

Original Research Article

Diversity and Distribution Patterns of Tick Fauna InBannu and Lakki Marwat, Khyber Pakhtunkhwa, Pakistan

ABSTRACT

Aims: This paper presents a comprehensive investigation into the tick fauna of Khyber Pakhtunkhwa, Pakistan, conducted across two districts. The study aimed to assess the diversity, relative abundance, and distribution of collected specimens to understand their patterns within the study area.

Location and Duration of Study: Tick specimens were Collected from the Bannu and Lakki Marwat districts of Khyber Pakhtunkhwa, spanning from August 2021 to June 2022.

Methodology: Visual encounter surveys were conducted, involving both daytime and nighttime observations, to collect specimens from diverse host animals such as cows, buffalo, goats, and dogs. Following the collection phase, a total of 312 specimens were carefully gathered and transported to the laboratory for further analysis. Upon arrival in the laboratory, the collected specimens were carefully arranged and preserved in Eppendorf tubes filled with a 70% ethanol solution. The preservation process aimed to maintain the integrity of the specimens for subsequent examination. The identification process involved a thorough morphological analysis, where authentic literature, updated taxonomic keys, and observation of morphological parameters were utilized.

Result: The specimens subjected to examination and identification belonged to the Ixodidae family of hard ticks, encompassing two genera: Hyalomma and Rhipicephalus. Within these genera, eight distinct species were recognized in the current study. From the Hyalomma genus, the identified species were *H.asiaticum*, *H.anatolicum*, *H.detrutum*, and *H.impeltatum*. The Rhipicephalus genus included *R.haemaphysaloides*, *R.microplus*, *R.sanguineus*, and *R.turanicus*. *R.microplus* emerged as the most dominant species, with a count of 126 individuals (SOR=100% and AQ=40.38%). Conversely, *H.detrutum*, *H.impeltatum*, *R.sanguineus* and *R.turanicus* were identified as satellite species. Additionally, *H.anatolicum*, *R.haemaphysaloides*, and nymphs were categorized as co-dominant. Moreover, this study reports the first-ever occurrences of *Hyalommaasiaticum* and *H.detrutum* in Khyber Pakhtunkhwa, specifically in the Lakki Marwat district. Furthermore, *R.haemaphysaloides* is possibly reported for the first time in both the Bannu and Lakki Marwat districts.

Conclusion: This research presents preliminary findings on the diverse tick population in regions where animal husbandry plays a crucial role in the local economy. The Lakki Marwat district exhibited the highest diversity indices compared to Bannu. The observed prevalence of ticks in the studied areas emphasizes their significant impact on domesticated animals. Cattle exhibit the highest infestation rates, followed by buffalo and goats. The current taxonomic work may require improvement.

Keywords: Tick fauna, Khyber Pakhtunkhwa, Bannu, Lakki Marwat, Species diversity, diversity indices, prevalence, infestation rates, taxonomic work.

