

Original Article

Species composition, distribution, and host associations of ixodid ticks in domestic animals on lowland pastures in North Macedonia

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ABSTRACT

Ixodid ticks (Acari: Ixodidae) are significant ectoparasites and vectors of pathogens that pose major veterinary and public health risks. Despite their epidemiological importance, comprehensive data on the tick fauna of North Macedonia remain limited. This study aimed to investigate the diversity, distribution, and host associations of ixodid ticks infesting domestic animals across the country. A total of 694 adult ticks were collected from sheep, cattle, goats, and dogs during field sampling conducted from May to September 2025 across 14 municipalities in five statistical regions. All specimens were morphologically identified to species level using established taxonomic keys. Eight tick species belonging to four genera were identified: *Rhipicephalus bursa* (35.2%), *Rhipicephalus sanguineus* sensu lato (30.3%), *Hyalomma marginatum* (25.4%), *Hyalomma scupense* (5.9%), *Hyalomma excavatum* (2.7%), *Hyalomma rufipes* (0.3%), *Ixodes ricinus* (0.1%), and *Haemaphysalis sulcata* (0.1%). Most ticks were collected from sheep (61.4%), followed by cattle (33.3%), dogs (4.3%), and goats (1%). Host association was evident, with *H. marginatum* dominating in cattle, *R. bursa* in sheep, and *R. sanguineus* s.l. in dogs and goats. The results confirm a diverse and well-established ixodid tick fauna in North Macedonia, consistent with patterns observed in the Western Balkans. The predominance of thermophilic Mediterranean species underscores the influence of climatic conditions and ecological factors on tick distribution. These findings provide baseline entomological data for the country and emphasize the importance of continuous monitoring and integrated One Health approaches to mitigate the risk of emerging and re-emerging tick-borne diseases.

1. Introduction

Ixodid ticks are globally recognized as obligate hematophagous ectoparasites of significant epidemiological and epizootiological importance. They serve as vectors and natural reservoirs for a broad spectrum of viral, bacterial, and parasitic pathogens in both humans and animals (Arnaudov et al., 2014; Latrofa et al., 2017). The global prevalence and expansion of tick-borne pathogens (TBPs) are driven by multiple complex factors, including regional climate warming, habitat changes, increased animal mobility, and human activities (Kapo et al., 2024; Ogden et al., 2013). These global drivers are particularly relevant in the Western Balkans, including North Macedonia, which is identified as a hotspot for tick activity and associated neglected infections due to environmental and socio-ecological conditions that create a favorable landscape for the establishment and expansion of various tick species (Kapo et al., 2025, 2024). In this region, domestic animals, specifically

sheep, goats, cattle, and dogs, are critical elements of the TBPs transmission cycle (Dreghiciu et al., 2025; Latrofa et al., 2017). These animals are highly exposed to tick infestations, particularly in the semi-intensive breeding systems commonly practiced locally, and often share pastures and tick fauna (Pavlovic et al., 2016). Moreover, domestic dogs play a fundamental role as shared tick hosts and potential carriers of tick vectors into the human environment (Dantas-Torres et al., 2012; Latrofa et al., 2017).

The confirmed endemic circulation of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in North Macedonia represents a profound zoonotic risk, with recent events like the 2023 cluster reinforcing its status as a documented and significant public health threat (Jakimovski et al., 2025; Kapo et al., 2024). Livestock serve as key asymptomatic hosts and reservoirs for CCHFV, as demonstrated by high seroprevalence rates found in the country (Mertens et al., 2015; Schuster et al., 2016). The principal tick vectors implicated in CCHFV transmission in the Balkan

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region, such as *Hyalomma marginatum* and *Rhipicephalus bursa*, are known to infest domestic animals in the country (Kapo et al., 2024). Furthermore, other zoonotic threats are present, including rickettsioses, evidenced by a reported Mediterranean spotted fever-like illness caused by *Rickettsia sibirica mongolitimonae* associated with a *Hyalomma* tick bite in the Skopje region (Jakimovski et al., 2022). Beyond their established role in public health, ixodid ticks in the Western Balkans function as critical vectors for a diverse range of pathogens with significant veterinary implications. The circulation of *Anaplasma*, *Ehrlichia*, *Babesia*, and *Theileria* species poses a persistent threat to the health of domestic animals, leading to substantial economic losses and undermining livestock productivity (Kapo et al., 2025).

