tick species	Male	Female	Sex ratio	p-value
<i>Haemaphysalis spp.</i>	9	29	1,2	0.003
<i>Hyalomma anatolicum</i>	6	17	1,2	0.001
<i>Hyalomma dromedarii</i>	16	39	1,2	0.042
<i>Rhipicephalus (Boophilus) microplus</i>	26	152	1,5	0.021
<i>Rhipicephalus sanguineus</i>	58	190	1,3	0.001 ^a

^a All calculated values were statistically significant.

Table 2 recorded the percentage infestation density of 8.60 ticks per domestic dog. The great majority of ticks belonged to *Rhipicephalus sanguineus* (45.76 %); other tick species were identified as *Rhipicephalus* (*Boophilus*) *microplus* (32.84 %), *Hyalomma dromedary* (10.15 %), *Hyalomma anatolicum* (4.24 %) and *Haemaphysalis spp* (7.01 %) respectively (Fig.2).

The summary of possible risk factor assessment was given in Table 3. Currently, no dog vaccine is available in Balochistan province, therefore the calculated values for this parameter

were recorded as non-significant ($p > 0.33$, OR = 0.93). Animal husbandry Department is present in Quetta but its policies are not fully implemented to control the tick on live stocks ($p > 0.08$, OR = 0.47). The role of NGOs is also not significant in our studies ($p > 0.45$, R = 1.75). The dog owner do not get their vaccination was found as another non-significant parameter ($p > 0.33$, OR = 0.93). The tick infestation (burden) was found as the statistically important parameter ($p < 0.00$, OR = 17.68). All these factors indicate that tick prevalence is increasing rapidly in the Quetta district.

TABLE 2. Total number of ticks and their percentage prevalence during May-July, 2019.

Name of tick species	Total No. of ticks	Percentage prevalence (%)
<i>Haemaphysalis spp.</i>	38	7.01
<i>Hyalomma anatolicum</i>	23	4.24
<i>Hyalomma dromedarii</i>	55	10.15
<i>Rhipicephalus</i> (<i>Boophilus</i>) <i>microplus</i>	178	32.84
<i>Rhipicephalus sanguineus</i>	248	45.76

TABLE 3. Summary statistics of possible risk factor assessment on ticks parasitizing on domestic dogs in Quetta district, Balochistan during a survey conducted in 2019.

Variables	Variable	Yes	No	OR ^a	p-value
Tick infestation on host animal	Yes	27	15	17.68 (2.82-20.70)	<0.00 ^d
	No	8	24		
Dog vaccination	Yes	32	10	0.93 (1.60-0.14)	>0.33
	No	28	14		
Owner vaccination ^a	Yes	3	39	0.46 (0.17-1.98)	>0.28
	No	6	36		
Tick and tick-borne knowledge ^b	Yes	28	14	2.31 (0.95-5.62)	>0.06
	No	19	22		
Government veterinary health control policies	Yes	15	29	0.47 (0.19-1.12)	>0.08
	No	22	20		
NGO ^c funding and role	Yes	5	37	1.75 (0.39-7.87)	>0.45
	No	3	39		
Animal movement across district	Yes	15	27	0.74 (0.30-1.78)	>0.50
	No	18	24		
Personal protection	Yes	6	36	0.83 (0.25-2.72)	>0.76
	No	7	35		

^a Is the dog owner vaccinated against ticks parasitizing on the dog?

^b Does dog owner ever received any information regarding TBDs?

^c Stands for Non-Government Organization.

^d The only significant value for which the multivariate model can be applied.

Figure 3 showed the tick prevalence in each month. *Rhipicephalus sanguineus* (n=21) and *Rhipicephalus* (Boophilus) *microplus* (n=21) were collected higher in number during April compared to other species. *Rhipicephalus* (Boophilus) *microplus* (n=77) was captured during May, while the lowest number seen

in *Hyalomma anatolicum* (n=2). Likewise, *Rhipicephalus sanguineus* was collected in the highest number (n=75). Peak infestation of the tick species was observed during July, where *Rhipicephalus sanguineus* was again caught in a large number (n=116) from the domestic dog.