INTRODUCTION

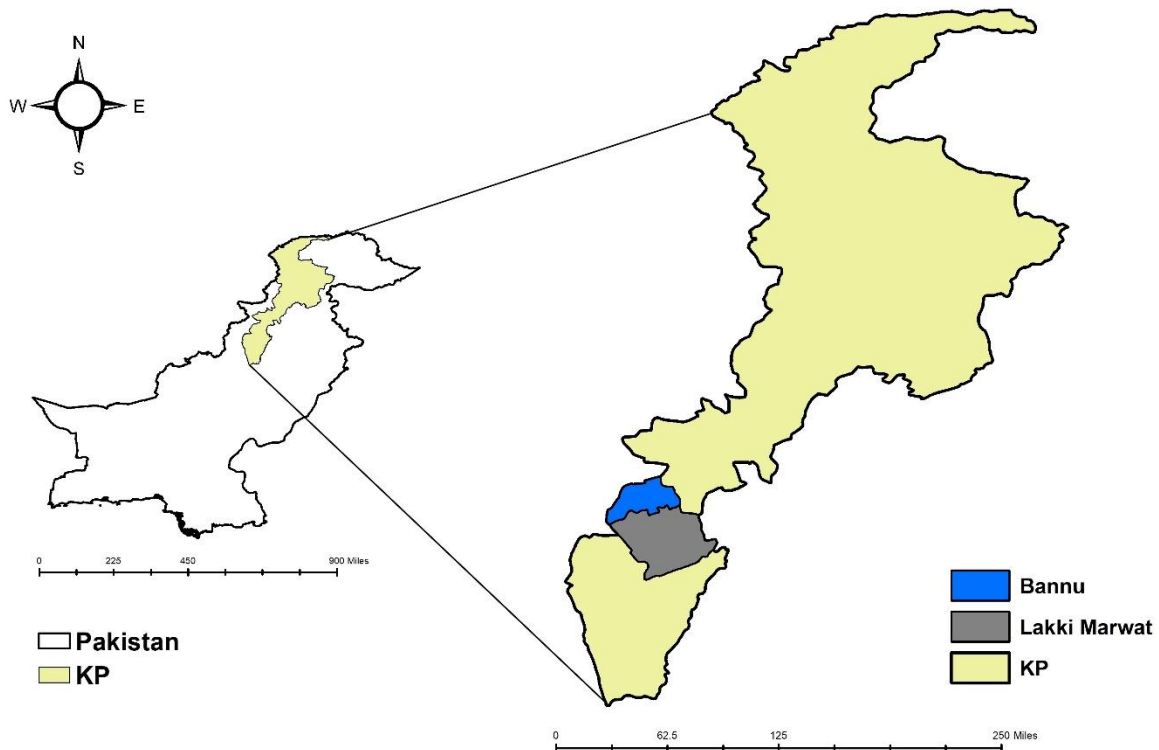
Ticks are arthropods categorized within the Phylum Arthropoda, Class Arachnida, Subclass Acari, Order Parasitiformes and Suborder Ixodida [44]. These creatures are categorized into three families: Argasidae (Soft ticks), Ixodidae (Hard ticks), and Nuttalliellidae (about which limited information is available [31,15]. Globally, the taxonomy encompasses nearly 900 tick species, with over 700 species falling under the Ixodidae (hard ticks) category and the remainder distributed within the Argasidae (soft ticks) [28]. Within Pakistan's specific regions, 53 tick species from the Argasidae and Ixodidae families have been documented [37]. The lineage of ticks traces back to the pre-mid Cretaceous period, with both Argasidae and Ixodidae families emerging in the middle of the Cretaceous era [34]. Ixodina is typified by the presence of a dorsal scutum, with nymphs and adults possessing a pair of stigmata positioned posteriorly to coxa IV. In contrast, Argasina is distinguished by the absence of a dorsal scutum, while nymphs and adults exhibit a pair of stigmata near coxa III [46]. These arachnids exhibit remarkable adaptability to diverse ecosystems, including humid temperate and tropical forests, open grasslands, deserts, continental cliffs, and remote islands hosting seabird colonies [19]. The distribution of ticks is influenced by various factors like vegetation, rainfall, temperature, predators, and the availability of hosts [47]. Host demographics and human management strategies play a significant role in tick prevalence globally [13]. Their life cycle encompasses four developmental stages: egg, larvae, nymph, and adult [49]. Importantly, all stages lack antennae and diverge from insects in terms of body segmentation, as they lack the conventional divisions of head, thorax, and abdomen [35]. In terms of respiration most tick life stages involve the conveyance of air from external openings in the stigmatal plates (spiracular) directly into tissues through trachea [48].

Ticks also serve as external parasites and are the primary carriers of diseases affecting both animals and humans on a global scale [32]. Approximately 10% of all tick species are recognized as transmitters of various pathogens [35,26]. They cause harm directly by consuming significant amounts of blood [18,33] and indirectly by inducing stress, allergies, irritation, weight loss, paralysis, and by transmitting various pathogenic microorganisms such as bacteria, fungi, protozoa, rickettsia, and spirochetes [16]. The influence of ticks on livestock is extensive, leading to considerable economic setbacks and posing a serious threat to cattle. Around 80% of the global cattle population is susceptible to diseases transmitted by ticks [21,12]. Beyond disease transmission, these parasites contribute to reduced milk and meat production, skin damage, diminished hide quality as well as induce fertility problems among livestock [45,17]. The economic losses inflicted by ticks on livestock are substantial, impacting hosts in various detrimental ways [36]. The economic impact of this challenge is evident as countries collectively expend between 13.9 and 18.7 billion dollars on vaccine procurement and dealing with the associated livestock deaths [23]. The inability to effectively manage tick populations stands as a major obstacle in maximizing livestock production [14].