In North Macedonia, the existing literature documents the presence of 15 species of ixodid ticks belonging to five genera: *Ixodes*, *Haemaphysalis*, *Dermacentor*, *Rhipicephalus*, and *Hyalomma* (Kapo et al., 2024). Localized studies, such as those conducted on sheep and cattle in the Kumanovo area, have identified seven species, with *I. ricinus* reported as the most abundant, along with *H. punctata*, *D. marginatus*, *Rh. sanguineus* s.l., *Rh. bursa*, and *D. reticulatus* (Pavlovic et al., 2014). Despite existing findings, a critical lack of integrated evidence remains regarding the tick fauna of North Macedonia, particularly data that concurrently evaluates species composition, geographic distribution, and host associations. To address this long-standing gap in regional surveillance, the primary objective of this study was to provide a comprehensive analysis of the ixodid tick landscape across the country's lowland pastures. By evaluating multiple domestic species, this research provides the evidence-based data essential for developing integrated One Health approaches to manage veterinary and public health threats.

2. Materials and methods

2.1. Study area

The study was conducted across North Macedonia, a landlocked and predominantly mountainous country situated in the central Balkans (25,713 km²; 40°–43° N, 20°–23° E). The national territory is characterized by rugged topography, deep river valleys, and an average elevation of approximately 850 m. The climate is transitional, ranging from Mediterranean influences along the Vardar River corridor to more pronounced continental conditions in the northern and high-altitude regions. Winters are typically cold and humid, whereas summers are warm and dry, with temperatures commonly exceeding 30–35 °C in lowland and southern areas.

2.1. Tick collection and identification

Ixodid ticks were collected through a passive, opportunistic sampling design from May to September 2025. The specimens were obtained by veterinarians in the field during routine diagnostic work and were then delivered to the Laboratory of Parasitology and Parasitic diseases at the Faculty of Veterinary Medicine in Skopje. The sampling strategy was guided primarily by the frequency of veterinary clinical requests and regional accessibility, targeting a host range that included cattle, sheep, goats, and farm dogs. To reduce sampling bias, each animal underwent a standardized, full-body examination with emphasis on anatomical sites where ticks commonly attach (ears, axillae, groin, perineal region, and tail base). Ticks were removed with fine-tipped forceps to preserve diagnostic structures, placed in vials containing 70% ethanol, and labeled with the host species, municipality, region, and sampling date. Samples were processed in the laboratory using a stereomicroscope (×10–×40). Species identification was based on established morphological keys (Estrada-Peña et al., 2017) using characters such as scutum ornamentation, basis capituli shape, coxal spurs, festoons, and the genital aperture. Members of the *Rhipicephalus sanguineus* complex were identified to the sensu lato level when species-specific features could not be reliably assessed.

2.2. Statistical analysis

All statistical analyses were performed in R (version 4.4.2; R Core Team, Vienna, Austria). Data management and visualizations were carried out in Microsoft Excel. Descriptive statistics included absolute counts, proportions, and 95% confidence intervals (95% CI), calculated using the Wilson method, which provides stable estimates even for small sample sizes or extreme proportions.

Differences in tick species composition among hosts and regions were examined using contingency tables and Pearson's chi-square test of independence. The chi-square statistic (χ^2), degrees of freedom, and corresponding *p*-values were reported for each analysis. All identified genera were included in the chi-square test; however, as *Ixodes* and *Haemaphysalis* were extremely rare (*n* = 1 each), post-hoc interpretations focused exclusively on the dominant *Hyalomma* and *Rhipicephalus*. Expected frequencies met chi-square assumptions in most cells; rare species were included in descriptive summaries but excluded from inferential tests when cell counts were too low.

