Research Article

Prevalence and Morphological Identification of Tick Infestation in Small Ruminants in Khyber Pakhtunkhwa, Pakistan

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Abstract | Ticks (Acari: Ixodidae) are obligate ectoparasites of diverse hosts that affect livestock globally and are carriers of several bacterial, viral, and protozoan infections that affect both animals and human. Ticks (Class Arachnida) are ectoparasites of a wide variety of vertebrates, including livestock, and wild animals. Ticks are arachnids of veterinary and medical importance because they can transmit various diseases. Accurate identification of tick species is crucial for effective disease surveillance, prevention, and control strategies. Morphological identification and epidemiology based on the examination of key external features remain a fundamental and widely used approach for tick taxonomy. This research work provides a comprehensive overview of the morphological characteristics used in the identification of ticks, focusing on the main genera and species of medical and veterinary significance. The purpose of the current study was to determine the prevalence of tick infestation, epidemiological characteristics, associated risk factors, and investigation of a variety of hard tick infestation in small ruminant populations in District Mardan, Buner, and Dir Lower, Khyber Pakhtunkhwa, Pakistan. A total of 5000 goats and sheep of various ages, breeds, sexes, and locations were inspected and examined for tick infestation. A total of 1774 goats and 1044 sheep were found to be tick infested, and the percentage prevalence was 59.37 for goats and 51.45 for sheep were recorded. Our studies showed tick diversity, infestation rate, and numerous factors (season, age, and gender of host) influencing tick infestation rate in goats and sheep population in District Mardan, Buner, and Dir lower, Khyber Pakhtunkhwa. Among the identified species *Haemaphysalis* and *Rhiphicephalus* were the most prevalent genera. To build a precise nationwide model of tick infestation, more study is required to confirm our findings in the area and to validate our findings in other locations of Pakistan. It is possible to boost domestic animal productivity while reducing the spread of deadly tick-borne illnesses in society by educating farmers about ticks and providing nearby veterinary services. A large-scale study is needed to explore the hard tick's diversity across the country and awareness is needed to minimize the risk of infection, especially among farmers and farms owners.

Links Researchers

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Introduction

Pakistan is an agricultural country and a large portion of Pakistani people work in animal husbandry. Especially in rural areas, 43% of workers are employed in the agriculture sector. Pakistan is home to 30.9 million sheep, 78.2 million goats, 41.2 million goats, 49.6 million cattle, and 41.2 million buffalo (Khan *et al*., 2022). After mosquitoes, ixodid ticks (Acari: Ixodidae) are the primary global carriers of infections with significant medicinal and veterinary value (Githeko *et al*., 2000). The backbone of Pakistan's economy is domesticated animals, yet ectoparasites have prevented farm animal production from reaching its full potential (Sajid *et al*., 2008). Ticks are hematophagous parasites that harm people, domestic animals, and wild animals worldwide. Anemia, allergic responses, paralysis, and toxicoses are only a few examples of the direct harm that they inflict on their hosts (Cabezas-Cruz *et al*., 2014).

Ticks and the infections they spread directly harm animals by lowering the quality of their hides, their live weight, and their milk supply. Ticks can also result in anemia, toxemia, and paralysis, so they pose a significant barrier to producing a healthy livestock business. In tropical places of the whole world where farmers raise cattle, ticks cause substantial economic losses. For the proprietors of resource-constrained, small-holder dairy farms in subtropical nations like Pakistan, the livestock (*Bubalus bubalis*; population: 40 million) and cattle (*Bos indicus* and *B. taurus*; population: 47.8 million) are essential sources of revenue (Ghafar *et al*., 2020).

