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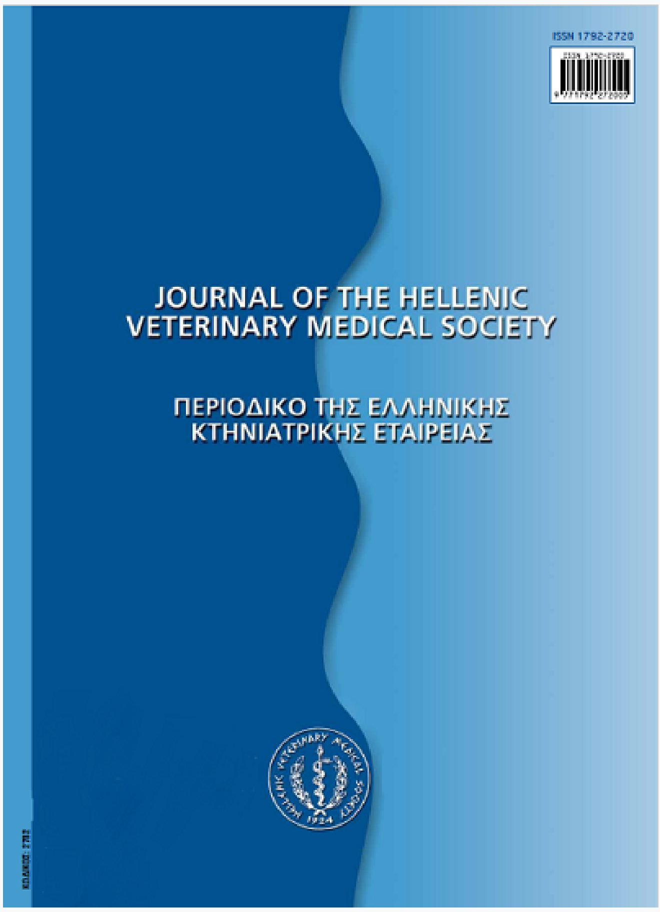

Distribution and abundance of ticks infesting livestock population along Karakorum highway from Mansehra to Gilgit, Pakistan

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Distribution and abundance of ticks infesting livestock population along Karakorum highway from Mansehra to Gilgit, Pakistan

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ABSTRACT. Ticks and tick-borne infections pose major economic threats to the livestock industry throughout the world. The present study reports the point prevalence of ticks in the free range livestock population of the plain and hilly areas of Khyber Pakhtunkhwa (Mansehra, Haripur, Shangala, Kohistan), and Gilgit Baltistan (Diamer, Gilgit, Astor) along the Karakoram highway. Through convenient and snowball sampling techniques, a total of 813 animals, consisting of 232 sheep, 163 cattle, 365 goats and 53 buffaloes were screened for their tick burden. During the study, the two tick species identified were *Hyalomma anatolicum* and *Rhipicephalus microplus.* The overall prevalence of tick infestation among the screened livestock was 75.03%, with the highest distribution in sheep (81.47%) followed, in order, by cattle (77.91%), goats (72.05%) and buffalo (58.49%). The district-wise prevalence of ticks was the highest in Haripur (85.58), followed, in order, by Gilgit (83.10%), Mansehra (81.14%), Batagram (81.05%), Shangala (77.78%), Kohistan (75.38%), Diamer (72.28%) and Astor (32.22%). The prevalence of ticks was found to be higher (85.67%) in younger livestock than adults (66.44%), and in females (80.33%) than males (66.44%). The present study provides the first report of the tick distribution in higher altitudes of northern Pakistan and confirms the presence of *Hyalomma* and *Rhipicephalus* ticks in the plains, as well as the hilly geoclimates of Pakistan. Furthermore, the data on risk factors allows us to make recommendations to restructure the existing husbandry system of northern areas of Pakistan with the aim of reducing the tick burden on livestock.