Pakistan constitutes a man-made northwestern political division within the Indo-Pakistan subcontinent rather than a naturally occurring geocological entity [25]. Spanning an area of 796,096 km² and positioned between latitudes 24°N and 37°N, as well as longitudes 61°E and 78°E, the country straddles two zoogeographical regions, namely Palearctic and Oriental [11,29]. Its climate is characterized as continental, displaying notable temperature fluctuations between winter and summer seasons [1]. The presence of ticks is more prominent during the summer and spring months compared to other seasons [10,3]. Given the Pakistan's agricultural nature and the vital role of livestock in its economy, a substantial portion of its landscape provides conducive environmental conditions for tick proliferation [43,41]. The country's location in a subtropical region contributes to its rich tick diversity [40]. Particularly impactful in rural communities, these arachnids pose significant challenges to small-scale farmers involved in milk and milk-related trade [24]. Notably, hard tick species also pose threats to both human and animal well-being,

acting as carriers for diseases like theileriosis, anaplasmosis, babesiosis, and Crimean Congo Hemorrhagic Fever (CCHF) [21,38]. This underscores the importance of thorough identification and characterization of tick species due to their potential threat to humans and animals. Further investigations are imperative to comprehend the interplay between tick-borne pathogens (TBP) and tick species [27].

Currently, the prevailing tick genera in Pakistan encompass a diverse range, Argas, Ornithodoros, Amblyomma, Boophilus, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, and Rhipicephalus [37]. This comprehensive representation accounts for a total of 53 distinct species, underscoring the richness and diversity of the tick population in the region. However, a noticeable gap exists in the comprehensive data regarding Ixodid ticks in the Khyber Pakhtunkhwa (KP) province. Recognizing the urgent need for accurate estimations of tick infestation, the present study strives to fill this knowledge gap by identifying various tick species and mapping their distribution across two districts in KP. This research aims to



contribute valuable insights into the prevalence and distribution of ticks in the region, serving as a foundational step toward developing effective strategies for tick management and mitigating their impact on both livestock and human health.

STUDY AREA

The present research study was carried out in two districts situated in southern Khyber Pakhtunkhwa, namely Bannu and Lakki Marwat (vid. Fig. 1). Both districts fall within the classification of a "hot semi-arid climate." The geographical coordinates for Lakki Marwat are approximately 33.2605278°N latitude and 70.914444°E longitude, while for Bannu, they are around 32.9854°N latitude and 70.6027°E longitude. In summer, the temperature range in both Lakki Marwat and Bannu is typically around 30°C to 45°C, reflecting the intense heat characteristic of the hot semi-arid climate. Conversely, during winter,

temperatures tend to remain milder, ranging from 5°C to 20°C in both districts. Livestock, including cattle, buffaloes, sheep, and goats, are prevalent in these districts, underscoring the crucial role of animal husbandry in the local economy. Over 43% of the population relies on agriculture and livestock for their livelihoods, highlighting the significance of these sectors in the socio-economic framework of the region.

Fig. 1. Map showing locations of the study area- Khyber Pakhtunkhwa, Pakistan

MATERIALS AND METHODS

The research was systematically carried out from August 2021 to June 2022, focusing on the collection and identification of tick specimens from a variety of host animals, namely cows, buffalo, goats, and dogs.

Ticks, being hematophagous ectoparasites, were extracted from host skin regions like the ear, neck, udder, abdomen, armpits, and testes. The collection tools involved surgical gloves, hairbrushes, Specialized tick tweezers, and magnifying glasses for on-spot examination ensuring minimal damage to the tick mouthparts, which are essential for their identification. Supplementary equipment like Eppendorf tubes, 70% ethanol, labeling tape, lamps, petri dishes, and compound microscopes, were utilized in this study. Ticks were primarily found embedded in the skin of the host animals, requiring specialized techniques like disturbance using fine hairbrushes to ensure their safe extraction. Headlamps were employed during low-light conditions, ensuring clear visibility, and reducing the chances of overlooking ticks. Data recording was facilitated using field notebooks, paper tape, and pencils, which ensured that all pertinent details related to each specimen were recorded.

Preservation and Identification of the Ticks

Following collection, all tick specimens were promptly transferred to Eppendorf tubes containing 70% ethanol, serving as an acarine preservative. They were later brought to the Entomology laboratory, Department of Zoology, University of Peshawar, where they were systematically laid out for scrutiny. The morphological identification process of the ticks was executed using a compound microscope, where each tick was placed on a glass slide and cross-referenced with the identification key "Ixodid ticks (Acarina, Ixodidae) of West Pakistan" by [30], as well as with a range of peer-reviewed journals and articles to ensure accuracy.

DATA ANALYSIS METHODS

Site Occupancy Ratio (SOR)

To determine the distribution status of tick species at different location sites, we used the Site Occupancy Ratio (SOR): Eq.1

$$SOR = \frac{n_{occupied\ sites}}{N_{total\ sites}} \times 100$$

Where $n_{occupied\ sites}$ represents the number of sites where a specific tick species was found, and $N_{total\ sites}$ is the total number of sites analyzed. The SOR values were then categorized as follows: rare (0–15%), sporadic (15–30%), common (30–50%), prevalent (50–70%), and ubiquitous (70–100%).

