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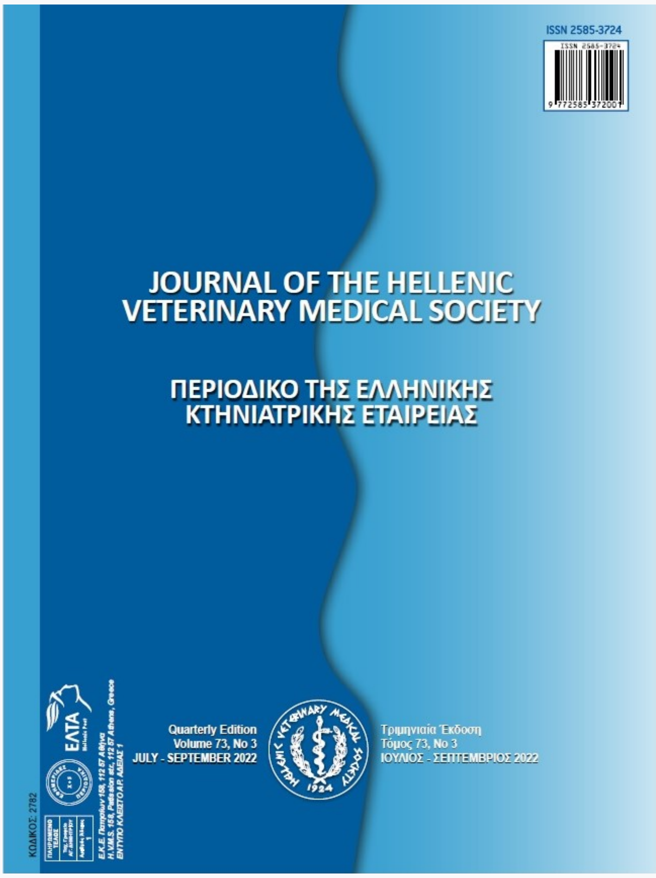

Epizootiology and associated risk factors of tick fauna infecting domestic livestock population in district Faisalabad, Punjab, Pakistan

M Maqbool, MS Sajid, Z Iqbal, M Saqib

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Epizootiology and associated risk factors of tick fauna infecting domestic livestock population in district Faisalabad, Punjab, Pakistan

M. Maqbool.[1], M.S. Sajid[1,2*], Z. Iqbal[1], M. Saqib[3]

[1]*Department of Parasitology, University of Agriculture, Faisalabad, Pakistan*

[2]*Department of Epidemiology and Public Health, University of Agriculture, Faisalabad, Pakistan*

[3]*Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan*

ABSTRACT: The current study was aimed to correlate the association of different risk factors with the hard tick (Acari: Ixodidae) infestation rate in livestock (cattle, buffalo, sheep, goat) population of district Faisalabad. Ticks were collected through the standard collection protocols andidentified under a stereomicroscope. Overall, 54%, 50%, 50%, and 49% tick infestation rateswere reported from cattle, buffalo, sheep, and goat, respectivelywith a higher prevalence of each in tehsil Tandlianwala (72%,65%,70%, and 57%, respectively). Among intrinsic factors (age and sex) higher prevalence was reported in young (7-12 months) and female animals. Significant association (P<0.05)of tick infestation was observed with the season being higher in summer. Statistically significant association (P<0.05)of tick infestation was observed with the extrinsic factors *viz*; feeding system (higher rate in grazing animals), housing system (higher rate in free-housed animals), type of farming (higher rate in free-ranged animals), farm structure (higher rate in animals kept on the uncemented floor) and hygienic measures (higher rate in animals with poor hygiene). The most prevalent tick species were *H. anatolicum,* (85%), *H. marginatum* (1.5%), *H.* (1.02%), and *R. microplus* (12%).The results of our study added information to the inventory of ixodids. The findings will help devise services for tick control in the selected areas and other regions having similar husbandry systems.

Keywords: Tick; Prevalence; Ecological Factor; Epidemiology

Corresponding Author:
M.S. Sajid,Department of Parasitology, University of Agriculture, Faisalabad, Pakistan
E-mail address: drsohailuaf@hotmail.com

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INTRODUCTION

Vector-borne diseases (VBDs) are considered among the main threats to animal and human health. Ticks (Acari: Ixodidae) are known as obligate hematophagous ectoparasites and are worldwide in distribution. Ticks are known as the first arthropod to be reported as vectors and the second most important vector after mosquitoes. Almost 900 species of ticks are identified till now and are divided into four main families: Ixodidae (703 sp.), Nuttalliellidae(1 sp.) Argasidae (194 sp.) and Laelaptidae(Monfared *et al*., 2015). Ticks act as vectors for disease transmission in vertebrates including 38 viruses belonging to six families, protozoal and bacterial diseases i.e. babesiosis, anaplasmosis, theileriosis, Lyme disease, louping ill, tick-borne fever, rocky mountain spotted fever, Crimean Congo Haemorrhagic Fever (CCHF), etc. (de la Fuente *et al*., 2016). Ticks and TBDs contribute a major share in decreased livestock and agricultural growth rates by decreasing production and increasing management and treatment costs. Tick infestation causes loss of blood and exposure to pathogens, damage to skins and hides. Cross-bred and exotic animals are more prone to tick infestation and act as reservoirs for disease transmission. The prevalence of TTBDs depends upon tick density, breed, season, age, management practices, and geographical area (Rehman *et al*.,2017).

