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Distribution of Ixodid Tick Species and Associated Risk Factors in Temporal Zones of Khyber Pakhtunkhwa Province, Pakistan

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SHF, MI, MIR conceived and designed the projecr and conducted research work. MHS, AA, AIA arranged and analyzed the data statistically. SHF, AIA wrote and reviewed the manuscript. SHF, SM arranged and performed GIS Mapping.

Key words
Prevalence, Temporal Zones, Ticks, Ixodid, Risk factors.

ABSTRACT

Distribution of various *Ixodid* tick species and risk factors associated with tick infestation and burden levels were studied in bovine from three distinct temporal zones of Khyber Pakhtunkhwa province of Pakistan. Twelve hundreds ticks were collected from four hundreds animals comprising of two hundred and fifty cattle and one hundred and fifty buffaloes. Descriptive statistics with Pearson's Chi-square test and regression model were applied to analyze the data. The results of study found *Rhepicephalus* the most prevalent genus followed by *Heamaphysalis, Hyalomma, Dermacenter* and *Amblyomma* with the prevalence of 78.50%, 10.33%, 10.08%, 0.67% and 0.42%, respectively. On species basis, *R. (Boophilus) annulatus, R. (Boophilus) microplus, Heamaphysalis aciculifer, R. appendiculatus,* and *R. decoloratus* were noted 41.67, 18.42, 9.83, 8.25, and 6.83% respectively, whereas least prevalence was noted as 0.42% in case of *A. pomposum* and *D. circumgguttatus;* 0.25% each was shown by *D. rhinocerinus, Heam. Excavatum,* and *H. impeltatum;* 0.17% exhibited by *Heam. Houyi and R. distinctus;* and 0.08% each displayed by *Heam. Parmata, and H. egyptium, H. rufipes, R. longus* and *R. parvas*. Risk factors analysis namely housing type, tick control, age and sex of animal presented significant ($P < 0.05$) association with tick infestation and burden while type of breed showed significant association with tick infestation but was non-significant with tick burden. Topography presented inverse behavior to that of breed with tick burden and tick infestation. On the other hand, geo-location was only factor exhibiting non-significant ly associated ($P > 0.05$) association with both dependent variables. The study concluded that *Rhepicephalus* was the most prevalent among the *Ixodid* genera whereas presumed risk factors were strongly associated with tick infestation and tick burden.

INTRODUCTION

Globally ticks are transmitting comparatively wider range of pathogenic bacteria, protozoa, rickettsiae, spirochaets, and viruses, and are considered the most important vectors of livestock, humans, and companion animal diseases (Ghosh *et al.*, 2007). These ticks carry different diseases like, protozoal *i.e.* babesiosis and theileriosis (Durrani and Kamal, 2008) and rickettsial; anaplasmosis (Kocan *et al.*, 2004). Ticks can cause paralyses, toxicoses, allergic reactions and are vectors of a broad range of viral, rickettsial, bacterial and protozoan pathogens. The ticks identified in Pakistan from various livestock species are; *Rhipicephalus sanguineus, Rhipicephalus (Boophilus) microplus, R (B) annulatus, Hyalomma anatolicum anatolicum, H. isaacii, H. aegyptium,* and *Dermacentor marginatus* (Kaiser and Hoogstraal, 1964). Ticks infestation is a somber issue of livestock in Khyber Pakhtunkhwa province of Pakistan (Perveen *et al.*, 2010). The ticks genera reported in Punjab province of Pakistan includes; *Hyalomma, Boophilus* (now included in genus *Rhipicephalus*), *Haemaphysalis* and *Rhipicephalus* (Durrani and Kamal, 2008) with varying prevalence in different regions of the province. The variation in tick prevalence in different areas is attributed to a variety of factors like geo-climatic

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conditions, association and life style of different species of animals, awareness/education of the farmers and farm management practices (Ghosh *et al*., 2007). Besides these, *Dermacentor* (Ghosh *et al*., 2007) and *Amblyomma* (Ali *et al*., 2013) have also been reported. Over 20 ixodid tick species are often found on humans exposed to infested vegetation, four of these are *Amblyomma* spps, seven *Dermacentor*, three *Haemaphysalis*, two *Hyalomma* and six are *Ixodes* spps (Estrada and Jongejan, 1999). Till now there is insufficient data about *Ixodid* ticks in Khyber Pakhtunkhwa (KPK) province. Keeping in view the dire need of tick infestation estimation, the current study is an effort to identify various tick species and their distribution along with their associated risk factors in three distinct temporal zones of KPK.