When the chi-square test indicated a significant association (*p* < 0.05), post hoc analyses of standardized Pearson residuals were performed to identify combinations contributing most strongly to the deviation from expected frequencies. Absolute residual values >2 were interpreted as statistically meaningful over- or underrepresentation.

Effect size for multi-category associations was quantified using Cramér's *V*, which adjusts the chi-square statistic for table size. Values were interpreted as follows: 0.1–0.3 indicated a weak association, 0.3–0.5 indicated a moderate association, and values >0.5 indicated a strong association. Statistical significance was set at *p* = 0.05 for all analyses.

3. Results

This section presents the species composition, geographic distribution, and host associations of ixodid ticks collected from domestic animals in North Macedonia. A total of 694 ixodid ticks were collected from domestic animals across North Macedonia, representing eight species within four genera. All collected specimens were adults, and males were more numerous than females (74.9%, 520/694, 95% CI 71.6–78.0 vs. 25.1%, 174/694, 95% CI 22.0–28.4). This sexual dimorphism was most pronounced in the genus *Hyalomma*, particularly in *H. marginatum*, where males represented 89.8% (*n* = 158) of the specimens compared to only 10.2% for females. In contrast, species like *Rh. sanguineus* s.l. exhibited a more balanced representation, though males still comprised the majority of specimens at most sampling sites (Table 1).

The most abundant species was *Rhipicephalus bursa* (35.2%, 244/694, 95% CI 31.7–38.8), followed by *Rhipicephalus sanguineus* sensu lato (30.3%, 210/694, 95% CI 27.0–33.8), *Hyalomma marginatum* (25.4%, 176/694, 95% CI 22.3–28.7), *Hyalomma scapense* (5.9%, 41/694, 95% CI 4.4–7.9), and *Hyalomma excavatum* (2.7%, 19/694, 95% CI 1.8–4.2). Less frequently detected species included *Hyalomma rufipes* (0.3%, 2/694, 95% CI 0.1–1.0), *Ixodes ricinus* (0.1%, 1/694, 95% CI 0.0–0.8), and *Haemaphysalis sulcata* (0.1%, 1/694, 95% CI 0.0–0.8).

Ticks were collected from 14 municipalities across five statistical regions. The Eastern region contributed the most significant proportion of samples (42.1%, 292/694, 95% CI 38.5–45.8), followed by the Northeastern (20.0%, 139/694, 95% CI 17.2–23.2), Pelagonia (15.8%, 110/694, 95% CI 13.3–18.8), Skopje (15.4%, 107/694, 95% CI 12.9–18.3), and Southeastern (6.6%, 46/694, 95% CI 5.0–8.7) regions (Fig. 1).

Species composition differed significantly between regions (χ^2 = 220.18, *df* = 8, *p* < 0.0001) (Table 2). The strength of this association was moderate (Cramér's *V* ≈ 0.40). In the Eastern region, *Rh. bursa* was dominant (44.9%, 131/292, 95% CI 39.3–50.6), whereas in the Northeastern region, *H. marginatum* predominated (59.0%, 82/139, 95% CI 50.7–66.8). The Pelagonia region was characterized by a marked predominance of *Rh. sanguineus* s.l. (81.8%, 90/110, 95% CI 73.6–87.9). In

Table 1
Sex distribution of adult ixodid ticks at the species level.

