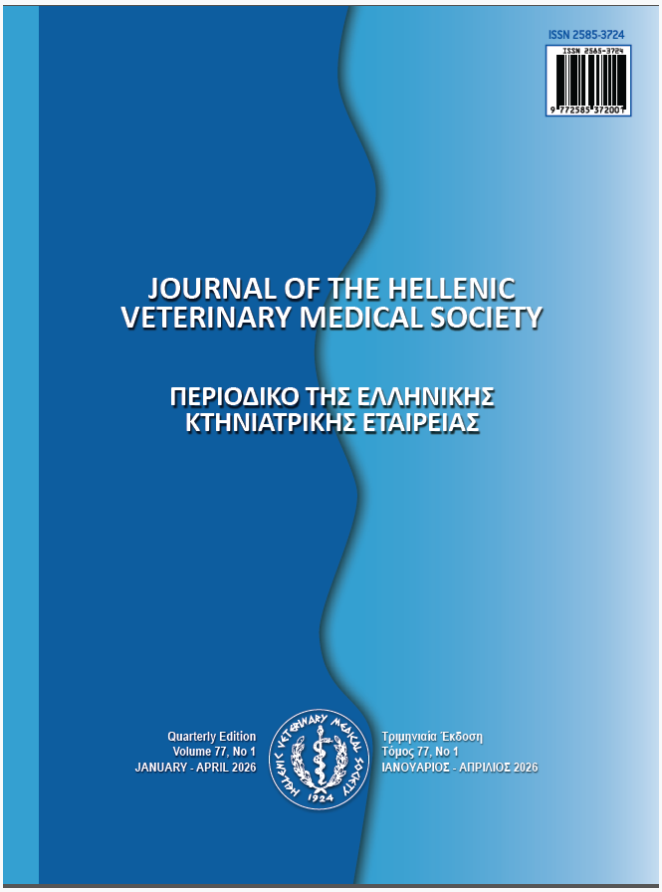
Journal of the Hellenic Veterinary Medical Society

Vol 77, No 1 (2026)



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doi: 10.12681/jhvms.39179

To cite this article:

Hussain, M., Manzoor, F., Ali, A., Ullah, S., Khan, H., Hussain, N., & Ahmed, H. (2026). Prevalence, Seasonal distribution, Risk factors, and Diversity of Ticks in Equines from Sindh Province, Pakistan. *Journal of the Hellenic Veterinary Medical Society*, *77*(1), 10099–10112. https://doi.org/10.12681/jhvms.39179

J HELLENIC VET MED SOC 2026, 77 (1): 10099-10112
ΠΕΚΕ 2026, 77 (1): 10099-10112

Research article
Ερευνητικό άρθρο

Prevalence, Seasonal distribution, Risk factors, and Diversity of Ticks in Equines from Sindh Province, Pakistan

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ABSTRACT: Ticks are blood-feeding ectoparasites that can infest various hosts and transmit various tick-borne pathogens. There have been no comprehensive studies on ticks infesting equines in Sindh province, Pakistan. The present study aimed to determine the prevalence, seasonal distribution, and diversity of ticks infesting equines (donkeys, mules, and horses) in the Sindh province, Pakistan. A total of 403 equines were examined for tick infestation, and a total of 152 ticks were collected from May 2023 to May 2024. The prevalence of ticks among equines was 37.5% (151/403). Equines in rural areas had higher (37.9%) tick infestation than urban (30.4%). Female equines (donkeys and horses) had a bit higher (40.8%%) infestation rate followed by males (39.9%) (donkey and horse) and mules showed no infestation. Infestation rates stratified by age group indicated that equines aged 6-10 years exhibited the highest prevalence (47.4%), followed by those aged 1-6 years (32.5%), 11-15 years (30.4%), and 16-20 years (30.0%). Horses showed the highest (64.5%) tick infestation rate, followed by donkeys (35.6%) and mules did not experience any tick infestation. Equines of brown color had the highest (43%) infestation rate. Equines that were not treated with acaricides had a higher (40%) infestation rate than acaricide-treated equines. The common preferred attachment sites for tick infestation were ears (14.6%) and thighs (14%). Female ticks were the most prevalent (17.4%), followed by males (15.4%) and nymphs (4.7%). Three tick genera including *Hyalomma* (31.5%), *Rhipicephalus* (3.7%), and *Haemaphysalis* (2.5%) were identified in the current study. *Hyalomma dromedarii* had the highest (15.9%) infestation rate followed by *Hyalomma anatolicum* (11.2%), *Hyalomma scupense* (4.2%), *Rhipicephalus haemaphysaloides* (2.7%), *Haemaphysalis bispinosa* (2.5%), *Rhipicephalus microplus* (1.0%), and *Hyalomma isaacii* (0.2%). Two species including *Hy. isaacii* and *Ha. bispinosa* were reported for the first time infesting donkeys in Sindh province, Pakistan. The highest infestation rate was observed in summer months from June to August, while seasonally, Summer (June-August) had the highest (54.5%) tick infestation rate followed by spring (March-May) (45.8%), autumn (September-November) (36%) and winter (December-February) (12%). Tick management in these areas should involve breed-specific studies, vaccination programs, seasonal control measures, and veterinary infrastructure development.