Ticks are prevalent in all areas of the world including Africa (Elghali and Hassan, 2012;Jongejan*et al*., 2020), Australia (Chandra *et al*., 2020), Europe (Mysterud*et al*., 2018), America (Boorgula*et al*., 2020), Asia (Sajid *et al*., 2007, 2011, 2017; Iqbal *et al*.,2013; Jabbar *et al*.,2015; Karim *et al*., 2017; Ramzan *et al*., 2018; Riaz *et al*.,2019; Ghaffar *et al*., 2020;Kamran et al., 2021; Shahid et al., 2022). The distribution of ticks in every region is associated with certain biotic and abiotic factors such as climate, habitat type, humidity, temperature, age, sex, breed, rainfall having a direct impact on the epidemiology of ticks and tick-borne zoonotic and non-zoonotic diseases (Thamm *et al*., 2009; Berman, 2011; Sajid *et al*., 2011; Taye *et al*., 2015;Ullahet al., 2019; Kifle et al., 2021; Kamran, 2021). Identification of these risk factors helpsrisk assessment and devising better and more effective control measures against ticks. A limited study has reported associations of these risk factors with the frequency of the tick infestation in livestock species in Pakistan (Sajid *et al*., 2011, 2020; Iqbal *et al*., 2013; Mustafa *et al*., 2014; Sultana *et al*., 2015; Farooqi *et al*., 2017; Ramzan *et al*., 2019; Ghaffar *et al*., 2020); however, there is a lack of systematic work in the in-

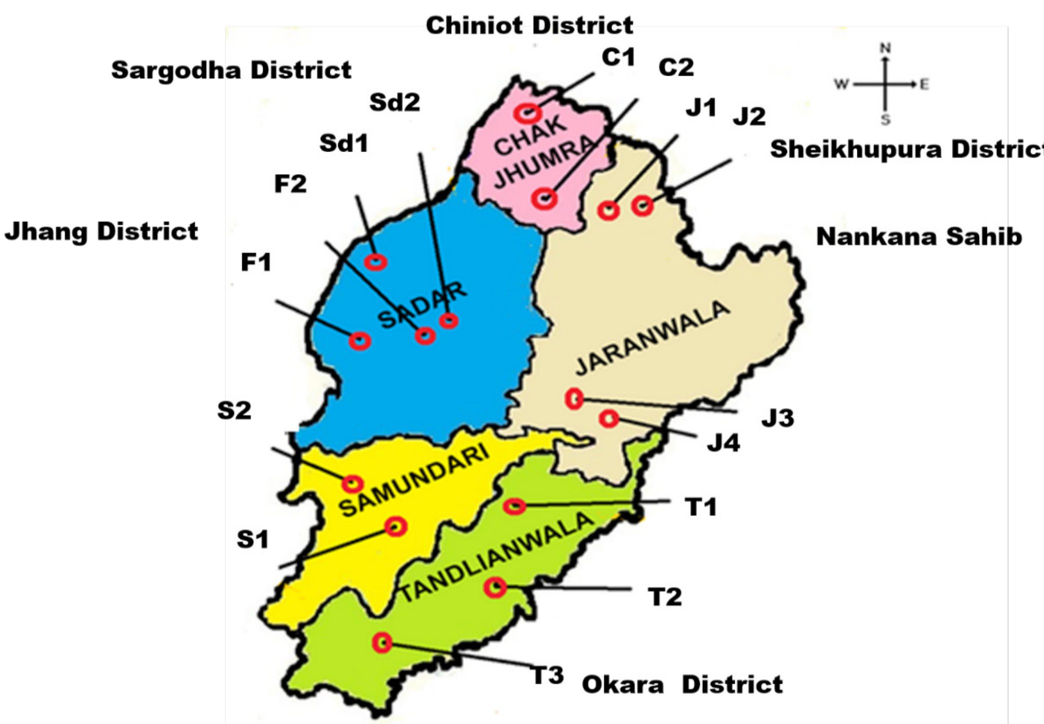

Figure 1 Physical map of Faisalabad district, Punjab, Pakistan showing the selected sites for a sampling of ticks from livestock population

vestigation of distribution and frequency of tick species in ruminants. The current study was designed to evaluate the epidemiology and associated risk factors of the tick population infesting domestic livestock in district Faisalabad, Punjab, Pakistan.

MATERIAL AND METHODS

Study Area

This study was conducted in district Faisalabad, Punjab Pakistan. District Faisalabad consists of six tehsils *viz;* Faisalabad Sadar, Faisalabad City, Samundri, Tandlianwala, Chak Jhumra and Jaranwala (Fig. 1). Four seasons are present in the selected study area i.e. spring (February to April), summer (May to July), autumn (August to October), and winter (November to January). District Faisalabad is situated at 186 meters (610 ft) above sea level, 30 km to the river Chenab, and 40 km to the river Ravi.

Epidemiological Survey

An epidemiological survey was conducted to determine the prevalence of ticks and their association with the ecological (temperature, humidity, rainfall, etc.), host-related (sex, age, species, and breed), and environmental (housing, feeding, and hygienic conditions) factors. For this purpose, a multiple-choice type questionnaire was prepared as described by Thrusfield *et al*. (2018) with some modifications and refined through formal and informal testing. Ecological parameters were procured from the Metrological Department of Punjab, Pakistan for the study year.

To determine the tick prevalence in the study areas, simple random sampling was used. The size of the sample was calculated by taking an expected prevalence of 50% at a 95% confidence interval and a precision level of 5% by using the formula as described by Thrusfield *et al*. (2018). To this end, sampling was done on monthly basis in each study sub-divisions.

Collection and Identification of Ticks

A total of 1536 animals, 384 from each livestock species (buffalo, cattle, sheep, goat), were screened. After restraining the animals, hair coats were examined carefully and systemically with hand to detect engorged ticks (Soulsby, 1982). Ticks were collected from the livestock population and their premises from the selected study areas. Hairs were clipped to clean the area around attachment and ticks were removed with the help of forceps. The forceps were placed on the mouthparts of the tick and removed gently without exerting undue pressure to secure their body parts. Soon after collection, ticks were placed in the labeled Mc Cartney sample collection bottles with screw-capped lids and transported safely to the Molecular Parasitology Lab., Department of Parasitology, University of Agriculture, Faisalabad, Pakistan for further processing. Identification of ticks was done under a stereomicroscope by using the keys of Walker *et al*. (2003).

