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Survey and determination of risk factors associated with *Boophilus Microplus* in cattle and buffalo of two distant ecological zones of Khyber Pakhtunkhwa: Pakistan

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The current study was designed to investigate the existing status of *Boophilus microplus* and its associated risk factors in two ecological zones viz: Zone-1 (Swat & Dir) and Zone-2 (Charsadda & Nowshehra) districts of Khyber Pakhtunkhwa province, Pakistan. A total of 600 ticks were collected from (n=400) animals (Cattle n=200 & Buffalo n=200) of mixed breed, age and sex from local dairy farms and domestic livestock owners. The overall percentage (33.33%) of *Boophilus microplus* in all four districts was found significant (P<0.05). Similarly the percentage of *Boophilus microplus* in Zone-1 i.e. (26.33%) and in zone 2 i.e. (40.33%) were significant (P<0.05). The overall percentage of *Boophilus microplus* was found higher in Zone-2 as compared to zone-1 however this was significant (P<0.05). Keeping in view of prevalence in the infested animals (gender wise and age wise), a significant (P<0.05) difference was observed in female and young animals in both zones. The specie wise infestation rate was recorded significant (P<0.05) for cattle as compared to buffalo. Among the risk factors involved as a result of the data collected based on questionnaire, a significant (P<0.05) increase was observed in muddy houses, grazing stock and animal owners with little or no awareness towards ticks and their associated problems. It may be concluded that damp, warm and unhygienic farm practices, young stock, female animals, grazing animals and those living in muddy houses were at higher risk of getting tick infestation and ultimately other related consequences.

Keywords: *Boophilus microplus*, Survey, Risk factors, Cattle and Buffalo, Khyber Pakhtunkhwa.

INTRODUCTION

Livestock population is profoundly affected by ticks in terms of blood loss (Anemia), hide destruction, toxicity, milk and meat production losses (Sajid et al. 2009). The United Nations Food and Agricultural Organization (FAO) estimated US \$ 7.0 billion global annual loss in food industry owing to tick infestation (Ali et al. 2013). An estimated daily 8.9 ml milk production loss and 1.0 gm weight loss may be attributed to the engorgement of single female tick (Rodriguez et al. 2004).

Cattle tick, *Rhipicephalus (Boophilus) microplus* is well known on account of its hostile character and fast transposition of other species of the same subgenus being prevalent in Latin America, (Evans et al. 2000), Middle East and Asia (Estrada-Pena et al. 2006) and East and South Africa (Tonnesen et al. 2004). *Boophilus microplus* also known as (blue tick) parasite is the tropical cattle tick. It is an important ectoparasite in farm animals globally and has been known as a vector for transmitting various pathogens of veterinary importance (Dantas-Torres et al. 2012;

Chen *et al.* 2014; Jabbar *et al.* 2015; Yu *et al.* 2015), including *Babesiosis*, *Anaplasmosis* (De Castro, 1997; Alim *et al.* 2012) and several bacterial, protozoal and viral diseases (Sonenshine, 1991).

So far little work has been done for investigating surveillance of *Boophilus microplus* in Khyber Pakhtunkhwa (KPK) province. Keeping in view the importance of tick (*Boophilus microplus*) infestation, the current study was designed as an effort to identify the tick Specie *Boophilus microplus*, its surveillance and inspecting risk factors associated with *Boophilus microplus* in cattle and buffalo of two distinct ecological zones of Khyber Pakhtunkhwa province of Pakistan.

MATERIALS AND METHODS

Study site and duration:

The study area included two ecological zones of Khyber Pakhtunkhwa (Pakistan), *i.e.* zone one consists of district Swat and Dir, whereas zone two consists of Charsadda and Nowshera. The ecological zones with dense livestock population were selected on the base of climate variation in order to make the data more reliable and representative. Among ecological zone one, district Swat lies between 35.22° N, and 72.4258° E latitude and 72.35 and 72.47° E longitude with

39°C to 7°C temperature extremes, having average rainfall of approximately 897 mm. District Dir lies at 35.19° N latitudes and 71.87° E longitudes with temperature climax of 40°C in July that falls to 11°C in January, whereas average annual rainfall goes to 1185 mm. In second ecological zone, district Charsadda, occupies 34.16° north and 71.75° east measuring 40 °C temperature extreme and an average annual rainfall of 460 mm. Whereas district Nowshera occupies 34.01° north and 71.98° east measuring 42 °C temperature with average annual 532 mm rainfall. The ticks were collected from cattle and buffaloes during the months of June to October in year 2018. The ticks were kept in pre-labeled falcon tubes with perforated lids. The research study was performed in the Entomology Laboratory, Department of Parasitology, University of Veterinary and Animal Sciences, Lahore.

