ISTANBUL UNIVERSITY CERRAHPASA

Epidemiological Assessment of Tick Infestations in Livestock and Domestic Fowl in Azad Kashmir: Prevalence, Species Diversity, and Implications for Disease Control

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Cite this article as: Ahmed, Z., Waqas, M., Hussain, A., Ahmed, I., Ahmed, I., Jabbar, A., Kiani, F. A., Kuthu, Z. H., Shamim, A., & Nawaz, M. (2025). Epidemiological assessment of tick infestations in livestock and domestic fowl in Azad Kashmir: Prevalence, species diversity, and implications for disease control. *Acta Veterinaria Eurasia*, 51, 0059, doi: 10.5152/actavet.2025.24059.

Abstract

Ticks, the blood-feeding arthropods, play a crucial role in transmitting diseases to livestock and pose serious economic losses. However, the diversity of tick species infesting domestic animals in Azad Kashmir, Pakistan, particularly in Bhimber, Mirpur, Kotli, and Bagh districts, is not well understood. This study aimed to assess the prevalence and identify tick species affecting livestock in these regions. A total of 1713 animals were examined, revealing 1209 cases of tick infestation. Morphological analysis of 1696 female ticks identified several species: *Rhipicephalus microplus*, *Rhipicephalus sanguineus*, *Haemaphysalis punctata*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum*, and *Nosomma monstrosum*, infesting cattle, buffalo, sheep, and goats. Infestation rates were 72.42% in sheep, 68.81% in goats, 40% in buffalo, and 73.14% in cattle. *H. punctata* was the most prevalent species in small ruminants, while *R. microplus* dominated infestations in cattle and buffalo. Principal Component Analysis (PCA) showed significant variations in tick species prevalence among districts, with PC1 explaining 56.13% of the variance. Additionally, in domestic fowl and their shelters, *Argas persicus* was the dominant tick species. The study highlights the high prevalence of ticks in both small and large ruminants, as well as domestic fowl. These findings are expected to raise awareness among livestock owners regarding the need for effective prevention and control measures in the affected regions.

Keywords: Argas persicus, Hyalomma anatolicum, Haemaphysalis punctata, Nosomma monstrosum, Rhipicephalus microplus, Rhipicephalus sanguineus

What is already known on this topic?

- *Tick populations in District Rawalakot have been identified, with only two to three species recognized through both morphological and molecular methods. However, limited information is available regarding the tick species present in other areas of Azad Kashmir. There is a need for further research to better understand the diversity and distribution of ticks across the region, as well as the potential health risks associated with them. Expanding studies to include more areas, utilizing both morphological and molecular identification techniques, and examining ecological factors could provide a clearer picture of the tick populations in Azad*

Introduction

Livestock, as the "spine of the agriculture sector," is an integral part of the rural economy of developing countries such as Pakistan. More than 70% of the population of Pakistan resides in rural areas of the country (Bibi et al., 2020; Ramzan et al., 2020; Rehman et al., 2017; Shah et al., 2015). However, the livestock sector in all the regions of Pakistan, including Azad Kashmir, is facing severe economic losses due to diseases caused by several bacterial, viral, and parasitic agents. Among parasites, ticks are known to transmit a variety of pathogens that cause many diseases in animals such as babesiosis, theileriosis, anaplasmosis, and cowdriosis (Ghafar et al., 2020a). Additionally, tick infestation may result in skin damage, weight loss, abortion, and mortality, leading to extensive economic losses (Jongejan & Uilenberg, 2004; Kaur et al., 2015). Therefore, we attempted to investigate the prevalence and distribution of tick species affecting livestock in Azad Kashmir, a critical issue given the economic importance of livestock in this agrarian region. Despite the known economic losses due to ticks, comprehensive studies focusing on this region are limited.

Despite the adverse impact of tick infestation on animal health and the economic well-being of small-scale farmers, comprehensive documentation of tick prevalence among domestic animals and poultry in Azad Kashmir is lacking (Sultana et al., 2015a). The majority of the region's population resides in rural areas, relying heavily on livestock for substantial income. The livestock statistics for the year 2019, as reported in the AJK Statistical Yearbook, indicate a population of 0.566 million cattle, 0.717 million buffalo, 1.807 million goats, 0.243 million sheep, and 4.33 million poultry (Government of AJK, 2019). The region encompasses diverse landscapes, including hilly areas and plains that are conducive to grazing,

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Received: August 2, 2024 • **Revision Requested:** September 5, 2024 • **Last Revision Received:** October 11, 2024 • **Accepted:** October 17, 2024 • **Publication Date:** January 22, 2025 • DOI: 10.5152/actavet.2025.24059

Available online at actavet.org

Kashmir. This would also aid in understanding their role as vectors of diseases and contribute to better public health strategies.