Ticks cause global output waste of between USD 22 billion and USD 30 billion annually, with the majority of these losses taking place in underdeveloped nations (Lew-Tabor and Valle, 2016). In livestock, ticks can result in economic losses due to tick burden, blood loss, injury to skin and mammary glands, tick poisons, or mortality or infirmity brought on tick born diseases (TBDs). Due to decreased milk production, decreased live weight gains in cattle, and damaged skins, these significant losses have a negative shock on the livelihood of farmers with limited resources (Dantas-Torres *et al*., 2008). Due to tick bites, tick infestations result in sores, infections, blood loss, and the possible spread of diseases. Tick-borne pathogens (TBPs) affect both human and animal health globally and include viruses, bacteria, protozoans, and helminths (Estrada-Peña *et al*., 2008). Diseases are caused by the former, whereas the latter are essential for keeping the vector healthy. Intracellular bacteria of the genus Rickettsia are responsible for tick-borne rickettsioses. A high temperature, rash, myalgia, headache, and lymphadenitis are some of the clinical symptoms (Onyiche *et al*., 2020). Animals and people can both contract infections from members of the genera *Anaplasma* and *Ehrlichia* (Family Anaplasmataceae) (Sharifiyazdi *et al*., 2017). Hard ticks of the genera *Amblyomma, Haemaphysalis, Hyalomma,* and *Rhipicephalus* are the primary local carriers of TBPs (Walker *et al*., 2003).

The province of Khyber Pakhtunkhwa, Pakistan is thought to be a hotspot for the development and reoccurrence of TBDs concern to both public health and veterinary medicine. Up to 75% of sheep, cattle, and goats in the area have been found to have tick infestations, compared to 58% of water buffalo. According to morphological identification, the tick species that were discovered to be infesting cattle the most commonly in Khyber Pakhtunkhwa Pakistan were *R. annulatus, R. microplus, Hy. aciculifer, R. appendiculatus*, and *R. decoloratus* (Farooqi *et al*., 2017; Nieto *et al*., 2012; Sajid *et al*., 2017).

Various risk factors have been linked to tick infection in livestock animals in past. For instance, various regions of the world have investigated how environment and host characteristics affect the dispersion of cow ticks. Only a few studies in Pakistan have identified the risk factors for tick infestation on livestock farms; therefore, examining these risk factors at the level of small-scale farmers may be crucial for developing a program for tick management that is both successful and affordable (Estrada-Pena, 2009; Sajid *et al.*, 2009; Iqbal *et al.*, 2013).

Nevertheless, accurate morphological identification of ticks takes knowledge, and it may be difficult with engorged or physically harmed specimens. As a result, other approaches, like the use of mitochondrial markers, cox1, and 16S rRNA, are suitable for molecular identification and inferring phylogenies of the tick species, which has resulted in the creation of the DNA barcoding system for ticks (Lv *et al.*, 2014; Duron *et al.*, 2015; Kasi *et al.*, 2020). There hasn't been any research done in Pakistan yet that looks at the molecular traits, genetic diversity, and epidemiology of hard ticks that infest dogs, except one morphological study that listed the three main genera of ixodid ticks (*Haemaphysalis, Hyalomma, and Rhipicephalus*) (Kasi *et al.*, 2020; Zeb *et al.*, 2019). The current study was designed to identify ticks morpho-molecularly in a randomized sample of livestock to evaluate their prevalence, spatiotemporal distribution patterns, associated risk factors, and molecular phylogenies in District Mardan, Buner, and Dir Lower, Khyber Pakhtunkhwa Pakistan.

Materials and Methods

Study area

The current study was in District Mardan, Buner, and Dir Lower, Khyber Pakhtunkhwa Pakistan from January 2022 to January 2023 (Figure 1). Mardan is located at an elevation of 310.42 meters (1018.44 feet) above sea level, Mardan has a Humid subtropical, no dry season climate. The district's yearly temperature is 18.94°C (66.09°F) and it is -1.95% lower than Pakistan's averages. Mardan typically receives about 127.16 millimeters (5.01 inches) of precipitation and has 140.52 rainy days (38.5% of the time) annually. District Buner is located on elevation of 944 meters (varies from 200 ft to 9550 ft) above sea level. The highest maximum temperature ranges from 40°C to 44°C during May to July. Annual rainfall ranges between 672mm to 1198mm. The elevation of Dir Lower ranges from 1200m to 2800m above sea level. The highest temperature ranges from 30°C to 35. Annual rainfall at Dir averages 58 inches, of which 400mm falls during the summer and twice that amount during the winter. High temperature and humidity throughout the summer time create ideal circumstances for tick development and infestation in this area.