Statistical Analysis

Collected data regarding the prevalence of ticks and their association with age, breed, sex, species, ecological parameters, housing type, feeding system, and hygienic conditions were analyzed using multiple logistic regression and odds ratios. The data were statistically analyzed using the SAS software package (SAS, 2010).

RESULTS AND DISCUSSION

Prevalence of tick species

Tick prevalence was recorded overone year (October 2018 to October 2019) in domestic animals of district Faisalabad, Punjab, Pakistan. Of the four domestic species *viz*; cattle, buffalo, sheep, and goat examined in this study; all were found infested with hard ticks. A high infestation rate was found in cattle (54%) followed in order by sheep (51%), buffaloes (50%), and goat (49%) as shown in Table 1. Tick infestation rates among the livestock species of different tehsils of the study district were found highest in tehsil Tandlianwala (66%) followed in descending order of abundance by Samundri, Jaranwala, Chak Jhumra, Faisalabad Saddar, and Faisalabad city (Table1).The taxonomic identification of the collected specimens revealed the presence of *H. anatolicum,* (85%), *H. marginatum* (1.5%), *H. truncatum* (1.02%), and *R. microplus* (12%) as depicted in Plate1. It is observed from the results that the tick prevalence varies among the livestock species which is not different from the reports published elsewhere (Gosh *et al*., 2007; Sajid *et al*., 2008; Dantas-Torres *et al*., 2012; Ashraf *et al*., 2013; Iqbal *et al*., 2013; Alam*et al*., 2013; Ali *et al*.,2013; OIE, 2014; Yun *et al*., 2015; Shabbir *et al*., 2016; Sajid *et al*., 2017; Riaz et al., 2019: Ramzan *et al*., 2020; Hussain et al., 2021; Shahid et al., 2022

The higher prevalence of ticks in cattle than buffalo might be due to the difference of thickness of their coat as it is thinner in the former and thicker in the latter. This might also be attributable to the preferen-

Table. 1. Prevalence of tick fauna in livestock population of district Faisalabad, Punjab, Pakistan.

Variables	Levels	Screened	Positive	Prevalence	CI 95%		Odds Ratio	P-Value
					Lower ratio	Upper ratio		
Species	Cattle	384	207	53.91	48.90	58.85	-	-
	Buffalo	384	193	50.26	45.27	55.25	-	-
	Goat	384	187	48.70	43.72	53.70	-	-
	Sheep	384	195	50.78	45.78	55.77	1.36	0.107
Tehsil (Cattle)	Chak Jhumra	79	38	48.10	37.25	59.09	-	-
	FSD City	46	19	41.30	27.82	55.86	-	-
	FSD Saddar	58	27	46.55	34.04	59.40	-	-
	Samundri	76	39	51.32	40.13	62.40	-	-
	Jaranwala	54	33	61.11	47.68	73.37	1.74	0.097
	Tandlianwala	71	51	71.83	60.56	81.35	-	-
Tehsil (Buffalo)	Chak Jhumra	68	31	45.59	34.06	57.49	-	-
	FSD City	51	20	39.22	26.59	53.04	-	-
	FSD Saddar	56	24	42.86	30.42	56.02	-	-
	Samundri	75	40	53.33	42.02	64.39	-	-
	Jaranwala	59	29	49.15	36.59	61.80	1.67	0.104
	Tandlianwala	75	49	65.33	54.07	75.44	-	-
Tehsil (Goat)	Chak Jhumra	79	35	44.30	33.65	55.37	-	-
	FSD City	48	15	31.25	19.38	45.32	-	-
	FSD Saddar	43	20	46.51	32.08	61.40	-	-
	Samundri	78	43	55.13	44.00	65.88	-	-
	Jaranwala	56	28	50.00	37.07	62.93	1.84	0.085
Tehsil (Sheep)	Chak Jhumra	71	35	49.30	37.81	60.84	-	-
	FSDCity	47	19	40.43	27.17	54.83	-	-
	FSD Saddar	49	21	42.86	29.61	56.93	-	-
	Samundri	76	36	47.37	36.35	58.59	-	-
	Jaranwala	64	30	46.88	34.92	59.10	1.73	0.103
	Tandlianwala	77	54	70.13	59.23	79.54	1.11	0.403
Species	*H. annatolicum*	782	668	85.42	82.82	87.77	0.14	0.000
	H. marginatum	782	12	1.50	0.8	2.59	-	-
	H. truncatum	782	8	1.02	0.4	1.93	1.50	0.4
	R. microplus	782	94	12	9.88	14.44	-	-

tial habitats of cattle and buffalo i.e. drier and muddy, respectively (Sajid *et al*., 2009). Besides this, genetic conformation might also play a significant impact on the tick infestation rates in various hosts (Jonsson *et al*., 2014). Buffalo's immune system has shown high sensitivity against tick proteins compared to cattle (Benitez *et al*., 2012). In small ruminants, only a handful of investigations is reported so far regarding tick infestation in Pakistan (Khan et al., 2019;Sajid *et al*., 2020;Hussain et al., 2021In the present study,insignificantly higher prevalence was reported in sheep (50.80%) than goat (48.70%); which is in agreement with the reports of Ramzan *et al*. (2018). Possible reasons for higher rates in sheep might be due to thinner skin (Tatchell, 1997), preferential habits of goats to be comparatively neater and cleaner (Pegram et al., 2004), and protective wool in sheep which might enable the ticks to hide and feed efficiently (Tatchll, 1997).