Sampling of animals:

A total of (n= 400) animals (Cattle n=200 & Buffalo n=200) of mixed breed were selected from four districts of province-Khyber Pakhtunkhwa (KPK) *i.e.* Swat, Dir, Charsadda and Nowshera. The animals were selected from local dairy farms and domestic livestock owners with no history of any acaricides application for at least for 3 months.

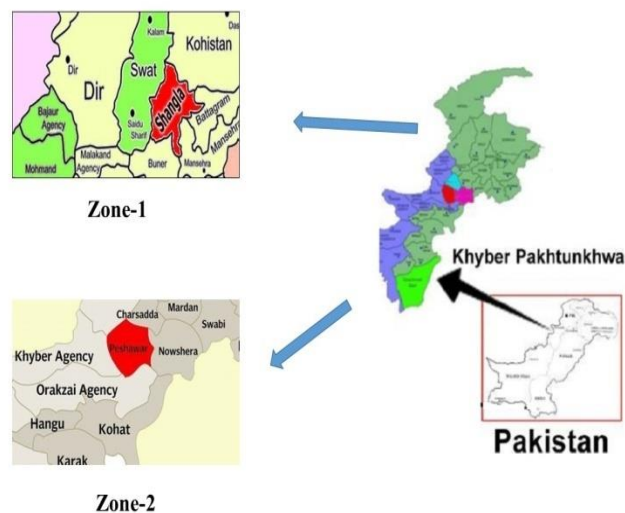


Figure-1: Map of Khyber Pakhtunkhwa, showing study areas

Epidemiological survey:

Various risk factors attributed to tick infestation based on specie, age, sex, housing type(concrete or muddy), different geographical location, exposure to anti-parasitic drugs, awareness of farmers, feeding methods that had alleged relation with infestation were recorded on a questionnaire as followed by (Thrusfield, 2007). Age wise categories of animals include young stock (cattle and buffalo) less than or equal to 1.5 years, and mature stock (cattle and buffalo) more than or 1.5 years.

Ticks sampling and identification:

Ticks were randomly collected from various body parts (ears, brisket, withers, knees, udder in case of female and testes in males along with the perennial region and tail) from infested bovine(irrespective of gender, age, breed, health status)with the help of forceps without injuring their mouth part by using convenient sampling technique as described by(Ali *et al.* 2013).The collected ticks were transported in pre-labelled falcon tubes with perforated lid to the Entomology laboratory, Department of Parasitology, UVAS Lahore, ticks were identified up to genus level on the basis of their morphological characteristics

using morphology key by Walker (2007). The tick’s identification includes following features. Short and broad mouth parts. Coxa-1having spur. Coxa-1 spur is distinct. Hypostomal teeth 4+4 Columns. Genital aperture posterior lip have a broad U-shape (Walker, 2007).

Statistical analyses

The data obtained for survey of *Boophilus microplus* and its associated risk factors, including age, sex, specie, housing type, various geographic locations, awareness of farmers, feeding methods (stall or grazing), were analyzed by using Chi square. Association between survey and risk factors were measured. All the data were analyzed by using SPSS software.

RESULTS

The data were statistically analysed using Pearson’s Chi square test. $P < 0.05$ were selected as significant. The result is presented as under. Figure 2. Shows the percentage of overall infested animals, the Pearson chi square value showed a significant ($P < 0.05$) difference between the infestation rate of *Boophilus microplus* between Buffaloes and Cattles.