MATERIALS AND METHODS

Study area

The study area included three temporal zones of province KPK *i.e.* district Buner, Mardan and Bannu. The temporal zones were selected based on climatic variation among the zones and districts densely populated with livestock were selected so as to make the data more reliable representative for the province. Temporal zone of district Buner lies between 34-9º and 34-43º N latitude and 72-10º and 72-47º E longitude with 44 to -2°C temperature extremes, having average rainfall of approximately 762 mm. District Mardan, the central zone, lies at 34° 05' to 34° 32' north latitudes and 71" 48' to 72° 25' east longitudes with highest temperature climax of 46.5°C in June that falls to 0.5°C in January, whereas average annual rainfall goes to 559 mm. District Bannu, southern zone, occupies 32.9861° north and 70.6042° east measuring 23.6 °C the average annual temperature with 327 mm of average rainfall. These zones have varying livestock populations and climatic variables.

Study population and sampling method

The ticks were collected from cattle and buffaloes during the months of June to September of year 2015. A total of 400 animals comprising of cattle (n=250) and buffaloes (n=150) inclusive of 100 tick free animals were studied. The total number of 1200 ticks (n= 400 ticks per zone) were collected from 300 animals (n=100 from each zone) by convenient sampling method. Additional 100 tick free animals were studied from the three regions to compare the association with possible risk factors (n=34 from Bannu, n=33 from Buner, n=33 from Mardan).

Epidemiological survey

The risk factors namely breed, sex of animal, housing system (wooden or concrete), different topographical locations, and exposure to acaricidals that had presumed association with tick infestation and tick burden were recorded using dichotomous questionnaire (Thrusfield, 2013). Age based categories of animals include bullocks/ cows (5–10 years), cattle young stock (3–5 years), buffalo bullocks (5–10 years) and buffalo young stock (3–5 years). The cattle breeds as important risk factor were recorded as Sahiwal (*B. indicus*), Achai (*B. indicus*), Friesian (*B. taurus*), Jersey (*B. taurus*), cross-bred (*Bos indicus* × *Bos taurus*) while those of buffaloes (*Bos bubalus babalis*) were included in the names of Nili Ravi, Kundi and Azakheli.

Ticks collection and storage

Ticks were collected at dawn and dusk during June to September months of year 2015. Tick's burden was divided into three categories *i.e.* low, moderate and high burden groups. Animals having 1-25, 26-50 and >50 ticks were designated as low, moderate and high burden groups, respectively (Ali *et al*., 2013). Ticks were collected from various body regions of animals *i.e.* dewlap, axillary region and perineum of animals with the help of forceps. The collected tick's specimens were stored in labeled disposable containers having 70% ethanol solution to prevent shattering and to preserve their morphological features.

Ticks identifications

The collected ticks' samples preserved in 70% ethanol were carried to Entomology Laboratory, Department of Parasitology, University of Veteriniary and Animal Sciences, Lahore. Identification of ticks was performed using morphological keys mentioning genus identification (Taylor *et al*., 2007), and specie level identification (Keirans and Litwak, 1989; Yeoman *et al*., 1967).

Statistical analysis

Data regarding ticks' prevalence and associated risk factors were analyzed with descriptive statistics, Pearson's Chi-square test (Aqib *et al*., 2017) and regression model test using SPSS (statistical package for social sciences) version 20. Risk factors were associated with the tick burden at P value < 0.05 was considered significant.

RESULTS

The initial confirmatory correlation and later on applying the binary logistic regression model with particular Hosmer Lameshow goodness of fit test at 95% confidence interval predicted 75% accuracy ability of the model. Moreover Hosmer and Lameshow test was found significantly fit for the current study at predictor value of

1.00. The current study concluded 100% variance (R^2 = 1.00) in dependent variables *i.e.* tick infestation and tick burden. The Omnibus tests of model coefficients predicted highly significant (P<0.01) correlation with all variables except age and location (Table I).