Among the identified tick species, *H. anatolicum* was the most prevalent species (85.4%; 668/782) followed in order by *R. microplus*, *H. marginatum,* and *H. truncatum*(shown in Plate 1which are not different from earlier findings (Sajid *et al*., 2009; Irshad *et al*., 2010; Ali *et al*., 2013; Burger *et al*., 2014; Mustafa *et al*., 2014; Sultana *et al*., 2015; Rehman *et al*., 2017; Karim *et al*., 2017; Sajid *et al*., 2017; Ramzan *et al*., 2020;Zaheer et al., 2021). Variable reports are available on the distribution of various tick species e.g. Iqbal et al. (2013) reported 75.56% prevalence of *H. anatolicum* and 24.44%of *R. microplus*, Ghaffar *et al*.

(2020) reported 90.6% prevalence of *H. anatolicum* in different agro geo-climatic zones of Pakistan.

*Hyalomma anatolicum*is having three-host life cycle and infests small and large ruminants including buffalo, cattle, sheep and goat, camel, pig, and wild animals species (Gosh et al., 2008;Guan et al., 2009) and is widely distributed in Palearctic, Afrotropical and Oriental regions (Vantansever, 2017; Hansford *et al*., 2019). These tick species are adapted to diversified ecology and are related to the transmission of many important pathogens including bacterial, viral, and protozoal pathogens of veterinary and public health importance as comprehensively reviewed by Sajid *et al.* (2018). *Hyalomma anatolicum*is the main vector for CCHF transmission and Pakistan has faced several outbreaks of CCHF in the recent past (OIE, 2014; Atif *et al*., 2017; Rehman *et al*., 2019; Yaqub *et al*., 2019; Rizwan *et al*., 2019).*Hyalomma marginatum* is having two host lifecycle andis known as the Mediterranean hyalomma tick. It is a potential vector for CCHF and *Theileria annulate* (Gargili et al., 2017; Alan et al., 2019). *Hyalomma truncatum* is a medically important tick species transmitting CCHF in humans along with Rickettsia conorii, Dugbe virus, and Rift Valley fever virus (Nchu and Rind, 2013; Goddard, 2016; Deka, 2018).In the current study, *H. marginatum* and *H. truncatum* were found in low numbers (1.50%; 12/782 and 1.02%; 8/782 respectively) these findings are not different from the findings of previous studies (Karim *et al*., 2017; Ramzan *et al*., 2019).

The other prevalent species found in the current study was *R. microplus* (12%; 94/782) and these findings are consistent with the findings of previous studies (Sing and Rath, 2013; Karim *et al*., 2017; Ramzan *et al*., 2020). *Rhipicephalus microplus* is a one-host tick and known as a vector for economically important TBDs (babesiosis and anaplasmosis) of bovines (Ramzan *et al*., 2020). Sing and Rath (2013) reported *R. microplus* prevalence in India and described that this species prefers hot and humid climates.In Pakistan, *H. anatolicum* and *R. microplus* are the potent vectors for the transmission of babesiosis, anaplasmosis, and theileriosis in bovines (Ali *et al*., 2013; Jabbar *et al*., 2015).

Age and gender-wise prevalence of ticks

Factors associated with the tick prevalence are divided into two categories i.e. intrinsic (age, gender, species) and extrinsic (housing, feeding type, farm structure, season, age, type of farming, and hygienic system) as reported earlier (Sajid et al., 2008, 2009, 2011, 2020; Iqbal et al., 2013) Animals of the study

Table.2. Age-wise and Gender wise prevalence of ticks in livestock population of district Faisalabad, Punjab, Pakistan.

Variables	Levels	Screened	Positive	Prevalence	CI 95%		Odds Ratio	P-Value
					Lower ratio	Upper ratio		
Gender (Cattle)	Female	251	149	59.36	53.20	65.32	-	-
	Male	133	58	43.61	35.36	52.13	1.23	0.276
Gender (Buffalo)	Female	258	138	53.49	47.38	59.52	-	-
	Male	126	55	43.65	35.18	52.41	1.34	0.115
Gender (Goat)	Female	**211**	116	54.98	48.22	61.60	-	-
	Male	173	71	41.04	33.89	48.49	1.32	0.14
Gender (Sheep)	Female	221	125	56.56	49.96	62.99	-	-
	Male	163	70	42.94	35.50	50.64	-	-
Age (Months) (Cattle)	0-6	77	32	41.56	30.96	52.78	1.48	0.113
	>6-12	174	107	61.49	54.10	68.51	-	-
	>12	133	68	51.13	42.65	59.55	-	-
Age (Months) (Buffalo)	0-6	79	30	37.97	27.79	49.02	1.5	0.103
	>6-12	163	93	57.06	49.36	64.50	-	-
	> 12	142	70	49.30	41.13	57.49	-	-
Age (Months) (Goat)	0-6	92	33	35.87	26.57	46.04	1.55	0.075
	>6-12	173	96	55.49	48.02	62.78	-	-
	> 12	119	58	48.74	39.84	57.70	-	-
Age (Months) (Sheep)	0-6	114	47	41.23	32.47	50.43	1.5	0.056
	>6-12	175	108	61.71	54.35	68.70	-	-
	> 12	95	40	42.11	32.49	52.20	-	-

area were divided into three categories based on age ranges i.e. 0-6 month (juvenile), >6-12 months (young stock), and > 1year (adults). A higher infestation rate ($P > 0.05$) was recorded in young stock (62% in sheep and 55% in goat) as compared to adults and juveniles(Table2) which is in agreement with the earlier findings (Rony *et al*., 2010; Kabir *et al*., 2011; Tsai *et al*., 2011; Sing and Rath, 2013; Mustafa *et al*., 2014; Sajid *et al*., 2017; Khalil *et al*., 2018; Burrow *et al*., 2019; Ghafar *et al*., 2020; Zeb *et al*., 2020;Shoaib et al., 2021) and in contrast with findings of (Sajid *et al*., 2009; Sherrard-Smith *et al*., 2012; Anderson *et al*., 2013; Rehman *et al*., 2017). The lower tick prevalence in adults might be related to acquired immunity gained through repeated tick exposures, better management and care as compared to calves, and appropriate husbandry and attention in small dairy farming (Sing and Rath, 2013; Burrow *et al*., 2019).Possible causes for the higher tick prevalence in younger animals include: (a) development of lesser immune response due to lesser exposure (b) softer skin and tissues of younger animals are favorable for ticks due to easy penetration of the mouthparts (Kabir *et al*., 2011; Singh and Rath, 2013; Khajuria *et al*., 2015; Lew-Tabor *et al*., 2017; Burrow *et al*., 2019).