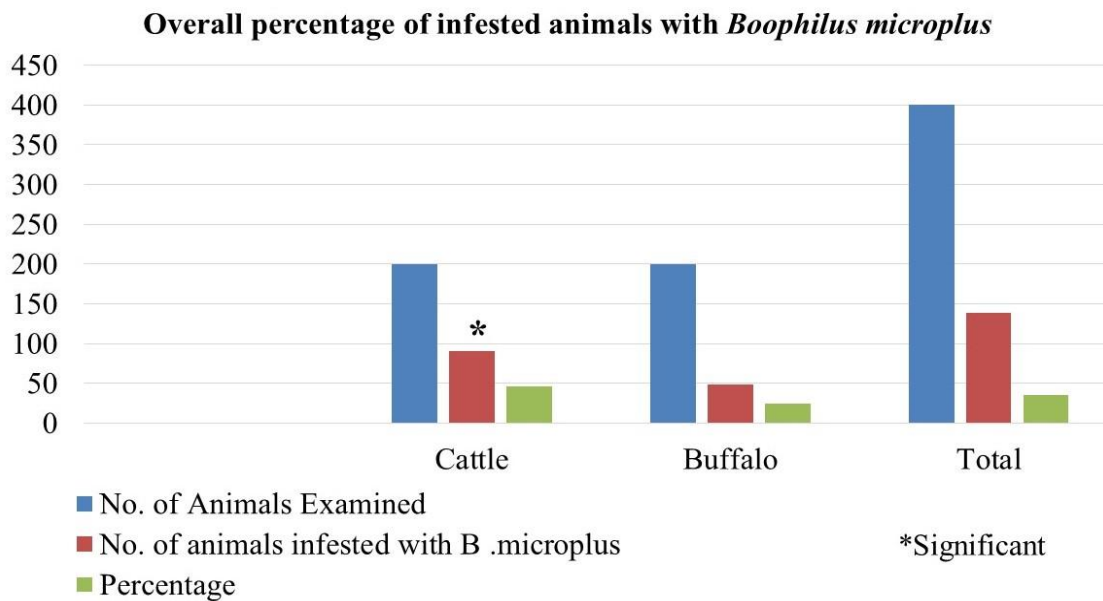


Figure 2: Overall percentage of infested animals with *Boophilus microplus*

Figure 3. Shows the percentage of infested animals with *Boophilus microplus* in districts swat and dir in ecological zone 1. The statistical analysis showed a significant ($P < 0.05$) difference

between the infestation rate of *Boophilus microplus* ticks in the observed animals i.e., Buffaloes and Cattles of both districts.

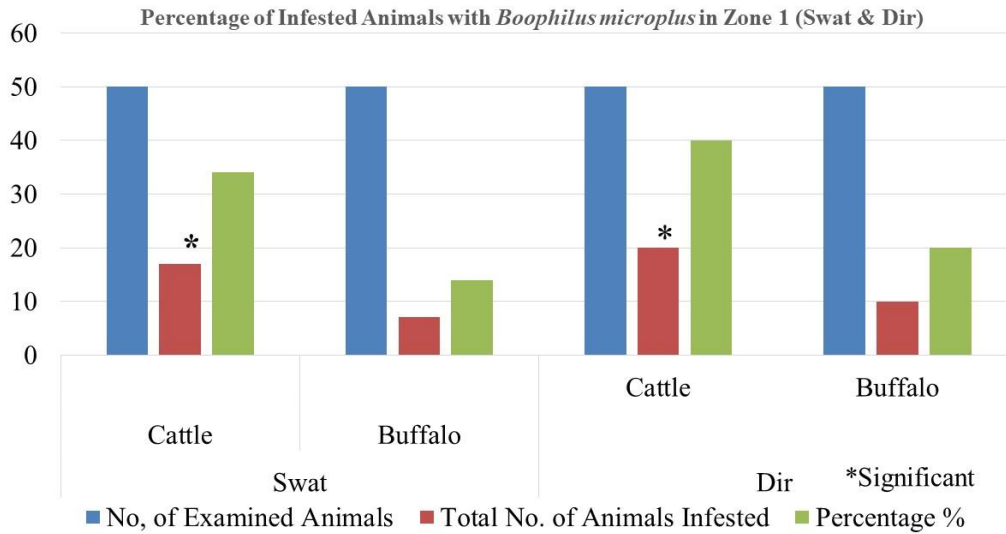


Figure 3: Percentage of Infested Animals with *Boophilus microplus* in Zone 1 (Swat & Dir)