Tick Species	Male Ticks	Female Ticks
<i>Rhipicephalus bursa</i>	178 (34.23%, 95% CI 30.28–38.41)	66 (37.93%, 95% CI 31.06–45.33)
<i>Rhipicephalus sanguineus</i> s.l.	128 (24.62%, 95% CI 21.11–28.50)	82 (47.13%, 95% CI 39.85–54.53)
<i>Hyalomma marginatum</i>	158 (30.38% 95% CI 26.59–34.47)	18 (10.34%, 95% CI 6.64–15.76)
<i>Hyalomma scupense</i>	41 (7.88%, 95% CI 5.87–10.52)	0
<i>Hyalomma excavatum</i>	13 (2.50%, 95% CI 1.47–4.23)	6 (3.45%, 95% CI 1.59–7.32)
<i>Hyalomma rufipes</i>	1 (0.19%, 95% CI 0.03–1.08)	1 (0.57%, 95% CI 0.10–3.18)
<i>Haemaphysalis sulcata</i>	1 (0.19%, 95% CI 0.03–1.08)	0
<i>Ixodes ricinus</i>	0	1 (0.57%. 95% CI 0.10–3.18)
Total	520	174

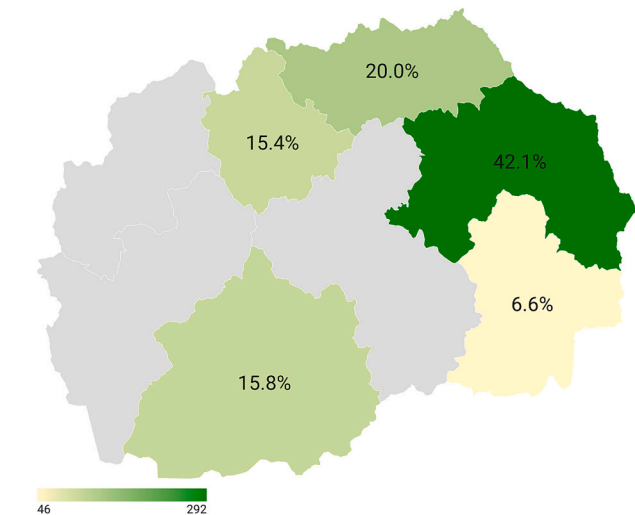


Fig. 1. Geographic distribution of collected ixodid ticks across the statistical regions of North Macedonia. The map illustrates the proportion of the total sample ($n = 694$) originating from each statistical region. Color intensity corresponds to the absolute number of ticks collected per region, with darker shades indicating higher counts (range: 46–292). Note: This distribution reflects the collected specimens and does not account for variations in sampling effort; therefore, it should not be interpreted as a measure of infestation prevalence or population-level risk.

the Southeastern region, *H. scupense* was the leading species (58.7%, 27/46, 95% CI 44.3–71.7), while in the Skopje region *Rh. bursa* again dominated (65.4%, 70/107, 95% CI 56.0–73.8). Post hoc standardized residuals ($|\text{residuals}| > 2$) indicated that *Hyalomma* species were over-represented in the Northeastern and Southeastern regions, whereas *Rhipicephalus* species were overrepresented in Pelagonia and Skopje regions.

Ticks were collected predominantly from sheep (61.4%, 426/694, 95% CI 57.6–65.0), followed by cattle (33.3%, 231/694, 95% CI 29.9–36.8), dogs (4.3%, 30/694, 95% CI 3.0–6.1), and goats (1.0%, 7/694, 95% CI 0.5–2.1). Host-associated species distribution showed a clear and highly significant structure ($\chi^2 = 414.78$, $df = 6$, $p < 0.0001$).

The degrees of freedom reflect the four host categories and two tick genera assessed (Table 3).

Among cattle, *H. marginatum* was dominant (60.2%, 139/231, 95% CI 53.6–66.5), followed by *H. scupense* (16.9%, 39/231, 95% CI 12.5–22.3), *H. excavatum* (8.2%, 19/231, 95% CI 5.3–12.5), *Rh. bursa* (7.4%, 17/231, 95% CI 4.7–11.5), *R. sanguineus* s.l. (6.5%, 15/231, 95% CI 4.0–10.4), and *H. rufipes* (0.9%, 2/231, 95% CI 0.3–3.2).