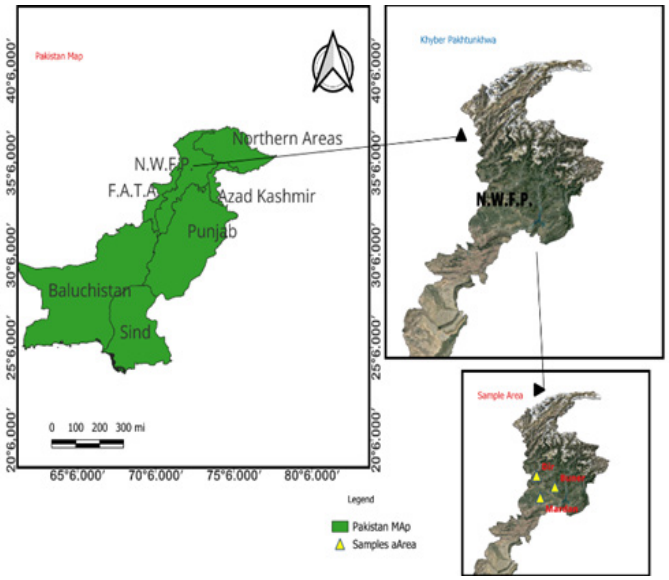

Figure 1: *Map of District Mardan, Buner and Dir Lower, Khyber Pakhtunkhwa Pakistan.*

Sample size, collection, and preservation

A total of 114 villages were chosen depending on the availability of permits. Using a prescribed methodology, they were screened for ticks (Ica *et al.*, 2007). Ticks were collected from different parts of the body and examined for species identification. The mouthparts of any ticks found were carefully removed with forceps to preserve their integrity (Soulsby Hilminthes, 1982). All parts of the host body (ear, mammary gland, genital organ, tail, and body) were deeply examined for the presence of the tick. After being separated from the body of the host, ticks were preserved in 70% ethanol. The collected ticks were kept in a safe tube in a refrigerator. Ticks have been collected in a cryogenic tube. Irrespective of the gender of animal ticks' collection has been done. A maximum of 10 and a minimum of 5 ticks were collected per host from different parts of the body.

Morphological identification

Samples are properly identified and sealed for storage in refrigerators. Using a stereomicroscope and a species-specific identification key, the morphological

characteristics such as scutum pattern, spiracle plate, coxa structure, adanal plates and capitulum shape were determined.

First, a gross identification of the ticks was performed. As a final step, the ticks were classified into different species depending on their morphology and basic structural characteristics. Such as the shape of the scutum, and its ventral structure. After genera identification of collected ticks, female adult ticks of each genus were separated by observing small areas of scutum on the anterior dorsum. All Ticks Identification whether male or female has been done by using a stereomicroscope and identified using different taxonomic keys like ticks of domestic animals in Africa: A guide to identification of species (Walker *et al.*, 2003; Kaiser *et al.*, 1963). The collected data were analyzed, and the abundance of each tick species was determined on the geological chart. Seasonal-wise prevalence variation of ticks has been shown.

Results and Discussion

The present results discover the prevalence of three tick genera and the ten species that are *Haemaphysalis punctata, Haemaphysalis sulcata, Rhipicephalus senegalensis, Rhiphicephalus lunulatus, Rhiphicephalus turanicus, Rhiphicephalus microlus, Hyalomma excavatum and hyalomma dromedarrii, Hyalomma anatolicum, Rhipicephalus sanguineus*. A total of 5000 goats and sheep of various ages, breeds, sexes, and locations were inspected and examined for tick infestation. A total of 1774 goats and 1044 sheep were found to be tick infested, and the percentage prevalence was 59.37 for goats and 51.45 for sheep were recorded as shown in Table 1. The unidentified and immature ticks on both hosts were neglected from discussion and analysis due to the difficult identification. The tick infestation rate was recorded in three different districts of Khyber Pakhtunkhwa with significant variation. For Goats the highest prevalence of tick infestation was recorded in Mardan while for sheep, the highest prevalence was observed in District Dir Lower (Figure 2).