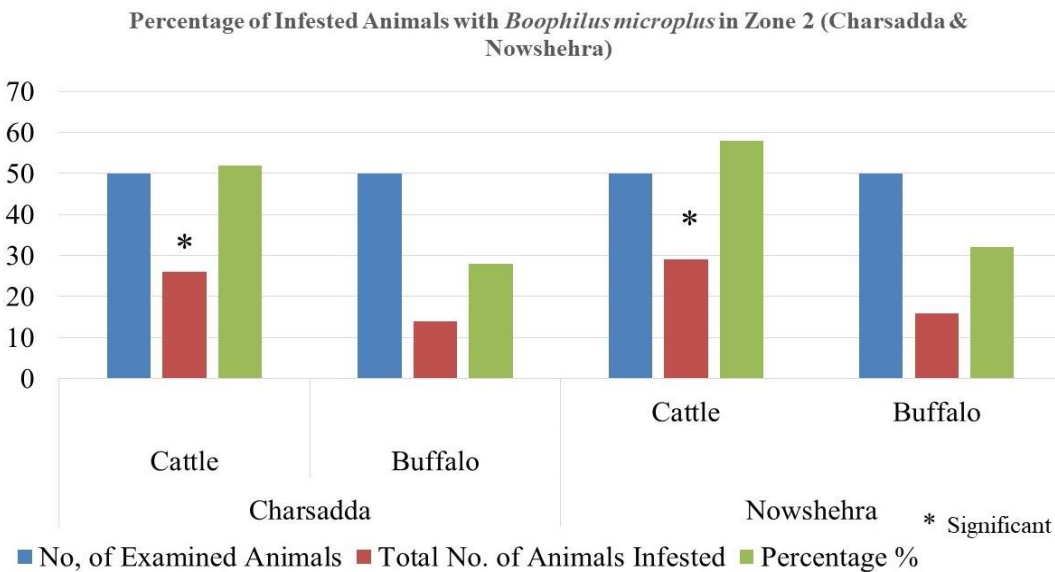


Figure 4: Percentage of Infested Animals with *Boophilus microplus* in Zone 2 (Charsadda & Nowshehra)

Figure 4. Shows the data of infested animals with *Boophilus microplus* in two districts i.e., Charsadda and Nowshehra of ecological zone 2. The statistical analysis showed a significant ($P<0.05$) difference between the infested animals of the two districts.

Table 1. Shows the percentage of *Boophilus microplus* among all collected ticks in Zone-1 i.e., district Swat, Dir. A total of 300 ticks of different species were collected ($n=150$) from each district Swat and Dir. In district Swat a total of 30 (20%) of *Boophilus microplus* ticks were investigated, whereas in Dir district this number was 49 (32.66%). The statistical analysis of the data showed a significant ($P<0.05$) difference between the infestation rate of *Boophilus microplus* in zones 1. Similarly in Zone-2 i.e. district Charsadda, Nowshehra.. In district Charsadda a total of 52 (34.66%) of *Boophilus microplus* ticks were investigated, whereas in Nowshehra district this number was 69 (46%). The statistical analysis of the data showed a significant ($P<0.05$) difference between the infestation rate of *Boophilus microplus* in zones 2.

Table 2. Shows the percentage of *Boophilus microplus* among all collected ticks in Zone 1 i.e., district Swat and Dir. A total of ($n=300$) ticks were

collected ($n=150$) from cattle's and buffaloes from each district. In district swat a total of 30 (20%) from both cattle and buffalo of *Boophilus microplus* were investigated, whereas in dir district this number was 48 (32%) from both cattle and buffalo. In district Charsadda a total of 52 (34.66%) of *Boophilus microplus* were investigated, whereas in Nowshehra district this number was 69 (46%). The statistical analysis of data showed a significant ($P<0.05$) difference in percent population of *Boophilus microplus* among other species of ticks in the two ecological zones.