Among intrinsic factors, the higher prevalence was recorded in the females (59% in cattle and 56% in sheep) as compared to males. (Table 2). However, numerically, female animals were found more prone to tick infestation as compared to male and this is related to findings of various scientists (Kabir *et al*., 2011; Iqbal *et al*., 2013; Rehman *et al*., 2017; Sajid *et al*., 2017; Zeb *et al*., 2020;Shahid et al., 2022). However, our findings are not in accordance with the study of Atif *et al.* (2012) and Shoaib et al. (2021) they reported higher prevalence in male animals. Higher infestation in females may be due to stresses in female

Table 3. Association of seasons with tick prevalence in livestock population of district Faisalabad, Punjab, Pakistan.

Variables	Levels	Screened	Positive	Prevalence	CI 95% Lower ratio	CI 95% Upper ratio	Odds Ratio	P-Value
Season (Cattle)	Winter	75	21	28.00	18.73	38.94	-	-
	Spring	245	131	53.47	47.20	59.66	-	-
	Autumn	17	14	82.35	59.11	95.31	3.12	0.000
	Summer	47	41	87.23	75.33	94.66	-	-
Season (Buffalo)	Winter	78	25	32.05	22.42	42.99	-	-
	Spring	219	103	47.03	40.48	53.66	-	-
	Autumn	27	18	66.67	47.56	82.36	2.44	0.003
	Summer	60	47	78.33	66.60	87.38	-	-
Season (Goat)	Winter	98	29	29.59	21.19	39.18	-	-
	Spring	202	105	51.98	45.09	58.82	-	-
	Autumn	25	14	56.00	36.42	74.26	2.23	0.007
	Summer	59	39	66.10	53.37	77.29	-	-
Season (Sheep)	Winter	78	21	26.92	17.97	37.56	-	-
	Spring	215	116	53.95	47.26	60.54	-	-
	Autumn	19	11	57.89	35.37	78.17	2.42	0.004
	Summer	72	47	65.28	53.77	75.58		
Months	November	251	47	18.73	14.26	23.91	-	-
	December	147	24	16.33	11.00	22.97	-	-
	January	135	21	15.56	10.16	22.42	-	-
	February	238	150	63.03	56.75	68.98	-	-
	March	290	206	71.03	65.61	76.04	-	-
	April	149	105	70.47	62.77	77.38	-	-
	May	41	32	78.05	63.52	88.73	4.82	0.001
	June	61	44	72.13	59.93	82.27	-	-
	July	47	37	78.72	65.34	88.66	-	-
	August	95	73	76.84	65.78	84.49	-	-
	September	54	33	61.11	47.68	73.37	-	-
	October	28	10	35.71	19.77	54.48	-	-

animals due to pregnancy, parturition, and milking which might lead to hormonal changes that lower the immune status and make them more prone to the infestation (Kabir *et al*., 2011). It has been observed that the male animals are used for draught and sacrificial purposes in Indo-Pakistan and have gained more care and management i.e. grooming which prevents a wide range of ectoparasitic attacks including ticks (Sajid *et al*., 2009; Kabir *et al*., 2011).

Ecological impact on the ticks and tick-borne diseases

Ecological changes are found to be an important factor affecting the epidemiology of vector-borne diseases. In the current study, ticks were available from the start of the summer season and a higher surge in prevalence was recorded during the mid-summer season from May to August while the lowest was in the winter season. Association of seasonal variation with tick infestation rate was also observed (P < 0.05) in the study district and higher prevalence was reported in summer (69%) followed by autumn (40%), spring (28.28%), and winter (17.85%) as shown in Table3; the findings are not different with those published elsewhere (Gosh *et al*., 2007; Sajid *et al*., 2007, 2009, 2011, 2017; Durrani and Shakoori, 2009; Roney *et al*., 2010; Ali *et al*., 2013; Iqbal *et al*., 2013; Sultana *et al*., 2015; Ali *et al*., 2016;Rehman et al., 2017; Zeb et al., 2020;Shahid et al., 2022).

The tick burden of an animal is related to breed, season, sex, nutrition, genotype, and ecological factors (Springell, 1974). Ecological factors including humidity, temperature, and rainfall are related to the rate of tick infestation in any area (Greenfield *et al*., 2011) along with other factors like feeding and housing system (Sajid *et al*. 2009, 2011). Changes in tick abundance patterns and phenological differences are directly related to the change in temperature, relative humidity, local climate change, farm management, and breed (Gharbi *et al*., 2013). Animals remained infested round the year with a higher prevalence rate in summer as reported by Durrani *et al*. (2008).The higher trend in summer is attributable to the suitable weather for reducing the generation time of the ticks and maximizing their fecundity (Jones et al., 2017). Temperature change adversely affects the tick population. It is predicted that a 2°C increase in temperature affects the habitat of four ticks (Estrada- Pena *et al*., 2012). Our findings are not different from those of Kabir *et al*. (2011) from Bangladesh who found 41.66% prevalence in summer compared to 31.5% in winter. Monthly prevalence results are similar to those published elsewhere having a higher prevalence of ticks during June, July, and August and lower infestation rates during December and January. Atif *et al*. (2012) has also reported a higher prevalence of ticks from July to August in district Sargodha Punjab, Pakistan which is not different from our findings. Later, Mustafa *et al*. (2014) also reported a higher prevalence during summer as compared to winter in district Sargodha, Punjab, Pakistan. In Pakistan, the temperature during the summer season is more suitable for tick growth, reproduction, activity, and development. Hence, higher prevalence has been recorded by different researchers during summers (Sajid *et al*., 2009, 2011, 2017; Sultana *et al*., 2015; Karim *et al*., 2017; Ramzan *et al*., 2020; Zeb *et al*., 2020;Shahid et al., 2022).