In sheep, *Rh. bursa* was the predominant species (53.3%, 227/426, 95% CI 48.6–57.9), followed by *R. sanguineus* s.l. (37.1%, 158/426, 95% CI 32.8–41.6) and *H. marginatum* (8.7%, 37/426, 95% CI 6.3–11.8). Rare species - *H. scupense*, *I. ricinus*, and *H. sulcata* - each occurred at $\leq 0.5\%$ ($\leq 2/426$, 95% CI 0.1–1.7).

In dogs and goats, infestations were exclusively caused by *Rh. sanguineus* s.l. (100%, 30/30, 95% CI 88.7–100.0; and 100%, 7/7, 95% CI 64.6–100.0, respectively).

A highly significant association was detected between tick genus and host species ($\chi^2 = 414.78$, $df = 6$, $p < 0.0001$; Cramér's $V = 0.61$). The effect size indicates a strong dependence between host type and tick genus.

Hyalomma were predominantly associated with cattle (86.1%, 199/231, 95% CI 81.1–90.1), whereas *Rhipicephalus* dominated in sheep (90.4%, 385/426, 95% CI 87.3–92.8), in dogs (100%, 30/30, 95% CI 88.7–100.0), and in goats (100%, 7/7, 95% CI 64.6–100.0).

Post hoc examination of standardized Pearson residuals ($|\text{residual}| > 2$ interpreted as meaningful) revealed very strong positive deviations for the *Hyalomma*-cattle combination and for *Rhipicephalus*-sheep, with residuals exceeding +5. All mismatched genus–host combinations showed substantial negative deviations (typically ≤ -4).

4. Discussion

This study represents a significant advancement in the understanding of the tick landscape in North Macedonia, addressing a long-standing gap in modern surveillance and providing much-needed updated baseline data. A primary strength of this research is its broad geographic scope, encompassing 14 municipalities across five statistical regions, coupled with the examination of four different domestic host species. By synthesizing these elements, the study offers a robust identification of the dominant ixodid species and their host associations. It appears that the confirmed tick community is profoundly influenced by the region's transitional Mediterranean-continental climate, which establishes the underlying ecological framework for distribution within the Western Balkans (Kapo et al., 2024).

The overall tick fauna in domestic farm animals is dominated by the thermophilic, open-habitat genera *Hyalomma* and *Rhipicephalus*. This observed predominance is consistent with the study's sampling design, which focused on lowland pasture environments during the peak summer activity period (May–September), ecologically selecting for these specific tick types (Todorov and Arnaudov, 2019). A key finding that reinforces the ecological specificity of the sampling is the scarcity of species typical of cooler, humid, or forested habitats (Todorov and Arnaudov, 2019). For instance, the rare presence of *Ixodes ricinus* (0.1%) and the complete absence of *Dermacentor* species are likely due to the study's timing and focus. *I. ricinus* avoids open pastures, preferring forested areas and high humidity (Krčmar, 2019; Todorov and Arnaudov, 2019). Similarly, the absence of *Dermacentor* spp. can be explained by their ecological preferences: for example, *D. marginatus* is typically found in warmer, drier steppe environments, whereas *D. reticulatus* thrives in cooler, more humid continental regions (Krčmar, 2019; Todorov and Arnaudov, 2019). Notably, *Ixodes* spp. and *Dermacentor* spp. usually exhibit bimodal seasonal activity, peaking in spring and autumn (Arnaudov et al., 2014; Krčmar, 2019; Todorov and Arnaudov, 2019). They are often inactive or absent during the hot mid-summer months, when *Hyalomma* and *Rhipicephalus* are most prevalent, which may result in their absence from collections focused on that period (Arnaudov et al., 2014; Krčmar, 2019; Todorov and Arnaudov, 2019).

Table 2
Distribution of ixodid tick species across statistical regions in North Macedonia. Species composition differed significantly between regions ($\chi^2 = 220.18$, $df = 8$, $p < 0.0001$; Cramér's $V \approx 0.40$).