Table 3. Shows the gender wise infestation of all observed animals in zone 1 and zone 2 with all tick species including *Boophilus microplus*. In zone 1 the number of infested males were 63 (31.5%) while in female this number was 137 (68.5%). In zone 2 a total of 49 (24.5%) male and 151 (75.5%) female animals were found infested. The statistical analysis of data showed a highly significant ($P<0.05$) difference between the infested male and female. The numbers of infested females were found significantly higher than males in both ecological zones, however the percentage of female animals were higher in zone 2 as compared to zone 1.

Table 1: Overall percentage of *Boophilus microplus* in Zone-1 and 2

District	No. of Ticks collected	<i>Boophilus microplus</i>	Percentage %	P- Value
Swat	150	30	20	0.0001
Dir	150	49	32.66	
Total	300	79	26.33	
Charsadda	150	52	34.66	0.0001
Nowshehra	150	69	46	
Total	300	121	40.33	

$P<0.05$ (Significant)

Table 2: Overall specie wise percentage of *Boophilus microplus* in Zone- 1 and 2

District	Specie	No, of Collected Ticks	Total No. <i>Boophilus microplus</i>	Percentage %	P- Value
Swat	Cattle	75	20	26.66	0.000
	Buffalo	75	10	13.33	
Dir	Cattle	75	29	38.66	
	Buffalo	75	19	25.33	
Charsadda	Cattle	75	31	41.33	0.000
	Buffalo	75	21	28	
Nowshehra	Cattle	75	43	57.33	
	Buffalo	75	26	34.66	

$P<0.05$ (Significant)

Table 3: Overall Gender wise Percentage of Infested Animals with all Ticks, in Zone-1 & Zone-2

Zone	Animals Examined	Male	Female	P-Value
1	200	63 (31.5%)	137 (68.5%)	0.0001
2	200	49 (24.5%)	151 (75.5%)	
Total	400	112 (28%)	288 (72%)	

$P<0.05$ (* Significant)

Figure 5. Depict the infested rate of animals on the basis of their age and association with gender. The number of animals less than 1.5 years of age were found with maximum frequency of ticks comparatively to those above 1.5 years in both ecological zones. The statistical analysis with Chi square test reveal a significant difference ($P < 0.05$) between lower and upper age group. Association between gender and age showed a higher number of tick infestation in female animals as compared to male in each zone to those above 1.5 years in both ecological zones. The statistical analysis with Chi square test reveal as significant difference ($P < 0.05$) between lower and upper age

group. Association between gender and age showed a higher number of tick infestation in female animals as compared to male in each zone.

Figure 6. Shows the association of management of animals with infestation rate of *Boophilus microplus*. The statistical analysis of the data showed a significant ($P < 0.05$) difference between the stall feeding and animals grazing openly in fields. The total infested animal in stall feeding was 36.69%, whereas animals with grazing showed 63.30%.

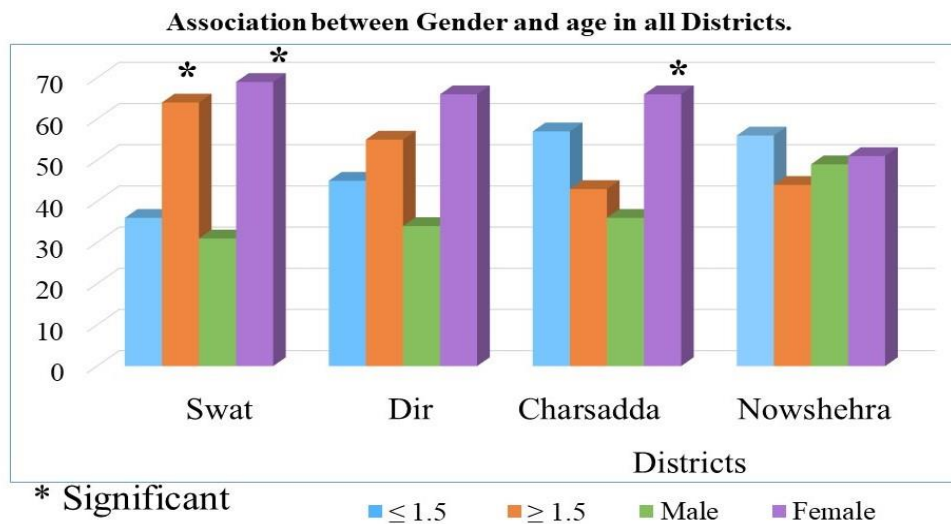


Figure 5: Association between Gender and age in all Districts.