Keywords: *Ticks; Khyber Pakhtunkhwa; Gilgit- Baltistan; Pakistan; Hyalomma anatolicum; Rhipicephalus microplus*

INTRODUCTION

Ticks (Acari: Ixodidae), blood imbibing ectoparasites of mammals, birds and reptiles, have an epidemic dissemination in tropical and sub-tropical regions (Gaisuddin et al., 1994; Vesco et al., 2011). Ticks are prevalent worldwide from Asia (Haque et al., 2011; Bilkis et al., 2011; Sajid et al., 2011; Razmi and Ramoon, 2012; Singh and Rath, 2013), Africa (Reye et al., 2012; Tiki and Mekonnen, 2011; Elghali and Hassan, 2012), North and South Americas (Guimaraes et al., 2001; Lohmeyer et al., 2011), Australia (Springell, 1974; Kamau, 2011) and Europe (Kirby et al., 2004; Scharlemann et al., 2008). Ticks not only cause direct losses, but also act as vectors for various pathogens of bacterial, rickettsial, protozoal, and viral origin (Petney et al., 2007). The direct production losses caused by ticks include loss of milk production, reduced weight gain (Peter et al., 2005; Sajid et al., 2007), low quality skins and hides (Jongejan and Uilenberg, 2004), and mortality (Niyonzema and Kiltz, 1986). Besides these production losses, ticks also cause clinical and sub-clinical infections such as anaemia, dermatitis, paralysis, otoacariasis (Drummond, 1983; FAO, 1998) and secondary bacterial infections (Ambrose et al., 1999). Various diseases vectored by ticks are babesiosis, theileriosis, anaplasmosis, lyme

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disease, tick typhus, Rocky Mountain spotted fever and Crimean-Congo haemorrhagic fever (Otim, 2000; Bell-Sakyi et al., 2004). During the last three decades tick-borne infections have increased globally (Patz et al., 2005). Production and economic losses are proportional to the tick burden on the body of host, which results in increased irritation, restlessness, weight loss and disease transmission (Gosh et al., 2007; Agarwal and Gupta, 2010). It has been estimated that economic losses due to vector transmitted diseases and cost of tick control worldwide is 7.0 billion dollars annually (Harrow et al., 1991; Garcia, 2003).

The distribution of ticks in any region is closely associated with certain biotic and abiotic features (Sonenshine, 1993). Tick dispersal in any area depends upon the environmental factors (Estrada-Peña, 2003) e.g. temperature of 27-39 °C and relative humidity range of 60-80% render ticks more active (Rodríguez and Domínguez, 1998); however, ticks can also survive at temperatures down to -18.5˚C (Clark, 1995; Vandyk et al., 1996, Schulze et al., 2001). In the past few decades, due to global warming, ticks have been reported in areas of higher altitudes which were previously tick-free. In Pakistan, studies conducted on prevalence of tick species across the country report the frequency distribution within the range of 6.99 to 80% in small and large ruminant population. However, studies on the tick distribution in the northern areas of Khyber Pakhtunkhwa (KPK) and Gilgit Baltistan (GB) are not available except a single study by Khan (1993) in Kaghan valley, almost two decades ago. The present study was aimed to explore the northern area of Pakistan for the distribution of tick fauna in livestock population.

MATERIALS AND METHODS

Study Area

The present study was conducted in selected areas of the provinces of KPK and GB including: Haripur (33.988 °N 72.95 5°E), Mansehra (34°20 ′N 73°12′E), Shangala and Batagram (34.41°N 73.1°E), Kohistan (34º 54´ and 35º 52´ N and 72º43´ and 73º57 E), Astor (35° 22’ N, 74° 51’ E) and Gilgit (35°55′19″ N 74°18′31″ E). The average distance between each study area ranged from 30 to 100 kilometers. In these areas, animals are reared under a traditional management system (i.e. farmers followed practices of their forefathers and deal with animals according to traditional beliefs and knowledge) in which the livestock is entirely dependent upon grazing. Variation in temperature, rainfall, and humidity levels of the study districts depends upon the differences in the landscape, climate and elevation of these areas from sea level (Anonymous, 2011). The present study was conducted during June which is the hottest month of summer season in Pakistan.