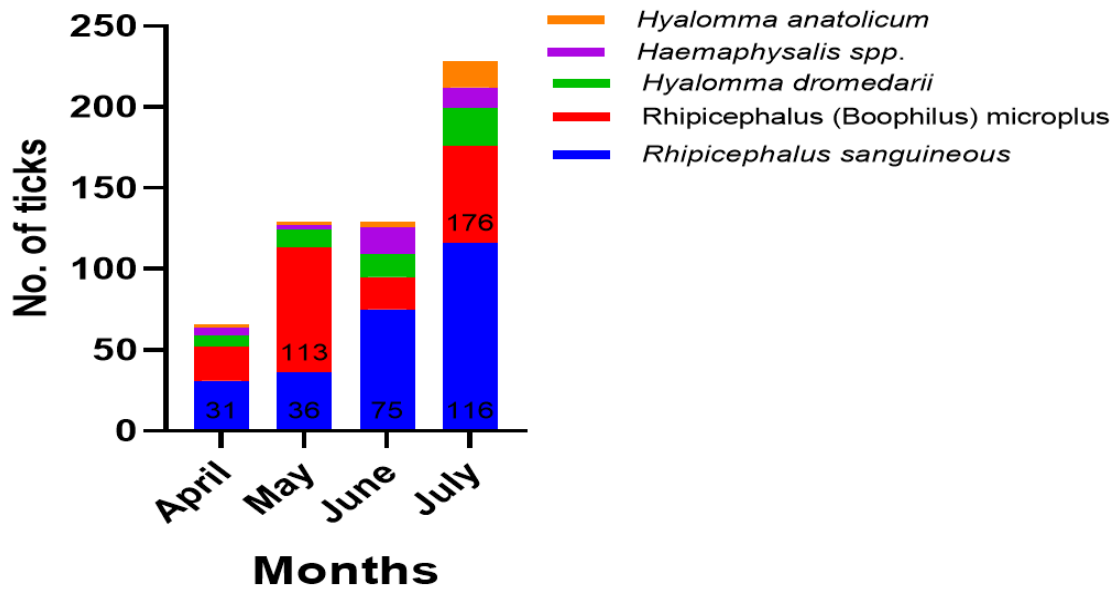


Fig.3. Monthly prevalence of tick species collected from April to July 2019. Significant relationship build between tick prevalence on monthly basis (Chi-square = 78.09, $p < 0.0001$).

Discussion

In this study, the most prevalent tick species was the *Rhipicephalus sanguineus* (45.76 %) found on dogs. As a host, the dogs favor the life-cycle of this brown colored tick. Our study correlates with the previous studies, where *Rhipicephalus sanguineus* was found to be one of the most abundant dogs tick with 92.5% prevalence [21]. According to recent updates [22, 23, 24] on the brown dog tick (i.e. *Rhipicephalus sanguineus*), it has been realized as the key vector for the rapid spread of *Babesia vogeli* and *Babesia gibsoni* in Taiwan. Moreover, Dantas-Torres [25] observed that *Rhipicephalus sanguineus* is the most abundant tick species throughout the globe and is considered to be one of the most prevalent ectoparasites on dogs. The present study is also in agreement with Changbunjong et al. [17] who has been reported that *Rhipicephalus sanguineus* as

one of the leading and dominant ectoparasites of dogs in different countries such as Africa, Asian Countries, Latin America, and Mediterranean Countries.