The study found significant difference (P<0.05) in each temporal zones of KPK province of Pakistan on genera and species level while sex and developmental stages of ticks were non-significantly (P>0.05) differed (Table II). Among genera *Rhepicephalus* was significantly prevalent (P<0.05) in each zone of KPK province followed by *Heamaphysalis, Hyalomma, Dermacente,* and *Amblyomma*. Zone wise, *Rhepicephalus* was higher in Bannu, *Heamaphysalis* in Mardan, *Hyalomma* in Bannu, while *Dermacenter* and *Amblyomma* were found greater in number in Buner district of KPK province. Among the tick species, *R(B). annulatus* was the most prevalent specie followed by *R(B). microplus* and *Heam. aciculifer* were highly prevalent. Besides these, other ticks species identified include; *R.appendiculatus*, *R. decoloratus*, *H. anatolicum*, *H. detritium*, *H. trancatum*, *R. evertsi*, *R. kochi*, *R. arnoldi*, *A. pomposum*, *D. circumguttatus*, *D. rhinocerinus, Heam. excavatum*, *H. impeltatum*, *Heam. houyi*, *R. distinctus*, *Heam. Parmata*, *H. egyptium*, *H. rufipes*, *R. longus* and *R. parvas*. The data regarding sex of tick presented non-significant (P>0.05) results in all temporal zones. Similar pattern was observed when developmental stages of ticks were studied (Table II).

Table I.- Prevalence (%) of Tick genera, species, sex and developmental stages in different temporal zones of KPK.

Variables		Northern Zone N (%)	Central Zone N (%)	Southern Zone N (%)	P- value
Tick genera	*Rhepicephalus*	314 (78.50)	306 (76.50)	322 (80.50)	0.001*
	Heamaphysalis	035 (08.75)	055 (13.75)	034 (08.50)	
	Hyalomma	038 (09.50)	039 (09.75)	044 (11.00)	
	Dermacenter	008 (02.00)	000 (00.00)	000 (00.00)	
	Amblyomma	005 (01.25)	000 (00.00)	000 (00.00)	
Tick species	*A. pomposum*	005 (01.25)	000 (00.00)	000 (00.00)	0.001*
	D. rhinocerinus	003 (0.750)	000 (00.00)	000 (00.00)	
	D. circumguttatus	005 (01.25)	000 (00.00)	000 (00.00)	
	Heam. Aciculifer	030 (07.50)	055 (13.75)	033 (08.25)	
	Heam. Parmata	001 (0.250)	000 (00.00)	000 (00.00)	
	Heam. Excavatum	002 (0.500)	000 (00.00)	001 (00.25)	
	Heam. Houyi	002 (0.500)	000 (00.00)	000 (00.00)	
	H. anatolicum	023 (05.75)	020 (05.00)	012 (03.00)	
	H. trancatum	000 (00.00)	000 (00.00)	028 (07.00)	
	H. detritium	011 (02.75)	019 (04.75)	003 (0.750)	
	H. egyptium	000 (00.00)	000 (00.00)	001 (0.250)	
	H. impeltatum	003 (0.750)	000 (00.00)	000 (00.00)	
	H. rufipes	001 (0.250)	000 (00.00)	000 (00.00)	
	R. annulatus	184 (46.00)	159 (39.75)	157 (39.25)	
	R. evertsi	005 (01.25)	009 (02.25)	008 (02.00)	
	R. microplus	072 (18.00)	074 (18.50)	075 (18.75)	
	R. decoloratus	041 (10.25)	024 (06.00)	017 (04.25)	
	R. distinctus	002 (0.500)	000 (00.00)	000 (00.00)	
	R. arnoldi	001 (0.250)	002 (0.500)	003 (0.750)	
	R.appendiculatus	008 (02.00)	038 (09.50)	053 (13.25)	
	R. longus	000 (00.00)	000 (00.00)	001 (0.250)	
	R. kochi	000 (00.00)	000 (00.00)	008 (02.00)	
	R. parvas	001 (0.250)	000 (00.00)	000 (00.00)	
Sexes of ticks	Male	084 (21.00)	078 (19.50)	076 (19.00)	0.761
	Female	316 (79.00)	322 (80.50)	324 (81.00)	
Developmen-tal Stage	Nymph	21 (05.25)	014 (03.50)	015 (03.75)	0.408
	Adult	379 (94.75)	386 (96.50)	385 (96.25)	

Table II.- Levene's test for between the subjects effects.