Table 1: *Percent prevalence of tick infestation in goats and sheep population in the study area.*

Hosts	Non-infested population	Tick-infested population	Total population	Prevalence (%)
Goats	1214	1774	2988	59.37
Sheep	985	1044	2029	51.45

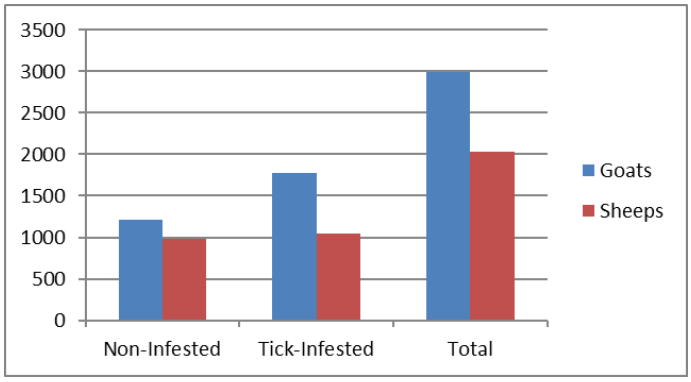

Figure 2: *Prevalence of tick infestation in goats and sheep population.*

District-wise tick prevalence

The results of tick species collected and identified from districts Mardan, Buner, and Dir Lower, Khyber Pakhtunkhwa confirmed the distinct distribution patterns. *Rhiphicephalus microplus, Haemaphysalis sulcata, Rhiphicephalus senegalensis, Rhipicephalus lunulatus,* and *hyalomma excavatum* were reported from the goats of district Mardan (Table 2, Figure 3). *Haemaphysalis punctata, Haemaphysalis sulcate,* and *Hyalomma dromedarri* were found in sheep of district Mardan (Table 3, Figure 4). *Rhiphicephalus microplus, Rhiphicephalus senegalensis,* and *Haemaphysalis suclcata* were reported in goats of district Buner (Table 2). *Haemaphysalis sulcate,* and *Haemaphysalis punctata* were present on sheep of district Buner (Table 3). *Haemaphysalis sulcata* and *Haemaphysalis punctata* were present in the sheep as well as in goats of district Dir Lower.

Table 2: *Prevalence of tick infestation in goat population in district Mardan, Buner and Dir Lower.*

District	Identified tick species								
	Rhiphicephalus microplus	*Haemaphysalis punctata*	*Haemaphysalis sulcata*	*Hyalomma excavatum*	*Rhiphicephalus turanicus*	*Rhiphicephalus senegalensis*	*Rhiphicephalus lunulatus*	*Hyalomma dromedarri*	*Hyalomma anatolicum*
Mardan	178	92	189	134	49	120	78	97	122
Buner	132	101	244	44	32	92	56	22	40
Dir Lower	102	244	332	0	0	27	17	0	0

Table 3: *Prevalence of tick infestation in sheep population in district Mardan, Buner and Dir Lowe.*

District	Identified tick species								
	Rhiphicephalus microplus	*Haemaphysalis punctata*	*Haemaphysalis sulcata*	*Hyalomma excavatum*	*Rhiphicephalus turanicus*	*Rhipicephalus senegalensis*	*Rhipicephalus sanguineus*	*Hyalomma dromedarri*	*Hyalomma anatolicum*
Mardan	72	101	98	47	86	120	39	42	92
Buner	61	201	144	20	67	52	0	0	0
Dir Lower	93	344	232	11	0	27	0	0	0