Selection of Animals

Convenience and snowball sampling techniques were applied in order to select the sample animals/farms along the Karakoram Highway screened during this study. A questionnaire was designed to acquire essential information relating to animals (sex, age and species), qualitative and quantitative abundance of ticks and/or other parasites and the environmental conditions, including management (Thrusfield, 2007).

Collection and Identification of Ticks

For collection of ticks, the whole body of the animal was screened. Ticks were removed with the help of a fine tweezer with minimum damage to the mouth parts (Soulsby, 1982), transferred to McCartney sample collection bottles containing 70% ethanol and labelled appropriately. After collection, specimens were brought to the Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan for taxonomic identification. Ticks were identified using stereomicroscope (IM-SZ-500 IRMECO Germany) according to standard keys (Walker et al., 2007).

Statistical Analyses

Prevalence and association of determinants was analyzed by using multivariate analysis (multiple logistic regressions) and odds ratio. Pairwise comparison was carried out by keeping buffaloes (animal species), *Rhipicephalus* spp. (tick species), males (sex), adults (age) and the Astor district (area) in group 2 of the analysis (Schork and Remington, 2010). All statistical analyses were conducted using SAS software package (SAS, 2010).

RESULTS

The overall prevalence of tick infestation in the

Table 1. Frequency distribution of hard ticks (Ixodidae) in different livestock species of Khyber Pakhtunkhwa and Gilgit-Baltistan, Pakistan

Variables	Levels	Animals Screened	Tick Positive	Prevalence (%)	95% Confidence interval		Odds Ratio	P-value
					Lower limit	Upper limit		
Animal Species	Sheep	232	189	81.47	76.07	86.06	1.39	0.167
	Cattle	163	127	77.91	71.06	83.78	1.33	0.289
	Goats	365	263	72.05	67.28	76.48	1.23	0.378
	Buffalo	53	31	58.49	44.94	71.13	-	-
Tick Species	*Hyalomma spp.*	813	309	38.01	34.72	41.38	1.03	0.782
	Rhipicephalus spp.	813	301	37.02	33.75	40.39	-	-
Sex	Female	483	388	80.33	76.61	83.69	1.19	0.105
	Male	330	222	67.27	62.07	72.18	-	-
Age	Young	393	311	79.13	74.90	82.94	1.11	0.322
	Adult	420	299	71.19	66.72	75.37	-	-
Province	KPK	498	403	80.92	77.29	84.74	1.23	0.063
	GB	315	207	65.71	60.34	70.80	-	-
Districts	Manshera	162	132	81.14	74.94	86.91	2.53	0.000
	Haripur	104	89	85.58	77.82	91.38	2.66	0.000
	Batagram	95	77	81.05	72.24	88.00	2.52	0.000
	Shangala	72	56	77.78	67.11	86.25	2.41	0.002
	Kohistan	65	49	75.38	63.86	84.69	2.34	0.003
	Diamer	83	60	72.28	61.95	81.10	2.24	0.003
	Gilgit	142	118	83.10	76.26	88.60	2.58	0.000
	Astor	90	29	32.22	23.19	42.38	-	-

livestock population of the study area was found to be 75.03% (610/813) with a higher (P>0.05) tick distribution in KPK than in GB. Only adult ticks of *Hyalomma anatolicum* and *Rhipicephalus microplus* were present on the screened livestock population. Animals/livestock species wise prevalence of ticks were 81.47% in sheep, 77.91% in cattle, 72.05% in goats and 58.49% in buffaloes. Similarly, animal numbers found positive for ticks species were 309 and 301 with *Hyalomma* and *Rhiphicephalus* spp. respectively. A total of 18907 adult ticks were collected from the four livestock species in the study areas. Higher number of ticks were collected from sheep (7590), followed in order by goats (6622), cattle (4004), and buffaloes (691). Distribution of ticks collected from the screened livestock species is given in Table 2. Tick distribution was found highest (P<0.05) in the Mansehra district followed by the Haripur, Batagram, Shangala, Kohistan, Diamer, Gilgit and Astor districts. Host age and sex were not found to be statistically associated (P>0.05) with the tick distribution in the study districts. Table 1 details the distribution of ticks by individual variables. Table 3 depicts the distribution of the two species of hard ticks infesting livestock in the various districts of KPK and GB.