What does this study add on this topic?

- *The current study aims to provide detailed information on the tick species present in the region. The results will not only identify the tick species infesting domestic animals but will also include an analysis of the species affecting the poultry population in the study area.*

influencing breed distribution based on atmospheric conditions (Awan et al., 2020). For instance, cross-bred, Australian, and Sahiwal cattle are predominantly raised in grazing-friendly areas such as Bhimber, Mirpur, and Kotli. The buffalo population, comprising Desi breeds, is distributed across all districts, with Neelum, Muzaffarabad, Bagh, and Rawalakot featuring an abundance of Desi buffalo breeds, while Nili Ravi buffalo are more prevalent in Bhimber, Mirpur, and Kotli. Goats have a favorable distribution throughout Azad Kashmir, while sheep are primarily concentrated in the hilly areas.

Besides the livestock sector of the country, the poultry industry provides employment to approximately 1.5 million people in Pakistan. Similarly, the current investment shared by this sector is approximately €4.2 billion (Rs 700 billion) (Government of Pakistan, 2019-2020). In the last two decades, a record development in commercial poultry production (broiler, layer, and breeder) has been reported. In addition to the emergence of commercial poultry, people are still raising domestic fowl in urban and rural areas of the country for egg and meat production (Zahid et al., 2021). Although the primary focus of poultry scientists was to improve production by improving management against poultry diseases, attention was not drawn towards ectoparasites and their control in domestic as well as commercial poultry.

The efficacy of tick-control strategies is contingent upon up-to-date data about the prevalence and distribution of ticks across various hosts. With this consideration, our study was meticulously crafted to comprehend the distribution patterns of ticks and their associated species infesting livestock in four distinct districts of Azad Kashmir – Bhimber, Mirpur, Kotli, and Bagh. This involved an extensive examination of tick infestation on domestic animals, encompassing sheep, goats, cattle, and buffalo, conducted on a large scale. Furthermore, a comprehensive assessment of domestic fowl and their habitats was undertaken to identify the attachment of tick species. The outcomes of this investigation are anticipated to contribute significantly to the formulation of effective control strategies against ticks and tick-borne diseases in the specified regions.

Materials and Methods

Ethical Considerations

In this investigation, we did not use any techniques that were hazardous to the target animals. Ticks, external parasites, were removed from the bodies of animals, which is a standard practice among farmers to protect their animals from external parasites. As a result, the current study did not require any permission from the animal care committee.

Description of Study Area

Ticks' prevalence and screening were carried out in four districts of Azad Jammu and Kashmir: Bhimber, Mirpur, Kotli, and Bagh. Bhimber, Mirpur, and Kotli are situated in the southern part, while Bagh is in the northern region. These locations are positioned at approximately 34.22°N latitude and 73.28°E longitude on the global map. The area experiences a moderate climate, with hot summers in the southern parts and cold winters in the eastern and northern regions. The average annual rainfall in the region ranges from 1300 to 1800 mm. The study site is known for its expansive grazing lands and diverse plant life, crucial for sustaining livestock. The region has a distinctive microclimate characterized by moderate to very low temperatures and significant rainfall, creating lush pastures. Additionally, the presence of the Jhelum River and various natural water reservoirs enhances the agricultural and ecological diversity of the area (Wikimedia Foundation, 2024). These environmental conditions create an ideal habitat for both livestock and the growth and development of parasites. Livestock rearing in the area primarily relies on free-range grazing during the summer months, supplemented with fodder, and a more concentrated diet during adverse weather conditions. The region lacks modern or commercial farming practices, and local pastoralists adhere to traditional rearing methods. Limited veterinary services, including deworming and dipping, contribute to a higher burden of ecto- and endo-parasitic infestations, showcasing a rich diversity that warrants comprehensive study (Zinsstag et al., 2010).

