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PREVALENCE OF IXODIDAE TICKS AND THEIR ASSOCIATION WITH DIFFERENT RISK FACTORS IN KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Ixodid ticks are in varying distribution patterns throughout Pakistan. They cause substantial losses to livestock industry in terms of hide loss and vector-borne disease transmission. This study was conducted in three districts of Khyber Pakhtunkhwa (KPK) from March 2018 to February 2019 with the aim of determining the prevalence and seasonal variation in tick species belonging to Ixodidae family in cattle population. A total of 1145 adult ticks were collected by a convenient method of sampling from 434 infested cattle of various breeds, age groups and sexes. Identification of ticks up to genus and species level was done with the help of specific morphological keys. The results showed higher prevalence of*Rhipicephalus (Boophilus) microplus* (77%) followed by *R. (B) annulatus* (70%)*, R. (B) decoloratus* (54%)*, H. anatolicum* (52%) and the leastprevalence was recorded in*R. (B) turanicus* (12%). The prevalence of all ixodid tick species with exception of *R. (B) annulatus* and *R. (B) decoloratus* was significantly associated (p<0.05) with districts, breed, age and sex of cattle. Tick prevalence was higher in Achai and Friesian breeds, young stock and male animals. Prevalence of tick infestation was at its peak during summer and rainy seasons and remained low during winter season. In conclusions, that cattle tick *R. (B) microplus* was the dominant tick species infesting cattle population of KPK province and tick burden presented the highestrecord in summer season.

Key words: Prevalence, Ixodid tick species, Cattle, Season, KPK.

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INTRODUCTION

Ticks are a major group of ectoparasites belong to Arachnida class. There are three main families of ticks. Family Ixodidae (hard ticks) accounting for 727 species, family Argasidae (soft ticks) having 211 species and family Nuttalliellidae with only one species(Barros-Battesti *et al*., 2006). In tropical and subtropical countries, livestock are affected mostly by tick infestation either by blood sucking directly or through transmission of toxins and pathogens indirectly, so they are harmful to economic(Jongejan and Uilenberg, 1994). They transmit pathogens like viruses, bacteria, protozoans and rickettsiae and have been ranked as the first vector of animal diseases while second as human diseases after mosquitoes (Zhou *et al*., 2009). Important hemoparasitic diseases like theileriosis, babesiosis and anaplasmosis are transmitted by ticks (Norval *et al*., 1984) and they result in lowered productivity (Sajid *et al*., 2007). *Rhipicephalus* (*Boophilus*) *microplus* and *R*. (*B*) *decoloratus* were the reported species of ticks in cattle of Cameroon (Silatsa *et al*., 2019).*Dermacentor reticulatus* and *Ixodes ricinus* were the prominent tick species in Lithuania (Sidorenko *et al*., 2021).

Hyalomma tick is the most prevalent tick genus followed by *Boophilus*, *Haemaphysalis* and *Rhipicephalus*, respectively in Kasur, Pakistan (Durrani and Kamal, 2008).*Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum anatolicum*are the tick species identified from various livestock species in Haripur, Mansehra, Kohistan and Shangla of KPK province andGilgit Baltistan province of Pakistan (Sajid *et al*., 2017). *Hyalomma anatolicum* and *R. (B) microplus* are thetick species from Gujranwala, Sheikhupura, Gujrat and Sialkot of Pakistan (Adegoke *et al*., 2020). *Hyalomma hussaini*, *R. (B) microplus*, *R. (B) annulatus, Hy. scupense* and *Hy. anatolicum* were the five identified tick species of hard ticks of family Ixodidae from five different zones of Pakistan (Ghafar *et al*., 2020). *Rhipicephalus (Boophilus) annulatus* is the most predominant species in three temporal zones of KPK, *R. (B) microplus* stands second followed by *Haem. aciculifer*. Besides these, *R. appendiculatus*, *R. decoloratus*, *H. detritium*, *H. anatolicum*, *H. trancatum*, *R. evertsi*, *R. arnoldi*, *R. kochi*, *A. pomposum*, *D. rhinocerinus*, *D. circumguttatus*, *H. excavatum*, *H. houyi*, *H. impeltatum*, *R. distinctus*, *H. aegyptium*, *Haem. parmata*, *H. rufipes*, *R. parvas* and *R. longus* are other