Management related Infestation rate of *Boophilus microplus*

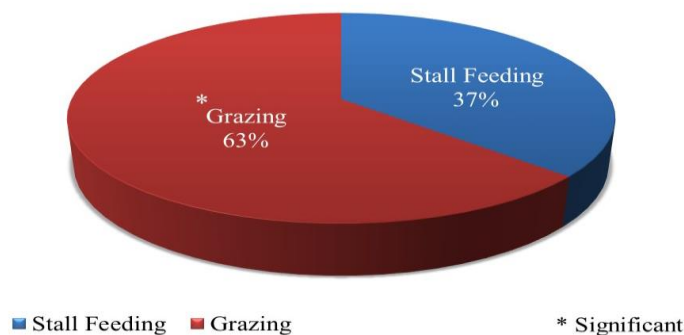


Figure 6: Overall Management related infestation rate of *Boophilus microplus*

Figure 7. Show the prevalence percentage of *Boophilus microplus* with the type of housing in which animals were kept. The statistical analysis showed a significant ($P < 0.05$) difference between the muddy and concrete housing system. Muddy houses showed an increase number 66.90% in comparison with concrete houses 33.03%.

Figure 8. Show association of awareness and education of farmers with overall infestation rate of ticks. The statistical analysis showed a significant ($P < 0.05$) difference between the animals kept by educated farmers 18.70%, while the livestock kept by illiterate owners showed a high infestation rate 29.49%.