Study Design and Tick Collection

A cross-sectional study was conducted from April 2022 to October 2023 to determine the prevalence of ticks infesting domestic animals, mainly sheep, goats, cattle, and buffalo. Animals from four districts of Azad Kashmir, namely Bhimber, Mirpur, Kotli, and Bagh, were used as the target population. The study duration spanned 18 months to capture the seasonal variation in tick populations, which fluctuate throughout the year based on environmental conditions. Animals were inspected thoroughly for the presence of ticks by physical examination. If found positive, ticks were detached from ears, back of the neck, udder, under the tail base, end of the tail (in terms of buffalo for the collection of *Nosomma* tick), and perineum using a standardized protocol (Soulsby, 1968). Ticks were removed carefully with the help

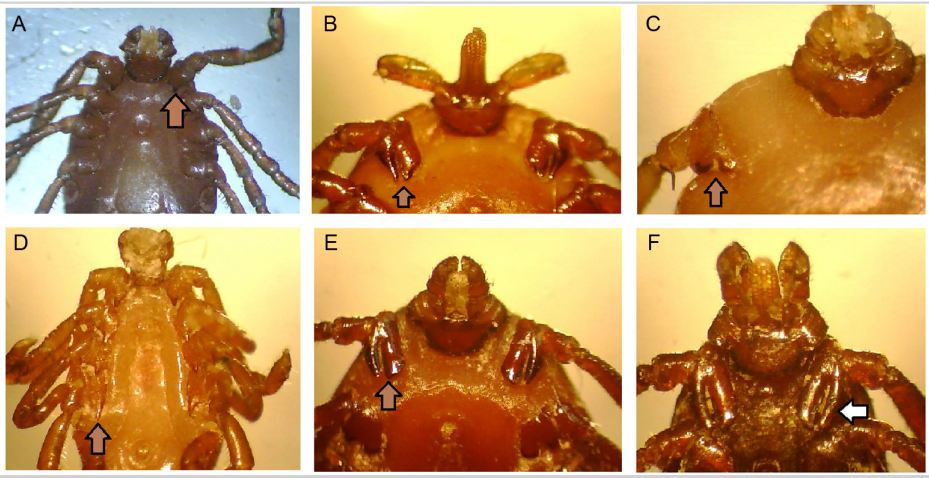

Figure 1.
Morphological Features of Tick Species. A, D: Pointed spur on coxa. B, E, and F: Longer and equal spurs on first coxa. C: Bifid first coxa.

of forceps to avoid any damage to the mouthparts or other body parts of the collected tick. Collected ticks were immediately placed in vials containing 100% ethanol and were labeled with the origin of the animal and its identification (sex, breed, and site of tick location on its body) (Wambura et al., 1998). In addition to this, Argasidae ticks were also collected from the domestic fowl and their shelters. Soon after collection, tick specimens were transferred to the Laboratory of Parasitology, Faculty of Veterinary and Animal Sciences, University of Poonch, Rawalakot, Azad Kashmir for further processing.

Morphological Examination of Ticks

Ticks were morphologically examined using a LABOMED CZM6 (Labo America, USA) stereo microscope. Tick species were identified with the help of taxonomic keys (Barker & Walker, 2014; Hoogstraal & McCarthy, 1965; Hosseini-Chegeni et al., 2019; Walker, 2003). A comparative analysis was generated between the body characteristics of ticks such as scutum pattern (length, shape), spiracles (shape), coxa, shape of capitulum, festoons (number, presence or absence), and eyes to differentiate between species as shown in Figures 1–3.

Principal Component Analysis (PCA) of Tick Species

The PCA was conducted to explore the underlying structure and patterns in the distribution of tick species across districts. The dataset, comprising counts of different tick species in various districts, was preprocessed by standardizing the numerical features to ensure comparability. Missing values were imputed with zeros, and the data were scaled for further analysis. The prcomp function in R was employed to perform PCA on the standardized data, generating principal components that capture the maximum variance in the original dataset. The biplot function was utilized to visualize the relationships between tick species and principal components. Loadings on each principal component were examined to understand the contributions of different tick species to the overall variance. Cumulative variance was calculated to assess the proportion of the total variance explained by each principal component.