Climate change has a direct effect on tick abundance as the major variables i.e. temperature and rainfall pattern has a positive statistical association with the tick activity, propagation rate, and fecundity (Githeko *et al*.,2000; Purse *et al*., 2005; Yakhchali and Hosseine, 2006). Studies have confirmed the delayed tick activity with an increase in altitude and decrease in temperature (Daniel *et al*., 2003). During past decades due to global warming, a higher tick infestation is reported at higher altitudes further elaborating the relationship between tick prevalence and temperature (Gray *et al*., 2009; Gilbert, 2010; Rafiq *et al*., 2017). Endemicity of the tick infestation might be due to the geographical changes and/or climatic conditions of the different study areas making acari able to maintain their survival where these were not prevalent earlier (Wikel, 2018).

Among other ecological factors, it has been observed that the humidity and rainfall patterns are having a direct relationship with the magnitude of ticks. The highest prevalence of ticks (78.72%) was observed during the summer season when rainfall and humidity levels are highest. The least abundance of ticks (15.56%) was observed in the winter season when rainfall and humidity levels were lowest (Fig. 2).

Association of feeding and farming systems with a tick infestation

In the case of extrinsic factors, the feeding system has a significant effect (P = 0.05) on tick prevalence. A higher prevalence was reported in the grazing animals (highest in sheep; 62.28%, and lowest in goats;

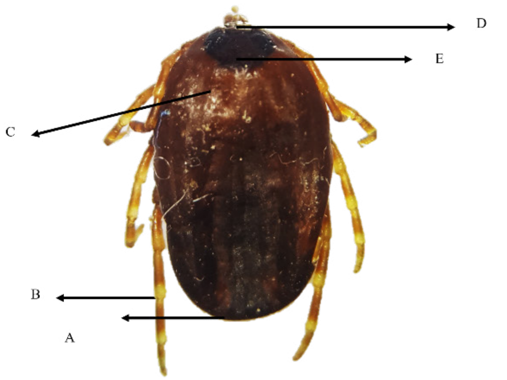

Figure 2 Stereomicrograph of *Hyalomma anatolicum*
A= Festoons are unclear, B= Pale rings, C= Shallow scapular grooves, D= long mouth parts, E= scutum id dark color

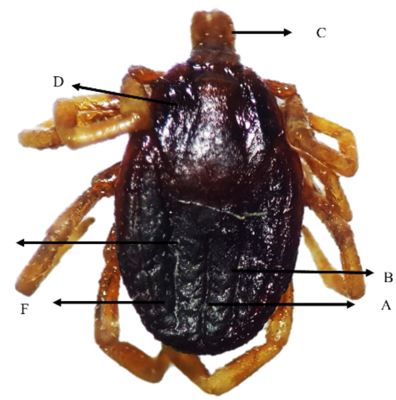

Figure 3 Stereomicrograph of *Hyalomma marginatum* A=Posteromedian groove, B= Long lateral grooves, C= Mouthpart, D= Apparent cervical field depression, E=Small Punctations size, F= Festoons

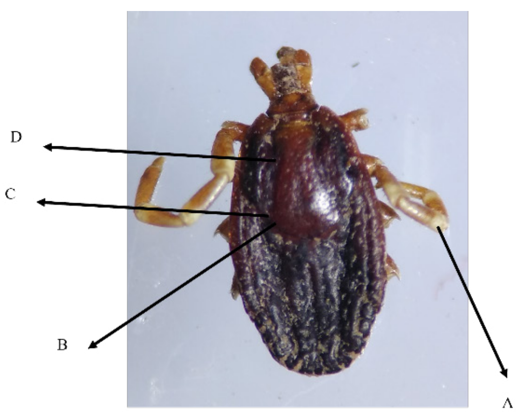

Figure 4 Stereomicrograph of *Hyalomma truncatum*
A=pale ring , B=Scutum posterior margin is distinctly sinuous, C=Punctation size is small, D=Scapular grooves profile is steep

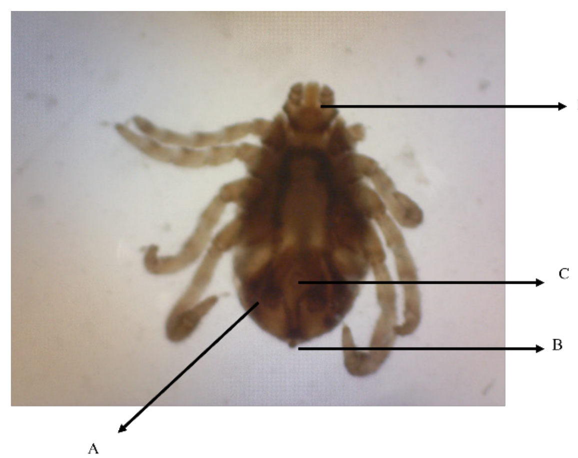

Figure 5 Stereomicrograph of Rhipicephalus microplus
A=Adenal plates , B=Caudal appendages, C=Ventral groove U shaped , D=short mouth parts

Plate 1. Stereomicrographs of identified tick species along with their identification points