Rhipicephalus (Boophilus) *microplus* and *Hyalomma dromedarii* were reported as the second and third dominant species followed by *Haemaphysalis* spp. and *Hyalomma anatolicum*. Our study aligns with one of the previous studies [26], which reveals that *Rhipicephalus* (Boophilus) *microplus* is the most prevalent tick species in China. Our study also correlates with the observations of Diab et al. [9], who recognized *Hyalomma dromedarii* as one of the abundant tick species in Saudi Arabia. According to Sofizadeh et al. [27] *Hyalomma dromedarii* usually causes tick infection in camels but it can also attack other hosts such as sheep, goat, cattle, horses and donkeys. Apart from these hosts [28, 29] observed

that dogs, wild rodents and many other animals can act as the occasional host for *Hyalomma dromedarii*. Sahu et al. [30] recognized that about 46.39 % of dogs were affected with three different tick species infection i.e., *Boophilus spp.*, *Rhipicephalus spp.*, and *Haemaphysalis spp.* The present study shows the abundance of the *Rhipicephalus spp.*, which aligns with the previous study [31] that the abundance of this genus is due to adaptation in harsh climatic. *Rhipicephalus sanguineus* is one of the important species of this genus that is present in both mountainous and plain regions with the ability to infest different domestic animals.

Our result demonstrates that the tick burden is prevalent in July which means the summer season is favorable for rapid growth. This is correlated with the findings of Juvenal and Edward [32], who reported that a decrease in temperature due to heavy rainfall can cause a drop in the density of the tick population. Furthermore, the collected specimen from the present study shows the greater abundance of female tick species as compared to the ratio of male species in July which indicates that the hot season is preferable for the breeding purpose and it agrees with the findings of Shemshad et al. [31]. The result of the present study revealed the season-wise occurrence of tick infestation which is greater in July as compared to April and is consistent with the reporting of Manan et al. [33] who recorded higher tick infestation during summer (August) and lower in the winter season (December and January).

The prevalence rate of tick species confirms that these ticks are the real source of health burning issues for the domestic dogs and their owners which is correlated with the previous studies [34]. The possible factor for this is unawareness of dog owners regarding vaccination, lack of knowledge about tick-borne pathogens along with diseases caused by them and lack of implementation of vaccination by the local government as well as negligence of NGOs working for animals. Jones et al. [35] observed that the owners of dogs are at higher risk of tick bite leading to an infestation of a tick than the people without pet dogs. As the ticks are the source of vector transmission of vulnerable diseases and our study aligns with the observation of Dantes-Torres et al. [1] who

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reported that the dogs can spread the ticks to the human-beings as well as environment surrounding them and can contribute for the transmission of TBDs. Our result corresponds by the observations of Sahu et al. [30] who described that the prevalence of tick species is common in stray dogs i.e. 58.33 % versus with pet dogs. This is consistent with the previous study conducted in Greece that the dogs living outdoors are much more vulnerable to tick infection in contrast to those who live indoors due to lack of vaccination (Latrofa et al. 2017).

The infestation of the tick can be reduced by the preventive measures other than the eradication of the tick population, which is an impossible factor. The ticks of the domesticated animal can be minimized by maintenance in vaccination, grooming at regular basis and application of acaricides. Besides, awareness and educating the public sector on factors associated with tick infestation and their prevention is significant. Furthermore, there is the requirement of studies emphasizing on the identification of tick species that attack humans, their life-cycle patterns, host searching behavior, the infectious stages of ticks that infest humans along with the association of TBDs and focusing studies to reach the risk factors that would assist in better knowledge of tick infestations accompanying to establish strategies for their reduction.

Conclusion

The Veterinary Department should make a comprehensive research on both domestic and non-domestic animals to study the interrelationship of tick and TBDs. Keeping the wide area of Balochistan, it is being proposed that the anti-tick vaccine campaign should launch for domestic dogs. It is also stressed to conduct studies on epidemiological and molecular biology to keep check and balance on the dispersal of tick species and TBDs in other districts to prohibit the illness spreading across the globe which imposes serious menace to domestic dogs as well as humans and to secure the animal welfare in terms of their health issues.

Ethical statement

The work is being done right before permission was taken from the ethics commission present

at the Department of Zoology, the University of Balochistan Quetta under registration number Zool/512-5-19.

Contribution of authors

SR, AI and KK designed this study. SR and KK revised the manuscript. SS adjusted the coordinates to draw the collection sites. AS performed SEM.

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Conflict of interest

The authors declared no conflict of interest.

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