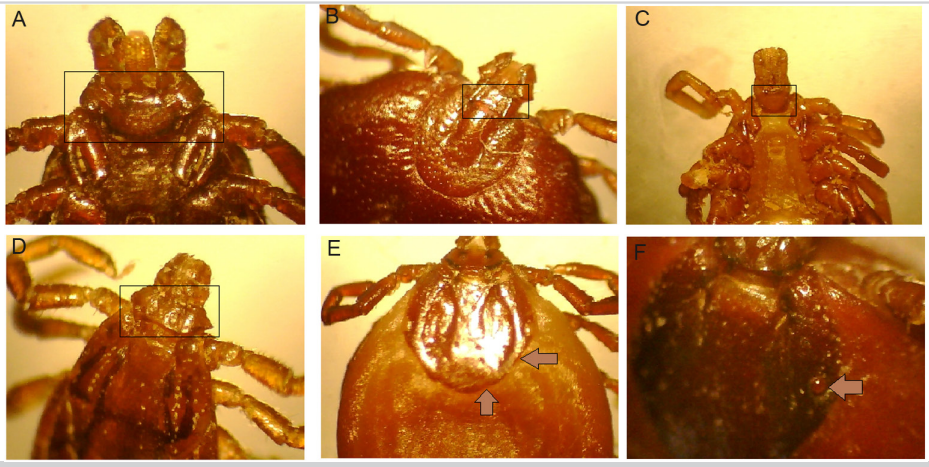

Figure 2.
Morophological Ffeaturs of Tick Species. A, D: Hexagonal basis capitulum (extends laterally). B: Rectangular basis capitulum. F: Convex eyes on scutum.

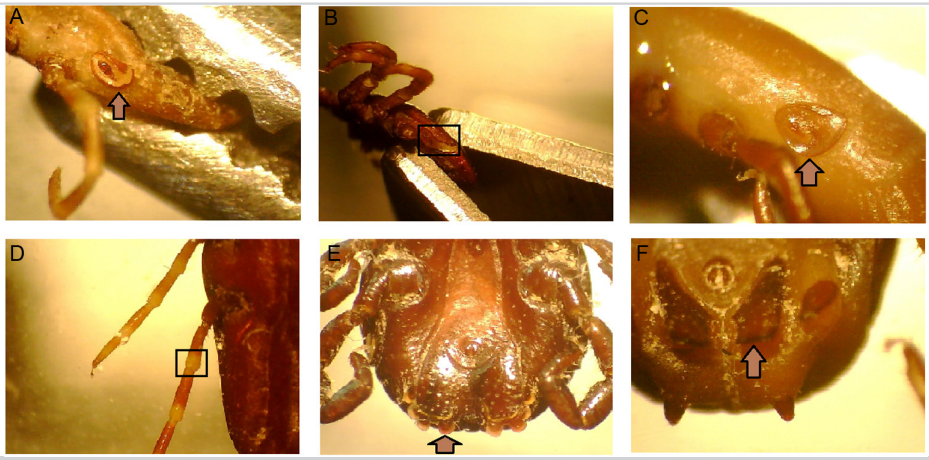

Figure 3.
Morphological Features of Tick Species. A, B, C: Different shapes of spiracles (coma and cigar-shaped). D: Yellow spots on legs. E, F: Adanal plates of male ticks.

Table 1.
Host-Wise Prevalence Rate of Ticks

Animal Species	Total Animals Observed	Infested	Non-Infested	Infected Percentage (%)
Cattle	525	384	141	73.14
Buffalo	200	80	120	40
Sheep	475	344	131	72.42
Goat	513	353	160	68.81
Total	1713	1209	479	71.62

Statistical Analysis

The statistical analysis employed in this study utilized the Statistical Package for Social Sciences, version 17.0.1 software (SPSS Inc.; Chicago, IL, USA) and the Prism-6 software package by GraphPad Software. The Chi-square tests available in the GraphPad Prism-6 software package were applied to assess the correlation between tick infestation and factors such as the breed of the targeted animals. Different graphical representations of data were conducted by using R language programming software version 4.3.1.

Results

Prevalence of Tick Infestation

A total of 1713 animals among four districts of Azad Kashmir were examined for tick infestation. Among the targeted animals, 70.57% (1209) were found positive for tick infestation. The percentage of female tick infestation was 73.14% for cattle, 68.81% for goats, 72.42% for sheep, and 40% for the buffalo population as shown in Table 1. In addition to this, some unidentified tick species, particularly in immature stages, were excluded from the study due to the challenges in their morphological identification. Future studies incorporating molecular techniques may offer more detailed insights.