Keyword: Tick infestation; Seasonal distribution; Equine; Tick diversity; Sindh; Pakistan

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Date of submission: **22-10-2024**
Date of acceptance: **22-12-2025**

INTRODUCTION

Pakistan is an agricultural country, where a significant part of the population is engaged in animal husbandry (Khan *et al*., 2022). Agriculture is crucial to Pakistan's economy, and more than 70% of the country's population depends on agriculture and agricultural activities (Karim *et al*., 2017). Ticks are blood-feeding parasites that must feed upon hosts to survive. Such hosts include mammals, birds, and reptiles. Ticks infest diverse hosts, causing a variety of diseases such as anaplasmosis, ehrlichiosis, rickettsiosis, and Q-fever (Boulanger *et al*., 2019; Ali *et al*., 2023a, b; Ullah *et al*., 2023). Various risk factors such as host, habitat, climatic conditions and human involvement play important role in tick distribution (Estrada-Peña, 2008). Global tick distributions are affected by biotic factors such as vegetation cover and host availability/management, whereas abiotic factors include rainfall, humidity, and temperature range (Estrada-Peña *et al*., 2012; Alam *et al*., 2022). These factors do not only affect tick abundance, and their spatial distribution but also directs critical transitions across the tick life cycle (Halsey & Miller, 2018).

There are approximately 122.4 million horses, 15 million mules, and 40 million donkeys in the world(Tedla *et al*., 2018). The livestock industry contributed up to 11.7% of Pakistan's GDP and 60.6% of total agriculture in 2020. Pakistan had approximately 0.4 million horses and 5.4 million donkeys in 2020 (Shah *et al*., 2020). A cross-sectional study was conducted on horses in several districts of Balochistan, revealing the presence of five hard tick species. The reported tick species were *Hyalomma anatolicum*, *Hyalomma dromedarii*, *Haemaphysalis bispinosa*, *Rhipicephalus haemaphysaloides,* and *Rhipicephalus microplus* (Kamran *et al*., 2021). Another cross-sectional study was conducted across nine districts in northern Khyber Pakhtunkhwa (KP), reporting five species including *Rhipicephalus turanicus sensu lato (s.l)*, *Rhipicephalus sanguineus*, *R. haemaphysaloides*, *R. microplus, Hy. anatolicum* (Ali *et al*., 2021). Hussain *et al*. (2023) reported equines infested by *Ha. bispinosa*, *R. haemaphysaloides*, *R. microplus,* and *Ixodes kashmiricus* in several agro-ecological zones of Punjab. Horses along with other hosts were heavily infested by ticks in the southern region of KP, the reported species on the equine include *Hyalomma scupense*, *Hy. anatolicum*, and *R. turanicus s.l* (Alam *et al*., 2022). Sindh Province is located in the southeastern region of Pakistan and is characterized by a diverse landscape that encompasses both urban centers and rural areas (Khahro *et al*., 2023). Comprehensive epidemiological studies on ticks in equines within Sindh Province are lacking, despite the potential influence of tick-borne diseases on equine health and production. Equines are essential for light tillage, sowing, and weeding in small-scale agricultural activities. They carry bags and tow carts, carrying materials such as wood, bricks, cement bags, gravel, sand, fuel, feed, and trash (Jamil *et al*., 2022). Surprisingly, despite their impact, equines are left with little regard and are frequently left untreated, which causes disease, wounds, and infections.

To the best of our knowledge, this is the first study conducted on equines in the Sindh region of Pakistan. Previous studies in Pakistan have focused largely on domestic animals and least on equids (Karim *et al*., 2017; Laus *et al*., 2013; Rehman *et al*., 2017). Furthermore, even though the climatic conditions favor the survival of tick species that feed on domestic animals, little data have been published on the frequency and distribution of ticks in equids in Pakistan (Ali *et al*., 2021). The current study aimed to investigate the prevalence, distribution and diversity of ticks in equines from Sindh, Pakistan.

MATERIALS AND METHODS

Study area

The study was conducted in different areas of Sindh, Pakistan from May 2023 to May 2024 (Figure 1). Sindh is situated in the western region of South Asia, sharing its eastern bordered by the Thar desert, to the west by the Kirthar mountains, to the north by Punjab, and the south by the Arabian Sea. Sindh has an area of 140,915 km^2, and is the third largest province of Pakistan, extending around 579 km from north to south and 442 km in its broadest direction from east to west. The climate of Sindh is warm, arid, and humid with hot summer and mild to cool winter. In the northern and higher parts, the lowest temperature drops to −1 °C in December and January, while the daytime temperature often reaches 50 °C between May to August. The highest average rainfall is 180 mm, received in July and August. The northern cold winds blow during the winter months of October to January, while the southwest monsoon winds start in mid-February and last until the end of September (https://en.climate-data.org/asia/pakistan/sindh-2230/).

Data Collection

In the current study, ticks infesting equines were collected through door-to-door visits to remote