Table 2. Tick Abundance in Screened Livestock Species of Gilgit Baltistan and Khyber Pakhtunkhwa, Pakistan

Sr. No. 1, 2, 3, 4	Livestock species	Range of tick Burden		Average
		Minimum	Maximum	
	Sheep	6	145	40.16
	Cattle	25	125	31.53
	Goats	19	130	25.18
	Buffaloes	10	60	22.29

DISCUSSION

The prevalence of tick infestation in ruminants

Table 3. Tick species-based distribution of hard ticks (Ixodidae) on different livestock hosts in the various districts of Khyber Pakhtunkhwa and Gilgit Baltistan, Pakistan

Variables	Levels	Animals Screened	Tick Positive	Prevalence (%)	Confidence interval 95%		Odds Ratio	P-value
					Lower limit	Upper limit		
Manshera	*Hyalomma*	162	71	43.83	36.33	51.54	1.16	0.440
	Rhipicephalus	162	61	42.96	30.44	45.30	-	-
Haripur	*Rhipicephalus*	104	48	46.15	36.74	55.78	1.17	0.571
	Hyalomma	104	41	39.42	30.38	49.05	-	-
Batagram	*Hyalomma*	95	40	42.11	32.49	52.20	1.08	0.737
	Rhipicephalus	95	37	38.95	29.54	49.02	-	-
Shangala	*Hyalomma*	72	31	43.06	32.00	54.66	1.24	0.481
	Rhipicephalus	72	25	34.72	24.42	46.23	-	-
Kohistan	*Rhipicephalus*	65	25	38.46	27.26	50.67	1.04	0.934
	Hyalomma	65	24	36.92	25.88	49.11	-	-
Diamer	*Hyalomma*	83	33	39.76	29.67	50.56	1.22	0.500
	Rhipicephalus	83	27	32.53	23.12	43.14	-	-
Gilgit	*Rhipicephalus*	142	64	45.07	37.03	53.31	1.19	0.413
	Hyalomma	142	54	38.03	30.32	46.22	-	-
Astor	*Hyalomma*	90	15	16.67	10.01	25.44	1.07	0.922
	Rhipicephalus	90	14	15.56	09.14	24.16	-	-

is much higher in the developing countries of Asia (Siddiqi and Jan, 1986; Khan et al., 1993; Sajid et al., 2007, 2008, 2009, 2011; Yi-Lun et al., 2011; Iqbal et al., 2013; Ali et al., 2013; Sultana et al., 2015) and Africa (Walker and Koney, 1999; Walker, 2003), where control measures are infrequently applied, than in Australia (Springell, 1974; Kamau, 2011), Europe (Papadopoulos et al., 1996; Hostis and Seegers, 2002) and the Americas (Guimaraes et al., 2001; Lohmeyer et al., 2011). Extensive research studies have been conducted on the prevalence of the tick fauna and associated risk factors in various districts of Pakistan. Seven tick species have been reported from sixteen districts of Pakistan, with a prevalence ranging from 6.99% to 86.50% in the bovine and caprine populations of various study areas during 1971 to 2015 (Iqbal et al., 1971; Khan et al., 1993; Zaman, 1997; Kakar and Kakarsulemankhel, 2008; Sajid et al., 2008; Durrani and Shakoori, 2009; Irshad et al., 2010; Perveen, 2011; Atif et al., 2012; Ali et al., 2013; Iqbal et al., 2013; Tasawar et al., 2014; Mustafa et al., 2014; Sultana et al., 2015). However, the northern areas of the country have been ignored due to harsh climate and the very difficult assess to small holder animal farmers in the remote hilly villages. This is the first attempt to conduct such a study in these areas. The two tick species, i.e. *H. anatolicum* and *R. microplus,* which were identified from the screened livestock population along the Karakaram highway, have also been reported earlier from other parts of the country (Ali, 1988; Khan et al., 1993, 1996; Wahid et al., 2004; Durrani, 2008; Sajid et al., 2008; Irshad et al., 2010; Atif et al., 2012; Iqbal et al., 2013; 2014; Mustafa et al., 2014; Tasawar et al., 2014). These two tick species have also been reported to infested deer, equine, swine, canine and humans. Further, these two ticks have been reported as vectors of viral, bacterial, rickesial and protozoal diseases of man and animals, including: Crimean-Congo hemorrhagic fever, babesiosis, theliriosis, Q fever, trypanosomiasis and ehrlichiosis (Morzaria et al., 1986; Jongejan and Uilenberg, 1994, Chinikar et al. 2009) as well as for *Staphylococcus* spp., *Coxiella burnetti*, tick-borne encephalitis virus, and *Ehrlichia* spp. (Yang et al., 1993; Anbalagan et al., 2014),