tick species identified(Farooqi *et al*., 2017). *Rhipicephalus microplus*, *R. haemaphysaloides*, *R. sanguineus*, *R. turanicus*, *R. annulatus*, *Haemaphysalis bispinosa*, *Hae. kashmirensis*, *Hae. montgomeryi*, *Hae. cornupunctata*, *Hae. sulcata*, *Hae. montomeryi*, *Hyalomma anatolicum*, *Hy. kumari*, *Hy. isacci*, *Hy. dromedarii*, *Hy. turanicum*, *Hy. scupense*, *Ornithodorus tholozani* and *Argas persicus* are the identified nineteen tick species infesting livestock in Pakistan. *Rhipicephalus haemaphysaloides*, *R. turanicus* and *Haemaphysalis montomeryi* are the tick species identified from KPK province (Karim *et al*., 2017). Therefore, this study was designed to find out the prevalence of tick species along with their seasonal distribution in districts Mardan, Swat and Kohat of Khyber Pakhtunkhwa province.

MATERIALS AND METHODS

Study Area: There are three zones (northern, southern and central) in KPK province based on climatic data such as temperature and rainfall(Farooqi *et al*., 2017). Three districts namely Swat, Kohat and Mardan were selected as the study areas from above zones respectively. These districts were selected based on temporal changes among the zones and the huge population of livestock in these districts. In the study areas, there are four seasons *viz.*winter (November-January), spring (February-April), summer (May-July) and autumn (August-October).

Study Population: The tick samples were collected from different cattle breeds in the selected districts of KPK province. Two periodical visits were made once a week to find out the seasonal prevalence of ticks in the three districts. These tick samples were collected from animals of local farmers and small holder farms. Ticks were collected from 434 infested cattle bya convenient method of sampling (Muhib *et al*., 2001). A total of 1145 ticks were collected from infested cattle (Mardan = 396, Kohat = 369 and Swat = 380). Cattle of both sexes, age and breed were examined, and therefore recorded as a sample unit. The examined breeds of cattle were Friesian, Cross-bred, Achai and Sahiwal.

Collection of ticks and storage: The study was carried out from March 2018 to February 2019. Tick samples were collected from infested animals. A questionnaire was used having information regarding date of collection, place of collection, details about animals(age, sex and breed) and body site of collection (Thrusfield, 2018). Ticks were collected from various body parts of animali.e.,axillary region, neck and back region, perineum and dewlap of animals with the aid of forceps and taking care of their mouthparts (Soulsby, 1982). 70% ethanol solution in labeled disposable tubes was used as preservative of ticks.

Identification of ticks: The preserved tick specimens were examined under stereomicroscope in the entomology laboratory, Department of Parasitology UVAS, Lahore. Identification upto genus and species level was done with the help of specific morphological keys (Karim *et al*., 2017; Keirans and Litwak, 1989).

Statistical Analysis: Data regarding tick prevalence and associated risk factors were analyzed using Pearson's Chi-square (χ^2)test. Specific software named statistical package for social science (SPSS) version 20 was used for Pearson's Chi-square (χ^2). The risk factors association with tick burden was considered significant at *p* value <0.05(Farooqi *et al*., 2017).

RESULTS

The study was conducted to determine the distribution of tick infestation in cattle population of KPK province. *Rhipicephalus* and *Hyalomma* were the two dominant genera in the present study. The identified species were *R. (B) microplus*, *R. (B) annulatus*, *R. (B) decoloratus*, *R. (B) turanicus* and *H. anatolicum*(Fig.2).