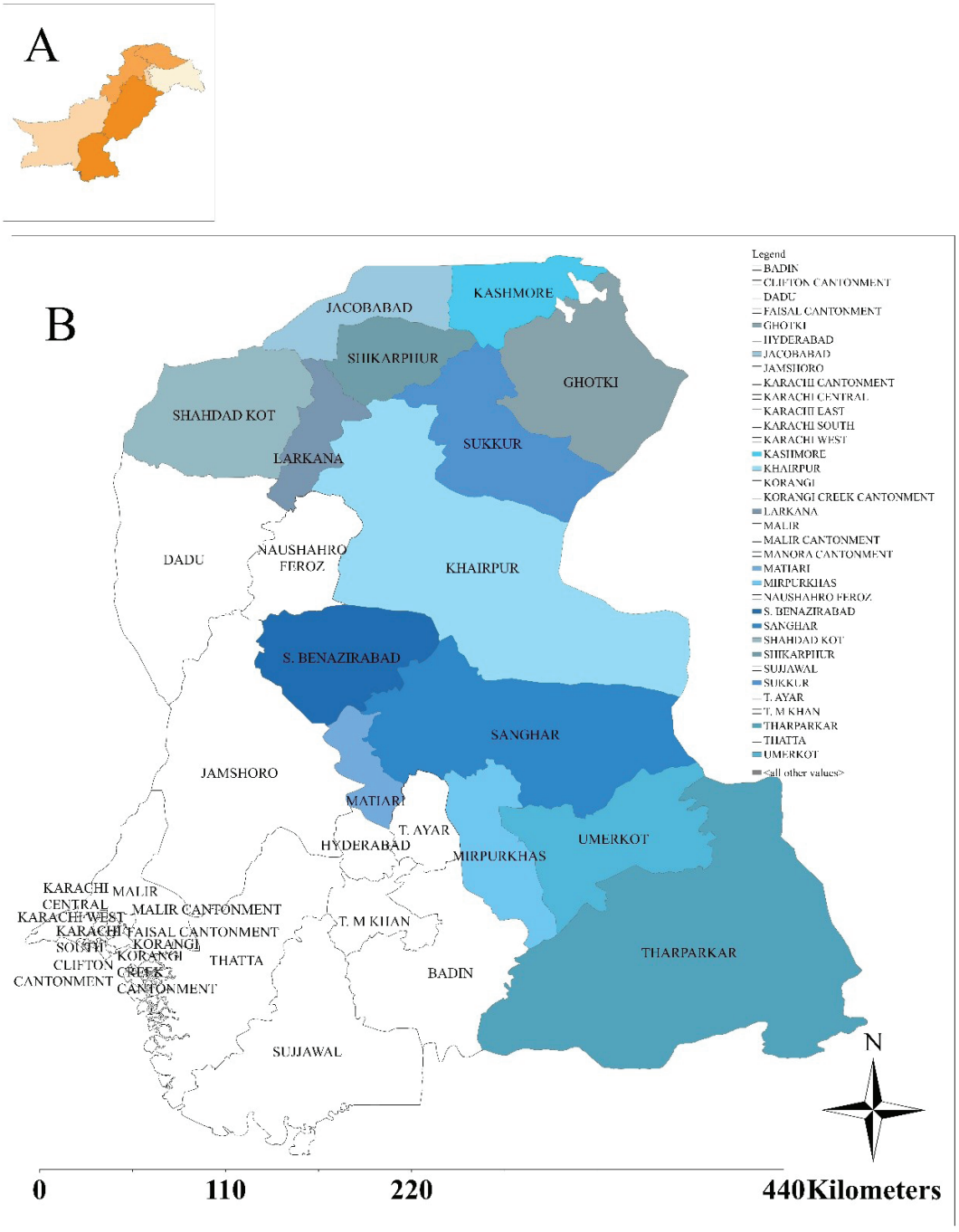

Figure 1. Map of the study area showing the highlighted regions representing sampling locations.

areas and visits to veterinary clinics. Each equine was visually examined to check for the presence of ticks. During this process, additional information was recorded, including the location, month, urban or rural setting, host species, age, sex, infestation status, number of ticks, affected body parts, herd type, body color, season, and the tick's life stage. Geographic coordinates (latitude and longitude) and any acaricide treatments were also noted.

Tick collection, preservation, and identification

Equines were thoroughly examined for tick infestation of the entire body of each animal, such as the ears, neck, abdomen, thighs, belly, legs, and anal regions of the donkeys, mules, and horses, with the permission of the owners to ensure that all the ticks present were collected. The same thorough examination and collection method was applied to all equines to maintain consistency in the sampling effort. The ticks were

removed cautiously with fine-toothed forceps from the host, avoiding damage to its body parts. The tick samples were tagged and transferred to tubes filled with 70% ethanol. After being collected, the samples were brought to the Department of Biosciences, Parasitological Lab, COMSATS University, Islamabad. Ticks were examined under a stereomicroscope, and tick species were determined via standard taxonomical keys (Hoogstraal & Trapido, 1966; Walker *et al*., 2000; Apanaskevich, 2003; Apanaskevich & Horak, 2008; Apanaskevich *et al*., 2008).

Data analysis

The accuracy and completeness of the dataset were ensured by the application of strict data-cleaning procedures before analysis. Sociodemographic data, including animal names (donkeys, mules, and horses), gender, age, locality (rural or urban), host species, animal infested or non-infested, location, month, season, animal color, number of ticks, body parts, herd type, acaricide treatment, and duration of tick's appearance after acaricide treatment were recorded on file in a Microsoft Excel sheet. Age groups were classified into four categories: 1-5 years, 6-10 years, 11-15 years, and 16-20 years. The prevalence of tick infestation was calculated as the number of positive animals divided by the total number of animals examined and multiplied by a hundred (Mohamed *et al*., 2023). After being collected in an Excel sheet, the data was imported and analyzed using IBM SPSS version 25. Furthermore, categorical variables were represented by frequencies and percentages, and the independence or association between variables was shown using Pearson chi-square. To determine whether to accept or reject the hypotheses, it was necessary to analyze the findings under $P<0.05$. In addition, graphs were created using GraphPad Prism (version 8.0). Tick diversities were determined via the Shannon diversity index (H'). Evenness was determined via Pielou's J equation (the value of the Shannon–Wiener index divided by the natural logarithm of the total number of species infested on hosts). The maximum potential diversity was subsequently computed (the natural logarithm multiplied by the total number of species in the population), and equitability was obtained by dividing the value of the Shannon–Wiener index by the maximum possible diversity (Mendes, Evangelista, Thomaz, Agostinho, & Gomes, 2008). Abundance refers to the number of species present on a given host. The tick burden was estimated by dividing the number of ticks on each host by the number of hosts sampled (Rehman *et al*., 2017).

Ethical Approval

Ethical approval (CUI/Bio/ERB/2024/42) was taken by the Institutional Review Board (IRB) of the Department of Biosciences, COMSATS University Islamabad.