Region	<i>R. bursa</i>	<i>R. sanguineus</i> s.l.	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. excavatum</i>
Eastern	131 (44.9%)	95 (32.5%)	55 (18.8%)	11 (3.8%)	0
Northeastern	22 (15.8%)	35 (25.2%)	82 (59.0%)	0	0
Pelagonia	9 (8.2%)	90 (81.8%)	5 (4.5%)	4 (3.6%)	2 (1.8%)
Southeastern	0	5 (10.9%)	8 (17.4%)	27 (58.7%)	6 (13.0%)
Skopje	70 (65.4%)	25 (23.4%)	7 (6.5%)	0	5 (4.7%)

Table 3
Host-specific distribution of ixodid tick species collected from domestic animals in North Macedonia. Species composition differed significantly between host species ($\chi^2 = 414.78$, $df = 6$, $p < 0.0001$; Cramér's $V = 0.61$).

Host	<i>Rh. bursa</i>	<i>Rh. sanguineus</i> s.l.	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. excavatum</i>	Total
Cattle	17 (7.4%)	15 (6.5%)	139 (60.2%)	39 (16.9%)	19 (8.2%)	231
Sheep	227 (53.3%)	158 (37.1%)	37 (8.7%)	2 (0.5%)	2 (0.5%)	426
Dogs	0	30 (100%)	0	0	0	30
Goats	0	7 (100%)	0	0	0	7

These species have been confirmed in North Macedonia by other studies (Kapo et al., 2024; Pavlovic et al., 2014).

The detection of *H. scupense* (5.9%) and *H. excavatum* (2.7%) in North Macedonia aligns with their known status as established livestock parasites within the diverse tick biodiversity of the Western Balkans (Kapo et al., 2024). The finding of *H. rufipes* is noteworthy as it is an African species, and its presence in Europe is generally attributed to transient introductions by migratory birds (Celina and Černý, 2025; Kapo et al., 2024). The study also recorded the rare presence of *Hae. sulcata* (0.1%), a species previously confirmed in North Macedonia (Kapo et al., 2024). This “desert-steppe” type species is generally restricted to warmer, southern regions, often favoring hilly pastures and higher altitudes (Todorov and Arnaudov, 2019).

Beyond this general composition, the study highlights critical spatial clustering: regional differentiation proved significant, with species composition varying meaningfully between geographical areas.

Region-specific residuals demonstrated clear ecological clusters. This spatial structuring is evident in the differential distribution of the dominant genera: *Hyalomma* are overrepresented in the Northeastern and Southeastern regions, whereas *Rhipicephalus* are dominant in Pelagonia and Skopje. The microclimate is a crucial determinant of the observed spatial patterns. Microclimatic parameters, specifically temperature, wind, and humidity, are critical factors that contribute to the increase or decrease in tick numbers (Dreghiciu et al., 2025; Ogden et al., 2013). A habitat's capacity to offer protection from extreme temperatures and desiccation creates a modified climate that directly controls tick mortality and host-seeking activity (Ogden et al., 2013). However, with a moderate Cramér's V of 0.40, the data suggests that regional factors do influence species presence, yet these distributions should not be viewed as absolute indicators of infestation risk. Since sampling was opportunistic, the higher intensity in certain regions may partially reflect the frequency of veterinary visits rather than an underlying ecological density.

The central finding of the study is the significant species-specific association between tick genera and host animals. While our results indicate significant relationships, these patterns must be interpreted within the context of the study's opportunistic sampling design. The strong association found between tick genera and their hosts (Cramér's

$V = 0.61$) underscores a robust biological preference in our sample; however, since the total number of animals examined was not recorded, these values represent the composition of the collected specimens rather than a definitive measure of population-level infestation prevalence.