It was found that the difference in prevalence of ixodid tick species was significant (p<0.05) among the study districts except *R. (B) annulatus* and *R. (B) decoloratus*. *Rhipicephalus(Boophilus) microplus* was the most prevalent tick species followed by *R. (B) annulatus, R. (B) decoloratus, H. anatolicum* and the least prevalent was *R. turanicus* species, respectively in the studied districts as shown in Table 1. The results showed a significant association (p<0.05) of tick infestation with all the cattle breeds except *R. (B) annulatus* and *R. (B) decoloratus*. *Rhipicephalus (Boophilus) microplus, R. (B) annulatus* and *H. anatolicum* were the predominant tick species in local breed (Achai) and *R. (B) decoloratus* and *R. (B) turanicus* in exotic breed (Friesian), respectively (Table 2). It was observed from the results that there was significant association (p<0.05) of tick species infestation with age and sex of the cattle among study districts except *R. (B) annulatus* and *R. (B) decoloratus* wherethere was non-significant association (p>0.05) as shown in Tables 3 and 4. The prevalence of tick species infestation was higher in young than adult cattle (Table 3). Similarly, male cattle were infested more with tick species than females in the studied districts (Table 4). It was observed from the study that there was higher ratio of male tick species than females except *R. (B) turanicus* where female ticks were higher in number than males as shown in Table 5. The study showed that there was significant association of tick infestation (p<0.05) with the seasons among the investigated districts. The prevalence of tick infestation presented higher in summer, followed by autumn, spring and lower in winter as shown in Table 6. Similarly, prevalence of tick species infestation recorded the highest in July followed by June

and August, respectively and the lowest one in December followed by January and February, respectively as shown in Fig. 3.

Fig. 1. Map of Pakistan showing three targeted districts of Khyber Pakhtunkhwa province (KPK) i.e., Mardan, Kohat and Swat. Tick samples were collected from these three districts.