RESULTS

Sociodemographic distribution of ticks and associated risk factors

A total of 403 equines were examined for tick infestation of which 37.5% were found tick-infested, resulting in the collection of 158 ticks (Table 1). Equines in rural areas had a higher infestation rate (37.9%) than the urban (30.4%). Female equines (40.8%) reported a bit higher infestation rate than males (39.9%). The mules were not reported with any tick infestation. Equines aged 6-10 years had the highest infestation rate (47.4%) followed by 1-5 years (32.5%), 11–15 years (30.4%), and 16-20 years (30%). Horses (64.5%) had the highest tick infestation rate followed by donkeys (35.6%), while mules had no reports of tick infestations. Brown-colored equines had the highest infestation rate (43%), followed by black-colored (37.5%) and white-colored (35.8%). Equines not treated with acaricide had a higher infestation rate (40%) than acaricide-treated equines (23%). Tick appearance was more likely 2 months after acaricide treatment (20.6%) than 4 months (22.2%). The study found that gender, age, animal type, acaricide treatment, and the duration of ticks after acaricide treatment were significant factors, whereas locality and animal color weren't significant.

Overall prevalence of tick species and tick attachment sites in equines

The attachment sites were varied in equines, being frequently reported on ears (14.6%) and thighs (14%), followed by the tail (6%), abdomen (2.7%), and neck (1.2%). Overall, tick infestation was observed across different body regions, with a higher prevalence of ticks on the ears and thighs compared to other parts. Attachment sites varied, with ticks most frequently found on the ears (14.6%) and thighs (14%), followed by the tail (6%), abdomen (2.7%), and neck (1.2%) of equines.

Overall prevalence of tick species and their life stages in equines

The current study enlisted seven species of *Hyalomma*, *Haemaphysalis,* and *Rhipicephalus* genera (Figures 2-3). *Hyalomma dromedarii* was the most common (n=64,15.9%) species followed by

Table 1. shows sociodemographic risk factors affecting the distribution of ticks in equines.

Variables	Categories	Non infested	Infested	Total	Percentage (%)	Chi-square (x^2)	P-value
Locality	Rural	236	144	380	37.9	0.555a	0.758
	Urban	16	7	23	30.4		
Gender	Female	122	84	206	40.8	19.619a	0.001
	Male	101	67	168	39.9		
	Intersex	29	0	29	0		
Age	1-5 years	85	41	126	32.5	12.957a	0.044
	6-10 years	81	73	154	47.4		
	11-15 years	57	25	82	30.4		
	16-20 years	28	12	40	30		
Herd type	Donkey	186	103	289	35.6	46.704a	0.000
	Horse	27	49	76	64.5		
	Mule	29	0	29	0		
Animal Color	Black	35	22	57	38.6	8.611a	0.072
	Brown	87	63	150	42		
	White	130	66	196	33.7		
Acaricide treatment	No	202	136	338	40	6.925a	0.031
	Yes	50	15	65	23		
Duration of tick appearance after acaricide treatment	After 2 months	23	7	34	20.6	10.938a	0.027
	After 4 months	26	8	36	22.2		

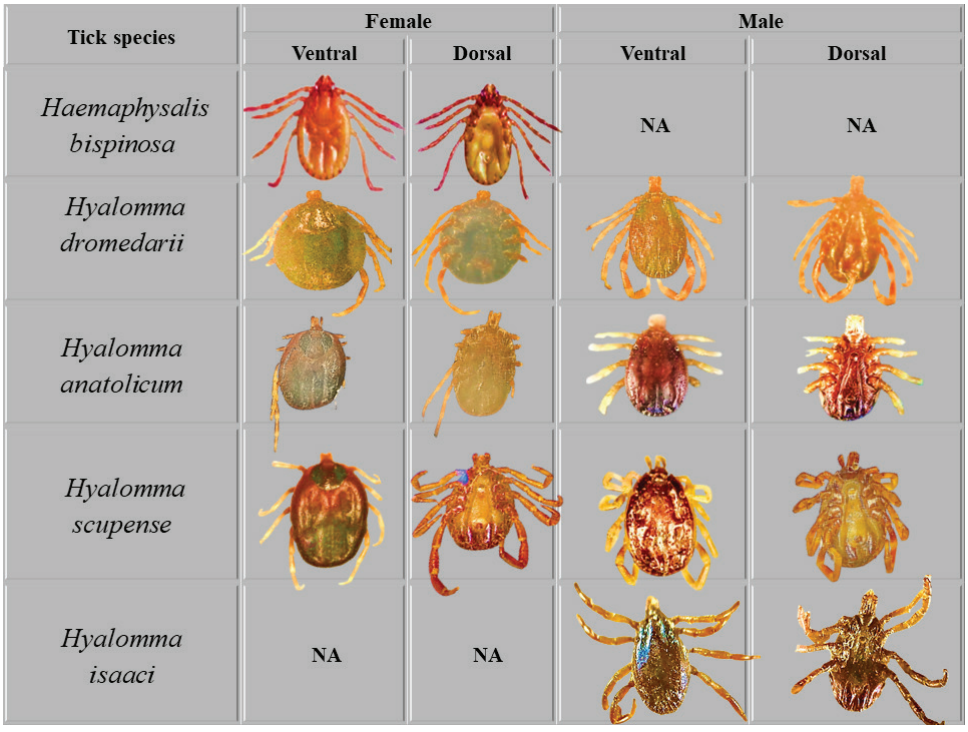

Figure 2. Enlisted species of Haemaphysalis and Hyalomma genera.