Identified Tick Species

Four genera of ticks were identified, namely *Hyalomma*, *Haemaphysalis*, *Rhipicephalus*, and *Nosomma*. However, six tick species were found to be infective for cattle, buffalo, sheep, and goat populations as shown in Table 2. *R. microplus*, *R. sanguineus*, *H. punctata*, *H. bispinosa*, *H. anatolicum*, and *N. monstrosum* were identified as the major tick species infesting animals in all four districts. *H. punctata* was the most abundantly observed species (46.64%), followed by *H. anatolicum* (19.08%), *R. microplus* (17.92%), *R. sanguineus* (9.69%), *N. monstrosum* (4.18%), and finally *H. bispinosa* (2.51%) as shown in Table 2.

Table 2.
Prevalence Rate of Tick Species Across the Study Area

Tick Species	Bhimber	Mirpur	Kotli	Bagh	Total	Percentage (%)
R. microplus	94	26	76	111	307	17.92
R. sanguineus	26	22	52	66	166	9.69
H. punctata	125	167	177	330	799	46.64
H. bispinosa	25	18			43	2.51
H. anatolicum	41	148	71	67	327	19.08
N. monstrosum	45	26	0	0	71	4.14

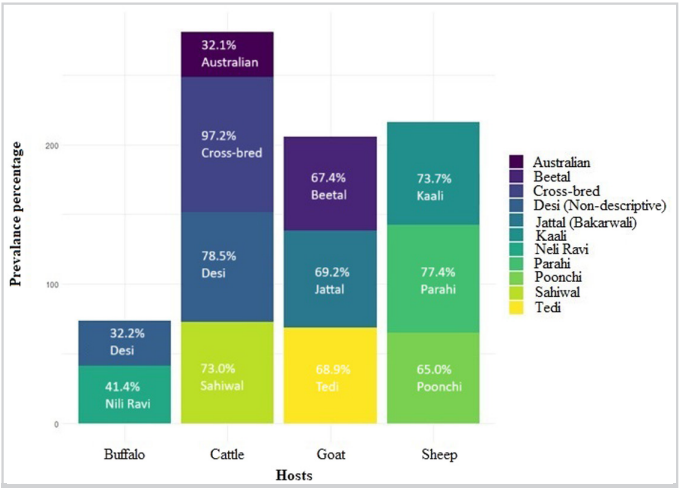

Figure 4.
Breed Wise Prevalence Across Total Animals.

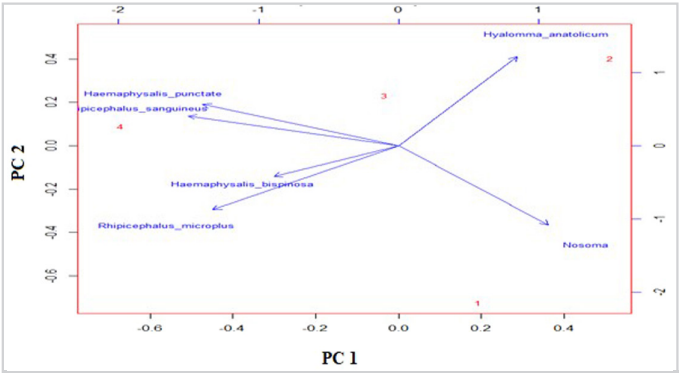

Figure 5.
PCA Biplot Representing RrelationshipS Among Different Screened Tick Species in this Study.

Breed-Wise Prevalence and Chi-Square Analysis of Tick Infestation

Elevated tick populations were observed across various goat breeds, with Tedi and Jattal breeds exhibiting high infestation, while the Beetal breed showed a 67.41% infestation rate as shown in Figure 4. Similarly, Parahi and Kaali breeds of sheep displayed infestation rates exceeding 70%, whereas the Poonchi breed, known for its large numbers, exhibited a comparatively lower infestation rate of 65.03%. Among buffalo breeds, both Neli Ravi and Desi breeds were investigated, revealing moderate tick infestation in Neli Ravi and a lower 32.25% infestation in Desi breeds. Cross-bred, Sahiwal, and Desi breeds of cattle, particularly the Cross-bred breed, demonstrated high tick infestation, while Australian cattle in Bhimber exhibited a lower prevalence of 32.11%.