Abundance Quotient (AQ)

To assess the relative abundance of tick species, we employed the Abundance Quotient (AQ): Eq.2

$$AQ = \frac{n_{individuals}}{N_{total\ individuals}} \times 100$$

In this equation, $n_{\text{individuals}}$ represent the number of specimens collected for a specific tick species, and $N_{\text{total individuals}}$ is the total number of specimens collected across all tick species. The AQ values were then classified into dominant (AQ > 10%), co-dominant (5–10%), and satellite (< 5%) categories based on their abundance in the overall sample.

Shannon-Wiener Index (H)

The Shannon-Wiener Index was calculated to find out the diversity of ticks. It can be calculated by the given expression: Eq.3

$$(H) = \sum_{i=1}^s p_i \ln(p_i)$$

RESULTS

The taxonomic examination of the collected tick specimens unveiled a singular family classification, Ixodidae, encompassing two distinctive genera: Hyalomma and Rhipicephalus. Further scrutiny within the genus Hyalomma revealed the presence of four distinct species, namely *Hyalomma asiaticum*, *Hyalomma anatolicum*, *Hyalomma detritum*, and *Hyalomma impeltatum*. Simultaneously, the genus Rhipicephalus exhibited a diversity of four species: *Rhipicephalus haemaphysaloides*, *Rhipicephalus microplus*, *Rhipicephalus sanguineus*, and *Rhipicephalus turanicus* (vid. Fig. 2).