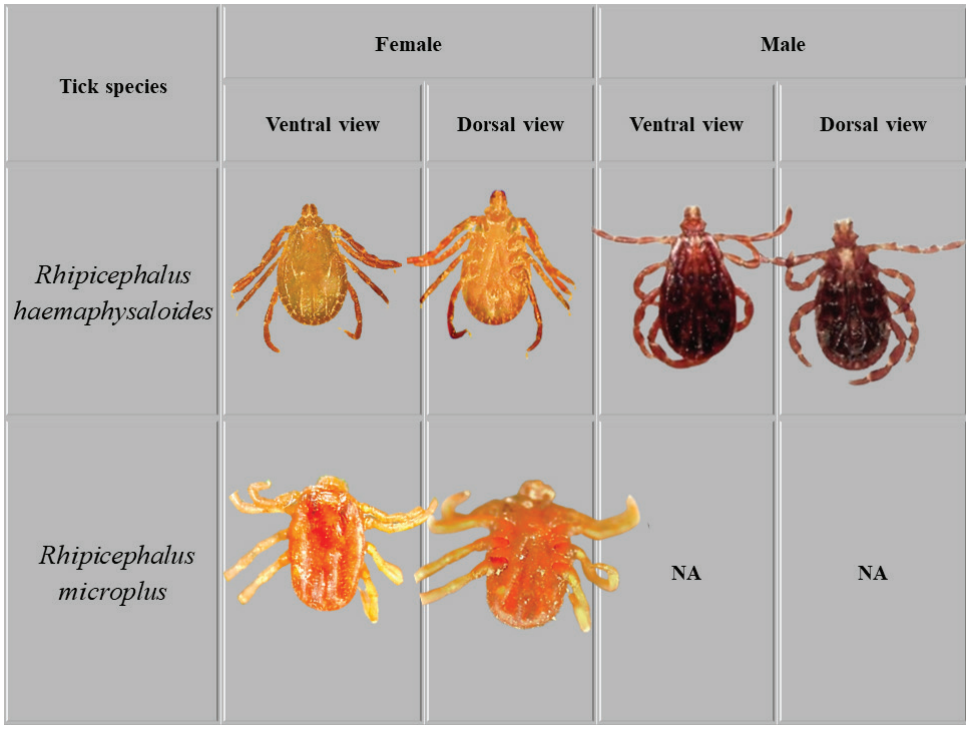

Figure 3. Enlisted enlisted species of the Rhipicephalus genus.

Hy. anatolicum (n=45, 11.2%), *Hy. scupense* (n=17, 4.2%), *Ha. bispinosa* (n=10, 2.5%), *Hy. isaacii* (n=1, 0.2%), *R. haemaphysaloides* (n=11, 2.7%), and *R. microplus* (n=4, 1.0%). Female ticks were the highest (n=75, 18.6%) followed by male ticks (n=58, 14.4%), and nymphs (n=19, 4.7%).

Diversity and abundance of tick species in different hosts

The Shannon–Wiener index equation was used to calculate the tick species diversity (Table 2). The species diversity and evenness varied according to the collection site. Based on 158 ticks in total, the distribution and abundance of tick species were evaluated in the current study. Of these, *Hy. dromedarii*

Table 2. shows the abundance (n) diversity (h') and equitability of tick species in the equines of Sindh, Pakistan. where: H = Shannon diversity index, pi = proportion of individuals of species i relative to the total number of individuals, n = total number of each species, Hmax=ln (total species), equitibility =H'/Hmax.

Categories	Species	N	Pi	ln (Pi)	(pi) ln (pi)	H'	Hmax	Pielou's J value (Evenness)
Tick species	*Hy. dromedarii*	64	0.421053	-0.865	-0.36421	1.467428	1.945910149	0.754109
	Hy. scupense	17	0.111842	-2.19067	-0.24501			
	Hy. anatolicum	45	0.296053	-1.21722	-0.36036			
	Ha. bispinosa	10	0.065789	-2.7213	-0.17903			
	Hy. isaaci	1	0.006579	-5.02388	-0.03305			
	R. haemaphysaloides	11	0.072368	-2.62599	-0.19004			
	R. microplus	4	0.026316	-3.63759	-0.09573			
Sum	7	158	1	-18.2816	-1.46743			

(H=Hyalomma, Ha=Haemaphysalis, R= Rhipicephalus)

was the most common species in the overall population, followed by *Hy. anatolicum, Hy. scupense*, *Ha. bispinosa*, *Hy. isaacii*. Furthermore, the population of *R. haemaphysaloides* was highly diverse compared with that of *R. microplus*. In Sindh Province, *Hy. dromedarii* was the most common tick species among equines. The Shannon diversity index (H') indicated a significant diversity of tick species, while the equitability score (0.75) showed a relatively even distribution across species.

Temporal patterns and dynamics of ticks in equines

The temporal patterns and dynamics of ticks in equines varied by month and season (Table 3). The overall monthly and seasonal prevalence was 37.5%. The tick infestation rate in January was 13%, but in February there were no infestations. Infestation rates were highest in July (64.3%), June (64.5%), and March (64.7%). There were moderate infestation rates in April (30%), May (45.4%), August (47.8%), and September (38.7%). The infestation rates decreased in October (37.5%), November (34.5%), and December (22.6%).

The seasonal infestation rates varied. Summer (June–August) had the highest (54.5%) tick infestation rate, followed by spring (March-May) (45.8%), autumn (September–November) (36%), and winter (December–February) (12%). Overall, the study revealed that infestation rates varied across different seasons and months, with significant differences observed, particularly during the spring and autumn months of March, September, and November. The chi-square analysis revealed significant associations between infestation and month ($\chi^2 = 61.671$, $p < 0.001$) and season ($\chi^2 = 13.070$, $p = 0.04$), which highlighted the impact of temporal and seasonal variables on tick infestation patterns in the study area.