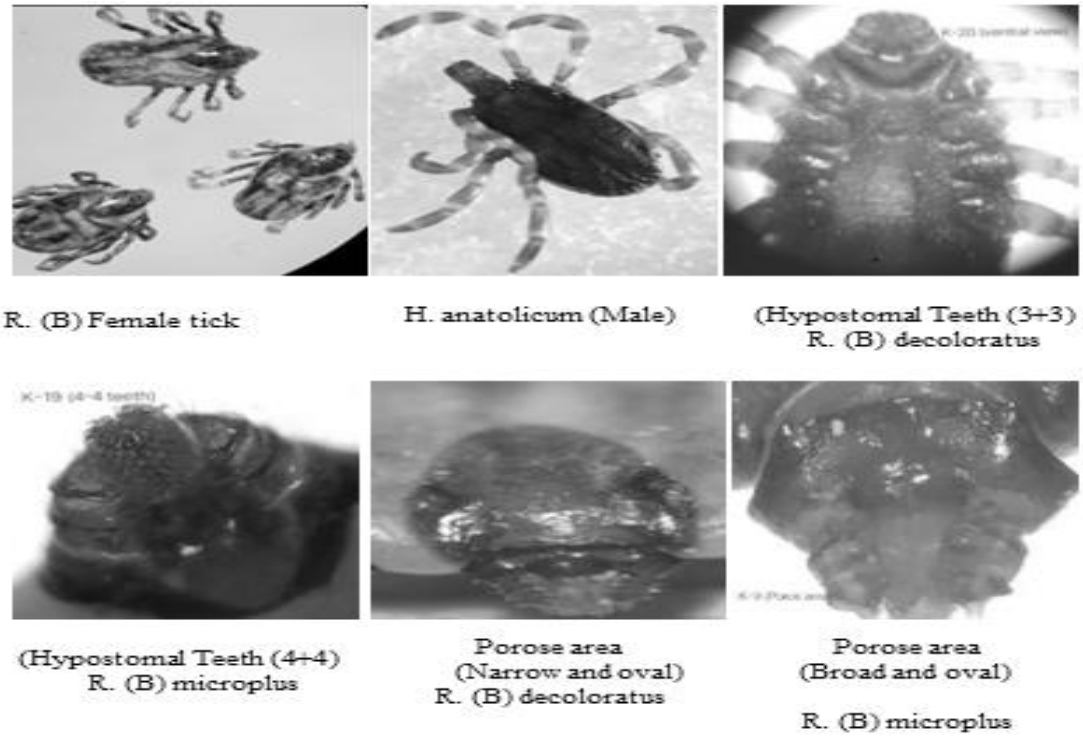

Fig. 2. Different ixodid ticks species of KPK. Basic morphological structures of tick like scutum, shape of porose area and hypostomal teeth configuration which are helpful in ixodid ticks species identification.

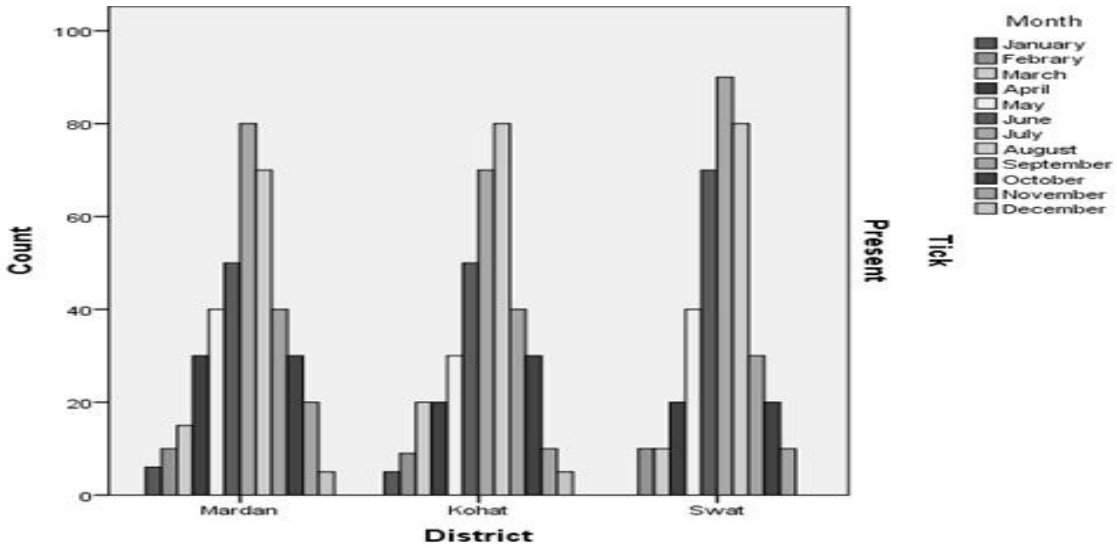

Fig. 3. Seasonal prevalence of ixodid ticks in three districts of KPK. The ticks were in abundance in summer, followed by autumn, spring and winter season, respectively in the studied districts. Month wise prevalence shows that tick prevalence was highest in July followed by August and June respectively and was lowest in December followed by November and October respectively.

Table 1. Prevalence of ixodid tick species in three districts of KPK, Pakistan.

Tick species	Mardan n(%)	Kohat n(%)	Swat n(%)	Total n(%)	p-value
R.(B) microplus	156(97.50)	122(93.10)	55(38.51)	333(76.70)	0.000
R. (B) annulatus	110(68.81)	96(73.31)	97(67.80)	303(69.80)	0.577
R. (B) decoloratus	80(50.00)	70(53.41)	83(58.01)	233(53.70)	0.374
H. anatolicum	40(25.00)	81(61.81)	105(73.41)	226(52.10)	0.000
R. (B) turanicus	10(06.30)	0.00	40(28.01)	50(11.50)	0.000

Note: H. =*Hyalomma*, B. =*Boophilus,* R. =*Rhipicephalus*

Table 2. Ixodid tick species prevalence in cattle breeds.