In the chi-square analysis for the Sheep category, varying degrees of association between host breeds and tick infestation were observed. Specifically, "Poonchi" and "Parahi" breeds showed no statistically significant association, with *p*-values exceeding .05 as shown in Table 3. In contrast, the "Kaali cross" breed exhibited a significant association with tick infestation ($p < .05$). For goats, no statistically significant associations were found among the observed breeds ("Beetal," "Tedi," and "Jattal/Bakarwali"), with all *p*-values exceeding .05. Similarly, buffalo breeds "Neli Ravi" and "Desi (non-descriptive)" displayed no significant association with tick infestation. However, in the cattle category, all four breeds – "Sahiwal," "Australian," "Cross-bred," and "Desi (non-descriptive)" - demonstrated a significant association with tick infestation ($p < .05$), suggesting a robust relationship between host breed and tick infestation status.

Male ticks, totaling 620, were examined to determine the male-to-female sex ratio, revealing a higher number of female ticks for all species. In addition to hard ticks, soft ticks prevalent in backyard poultry were investigated. Examination of poultry birds and their nest boxes revealed the presence of ticks, primarily in cracks and crevices of shelters. A total of 281 ticks from Bhimber, 179 from Bagh, 95 from Mirpur, and 43 from Kotli were collected, with *Argas persicus*

Table 3.
Breed-wise Prevalence Rate of Tick Species

Host	Breed	Observed	Expected	$(O-E)^{2/E}$	Results
Sheep	Poonchi	57	47.53	9.97	**NS**
	Pahari	168	148.93	5.04	**NS**
	Kali	70	38.54	39.5	**S**
Goat	Beetal	60	44.16	4.11	**NS**
	Tedi	104	72.2	16.64	**NS**
	Jattal/Bakarwali	189	131.49	20.76	**NS**
Buffalo	Neli Ravi	70	92.33	9.97	**NS**
	Desi (non-descriptive)	10	18.67	5.04	**NS**
Cattle	Sahiwal	54	36.19	8.92	**S**
	Australian	35	49.42	7.14	**S**
	Cross-bred	138	110.89	19.48	**S**
	Desi (non-descriptive)	157	122.5	45.79	**S**

Note: S indicates statistical significance, meaning there is a significant association between the variables. NS indicates that there is no significant association between the variables. The decision is based on a significance level of 0.05.

identified as the dominant species infesting poultry birds across all four districts of Azad Kashmir.

Principal Component Analysis

We applied PCA to examine the distribution of tick species across districts. The analysis revealed four principal components (PC1 to PC4) with varying contributions to the overall variance. PC1, explaining 56.13% of the variance, likely signifies major differences in tick species composition among districts. PC2 (26.39% variance) captures additional variation, contributing to an 82.52% cumulative understanding. PC3 (17.48% variance) and PC4 (no variance) provide diminishing insights. The biplot visually represents relationships among tick species and their contributions to the principal components, aiding in the interpretation of major contributors to observed variance, as shown in Figure 5.

Discussion

In this comprehensive study, we undertook a thorough baseline examination of tick infestations across various domestic animals in Azad Kashmir. These findings have significant implications for controlling tick infestations and the diseases they transmit. The diversity of tick species identified among cattle, buffalo, sheep, goats, and domestic birds underscores the ecological suitability of the region for tick development and dissemination (Patra et al., 2020; 2022).

Our findings revealed varying infestation rates among different livestock species. This pattern is consistent with earlier studies, attributing the lower tick infestation rates in buffaloes to the vigilant care provided by farmers, who often have smaller herds and promptly remove ticks upon detection (Ahmad et al., 2019; Ghafar et al., 2020b; Ramzan et al., 2020). The thin skin of cattle, coupled with favorable habitat conditions, likely contributes to their higher susceptibility to tick infestations (Khan et al., 2022).

Moreover, our study revealed that breed susceptibility plays a critical role in tick infestations, as crossbred cattle were identified as more vulnerable. Previous studies have highlighted that crossbred cattle exhibit higher susceptibility compared to indigenous breeds. For instance, research in Benin indicated that Girolando cattle, a crossbreed, were the most sensitive to tick infestations, particularly *R. microplus*, while Borgou cattle showed the least susceptibility due to their thicker skin and genetic resistance (Yessinou et al., 2018). Similarly, a study in Nigeria found that purebred N'dama cattle were less infested with ticks compared to crossbred genotypes, suggesting that resistance to tick infestation is an acquired trait influenced by repeated exposure (Nwachukwu et al., 2020). Additionally, research comparing zebu (Bos indicus) and crossbred cattle demonstrated that pure zebu cattle had significantly lower tick burdens, reinforcing the notion that indigenous breeds possess inherent resistance mechanisms (Wambura et al., 1998). These findings underscore the importance of breed selection in managing tick infestations in cattle.