The genus *Hyalomma* was predominantly associated with cattle (86.1%), with *H. marginatum* (60.2%) serving as the dominant species in this host group. This finding is epidemiologically significant, as *H. marginatum* is a primary vector and a natural reservoir for CCHFV in Europe (Celina and Černý, 2025; Sherifi et al., 2018). Recent predictive models indicate that *H. marginatum* is expanding its range northward and to higher elevations, driven by warming temperatures and reduced climatic barriers to overwintering success (Celina and Černý, 2025; Ogden et al., 2013). North Macedonia's position as a climatic transition zone, bridging Mediterranean and continental influences (Kapo et al., 2024), suggests that future warming could facilitate the expansion of *H. marginatum*, potentially amplifying the risk of CCHFV transmission (Jakimovski et al., 2025). This ecological shift is critically important given the country's status as a CCHFV-endemic hotspot, as evidenced by high seroprevalence rates in local livestock (Mertens et al., 2015; Schuster et al., 2016).

Similarly, *Rhipicephalus* species were strongly overrepresented on sheep and dogs. The standardized residuals observed, showing substantial positive deviations for *Rhipicephalus*-sheep and *Rhipicephalus*-dogs, further underscore this specific ecological structuring.

The most abundant species overall, *Rh. bursa* (35.2%) was the most dominant tick on sheep (53.3%), consistent with its established role as the principal tick of small ruminants across the Western Balkans (Arnaudov et al., 2014; Chaligiannis et al., 2016). As a thermophilic species, *Rh. bursa* is largely restricted to the Mediterranean region, where the climate provides favorable conditions for its development; its distribution is primarily limited by reduced humidity (Arnaudov et al., 2014). Beyond its veterinary importance as a vector for protozoan pathogens such as *Babesia ovis* and *Anaplasma ovis* (Dantas-Torres et al., 2012; Kapo et al., 2025; Saratsis et al., 2022), the association of regional CCHFV strains with *R. bursa* suggests a specialized ecological role for this species in viral circulation within the Balkan Peninsula (Sherifi et al., 2018).

In this study, *Rh. sanguineus* s.l. exhibited an exclusive association

with dogs and goats (100%). This highly adaptable species thrives in both urban and rural environments, a capacity that facilitates its successful establishment across diverse geographical regions (Ghodrati et al., 2025; Kadriaj et al., 2016; Latrofa et al., 2017). Traditionally viewed as a Mediterranean tick, its seasonal biological activity is increasingly influenced by climate change and the movement of domestic dogs (Ghodrati et al., 2025). The recent molecular evidence of *Ehrlichia canis* in symptomatic pet dogs from the Skopje region provides critical epidemiological insight into the active pathogens harbored by these vectors (Petrov et al., 2021). Additionally, the association of *Rh. sanguineus* s.l. with dogs and goats presents a significant risk for the transmission of zoonotic rickettsioses (Dantas-Torres et al., 2012). This specialized host-vector relationship is particularly concerning in areas where these animals live in close proximity to human settlements, as it facilitates the spillover of pathogens into the domestic environment (Dantas-Torres et al., 2012). Consequently, our findings emphasize that proactive ectoparasite management on canids remains a critical component of disrupting the transmission cycle of these important zoonotic and veterinary agents (Dantas-Torres et al., 2012; Diakou et al., 2022).

Tick populations in North Macedonia exhibit specific ecological structures, characterized by significant host associations. These consistent relationships ensure that vectors regularly encounter reservoir-competent hosts, providing the environmental stability necessary for the sustained maintenance of pathogen cycles (Ogden et al., 2013). While this study focuses on the vector landscape rather than direct pathogen detection, the high prevalence of these specific ticks in lowland pastures indicates a significant potential for the active circulation of various veterinary and zoonotic agents (Todorov and Arnaudov, 2019). Consequently, reducing these risks requires an integrated One Health approach that synthesizes climate monitoring, livestock management, and proactive ectoparasite control to disrupt the transmission cycles involving animals, humans; and their shared environment (Dantas-Torres et al., 2012).