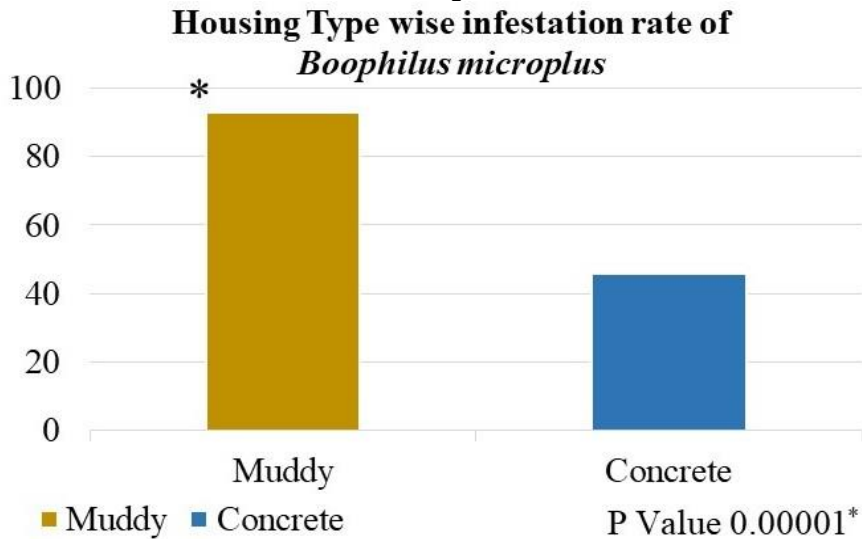


Figure 7: Housing Type wise infestation rate of *Boophilus microplus*

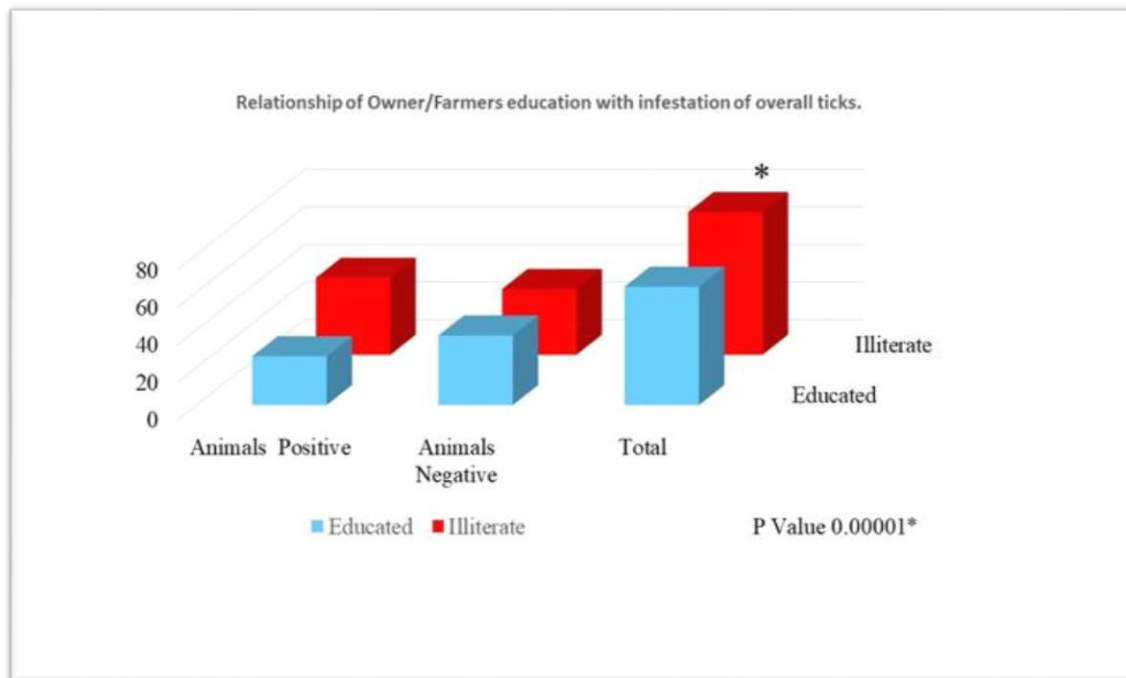


Figure 8: Relationship of Owner/Farmers education with infestation of overall ticks.

The present study was conducted in order to record the survey of *Boophilus microplus* in two different ecological zones of Khyber Pakhtunkhwa province, Pakistan. The result upon statistical analysis reveals an overall higher infestation rate in animals infested with *Boophilus microplus* i.e. (46% and 23.5% Vs 60.5% and 17.8%) in cattle and buffaloes respectively that was found statistically significant ($P < 0.05$), (Figure-2) and comparable with results as studied by (Asmaa et al. 2014). This high rate of infestation might be due to exposure to unhygienic conditions, quality of their skin and rearing mostly exotic breed of cattle. Similar findings were also reported by (Khan et al. 2013) who found high Tick Infestation Rate (TIR) i.e., (33.36%) in cattle than buffaloes (22.58%).

Figure-3 shows the percentage of infested animals in zone-1. The infestation percentage was (34% and 14%) in cattle and buffalo respectively in Swat District, whereas in Dir District it was (40% and 20%) for cattle and buffalo respectively. The statistical analysis showed a significant ($P < 0.05$) difference between the infested cattle of Dir and Swat Districts. This higher infestation rate in lower Dir could be attributed to higher annual average rain fall and higher average temperature than Swat District. Similarly the data of zone 2 showed a higher tick infestation rate in Nowshehra i.e., (57.33%) and (33.33%) for cattle and Buffalo while in Charsadda i.e., (41.33%) and (28%) (Figure-4). A statistically significant ($P < 0.05$) increase was observed in the percentage of cattle of Nowshehra as compared with Charsadda. Similar results i.e. (34.79%) were reported by (Khan et al. 2013) who reported a statistically significant higher number of tick infestation of *Boophilus microplus* in lower humid and temperate districts of Khyber Pakhtunkhwa.