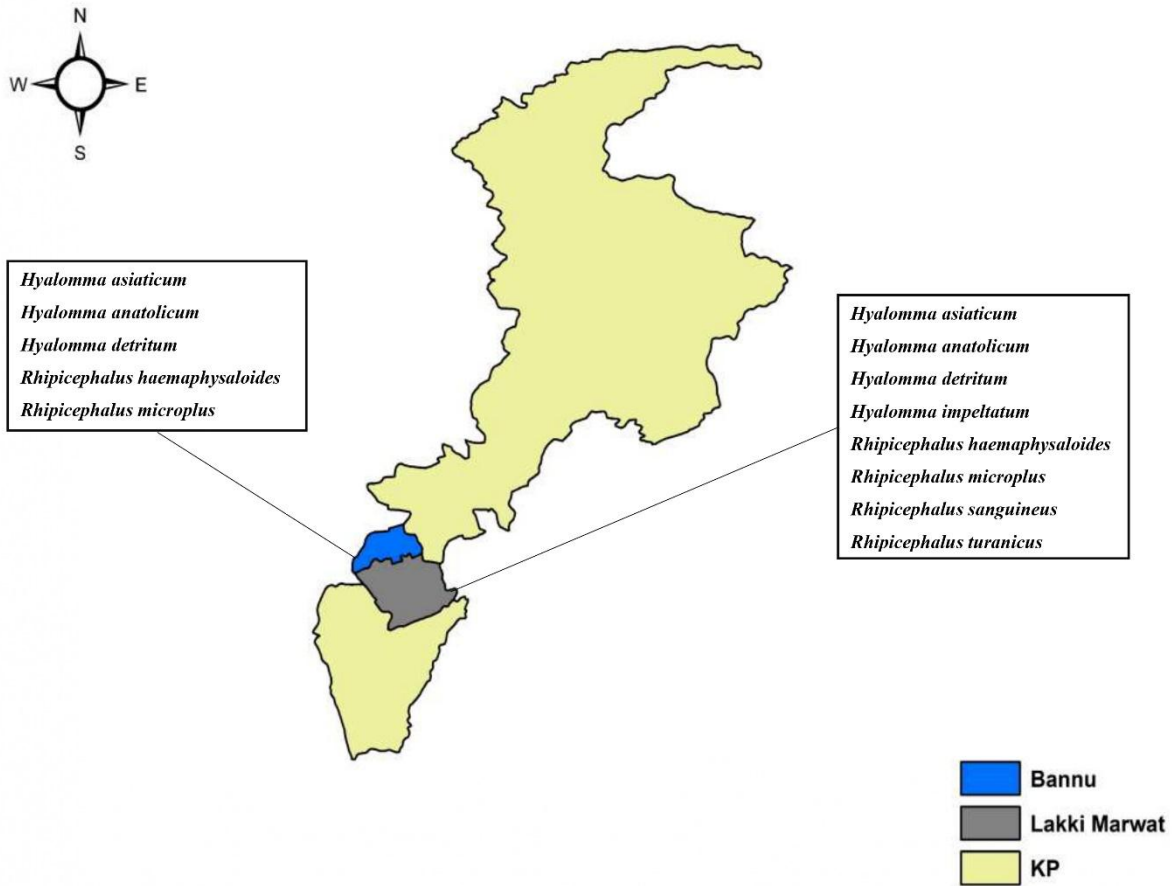


Fig. 2. Geographical distribution of Ticks in Khyber Pakhtunkhwa, Pakistan

Abundance of Tick Species

The bar graph provides an overview of the distribution and abundance of various tick species in two specific districts. The data highlights the prevalence of *Rhipicephalus microplus*, the significant presence of *Hyalomma asiaticum*, *Hyalomma anatolicum*, *Rhipicephalus haemaphysaloides*, and the comparatively smaller populations of *Hyalomma detritum*, *Rhipicephalus turanicus*, *Rhipicephalus sanguineus*, and *Hyalomma impeltatum*. Additionally, Nymphs were collected from both districts, with a total count of 25, although they are not represented in the graph.

Based on the scrutiny of the 312 collected ticks, it is evident that *Rhipicephalus microplus* is the most prevalent species, with a count of 126 individuals (vid. Fig. 3). This species constitutes 40.38% of the total collected ticks, indicating its dominance in both districts. The second most abundant species is *Hyalomma asiaticum*, with a notable count of 79 individuals (vid. Fig. 3). This species also demonstrates a widespread presence in both districts Bannu and Lakki Marwat, highlighting its adaptability and successful establishment in the local ecosystem. In the third position in terms of abundance, *Hyalomma anatolicum* is observed with a count of 31 individuals (vid. Fig. 3). Furthermore, *Rhipicephalus haemaphysaloides*

documented with a count of 22 individuals in the studied regions (vid. Fig. 3). Although its population size is smaller compared to the dominant species, its presence signifies its ecological importance in the studied regions. Additionally, the dataset records the presence of *Hyalommadetritum*, with 15 individuals observed in districts Bannu and Laki Marwat (vid. Fig. 3). *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* are observed with counts of 7 and 5 individuals, respectively, exclusively in district Laki Marwat, indicating their relatively lower population densities (vid. Fig. 3). Finally, *Hyalomma impeltatum* is observed in the district of Laki Marwat, with a count of only 2 individuals (vid. Fig. 3). Despite the lower numbers, the identification of the last three species adds to the overall species diversity and emphasizes its existence in the sampled area. These findings significantly contribute to our understanding of the local tick fauna and offer valuable insights for future research works.