Host-wise species distribution among different equine hosts

The distribution of various host species in the study area was clearly shown on a map (Figure 1), and different tick species presented different equine preferences (Table 4). *Hy. dromedarii* had the highest distribution in horses (10.9%) followed by donkeys (5%). *Hy. anatolicum* was highly distributed among donkeys (10.4%) and was present in horses with the

Table 3. shows temporal patterns and dynamics of ticks in equines.

Variable	Category	Non infested	Infested	Total	Percentage (%)	Chi square (x^2)	df	P-value
	January	24	7	31	22.6			
	February	31	0	31	0			
	March	6	11	17	64.7			
	April	14	6	20	30			
	May	40	6	46	13			
	June	19	12	31	38.7			
Month	July	36	19	55	34.5	61.671[a]	11	0.000
	August	45	41	86	47.8			
	September	11	20	31	64.5			
	October	10	6	16	37.5			
	November	10	18	28	64.3			
	December	6	5	11	45.45			
	Total / Mean infestation rate	252	151	403	37.5%			
	Summer	151	98	249	39.4			
	Autumn	45	12	57	21.1			
Season	Winter	20	24	44	54.5	13.070[a]	3	0.04
	Spring	36	17	53	32.1			
	Total / Mean infestation rate	252	152	403	37.5%			

Table 4. shows the host species wise distribution of ticks related to different equine hosts.

Genus	Tick Species	Infestation Prevalence Rate				X^2	P-Value
		Donkey (N, %)	Horse (N, %)	Mule (N, %)	Total (N, %)		
Hyalomma	*H. dromedarii*	20 (5.0)	44 (10.9)	0	64 (15.9)	145.9	0.000
	H. scupense	16 (4.0)	1 (0.25)	0	17 (4.2)		
	H. anatolicum	42 (10.4)	3 (0.7)	0	45 (11.2)		
	H. isaaci	1 (0.25)	0 (0.0)	0	1 (0.2)		
Haemaphysalis	*Ha. bispinosa*	9 (2.2)	1 (0.25)	0	10 (2.5)		
Rhipicephalus	*R. haemaphysaloides*	11 (2.7)	0 (0.0)	0	11 (2.7)		
	R. microplus	4 (1.0)	0 (0.0)	0	4 (1.0)		
Life cycle	Male	15 (3.7)	43 (10.7)	0	58 (14.4)		
	Female	30 (7.4)	45 (11.2)	0	75 (18.6)		
	Nymph	9 (2.2)	10 (2.5)	0	19 (4.7)		

(H=Hyalomma, Ha=Haemaphysalis, R= Rhipicephalus, X^2=Chi square value)

least prevalence (0.7%). *Hy. scupense* predominantly infested donkeys (4%) but least infestation in horses (0.25%). *Ha. bispinosa* infested donkeys (2.2%) and horses (0.25%). *R. haemaphysaloides* (2.7%), *R. microplus* (1%), *Hy. isaacii* (0.25%), were only observed in donkeys and these three species showed no infestation in horses and mules. A total of seven tick species were collected. All were found infesting donkeys, while only four species (Hyalomma dromedarii, H. anatolicum, H. scupense, and Haemaphysalis bispinosa) were recorded on horses. No tick infestations were detected in mules. Primarily ticks that were associated with horses include *Hy. dromedarii* and *Ha. bispinosa*, while ticks that infested both donkeys and horses include *Hy. dromedarii*, *Hy. scupense*, and *Hy. anatolicum*. The current study reported *Hy. isaacii* infesting donkeys, for the first time in Sindh, Pakistan.

Spatial distribution of equine ticks in the Study area

The tick distribution in equines varied across the study area (Table 5). In Ratnaur and Sanghar, all equines except mules, were highly (100%) infested, followed by Shikarpur (90%), Umerkot (83.3%), Nawabshah (83%), Mirpurkhas (80%), Bhalwah (71.4%), Pir Jo Goth (59.1%), Jacobabad (45.45%), Ratan Jo Tar (45.45%), Naukot (37.5%), Larkana (37.5%), Thari Mirwah (37.5%), Islamkot (33.96%), Nangarparkar (30.76%), Bichho Jo Tar (30%), Ghotki (28.57%), Nara (28.57%), Kashmore (22.58%), and Gadeji (5%). No tick infestations were reported in the KotDigi, Ranipur, and Sobhodero regions. The chi-square analysis (χ^2) revealed borderline significant relationships between infestation and location (P- value < 0.05).

Ticks burden assessment in equines

The burden of tick species on different equine populations, including donkeys, horses, and mules, was calculated. *Hyalomma dromedarii* had the highest burden, with 0.58 ticks per horse and 0.07 ticks per donkey. Donkeys showed a wider diversity of tick species compared to horses. *Hyalomma scupense* had a low burden on horses (0.01 ticks per horse) and slightly higher on donkeys (0.06 ticks per donkey). *Hyalomma anatolicum* infested donkeys at 0.14 ticks per donkey and horses at 0.04 ticks per horse. *Haemaphysalis bispinosa* showed the lowest burden on both horses (0.01 ticks per horse) and donkeys (0.03 ticks per donkey), while *Hyalomma isaacii* had a minimal burden, infesting only a single donkey. *Rhipicephalus haemaphysaloides* was recorded on donkeys (0.04 ticks per donkey) but was absent on horses.

The impact of these species varied among equine hosts, with donkeys generally showing higher sensitivity to some species than horses and mules. Individually, *Haemaphysalis bispinosa*, *Hyalomma isaacii*, *R. haemaphysaloides*, and *R. microplus* had relatively low burdens, but together they contributed to the overall tick burden on equines. No infestations were observed in mules in this study (Table 6).