In addition, our investigation unveiled the presence of *Nosomma* ticks in buffaloes, adding a significant dimension to the knowledge of tick diversity in the region. Previously, evidence of the epidemiological existence detected through molecular identification and characterization of the *N. monstrosum* tick was well documented (Aiman et al., 2022) in a neighboring region of our study area. Here, screening this tick species through morphological identification in buffaloes from Bhimber and Mirpur districts marks a crucial milestone, considering the limited reports on this tick species in the area. While morphological identification served as the primary method in this study, it is acknowledged that molecular identification could provide more precise species confirmation, particularly for rare species such as *N. monstrosum*. The limited occurrence of *Nosomma* in this region warrants further investigation using molecular techniques to enhance the accuracy of species identification. Future studies can incorporate molecular methods to improve species resolution, especially for species that are difficult to identify morphologically.

In the realm of domestic fowl, our study identified *Argas persicus* as the predominant tick species. The prevalence of *Argas persicus* as a significant ectoparasite in domestic fowl has been well-documented across various regions, including Pakistan, India, South America, and China. Studies have reported *A. persicus* as a predominant tick species, highlighting its genetic diversity and the impact of environmental factors on its prevalence in poultry farms (Zahid et al., 2021). Research in South America has shown the widespread distribution of *A. persicus* in subtropical regions, emphasizing its ability to thrive in diverse climatic conditions and the need for control measures to mitigate economic losses (Busi et al., 2024; Muñoz-Leal et al., 2018). Chinese studies have focused on the ecological aspects of *A. persicus*, examining its seasonal dynamics and genetic variation, as well as its health impacts on poultry, underscoring the necessity for targeted control strategies (Chen & Liu, 2022; Qi et al., 2023). In addition, the observed host spectrum of *A. persicus* also aligns with earlier reports conducted in Pakistan, showcasing its prevalence in domestic fowl (Khan et al., 2001; Zahid et al., 2021). The high *A. persicus* index in domestic fowl further corroborates its substantial presence in this host, underscoring the need for targeted control measures.

Despite the dearth of scientific reports on tick prevalence in neighboring regions, our study stands out for its focus on Districts Bhimber, Mirpur, Kotli, and Bagh (Sultana et al., 2015a). The comparative analysis reveals substantial differences in sample size, the number of animals investigated, tick species identified, and the duration of sampling compared to previous studies (Sayyad et al., 2016; Sultana et al., 2015a, 2015b). Our extensive sampling, spanning 18 months and encompassing a larger population of domestic animals, provides a more comprehensive understanding of the tick species prevalent in Azad Kashmir.

Altogether, our epidemiological assessment of tick infestations in livestock and domestic fowl in Azad Kashmir contributes valuable insights into the prevalence and diversity of tick species. The observed patterns in infestation rates among different animal species, the dominance of specific tick species, and the identification of *N. monstrosum* in buffaloes enrich our understanding of tick dynamics in this region. These findings serve as a foundation for targeted disease control strategies and emphasize the importance of raising awareness among livestock owners to mitigate the impact of tick-borne diseases.

Conclusion and Recommendations

In the current study, prevalence of tick infestation was carried out in buffalo, cattle, sheep, goats, and domestic poultry in four districts of Azad Kashmir. A total of seven tick species belonging to five genera were identified. All of the identified tick species are known to transmit

numerous tick-borne pathogens to domestic animals, thereby posing serious threats to the community. The results of our study could help to improve locally appropriate tick control strategies based on habitation, animal host, and tick species in Azad Kashmir.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: In this study, the authors did not use any techniques that were hazardous to the target animals. Ticks, external parasites, were removed from the bodies of animals, which is a standard practice among farmers to protect their animals from external parasites. As a result, the current study did not require any permission from the animal care committee.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.N.; Design – M.N., Z.A., A.S.; Supervision – M.N.; Resources – M.N., Z.A.; Materials – M.W., Imtiaz A.; Data Collection and/or Processing – A.J., F.A.K., Imran A., A.H.; Analysis and/or Interpretation – Z.A., M.N.; Literature Search – A.S., M.W.; Writing Manuscript – M.N., Z.A., M.W.; Critical Review – Z.H.K.

Declaration of Interests: The authors have no conflict of interest to declare.

Funding: This study was funded by the Higher Education Commission of Pakistan (Grant no: HEC-NRPU: 20-16981).

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