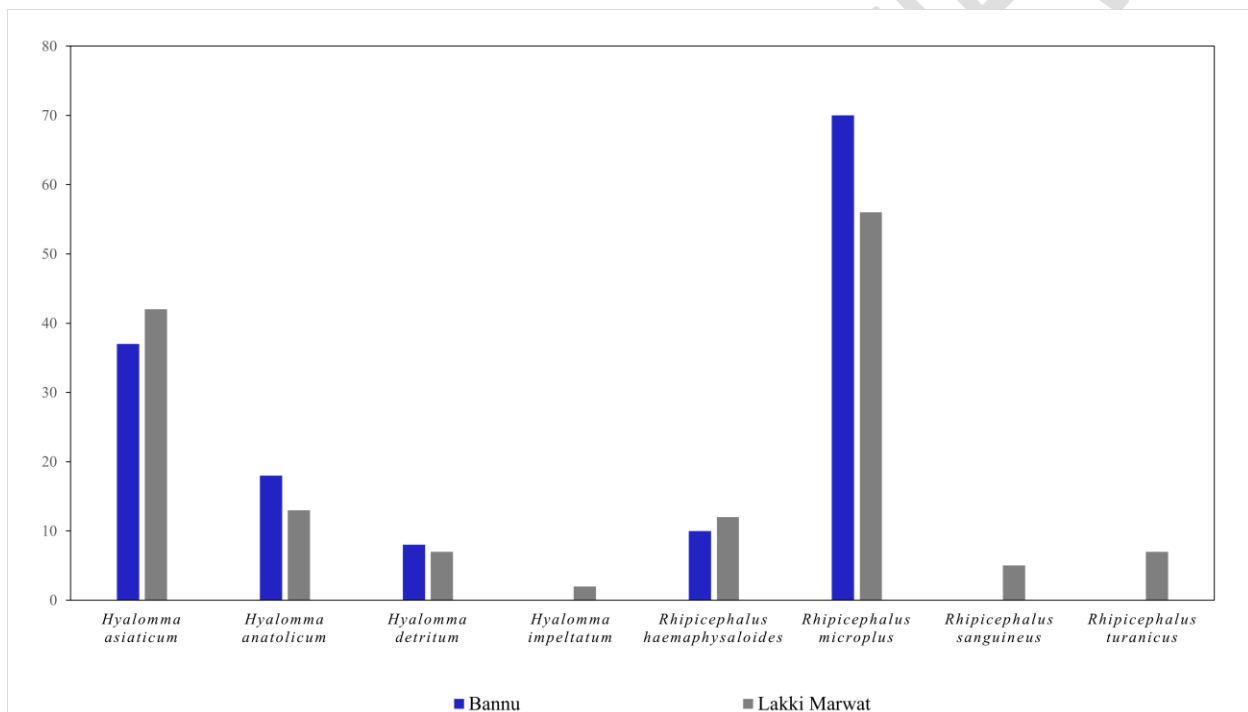


Fig. 3. Relative abundance of Ticks found in the two districts

Host Specific Tick Infestation

The bar graph provides a comprehensive cross-comparison of tick infestation rates among various host animals, revealing notable trends and variations (vid. Fig. 4). *Rhipicephalus microplus* emerges as the dominant species, infesting both cows and buffaloes to a notable extent, emphasizing its prevalence in ruminant populations. In contrast, the exclusive occurrence of *Hyalomma impeltatum* in goats, with a total of 2 specimens, and *Rhipicephalus sanguineus* in dogs, with a recorded count of 5 specimens, suggests a more targeted host preference. Among the *Hyalomma* species, *Hyalomma asiaticum* displays a higher infestation rate in cows, boasting 43 specimens compared to 36 in buffaloes, indicating a clear inclination towards the bovine host. Similarly, *Hyalomma anatolicum* and *Hyalomma detritum* consistently exhibit a higher prevalence in cows compared to buffaloes. Interestingly, *Rhipicephalus haemaphysaloides* deviates from this pattern, showing a higher infestation rate in buffaloes compared to cows and goats. The exclusive occurrence of *Rhipicephalus turanicus* in cows contributes to the overall diversity of tick infestations among different hosts. These comparisons highlight the intricate relationships between tick

species and their host preferences, providing valuable insights for understanding ecological dynamics and implementing targeted control strategies.