Variable	Dependent variables	Type III sum of square	df	Mean square	F	P-value
Location	Tick infestation	0.001436	1	0.001436	0.020275	0.887
	Tick Burden on animal	0.24071	1	0.24071	0.396622	0.529
Housing	Tick infestation	2.507594	1	2.507594	35.41049	0.000
	Tick Burden on animal	8.051092	1	8.051092	13.26591	0.000
Tick control	Tick infestation	5.510485	1	5.510485	77.81523	0.000
	Tick Burden on animal	17.41349	1	17.41349	28.69247	0.000
Topography	Tick infestation	0.013589	1	0.013589	0.191891	0.662
	Tick Burden on animal	2.537108	1	2.537108	4.180432	0.042
Age	Tick infestation	1.969907	2	0.984954	13.90883	0.000
	Tick Burden on animal	9.438127	2	4.719064	7.775674	0.000
Breed	Tick infestation	3.249468	7	0.46421	6.555245	0.000
	Tick Burden on animal	7.902172	7	1.128882	1.860076	0.075
Specie	Tick infestation	0	0	NA	NA	NA
	Tick Burden on animal	0	0	NA	NA	NA
Sex	Tick infestation	0.737408	1	0.737408	10.41316	0.001
	Tick Burden on animal	4.361623	1	4.361623	7.186713	0.008

Table III.- Odd ratios of different factors responsible for tick infestation in large ruminants in KPK, Pakistan June to September in 2015.

Variables	B	S.E	Sig	Exp (B)	95.0% C.I. for Exp (B)	
					Lower	Upper
Location	0.137	1.450E3	0.809	1.147	0.960	1.46
Species	-1.346	5.090E3	0.000	0.260	0.031	0.45
Breed	0.168	998.697	0.000	1.183	0.760	1.92
Age	-0.043	1.454E3	0.664	0.958	0.614	1.32
Sex	0.120	2.487E3	0.000	1.127	0.831	1.41
Housing	-0.956	2.439E3	0.000	0.384	0.051	0.67
Tick control	0.574	2.327E3	0.000	1.776	1.134	2.75
Topography	0.485	2.718E3	0.001	1.623	1.113	2.68

The "Levene's test for between subject effects" was applied on collected information regarding presumed risk factors like; housing type, tick control, age and sex of animal, which showed significant association ($P<0.05$) with both tick infestation and burden. The data regarding breeds was found significantly ($P<0.05$) associated with tick infestation but with tick burden it was noted non-significant ($P>0.05$). The topography was noted vice versa of breed's pattern of association with tick burden and infestation in that tick infestation was non-significantly associated (P>.0.05) whereas tick burden was significantly ($P<0.05$) associated with topography. Apart from all only the geo-location was not found risk factor in occurrence of tick infestation (Table III).

DISCUSSION

The higher prevalence of *Rhepicephalus* and *Heamaphysalis i.e.* 78.5 and 10.33%, respectively, are in line with the finding of Islam *et al.* (2006) who reported *Rhepicephalus (Boophilus*) the most predominant genus followed by *Heamaphysalis* in three distinct topographic zones of Bangladesh, *viz.* flood plains, hills and steppe 'Barind'. The justification of higher prevalence of above stated genera was correlated with high to moderate rainfall as quoted by Islam *et al.* (2006) coincides with current study. The higher tick intensity is also justifiable with progressing rainy season that make ticks' propagation suitable because of warm and humid environment (Lima *et al.*, 2000). The decreasing pattern of prevalence of *Hyalomma* in current study was associated with rainy to semi-arid characteristics of temporal zones which is supported by Islam *et al*. (2006) and Harwood and James (1979).