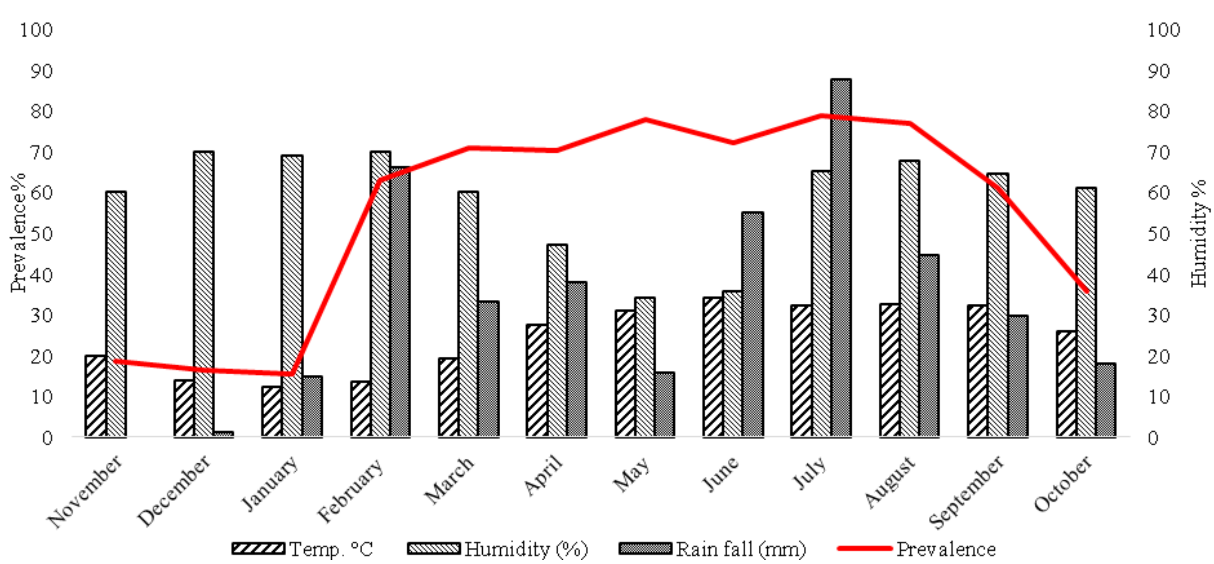

Figure 6 Association of ecological factors with tick infestation rate in livestock population of district Faisalabad, Punjab, Pakistan.

59.09%) followed in descending order by mixed feeding system (highest in cattle; 57.26%, and lowest in goats; 41.94%) and stall-feeding system (highest in sheep; 39.76%, and lowest in goats; 36.90%) as shown in Table 4. These findings are not different from the findings of Iqbal *et al*. (2013) who reported a higher prevalence in grazing animals from district Toba Tek Singh, Punjab, Pakistan. Sajid *et al*. (2017) reported a higher prevalence in grazing and mixed feeding systems compared to the stall-feeding system. Increased prevalence in the grazing system may be attributed to the higher probability of getting exposure to the questing ticks in grazing as compared to the stall-feeding animals (Ghosh *et al*., 2007; Iqbal *et al*., 2013; Rehman *et al*., 2017; Zeb *et al*., 2020).

A significant positive statistical association ($P < 0.05$) was reported between the rate of tick infestation and the farming system. Animals were divided into two categories i.e. free-ranged and farmed and higher tick prevalence was reported in free-ranged animals (highest in cattle 62.11% and lowest in goat 56.43%) followed by farmed (highest in cattle 42.04% and lowest in goat 35.66%) as described in Table 4. Free housed animals showed higher prevalence as compared to tethered animals during the current study which is not in correlation with the finding of Iqbal *et al*. (2013) they reported higher prevalence in tethered animals that may be due to the confinement of livestock population into a specific area by reducing the movement and increase the chance of tick prevalence, low immune response due to tethering stress faced by animals (Sajid *et al*., 2009; Iqbal *et al*., 2013).Animals kept in open housing system (free-ranged) showed higher prevalence in contrast to those in the closed housing system and these findings are in contrast with the finding of Iqbal *et al*. (2013) who found 52.27% prevalence in a closed housing system as compared to 13.64% in the open housing system. Rehman *et al*. (2017) also reported a higher prevalence in closed farming. This high prevalence in open farming may be due to lack of proper management, acaricidal application, and cleaning as compared to closed housing (Muhammad *et al*.,2008).

Table 4. Association of feeding system and type of farming with a tick prevalence rate in livestock population of district Faisalabad, Punjab, Pakistan.

Variables	Levels	Screened	Positive	Prevalence	CI 95%		Odds Ratio	P-Value
					Lower ratio	Upper ratio		
Feeding System (Cattle)	Stall feeding	94	35	37.23	27.91	47.33	1.63	0.038
	Grazing	173	105	60.69	53.27	67.77	-	-
	Both	117	67	57.26	48.18	66.00	-	-
Feeding System (Buffalo)	Stall feeding	97	37	38.14	28.89	48.10	1.56	0.05
	Grazing	161	96	59.63	51.91	67.00	-	-
	Both	126	60	59.63	39.00	56.34	-	-
Feeding System (Goat)	Stall feeding	84	31	36.90	27.11	47.59	1.6	0.05
	Grazing	176	104	59.09	51.71	66.18	-	-
	Both	124	52	41.94	33.48	50.76	-	-
Feeding System (Sheep)	Stall feeding	83	33	39.76	29.67	50.56	1.57	0.05
	Grazing	167	104	62.28	54.74	69.39	-	-
	Both	134	58	43.28	35.08	51.77	-	-
Type of Farming (Cattle)	Farmed	157	66	42.04	34.50	49.87	1.48	0.03
	Free ranged	227	141	62.11	55.67	68.25	-	-
Type of Farming (Buffalo	Farmed	146	54	36.99	29.44	45.04	1.58	0.018
	Free ranged	238	139	58.40	52.06	64.55	-	-
Type of Farming (Goat)	Farmed	143	51	35.66	28.13	43.77	1.58	0.017
	Free ranged	241	136	56.43	50.11	62.60	-	-
Type of Farming (Sheep)	Farmed	138	52	37.68	29.89	45.98	1.54	0.022
	Free ranged	246	143	58.13	51.89	64.18	-	-

Table 5. Association of farm structure and housing system with tick prevalence in livestock population of district Faisalabad, Punjab, Pakistan.