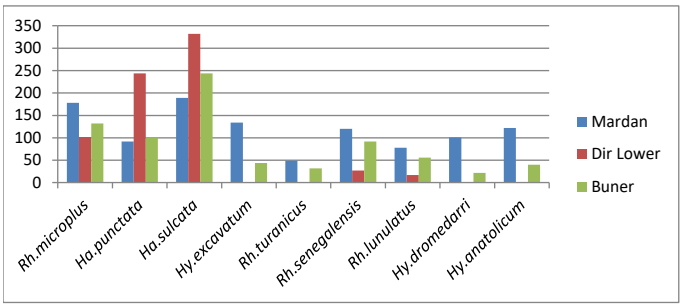

Figure 3: *District wise prevalence of tick infestation in goat population.*

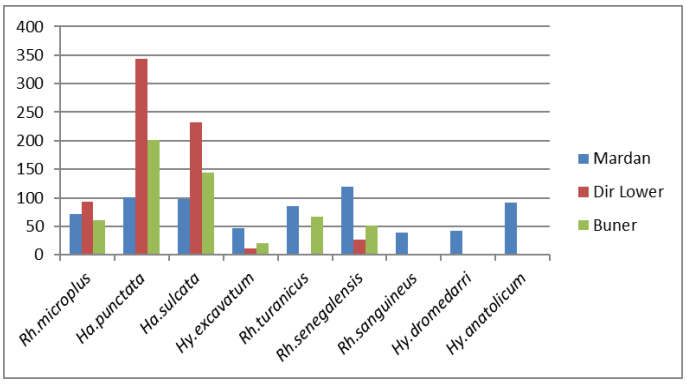

Figure 4: *District wise prevalence of tick infestation in sheep population.*

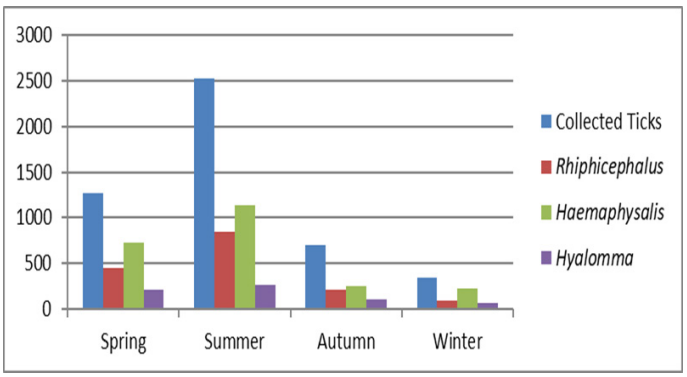

Figure 5: *Seasonal prevalence of tick infestation in sheep population.*

Seasonal prevalence of ticks

Tick prevalence showed different variations in the four seasons. Summer was the most prevalent and maximum number of ticks has been collected in the summer which was about 2527 ticks followed by spring in which 1273 ticks were collected. In autumn season which was less prevalent, the collected ticks count was 702. Winter was the less prevalent season and hardly 339 ticks were collected throughout the winter (Figure 5).

Genera-based prevalence of tick infestation

The high infestation rate of *Haemaphysalis punctata,* and *Haemaphysalis sulcata* was recorded in district Dir (lower) in sheep. The dominant tick genera of Dir Lower was *Haemaphysalis*. *Rhiphicephalus* and *Hyalomma* were the dominant species identified in district Mardan. District Buner dominant tick species was also *Haemaphysalis*.

In the research investigation, distinct tick species were recorded amongst different host animals. Hard tick's higher prevalence in goats and sheep has several contributing factors such as climate conditions, average temperature, relative humidity, vegetation type, and landscape which provide favorable environments for the development of many tick's species (Bacon *et al.*, 2022). Various conditions which may affect the reproduction and development of ticks in a specified locality include humidity, temperature, and precipitation (Greenfield, 2011).

Among the ticks collected and identified from goats and sheep ten tick species were identified from three different districts of Khyber Pakhtunkhwa, Mardan, Buner, Dir Lower. These ten tick species have belonged to three genera *Hylomma, Haemaphysalis,* and *Rhiphicephalus*. In our findings, the prevalence of *Haemaphysalis* and *Rhiphicephalus* were recorded higher. A similar study was conducted by Farooqi *et al.* (2017) in which the occurrence of hard tick species was investigated in different ecological zones of Khyber Pakhtunkhwa and documented *Haemaphysalis* and *Rhiphicephalus* as the abundant genera while the least abundant tick genus was *Hyalomma* (Farooqi *et al.*, 2017).