Some of livestock associated ticks genera like *Hyalomma*, *Rhipicephalus* and *Amblyomma* were reported in previous study conducted by Muhammad *et al*. (2008). However five genera, *Rhepicephalus, Hyalomma, Heamaphysalus, dermacenter* and *Amblyomma*, identified in this project were also reported in neighbor countries like India and Bangladesh (Ghosh *et al*., 2007). Not to this only, Turkey was also reporting *Ixodes*, *Haemaphysalis, Hyalomma, Dermacentor,* and *Rhipicephalus* as major ticks' genera (Aydin and Bakirci, 2007). These genera are reported as found wide spread in the Anatolia region of turkey. The suitable hosts for these vectors and their resultant maladies are reported to be cattle, sheep, horses and dogs. The reason for the presence of these genera in Pakistan can be attributed to the practice of mixed animal's species rearing in majority of animals facilities over the country. The prevalence rates vary between Turkey and Pakistan, which might be due to the variations in the climatic dynamics between the two regions. Climatic conditions dictate the dynamics of ticks and tick-borne diseases by influencing the distribution of ticks and their seasonal occurrence. Steadfast foretelling models are required to determine the direct effect of climatic shift on the burden of ticks and tick-borne diseases. The bio-ecology of TBDs is complicated, as is the impact of climate on spatial and regional variability in ticks and tick-borne diseases. The comparative impact of climate is frequently difficult to discriminate from variability in other determinants that are not directly climatic. Climate variations may influence not only tick biology but also indirectly affects the host ecology and abundance, causing appearance of TBDs in some regions and their disappearance in other areas (Ahmed *et al*., 2007). The data on tick distribution in Pakistan compared to other countries is still scarce.

The tempral zones of KPK province of Pakistan exhibited non-significant difference with tick burden and tick infestation which is contradicted to the studies conducted in other provinces of Pakistan (Durrani and Kamal, 2008; Khan *et al*., 1993; Sajid *et al*., 2008). The justification of this contraindication might be the fact of free movement of animals across the districts, higher number of small herds with unawareness of tick control measure, and mixed species rearing in KPK that makes no significant difference of tick infestation and burden among the temporal zones.

The significant association of housing type *i.e.* wooden and concrete type with tick infestation and burden of current study is line with the findings of Muhammad *et al*. (2008) and Chhabra (1992). The significant association is attributed to cracks and crevices present in wooden houses that provide hiding space for the ticks. Tick control measure was also important risk factor that presented predominant association coinciding with results of Shimizu *et al*. (2000) stating application of acaricides against theileriosis on animal body a good practice in its control.

A non-significant correlation of topography with tick burden whereas its significant association with tick infestation was also supported by findings of Kumsa *et al*. (2012), Fourie and Kok (1992) and Heath *et al*. (1977). The higher tick burden is justifiable with movement of infested animals from highlands to low lands that supports tick propagation. The higher tick infestation and burden in cattle significantly differing to that of buffaloes is attributed to thin hairy coat of cattle that makes cattle more prone to tick infestation (Sajid *et al*., 2009). Higher tick infestation in exotic breeds and crossbreds compared to indigenous breeds, within specie, was also reported by L'Hostis *et al*. (2006) and Atif *et al*. (2012). Wambura *et al*. (1998) have also reported higher tick resistance in *B. indicus* than *B. taurus* and their crosses. This resistance pattern was explained by higher serum complements in indigenous breeds (Sajid *et al*., 2009). The inherited traits in *B. indicus* also explain resistance in indigenous breeds (Jongejan and Uilenberg, 2004).

The higher susceptibility of older animals (7-9 year), followed by younger (1-3year), and young adult age (4-6year) to tick infestation and burden resembled to findings of studies carried out by Manan *et al*. (2007), Vatsya *et al*. (2007) and Kabir *et al*. (2011). The buildup of immunity by repeated exposure in adult animals explains less infestation compared to younger animals. Kabir *et al*. (2011) reported young cattle to be 2.23 times more susceptible to tick infestation than adult cattle in Pakistan. Higher susceptibility of older animals to tick infestation and burden is explainable by poor body conditions which directly affect the immune status of the animals. Similar justification has been reported by Rony *et al*. (2010).

The current study reported tick infestation and burden form external body along with risk factors that will be extended to gut and blood specimens to identify the ticks in next study.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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