Prevalence of these species of ticks has already been reported in wide range of farm animals from the hilly and plain areas of Malaysia (Mariana et al., 2008). The results of present study indicate a relatively higher prevalence of ticks in the livestock populations of the plain and hilly areas of Pakistan as compared to earlier studies conducted in other parts of the country in which the prevalence ranged from 14% to 54.76% (Iqbal et al., 1971; Sajid et al., 2007, 2008; Ramzan et al., 2008; Sajid et al., 2009; Irshad et al., 2010; Sajid et al., 2011; Atif et al., 2012; Iqbal et al., 2013).

The key factors which favour the growth and development of ticks in particular region are: temperature, humidity, rainfall (Greenfield et al., 2011), vegetation (Gray, 2002), host availability, season, habitat (Teel et al., 1996), altitude (Jouda et al., 2004; Burri et al., 2007; Cadenas et al., 2007), breed, age, sex, stage of lactation, gestation period, nutritional status of the animal (Swai et al., 2005; Alonso et al., 2007; Yacob et al., 2008), body condition (Rony et al., 2010), method of application of acaricides (Bianchi et al., 2003) and husbandry practices (Sajid et al., 2011). In addition, tick survival and activity are dependent on many factors, primarily, humidity and moisture contents. To this end, mat depth (depth of soft soil which is usually measured by ruler) is another important factor which helps to maintain a better microclimatic condition suitable for tick survival, as more depth of met retains more water (Milne, 1948) and provides more niches for ticks. Removal of met layer/thickness causes desiccation of ticks thus reducing tick abundance. In addition to this, a humidity of above 80% is essential for tick survival and levels below this have a detrimental effect on tick survival (Macleod, 1934, 1935; Milne, 1948). It is therefore believable that humidity is an important determinant of tick survival. Humidity also has a profound effect on tick activity, primarily controlling the amount of time a tick can spend questing (Greenfield, 2011). Tick growth and development is temperature dependent and tick activity is greatly affected by changes in temperature. It has been reported that at temperatures $<7^0$C, ticks remain inactive, only venturing out of the mat and up the vegetation to quest for a host when temperatures increase (Macleod, 1939). Further, an increase in temperature not only causes an increase in the distribution of ticks but is also linked to the encroachment of ticks into higher altitudes not previously colonized by ticks (Danielov *et al*., 2008). The increase and decrease in temperature is also associated with tick diapause and shift in nymph and adult activity. It is reported that lower temperatures ($16.0–20.9^0$C) significantly reduced the number of ticks recovered during dragging. Increase and decrease in temperature, soil moisture and cloud coverage can also affect tick questing. Studies have shown that higher humidity is not favorable for ticks, as they become prone to over saturation, thus limiting their activities (Lee, 1946; Milne, 1948; Arthur, 1962). Gray (2002) reported that vegetation structure plays a large role in the presence and the absence of ticks. Ticks are often recorded in woodland habitats, as these provide a dense shrub layer; habitat with bracken, however, will also provide a suitable habitat. Vegetation height is also important factor which affects tick prevalence as higher heights are associated with a decrease in tick abundance. It is reported that the tick abundance is higher at 151-300mm high vegetation. Presence and absence of host in an area also affect occurrence of ticks. So, presence of ticks is not static as it depends on a number of factors.