Table 5. shows spatial distribution of Ticks in equines in various regions of Sindh.

Village/Cities	Non infested	Infested	Total	Percentage (%)	Chi-square (x^2)	df	P-value
Bhalwah	4	10	14	71.4			
Bichho Jo Tar	14	6	20	30			
Gadeji	17	3	20	15			
Ghotki	10	4	14	28.57			
Islamkot	35	18	53	33.96			
Jacobabad	6	5	11	45.45			
Kashmore	24	7	31	22.58			
KotDigi	3	0	3	0			
Larkana	10	6	16	37.5			
Mirpurkhas	1	4	5	80			
Nangarparkar	9	4	13	30.76			
Nara	50	20	70	28.57	90.552[a]	22	0.000
Naukot	15	9	24	37.5			
Nawabshah	1	5	6	83			
Pir Jo Goth	9	13	22	59.1			
Ranipur	8	0	8	0			
Ratan Jo Tar	6	5	11	45.45			
Ratnaur	0	6	6	100			
Sanghar	0	4	4	100			
Shikarpur	1	9	10	90			
Sobhodero	22	0	22	0			
Thari Mirwah	5	3	8	37.5			
Umerkot	2	10	12	83.3			

Table 6. shows the burden of tick species in the Sindh region, Pakistan equines.

Tick Species	Tick Burden per individual			Total Burden of particular species on overall equines
	Donkey (N=289)	Horse (N=76)	Mule (N=29)	
Hy. dromedarii	0.07	0.58	0	0.65
Hy. scupense	0.06	0.01	0	0.07
Hy. anatolicum	0.14	0.04	0	0.18
Ha. bispinosa	0.03	0.01	0	0.04
Hy. isaaci	0.003	0	0	0.003
R. haemaphysaloides	0.04	0	0	0.04
R. microplus	0.01	0	0	0.01

DISCUSSION

To the best of our knowledge, this is the first epidemiological study on equines in Sindh province, Pakistan. Notable tick species identified include *Hy. anatolicum*, *Hy. scupense*, *Ha. bispinosa*, *R. haemaphysaloides*, *R. microplus*, and *Hy. isaacii*. This study is the first to report *Hy. isaacii* and *Ha. bispinosa* in donkeys in Sindh, although these species are also found in other regions of Pakistan, including Balochistan (Kamran *et al*., 2021), Khyber

Pakhtunkhwa (Alam *et al*., 2022; Ali *et al*., 2023b), Kashmir (Karim *et al*., 2017), and Punjab (Ghafar *et al*., 2020; Hussain *et al*., 2023).

Female equines reported higher infestation rates than males. Our findings differed from those published by Kamran *et al*., (2021); Onyiche *et al*., (2022), who found that males have a larger tick infestation than females (Kamran *et al*., 2021; Onyiche *et al*., 2022) while other studies showed consistency with our results (Bartolomé Del Pino *et al*., 2016; I. Hussain, Rather, & Ahanger, 2019). Female ticks require a blood meal to reproduce, as they need the nutrients for egg production. They tend to seek hosts that provide a suitable environment for feeding and reproduction. The age range of 6-10 years had the highest infestation followed by 11–15 years and 16-20 years. Our results were consistent with prior research (Dantas-Torres, Chomel, & Otranto, 2012; Ferede, Kumsa, Bsrat, & Kalayou, 2010). The increased incidence of parasitic infestations in old age equine populations is due to a combination of various factors. Firstly, chronological maturation goes along with a progressive loss of immunological competence that limits the host's capability to resist ectoparasitic invasions (Horohov *et al*.,2009). Secondly, older animals are likely to have had years of repeated exposure to tick-infested environments over their entire lifetime. Thirdly, age-related physical impairments may reduce grooming efficiency, therefore reducing the capacity to remove attached ticks. Finally, a preference for resting on vegetation cover or in other areas, where the animals spend more time, may increase the risk of infestation even more.

Horses had the highest infestation rate, followed by donkeys, demonstrating their susceptibility to tick infestation. Our results were consistent with the literature, indicating that horses had the highest tick infestation rate (Kamran *et al*., 2021; Labruna *et al*., 2001; Teglas *et al*., 2005). There were no reports of tick infestations in mules in our study, which might be attributable to unique circumstances, such as how their owners cared for them and how they were managed. Mules received frequent care from their owners because they were adapted to heavy workloads. Mules, unlike horses or donkeys, are regularly moved and are rarely kept immobile in one place for long periods. This mobility decreases their exposure to tick-infested habitats. Our results differ from those of earlier studies conducted in Khyber Pakhtunkhwa (Ali, *et al*., 2021).

Brown-colored equines had the highest infestation rate, followed by black-colored and white-colored equines. Our results demonstrated no association with animal color, which differs from the findings of previous studies Kyari *et al*., (2019). Equines of different colors might exhibit varying behaviors that affect their exposure to tick habitats. Equines not treated with acaricides had a higher infestation rate than acaricide-treated equines. Our findings were consistent with (Kyari *et al*., 2019). The higher tick infestation rate found in equines without any intervention with acaricide is most likely the result of the absence of effective chemical control. Conventional types of acaricides primarily work by causing the direct death or repulsion of ticks from the host and reducing tick burdens and the tick life history, not through the host's immunological response (Meneghi *et al*., 2016; Kitsou *et al*., 2021). In contrast, the equines that are not subjected to treatment with acaricides continue to be constantly exposed to ixodid ticks and, therefore, reprove a higher probability of recurrent infestations accompanied by high cumulative tick burdens. While some development of acquired resistance or partial immunity to tick salivary components may be observed upon repeated exposure, and this is not necessarily insignificant in successfully limiting the development of marked levels of infestation in tick-free equine populations, such a natural defense is generally insufficient to compensate for the lack of chemical prophylaxis, and does not prevent high levels of infestation from occurring in unprotected equine populations (Wikel, 1996).