Tick species	Breed	Mardan n(%)	Kohat n(%)	Swat n(%)	Total n(%)	p-value
R. (B) microplus	Friesian	35(100.00)	14(82.40)	14(43.81)	63(75.00)	0.000
	Crossbreed	66(95.71)	60(93.81)	22(31.90)	148(73.30)	0.000
	Achai	45(100.00)	38(97.41)	14(42.42)	97(82.90)	0.000
	Sahiwal	10(90.90)	10(90.91)	05(55.62)	25(80.60)	0.078
R. (B) annulatus	Friesian	24(68.60)	10(58.81)	23(71.92)	57(67.90)	0.644
	Crossbreed	48(69.60)	45(70.31)	46(66.72)	139(68.80)	0.890
	Achai	33(73.30)	31(79.51)	23(69.72)	87(74.40)	0.625
	Sahiwal	05(45.50)	10(90.91)	05(55.61)	20(64.50)	0.067
R. (B) decoloratus	Friesian	20(57.10)	08(47.11)	22(68.81)	50(59.50)	0.315
	Crossbreed	36(52.20)	29(45.30)	35(50.71)	100(49.50)	0.709
	Achai	20(44.40)	25(64.11)	21(63.61)	66(56.40)	0.119
	Sahiwal	04(36.41)	08(72.70)	05(55.61)	17(54.80)	0.230
H. anatolicum	Friesian	12(34.30)	08(47.11)	25(78.11)	45(53.60)	0.001
	Crossbreed	12(17.41)	37(57.80)	49(71.00)	98(48.50)	0.000
	Achai	14(31.11)	28(71.80)	26(78.81)	68(58.10)	0.000
	Sahiwal	02(18.20)	08(72.71)	05(55.60)	15(48.40)	0.033
R. (B) turanicus	Friesian	10(28.60)	0.00	10(31.21)	20(23.80)	0.035
	Crossbreed	0.00	0.00	16(23.20)	16(07.90)	0.000
	Achai	0.00	0.00	09(27.30)	09(07.70)	0.000
	Sahiwal	0.00	0.00	05(55.60)	05(16.10)	0.001

Table 3. Ixodid tick species prevalence according to the age of cattle.

Tick species	Age	Mardann(%)	Kohat n(%)	Swat n(%)	Total n(%)	p-value
R. (B) microplus	Young	34(100.00)	31(91.20)	15(46.91)	80(80.00)	0.000
	Adult	122(96.80)	91(93.81)	40(36.00)	253(75.70)	0.000
R. (B) annulatus	Young	22(64.70)	26(76.51)	24(75.01)	72(72.00)	0.502
	Adult	88(69.80)	70(72.22)	73(65.81)	231(69.20)	0.595
R. (B) decoloratus	Young	17(50.00)	17(50.01)	21(65.61)	55(55.00)	0.342
	Adult	63(50.00)	53(54.61)	62(55.90)	178(53.30)	0.634
H. anatolicum	Young	09(26.51)	22(64.70)	24(75.02)	55(55.00)	0.000
	Adult	31(24.61)	59(60.80)	81(73.01)	171(51.20)	0.000
R. (B) turanicus	Young	05(14.71)	0.00	12(37.50)	17(17.00)	0.000
	Adult	05(04.01)	0.00	28(25.21)	33(09.90)	0.000

Table 4. Sex wise prevalence of ixodid tick species in cattle.