Variables	Levels	Screened	Positive	Prevalence	CI 95%		Odds Ratio	P-Value
					Lower ratio	Upper ratio		
Farm Structure (Cattle)	Cemented	87	32	36.78	27.16	47.27	1.6	0.036
	Uncemented	297	175	58.92	53.25	64.42	-	-
Farm Structure (Buffalo)	Cemented	73	25	34.25	24.06	45.66	1.63	0.05
	Uncemented	301	168	55.81	50.16	61.36	-	-
Farm Structure (Goat)	Cemented	68	21	30.88	20.78	45.28	1.7	0.043
	Uncemented	316	166	52.53	47.02	58.00	-	-
Farm Structure (Sheep)	Cemented	73	23	31.51	21.65	42.81	1.75	0.029
	Uncemented	311	171	54.98	49.42	60.45		
Housing System (Cattle)	Free	143	99	69.23	61.31	76.38	1.29	0.004
	Tethered	134	51	134	30.13	45.60	-	-
	Both	107	57	53.27	43.79	62.57	-	-
Housing System (Buffalo)	Free	174	101	58.05	50.61	65.22	1.5	0.05
	Tethered	119	46	38.66	30.23	47.63	-	-
	Both	91	46	50.55	40.34	60.72	-	-
Housing System (Goat)	Free	198	114	57.58	50.61	64.33	1.53	0.06
	Tethered	101	38	37.62	28.59	47.36	-	-
	Both	85	35	41.18	31.09	51.85	-	-
Housing System (Sheep)	Free	190	109	57.37	50.25	64.26	1.54	0.047
	Tethered	105	39	37.14	28.31	46.68	-	-
	Both	89	47	52.81	42.43	63.01	-	-

Table 6. Association of hygienic system with tick's prevalence in livestock population of district Faisalabad, Punjab, Pakistan.

Variables	Levels	Screened	Positive	Prevalence	CI 95%		Odds Ratio	P-Value
					Lower ratio	Upper ratio		
Hygienic System (Cattle)	Poor	174	114	65.52	58.22	72.30	1.62	0.05
	Good	136	63	46.32	38.06	54.74	-	-
	Excellent	74	30	40.54	29.82	51.99	-	-
Hygienic System (Buffalo)	Poor	221	127	57.47	50.87	63.87	1.87	0.043
	Good	111	50	45.05	35.98	54.37	-	-
	Excellent	52	16	30.77	19.40	44.23	-	-
Hygienic System (Goat)	Poor	204	120	58.82	51.97	65.43	1.83	0.016
	Good	99	41	41.41	32.03	51.30	-	-
	Excellent	81	26	32.10	22.63	42.83	-	-
Hygienic System (Sheep)	Poor	221	131	59.28	52.70	65.61	1.88	0.028
	Good	106	46	43.40	34.20	52.95	-	-
	Excellent	57	18	31.58	20.54	44.45	-	-

Association of tick abundance with the farm structure and housing system

Based on the farm structure, animals were divided into two groups viz; (a) animals kept on the cemented floor and,(b) those kept on the uncemented floor. A higher tick infestation rate was observed in animals kept on the uncemented floor (58.92% in cattle, and 52.53% in goats) than in those kept on the cemented floor (36.78% in cattle, and 30.88% in goats). Statistically, a positive association ($P<0.05$) was found between the farm structure and tick infestation rate (Table 5) which is in agreement with the finding of Iqbal *et al*. (2013) and Rehman *et al*. (2017). Possible causes for higher prevalence of ticks in animals reared on uncemented floors could be (a) favorable niches for the growth of disease vectors, (b) hygienic measures are compromised due to the cracks and slits in the floors, and (c) crevices and the dung-cake heaps provide the best oviposition and off-host nidicolous questing sites for the hard ticks (Muhammad *et al.,*2008;Nicholson et al., 2019).

Hygienic system and tick abundance

The hygienic condition of animals was statistically associated (P<0.05) with tick prevalence. Associations of all the extrinsic factors with the frequency of ticks are shown in Table 6. The qualitative evaluation of the hygienic system was recorded in a semi-quantitative way by numbering from 1-10 (1-4 for poor, 5-8 for good, and 8-10 for excellent) keeping in view the variables like surface coat condition, grooming (Van der Waal et al 2017).Irrespective of the host species, animals kept in poor hygienic conditions showed the highest prevalence of ticks (65.52% in cattle and 57.47% in buffalo) followed in descending order of abundance by those having good hygiene conditions (46.32% in cattle, and 41.41% in goats) and excellent hygiene conditions (40.54% in cattle, and 30.77% in buffalos). Possible reasons for the highest prevalence rate of the hard ticks in the animals reared in poor hygienic conditions may be the presence of dung masses and heaps which contribute towards the higher humidity levels due to the absence of sunlight and provide the best ecology for the vector population (Muhammad *et al.,*2008). Animals that are reared in poor hygienic systems might not be undergone regular grooming or preventive therapeutic management which might have caused the highest abundance of ticks in these animals (Wikel, 2018).

CONCLUSION

The study concludes that *H. anatolicum* is the most prevalent tick species followed in descending order of abundance by *R. microplus, H. marginatum,* and *H. turncatum* in the domestic ruminant population of district Faisalabad, Punjab, Pakistan. Age, the sex were the intrinsic factors, and season, feeding, housing, farming systems, farm structure, and hygienic measures were the extrinsic factors positively associated with the tick infestation rate in the study district.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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