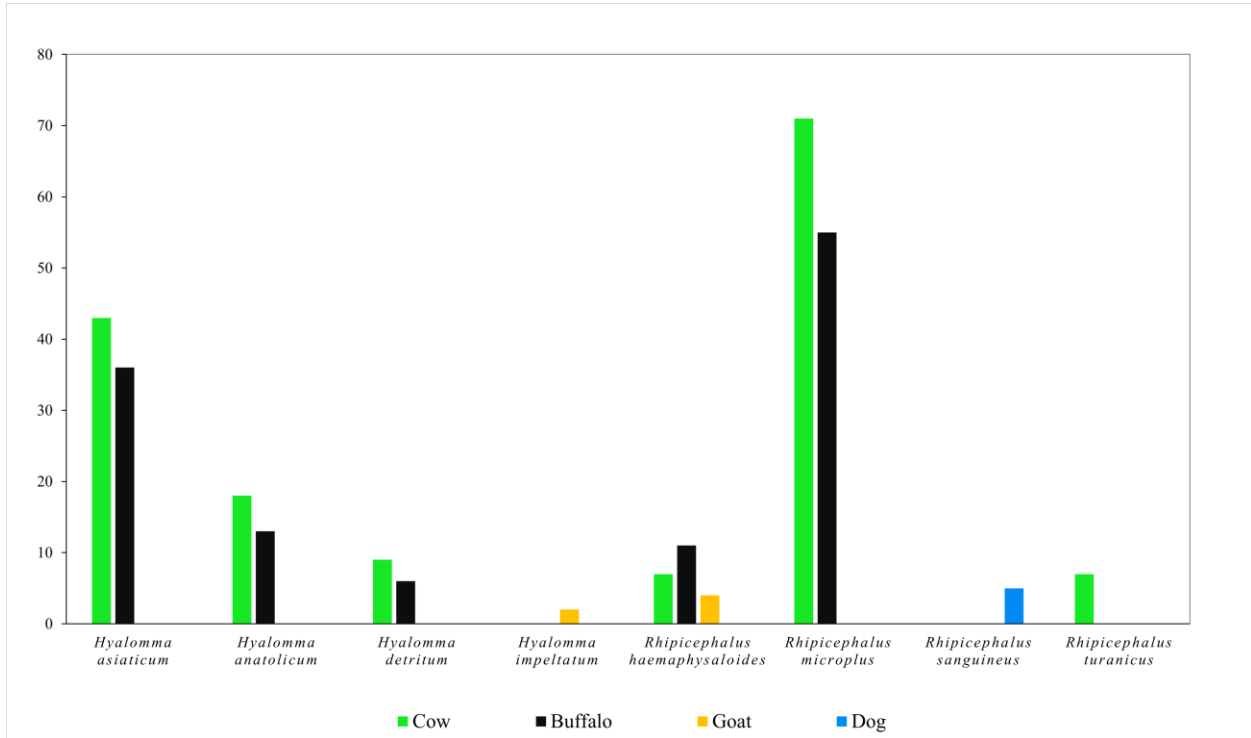


Fig. 4. Infestation of Ticks found among host animals

UNDER PEE

UNDER PEER REVIEW

Diversity Indices

In our examination of tick species within the geographical boundaries of Bannu and Lakki Marwat districts, our study conducted a thorough identification and analysis. The resulting data, delineated in Table 1 and Table 2, offers valuable insights into the biodiversity of tick populations within the respective districts. The analysis employs the Shannon-Wiener Index (H value) as a robust metric, encapsulating

Table 1. Diversity indices of the tick species in Bannu district

both the richness and evenness of tick species distribution.

Species	Individuals	P_i	$\ln(P_i)$	$P_i \ln(P_i)$
<i>Hyalomma asiaticum</i>	37	0.24183	-1.41952	-0.3432826
<i>Hyalomma anatolicum</i>	18	0.11765	-2.1400662	-0.2517725
<i>Hyalomma Detritum</i>	8	0.05229	-2.9509964	-0.1543005
<i>Rhipicephalus haemaphysaloides</i>	10	0.06536	-2.7278528	-0.178291
<i>Rhipicephalus microplus</i>	70	0.45752	-0.7819427	-0.3577516
Nymphs	10	0.06536	-2.7278528	-0.178291
H				1.463689192

Table 2. Diversity indices of the tick species in Lakki Marwat district

Species	Individuals	P_i	$\ln(P_i)$	$P_i \ln(P_i)$
<i>Hyalomma asiaticum</i>	42	0.26415	-1.3312346	-0.3516469
<i>Hyalomma anatolicum</i>	13	0.08176	-2.5039548	-0.2047259
<i>Hyalomma Detritum</i>	7	0.04403	-3.1229941	-0.1374903
<i>Hyalomma Impeltatum</i>	2	0.01258	-4.375757	-0.055041
<i>Rhipicephalus haemaphysaloides</i>	12	0.07547	-2.5839976	-0.1950187
<i>Rhipicephalus microplus</i>	56	0.3522	-1.0435525	-0.3675405
<i>Rhipicephalus sanguineus</i>	5	0.03145	-3.4594663	-0.1087882
<i>Rhipicephalus turanicus</i>	7	0.04403	-3.1229941	-0.1374903
Nymphs	15	0.09434	-2.360854	-0.2227221
H				1.78046383

The relative abundance and distribution of the species is shown in Table 3. The calculations are based on the total population of eight species across two different districts. *R. microplus* was the most dominant species found at all the study sites with SOR=100% and AQ=40.38%, while species like *H. detritum*,

H. impeltatum, *R. sanguineus* and *R. turanicus* were identified as satellites. Additionally *H. anatolicum*, *R. haemaphysaloides* along with Nymphs were classified as Co-dominant.

Table 3. Distribution and relative abundance of tick species in the two districts

S.No.	Species name	Total number of specimens	Distribution (SOR)	Status	Relative abundance (AQ)	Status
1.	<i>H. asiaticum</i>	79	100%	Ubiquitous	25.32%	Dominant
2.	<i>H. anatolicum</i>	31	100%	Ubiquitous	9.93%	Co-dominant
3.	<i>H. detritum</i>	15	100%	Ubiquitous	4.80%	Satellite
4.	<i>H. impeltatum</i>	2	50%	Prevalent	0.64%	Satellite
5.	<i>R. haemaphysaloides</i>	22	100%	Ubiquitous	7.05%	Co-dominant
6.	<i>R. microplus</i>	126	100%	Ubiquitous	40.38%	Dominant
7.	<i>R. sanguineus</i>	5	50%	Prevalent	1.60%	Satellite
8.	<i>R. turanicus</i>	7	50%	Prevalent	2.24%	Satellite
9.	Nymphs	25	100%	Ubiquitous	8.01%	Co-dominant

DISCUSSION

Tick identifications were carried out through morphological analysis utilizing identification keys. Identification of the tick specimens revealed that the collected ticks belong to one family, Ixodidae and a total of eight tick species distributed across two genera: Rhipicephalus and Hyalomma. Within the genus Hyalomma, four distinct species were identified: *Hyalomma asiaticum*, *Hyalomma anatolicum*, *Hyalomma detritum*, and *Hyalomma impeltatum*. The genus Rhipicephalus presented four species: *Rhipicephalus haemaphysaloides*, *Rhipicephalus microplus*, *Rhipicephalus sanguineus*, and *Rhipicephalus turanicus*.