Tick species	Sex	Mardan n(%)	Kohat n(%)	Swat n(%)	Total n(%)	p-value
R. (B) microplus	Male	48(100.00)	41(95.31)	17(43.62)	106(81.50)	0.000
	Female	108(96.40)	81(92.00)	38(36.51)	227(74.70)	0.000
R. (B) annulatus	Male	35(72.91)	35(81.40)	30(76.92)	100(76.90)	0.632
	Female	75(67.00)	61(69.31)	67(64.42)	203(66.80)	0.772
R. (B) decoloratus	Male	27(56.20)	24(55.81)	27(69.22)	78(60.00)	0.372
	Female	53(47.30)	46(52.31)	56(53.81)	155(51.00)	0.606
H. anatolicum	Male	13(27.11)	30(69.81)	31(79.50)	74(56.90)	0.000
	Female	27(24.11)	51(58.00)	74(71.22)	152(50.00)	0.000
R. (B) turanicus	Male	03(06.20)	0.00	13(33.31)	16(12.30)	0.000
	Female	07(06.20)	0.00	27(26.00)	34(11.20)	0.000

Table 5. Sex ratio of ixodid tick species.

S. No	Tick Species	Male	Female	M:F	Total
1	*R. (B) microplus*	200	133	1.50:1	333
2	*R. (B) annulatus*	198	105	1.89:1	303
3	*R. (B) decoloratus*	143	90	1.6:1	233
4	*H. anatolicum*	140	86	1.63:1	226
5	*R. (B) turanicus*	20	30	1:1.5	50
6	Total	701	444	1.74:1	1145

Table 6. Seasonal prevalence of ixodid tick species.

Tick Species	Winter n(%)	Spring n(%)	Summer n(%)	Autumn n(%)	p-value
R. (B) microplus	24(07.20)	69(20.71)	130(39.00)	110(33.00)	0.000
R. (B) annulatus	13(04.30)	20(06.61)	150(49.51)	120(39.60)	0.002
R. (B) decoloratus	08(03.40)	15(06.41)	120(51.50)	90(38.60)	0.004
H. anatolicum	16(07.11)	40(17.70)	90(39.80)	80(35.40)	0.023
R. (B) turanicus	0.00	0.00	30(60.00)	20(40.00)	0.007

DISCUSSION

The results showed that*Rhipicephalus (Boophilus) microplus* was the most prevalent tick species followed by *R. (B) annulatus, R. (B) decoloratus, H. anatolicum* and the least prevalent was *R. (B) turanicus* species respectively over the studied districts. Similar study was done by Mustafa *et al*.(2014) who reported that there was higher prevalence in*R. (B)microplus* (22.59%) followed by *R. (B) annulatus* (17.15%) and *H. anatolicum* (9.03%). Farooqi *et al*. (2017),studied the prevalence of ixodid ticks in three temporal zones of Khyber Pakhtunkhwa (KPK)and found that *R. (B) annulatus* (41.67%) was the predominant tick followed by *R. (B) microplus* (18.42%),*R. appendiculatus* (8.25%) and *R. decoloratus* (6.83%). They observed that

sex and age of the animal were significantly (p<0.05) associated with tick infestation which correlates with this study. A number of other researchers studied the prevalence of ixodid tick species in different regions of the world likeSajid *et al*. (2009), Telmadarraiy *et al*. (2010),Kabir *et al*. (2011), Atif *et al*. (2012), Asmaa *et al*. (2014), Mandloi *et al*. (2016), Tesgera *et al*. (2017), Shanan *et al*. (2017), Ghashghaei *et al*. (2017), Kaur *et al*. (2017), Silatsa *et al*. (2019), Ghafar *et al*. (2020) and Sidorenko *et al*. (2021) which coincide with this study.

The results showed that tick infestation was significantly associated (p<0.05) with age of the cattle among the studied districts of KPK. Young animals were affected more with tick species infestation than adults. These findings are in agreement with the studies conducted by Kabir *et al*. (2011), Dehuri *et al*. (2017) and Godara *et al*. (2018). However, the results of this study disagree with those of Manan *et al*. (2007) and Vatsya *et al*.(2007) according to whom tick infestation were more in adult than young cattle. Young cattle have less developed immune system as a result of less exposure to tick vectors and the farmers give more attention to adults and lactating animals than young animals are among the possible reasons for higher tick infestation in young cattle. On the other hand, justifications for above studies with higher prevalence of tick species infestation in adult than young animals can be ascribed to immune system less performant in adults due to poor body status and less hygienic conditions(Farooqi *et al*., 2017; Rony *et al*., 2010).