Table-1 shows the prevalence percentage of *Boophilus microplus* in zone 1 and 2. The percentage of *Boophilus microplus* in district Swat was (20 %) whereas in Dir district this number was (32.66 %). This was statistically significant ($P < 0.05$). Similarly the prevalence percentage of *Boophilus microplus* in zone-2 showed a statistically significant ($P < 0.05$) increase in the percentage in district Nowshehra (45.33%) as compared to Charsadda (34.66%). Similar findings were reported by (Iftikhar Ahmad et al. 2014). This high percentage may be due to high humidity and temperature which favors the population of ticks in Charsadda and Nowshehra. Our results are in agreement with (Khan et al. 2013) who found (18.63%) comparatively less

prevalence percentage in cold and hilly areas of Khyber Pakhtunkhwa such as Malakand, Shangla and Swat.

The data regarding percentage of *B. microplus* in zone-1 was statistically higher in cattle as compared to buffalo. This high prevalence percentage of *Boophilus microplus* may be due to favoring temperature, average rainfall in the study area for the prevailing tick population and their eggs or larval stages. This was supported by (Khan et al. 2013) who reported similar results regarding the hilly areas. Similarly a statistically significant results ($P < 0.05$) was found in the population percentage of *Boophilus microplus* in Nowshehra as compared to Charsadda in both cattle and buffalo. This higher number of tick prevalence percentage in zone-2 might be credited to high relative humidity, topographical difference, temperature difference and lower altitude of zone-2 which caused the tick population to flourish in these ideal circumstances. The current findings of the study are in agreement with those reported by (Khan et al. 2013) who reported a high tick infestation rate in cattle (41.72%) in hot arid plane area while (20.40%) in areas of high altitude.

The data regarding the overall gender wise prevalence (Table- 5) which showed a significant difference ($P < 0.05$) between male and female species in both ecological zones. The high prevalence percentage in female as compared to male specie may be due to severe exposure to ticks and their larvae and a relatively high stress in females especially during pregnancy and parturition. The current findings of our study relating gender was in line with (Kabir et al. 2011) who found a significantly higher percentage of ticks in females (Lloyd, 1983) reported that high level of prolactin and progesterone hormones during pregnancy and after parturition are the predominant cause of high infestation rate in female animals.

Prevalence of *Boophilus microplus* was significantly ($P < 0.05$) higher in young stock followed by adult. (Khan et al. 2013) also found higher tick infestation rate (TIR) in young buffaloes as compared to adults. (Figure-5). The current findings are also in line with those stated by (Kabir et al. 2011) who reported 2.23 times more infestation in young stock as compared to adult. The basic mechanism for this high rate of infestation in young stock is exactly unknown however this could be attributed to the fact that young stock are at a greater risk due to their immune status and their resistance.

Among management wise prevalence a significant increase was observed in grazing animals (Figure-6), this was supported by (Kabir et al. 2011) who found a higher infestation rate among grazing animals as compared to stall feeding. Higher infestation rate in grazing animals might be due to increased risk of free roaming ticks in fields and open grazing areas. Animals with stall feeding were at lower risk due to regular washing of brans, farm premises and other cleansing practices. Another risk factor associated with tick burden was type of housing and awareness among livestock owners. In our study significant ($P < 0.05$) difference was observed in animals rearing in muddy houses compared with those in concrete (Figure-7). This could be due to fissures and cervices in muddy houses which could be hiding place for ticks and the accessibility of various acaricides (Farooqi et al. 2014). Furthermore, the education status of farmers and awareness regarding the catastrophic consequences of tick infestation is also a significant risk factor. In our study the majority of farmers interviewed were illiterate having no or little understanding of tick infestation and the subsequent usage of acaricides or other routine practices performed at the farm and the results were found statistically significant ($P < 0.05$) (Figure-8).

CONCLUSION

It is concluded that the infestation rate of ticks including *Boophilus microplus* in zone-2 was significantly higher than ecological zone-1. The infestation rate was higher in cattle as compared to buffaloes in all districts of both zones. The population of females and young stock were at higher risk for getting infection.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Muhammad Qasim, Mian Abdul Hafeez. Conceived and designed the idea of research experiment. Muhammad Qasim, Collected the data and performed the experiments. Muhammad Nisar, Aftab Ahmad Anjum. Analyzed

the data and performed a statistical analysis, interpreted the results, and contributed to the discussion. Muhammad Qasim, Mian Abdul Hafeez. Contributed writing the manuscript, all the authors check the proof reading of manuscript.

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