In the present study the predominant occurrence of tick infestations was noted in cattle, potentially attributed to their thinner skin and the conducive habitat and climatic conditions for ticks in Pakistan [24]. Our findings, indicating lower tick infestation rates in buffaloes compared to cows, align with previous research that consistently reported a lesser prevalence of tick infestation in buffaloes compared to cattle [39,16,2]. The lower rate of tick infestation in goats was maybe due to pasturing in steep and rocky habitats that limit contact with other species of livestock [5].

In the context of this study, we can conclude that *Rhipicephalus microplus* a prevalent species in KP province, particularly in Bannu district. According to the study by [42], *Rhipicephalus microplus* is one of the most abundant species in district Bannu, with a total sample size of 1474, aligning with our current

findings. Consistent with our results, earlier studies by [15,4,8,9] confirmed the presence of *Rhipicephalus microplus* in districts Bannu and Lakki Marwat.

Regarding *Hyalomma asiaticum*, which is present in both of our study locations was reported from Peshawar, Mardan and Bannu by [20], while our study possibly marks the first reporting of *Hyalomma asiaticum* in district Lakki Marwat.

As shown above (Table 3.), *Hyalomma anatolicum* was found to be a co-dominant species in our study sites. Existing literature supports its wide distribution in both districts Bannu and Lakki Marwat, as observed in studies by [22,15,42,4,8,9].

Hyalomma detritum found in both study sites, i.e., Bannu and Lakki Marwat, in our findings, showcasing its adaptability to the region. However, previous findings by [15] only confirm its presence in Bannu, with no reports found from district Lakki Marwat in earlier studies. *Rhipicephalus sanguineus* appears to be a sporadic species, limited to Lakki Marwat. In contrast, previous literature reports its presence in both Lakki Marwat and Bannu, as noted by [22,4]. Additionally, [7] reported its presence in rural areas, specifically Mardan and Charsadda.

Similarly, in our current survey, *Rhipicephalus turanicus* and *Hyalomma impeltatum* were exclusively identified in District Lakki Marwat. Previous research validates the presence of *Rhipicephalus turanicus* in Bannu, as reported by [42] and [4] also documented its occurrence in both Bannu and Lakki Marwat. Additionally, [9] affirmed its existence in Bannu and Karak. Conversely, data on *Hyalomma impeltatum* limited in Khyber Pakhtunkhwa; [15], recorded its presence in district Bannu, while [6] reported its occurrence in districts Bannu and Karak, respectively.

Lastly, *Rhipicephalus haemaphysaloides*, which was previously documented exclusively in selected districts of KP, specifically Peshawar, Charsadda, and Mardan in the study conducted by [6], lacks previous data confirming its presence in the Bannu and Lakki Marwat districts. This underscores our current findings, which serve as the inaugural report of *R. haemaphysaloides* in these localities.

CONCLUSION

Hard ticks are widely distributed not only in Pakistan but also across diverse global regions. Their significance lies in serving as highly effective vectors for a range of pathogens, including protozoa, bacteria, and viruses. This study offers initial insights into the presence of diverse tick fauna in regions where animal husbandry significantly contributes to the local economy. The prevalence of ticks observed in the investigated areas highlights the substantial impact on domesticated animals, with cattle demonstrating higher infestation rates, followed by buffalo and goats.

Despite the negative impact of ticks on the local ecosystem, substantial knowledge gaps persist, particularly regarding the identification of various tick species in the unexplored regions of Khyber Pakhtunkhwa (KP). The findings of this study underscore the considerable potential of KP in revealing undiscovered tick species, potentially expanding scientific knowledge with the addition of new species. Beyond contributing to our current understanding, this study lays a robust foundation for future, in-depth exploration, and identification of ticks within this specific geographical region. Ongoing research in this area is pivotal for advancing our knowledge of tick diversity and ecology, ultimately facilitating the development of more effective strategies for tick-borne disease management and control in the region.

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