The reasons of high prevalence of ticks in the livestock population nurtured along the Karakoram highway of Pakistan could be due to different factors. Geography and climate of the area are among those factors which are linked with variables such as: temperature, rainfall, humidity, vegetation, landscape and altitude and the role of these factors leading to a higher abundance of ticks has been reported (Estrada-Pena, 2003). Recently, it has been reported that the height of the study area from the sea level has negative correlation with the tick distribution and abundance (Qviller et al., 2013). Probably, at the higher altitudes, environmental temperature does maintain the optimum range required for the growth and development of ticks (Perret et al., 2000; Jouda et al., 2004; Randolph, 2004). Further, at higher altitudes the micro climatic conditions diminish the body reserves of the ticks, which ultimately lead to delayed embryogenesis (Daniel, 1993). During the past decade, increases in the climatic temperature might have resulted in the higher abundance of ticks even at higher altitudes (Burri et al., 2007; Cadenas et al., 2007). Climate alteration is a believable justification for changes in the distribution and severity of tick infestation at higher altitude (Coulson et al., 2009). In other words, environmental changes favour settlement of ticks at higher altitude where they had not colonized earlier (Danielova et al., 2008). Vegetation and forest make another microclimatic component of the area that have significant role in higher distribution of ticks as it provides covering layer for lodging of ticks (Gray, 2002). Hilly areas of GB and KPK are densely shielded by shrubs, bushes and forests which provide appropriate resources for the exophilic questing behaviour of ticks.

Season plays vital role in the tick distribution as has been reported from all over the globe with highest prevalence in summer (Singh and Singh, 1999; Hussian and Kumar, 1991; Vatsya et al., 2008; Haque et al., 2011). In Pakistan too, summer season has been reported most favorable for the growth, propagation and infestation of ticks on the livestock population from various regions of the country (Sajid et al., 2007; Durrani and Shakoori, 2009; Sajid et al., 2009, 2011; Atif et al., 2012; Iqbal et al., 2013) Current point prevalence was determined in the summer when the weather conditions are hot and humid which favour tick growth and development (Gosh et al., 2007). Probably, nomadism also contributes towards higher distribution of tick infestation in the area as in our country animal keepers/farmers from the warmer areas of Punjab, Sindh and KPK migrate with their livestock in summer season to the hilly areas like GB where temperature is much lower during summer season. This activity favours successful transmission of ticks from the immigrant livestock to the indigenous animals of the area.

Husbandry practices are also correlated with tick abundance and distribution. In this context, mixed grazing of different animal species on the same pasture and/or mixed housing provides maximum opportunity to ticks to infest a large population at one time. In Pakistan, animal sheds are made of bricks and stones with mud which provides cracks and crevices that is suitable for the nidiculous questing behaviour of ticks (Soulsby, 1982). Lack of awareness about the treatment of infested animals and lack of veterinary facilities are also noteworthy reasons for the higher tick infestation in these areas. Most of the interviewed farmers were illiterate and had a misconception that ticks do not impact on animal health and production. On the basis of the results of the present study, some recommendations for farmers and researchers are: (a) prophylactic treatment of the livestock populace must be administered at the start of warm season (March-April) (b) young and female animals need extra care (c) dairy and livestock development organizations must plan an awareness campaign in these areas to explain the possible threats of ticks and tick-borne diseases to the livestock. The present study is the first report of the successful adaptation and settlement of ticks at the high altitudes of northern Pakistan.

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