Sex of the animal is another important determinant of tick infestation. In this study there was higher prevalence of tick species infestation in male than female cattle. These findingsare consistent with those of Sajid *et al*. (2009) and Musa *et al*. (2014) according to whom male cattle were infested more with tick species than female cattle. Female cattle are kept by farmers for milk production, so receive more attention than male which are kept for meat and drought purposes and receiving less attention is a justification of higher tick infestation in male than female cattle (Sajid *et al*., 2009). However, our results do not coincide with those reported by Rony *et al*. (2010),Kabir *et al*. (2011), Asmaa *et al*.(2014) and Tafesse and Amante (2019) who reported that male cattle are affected less with tick infestation than female.

In the current study, the results revealed that there was mixed tick species infestation in cattle breeds. *Rhipicephalus (Boophilus) microplus, R. (B) annulatus* and *H. anatolicum* infestationswere higher in local breed (Achai) than exotic breeds. This study matches with the work done by Kabir *et al*.(2011)who reported higher tick infestations in local cattle (43.82%) than crossbred (24.13%). As the exotic breeds are used for higher milk and meat production, the farmers give more attention and care to these breeds than local breeds. So, this can be the reason for higher tick infestation in local than exotic breeds. On the other hand,*Rhipicephalus (Boophilus) decoloratus* and *R. (B) turanicus* tickspecies infestations were recorded higher in exotic breed (Friesian) than local breeds. This correlates with the findings of Sajid *et al*.(2009),Atif *et al*.(2012) andFarooqi *et al*.(2017). Justification for this higher tick infestation in exotic breeds is that indigenous breeds have developed better resistance than European breeds due to their constant exposure (Jongejan and Uilenberg, 2004;Sajid *et al*., 2009).

It was observed from the study that male ticks were higher in ratio than female ticks except *R. (B) turanicus*. This is in full agreement with Telmadarraiy *et al*. (2010) and Tadesse and Sultan (2014). The adult female ticks drop off to land after taking the blood meal and lay eggs while males remain attached for several months on host to start feeding, mating with other female ticks, mature and finally drop off to ground so males remain attached to host for longer time than female ticks. This fact could be the possible reason for male tick dominance than females as discussed in Gebre *et al*. (2001).

According to the seasonal prevalence of ticks it was clear from the results that the ticks were in abundance in summer, followed by autumn, spring and winter season respectively in the investigated districts. Tick number was higher in the month of July and was lower in December. The findings of this study correlate with Kumar *et al*. (2004),Islam *et al*. (2006), Vatsya *et al*. (2007),Sajid *et al*. (2009), Kabir *et al*. (2011), Mustafa *et al*. (2014), Asmaa *et al*. (2014), Negi and Arunachalam (2019) and Al-Mayah and Abdul-Karim (2020)who found similar results and justifications about the seasonal prevalence of tick infestations in animals during their studies.This higher prevalence of tick infestation in summer and autumn seasons could be assigned to higher temperature and humidity in environment due to heavy rains (rainy season) which favor the growth of ticks. Similarly, reason for lower number of ticks in winter season is the decreased temperature, dry weather and short- day which retard tick growth,while in spring season tick start increasing their number as the temperature increases (Sajid *et al*., 2009; Mustafa *et al*., 2014).

Conclusion: The study concludes that *R. (B) microplus* was the most prevalent tick species followed by *R. (B) annulatus, R. (B) decoloratus, H. anatolicum* and the least prevalent was *R. turanicus* species respectively among the studied districts of KPK province. Districts, breed, age and sex of cattle were found significantly (p<0.05) associated with tick infestation except *R. (B) annulatus* and *R. (B) decoloratus*. Tick prevalence was the highest in Achai and Friesian breeds, young stock and male animals. There was higher male tick ratio than

female. Prevalence of tick species were at their peak during summer and rainy seasons and remained low during winter season.

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