The *Hyalomma* genus had the highest infestation rate. *Hy. dromedarii* had the highest infestation rate followed by *Hy. anatolicum*, *Hy. scupense, Ha. bispinosa*, *R. haemaphysaloides*, *R. microplus* and *Hy. isaacii.* Our findings were in line with other research reporting a high prevalence of *Hyalomma* ticks on horses (Khosravi *et al*., 2012; Kamran *et al*., 2021; Alam *et al*., 2022). *Hyalomma dromedarii* is a camel-specific tick, the reason behind the equid's infestation by *Hy. dromedarii* was a similar habitat shared between camels and equines in the current study area. Some species might be more efficient or opportunistic in finding suitable hosts than others. The Shannon diversity index revealed that *Hy. dromedarii*, which had the highest diversity and equitability score, presented an equal distribution of ticks across different species. Our findings were consistent with earlier research showing a high prevalence of *Hy. dromedarii* (Ghafar *et al*., 2020;

S. Hussain *et al*., 2022). Several tick species were observed in the research region on equines (Bacon *et al*., 2022). The diversity of tick species might be due to climatic conditions, which are favorable for the development of many tick species (Ogden, Ben Beard, Ginsberg, & Tsao, 2021).

Among the identified ticks, the number of female ticks was higher than male ticks. Our findings were in line with previous research conducted in Pakistan (Ahmad *et al*., 2022; Khan *et al*., 2023; Hussain *et al*., 2023). Female ticks stay more on hosts as compared to male ticks; this is due to the large amount of blood needed for their egg maturation. Most of the recovered ticks were in the adult stage, with no larvae observed, a pattern that has been reported in several previous studies (Tirosh-Levy *et al*., 2018; S. Ali *et al*., 2020b; Aiman *et al*., 2022). The absence of larvae on equines may be due to host specificity, as the larvae of hard ticks generally prefer smaller-sized vertebrate hosts (Guglielmone & Nava, 2017). This may be attributed to the thinner skin, which facilitates tick mouthpart penetration and provides richer blood supply.

Seasons and months had varied rates of infestation; significant variations in the incidence of infestation were found, especially in the summer months from June to August, because several variables may have contributed to the increased tick infestations in equines over the winter in our study area. One important element might be the availability of conducive circumstances for tick survival and multiplication throughout the winter months. Climate change, which causes warmer winters and high plant cover, provides excellent homes for ticks, and potential changes in host availability or species dynamics may contribute to this phenomenon (Hussain *et al*., 2023). Certain locations, including Bhalwah, Mirpurkhas, Nawabshah, Pir Jo Goth, Ratnaur, Sanghar, and Shikarpur, presented significant infestation rates, whereas other areas, such as KotDigi, Ranipur, and Sobhodero, presented no infestations. Furthermore, the rate of tick infestation varies according to the local climate and environmental conditions, such as humidity, temperature, and the presence of host animals (Zając *et al*., 2021).

The tick species burden varied among hosts, with *Hy. dromedarii* and *Hy. anatolicum* infesting horses most heavily, while other tick species showed different patterns of infestation in donkeys and mules. These findings emphasize the importance of host-specific interactions in tick ecology and disease transmission. In Pakistan, tick infestations in equines have varied, with some studies, such as in Lahore, reporting no tick-infested horses (Rubina Hassan *et al*., 2005). The overall reported tick infestation prevalence in donkeys and other animals in Peshawar was 13.37% (Manan *et al*., 2007). In 2010, researchers recorded a prevalence of 4.2% in donkeys in Khyber Pakhtunkhwa (Perveen *et al*., 2010), while the overall tick infestation prevalence in equids in Punjab was 4% (Goraya *et al*., 2013). The tick species burden varied across different hosts, with *Hy. dromedarii* and *Hy. anatolicum* infest horses the most, whereas other tick species affect donkeys and mules differently.

The distribution and abundance of tick species can vary by region, leading to higher infestation rates in equines where these ticks are more prevalent. While the study aimed to collect all ticks from the equines, some limitations should be noted. The sampling was conducted in various areas, which means that the presence of ticks and their abundance could vary depending on the location and presence of animals at the time of sampling. Additionally, small or hidden ticks may have been missed despite thorough examination. However, efforts were made to minimize these issues by applying a consistent and comprehensive sampling approach across all the examined animals. To effectively apply control measures and minimize the adverse impacts of tick infestations on animal health and production, it is important to understand the epidemiology, seasonal distribution, and risk factors for tick infestations in equines in Sindh, Pakistan.

CONCLUSION

The current study offers a basis for future studies in Sindh Province, Pakistan. This study revealed that factors such as herd type, acaricide treatment, and herd color significantly influence tick infestations in equines. Female equines in rural areas have a higher infestation rate. *Hyalomma dromedarii* is the most prevalent tick species. Equines had significant variability across different months and seasons. Horses, and donkeys, experience varying levels of infestation from different tick species, with *Hy. dromedarii* and *Hy. anatolicum* imposing the highest burden while mules showed no infestation. Understanding the epidemiology of tick infestations in equines is crucial for implementing effective control measures and mitigating their impact on animal health and productivity.

ACKNOWLEDGMENTS

We would like to thank Dr. Rizwan Ahmed Khan, and my uncle: Peer Dino Bhanbhro for their continuous support for continuing this work. Their steadfast support reflects the spirit of community and cooperation that drives our work.

CONFLICT OF INTEREST

The authors state that they have no known conflicting financial interests or personal ties that might have influenced the work described in this study.

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