These ticks species belonged to the above genera *Rhiphicephalus, Haemaphysalis, and Hyalomma* are

known to be facilitators of several tick-borne infections and have been reported previously (Telmadarraiy *et al*., 2015; Mans *et al*., 2015; Ghafar *et al*., 2020; Estrada-Peña *et al*., 2008). Tick fauna associated with the study has previously been described in South Africa (Jongejan *et al*., 2020); Lebanon (Dabaja *et al*., 2017) and Pakistan (Muhammad *et al*., 2008).

Our results showed that goats were infested with ticks at 59.37% while sheep were infested with ticks at 51.45%. The highest tick infestation was observed in the sheep than in goats of district Dir Lower. This pattern has been also recorded in some studies of tick infestation of animals in Pakistan (Rashid *et al*., 2018; Ramzan *et al*., 2019). The highest tick infestation was observed in the goats than in the sheep of district Mardan which was by the results of Rehman *et al*. (2017). But this report was opposite in Dir Lower case as there were higher tick infestations in sheep than goats. The relatively high infestation of ticks may be due to their skin as they have thin skin as compared to other animals. This may be due to hilly and semi-hilly areas while plain areas have the highest infestation recorded in goats. The highest distribution of hard ticks in our study could be due to prevailing rainfall, relative humidity, host availability, condition of host body, acaricides application, breed, age, sex, average temperature, altitude, vegetation type, landscape of area animal husbandry practices (Estrada- Peña, 2003; Burri *et al*., 2007; Gray, 2008; Rony *et al*., 2010; Sajid *et al*., 2011).

In the current study sheep and goats in the 1-2 years age group were more infected than those older than 2 or more-year age group. Our finding results agree with previous findings that the younger animals shows more vulnerability to tick infestation than the adult hosts (Abera *et al*., 2010). The possible justified answer to this might be that after continuous exposure to tick infestation, the adult host has developed resistance also because of the soft skin of younger animals. The younger animals were also directly connected with their parents and other animals through which the tick transfer occurred.

The high prevalence rates of tick infestation in all three districts were recorded throughout the summer which are likely due to the humidity, moisture, and high temperature. It was according to the previous studies which also shown peak infestation rates in summer (Gul *et al*., 2021; Ullah *et al*., 2018).

Furthermore, the current results showed that the female tick's prevalence was more as compared to the prevalence of male ticks previously reported by Mustafa *et al*. (2014). Tick prevalence was somewhat higher in female sheep and goats than in males. This was against some previous studies (Shoaib *et al*., 2021).

Conclusions and Recommendations

The present study showed the prevalence of ticks and identified ten (10) tick species that belonged to three main genera *Haemaphysalis*, *Rhipicephalus,* and *Hyalomma* were the predominant tick species in small ruminant populations in district Mardan, Buner, and Dir Lower, Khyber Pakhtunkhwa, Pakistan. These ticks feed on blood and transmit various diseases affecting both domestic animals and humans directly or indirectly. It is evident from our study that tick infestation has an existing animal population and is a major threat to both the human and animal population in the area. It is recommended that necessary control measures and awareness campaigns should be adopted and implemented at the government level to control the problem of tick infestation in both small and large ruminant populations in the province of Khyber Pakhtunkhwa, Pakistan.

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Novelty Statement

This research and experimental work on "Prevalence and Morphological Identification of Tick Infestation in Small Ruminants in Khyber Pakhtunkhwa, Pakistan" is original and new in the field of zoology in Khyber Pakhtunkhwa, Pakistan.

Author's Contribution

Zulfiqar Ali: Investigation, writing-original draft preparation.
Asad Ullah: Supervision.
Shumaila Gul: Project administration.

Maryam Begum: Resources.
Raheela Taj: Formal analysis.
Tahira Tayyeb: Methodology.
Maiz ur Rahman: Visualization.
Muhammad Owais Khan: Data curation.
Rafiq Ullah: Conceptualization.
Imad Khan and Ali Gohar: Writing-review and editing.
Shakirullah Khan and Khudija Ghani: Softwar.
Muneeb Islam: Validation.

Statement of conflict of interest

The authors declared no potential conflicts of interest with respect to research, authorship, and/or publication with the work submitted.

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