Climate change influences tick ecology through multiple, interconnected mechanisms. Rising temperatures accelerate tick life cycles, reducing generation time and increasing reproductive potential (Ogden et al., 2013; Omeragic, 2011; Omeragić et al., 2022). Warmer winters improve overwintering survival, particularly for thermophilic species like *Hyalomma* (Celina and Černý, 2025; Dantas-Torres et al., 2012; Krčmar, 2019). These changes enable a northward expansion, particularly for Mediterranean species previously restricted by cold tolerance limits (Celina and Černý, 2025; Ogden et al., 2013). In North Macedonia, which already experiences Mediterranean-continental climatic transitions (Kapo et al., 2024), future warming may further establish *Hyalomma* populations at higher elevations and extend their seasonal activity periods (Celina and Černý, 2025; Jakimovski et al., 2025).

4.1. Limitations

Despite these significant findings regarding tick diversity, several limitations of this study must be acknowledged. First, the data was generated through an opportunistic sampling design based on specimens submitted by field veterinarians, rather than a systematic surveillance. Consequently, the total number of animals examined per host species and region was not recorded, preventing the calculation of a denominator. It appears likely that the reported distributions and host associations reflect the relative abundance within our specific sample rather than absolute infestation prevalence or population-level risk. Given the descriptive nature of these data, the results should be interpreted as evidence of vector presence and diversity across the lowland pastures of North Macedonia, rather than a definitive measure of environmental infestation intensity.

The sampling was unevenly distributed across the country's statistical regions, potentially resulting in an incomplete representation of national tick diversity and distribution patterns. A key finding of this

collection, which differs from broader regional surveys, is the scarcity of *I. ricinus* and the complete absence of *Dermacentor* spp. The scarcity in this study is likely attributable to its specific focus on lowland pasture animals, an environment conducive to the occurrence of the observed *Hyalomma* and *Rhipicephalus* spp. (Krčmar, 2019; Todorov and Arnaudov, 2019). Areas such as mountainous and forested zones are particularly underrepresented. The lack of molecular characterization prevents fine-scale taxonomic resolution, particularly for the *Rh. sanguineus* s.l. complex, which may comprise multiple species with distinct vector competences and ecological niches (Ghodrati et al., 2025). The study focused exclusively on adult ticks, excluding immature stages that could offer further insights into tick population dynamics and seasonal activity. It must be noted that while a systematic whole-body search was performed, the difficulty of achieving complete tick clearance from live, conscious livestock, particularly from cryptic sites such as the ear canal and inguinal folds, may have introduced a minor bias toward the collection of more accessible specimens. Furthermore, the cross-sectional design captures only a single time point, precluding assessment of temporal variation in tick abundance or species composition. Lastly, the lack of molecular analyses prevents the detection of potential TBPs and genetic variants. Future research should incorporate larger, more geographically balanced samples, include all life stages, and employ molecular pathogen identification to achieve a more comprehensive understanding of tick ecology and associated disease risks in North Macedonia.

5. Conclusion

This study establishes an updated baseline of the ixodid tick fauna inhabiting lowland pastures in North Macedonia, identifying eight species across four genera. The documentation of a diverse and well-established tick population is consistent with patterns observed across the Western Balkans, a region recognized for high tick activity. While the presence of species such as *H. marginatum* and *Rh. bursa* is ecologically significant due to their roles as competent vectors in the region, the scope of this work remains strictly entomological.

Our findings characterize current species composition and host associations, providing a necessary foundation for an integrated strategy encompassing surveillance, research, and control. The evidence so far suggests that addressing potential tick-borne threats will require a co-ordinated One Health approach to link human, animal, and environmental health sectors. Future efforts should prioritize continuous surveillance through systematic sampling and the integration of ecological data to facilitate early detection of emerging risk trends. Furthermore, subsequent longitudinal studies incorporating molecular pathogen screening are required to definitively bridge the gap between the documented vector distribution and actual public health or veterinary risks. Collectively, these actions provide a framework for an evidence-based national strategy to mitigate the risks associated with tick populations in North Macedonia.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used Grammarly in order to improve the language and readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Bojana Chapkunovska: Writing – original draft, Visualization, Investigation, Formal analysis. **Ljubica Rashikj:** Resources, Investigation, Formal analysis. **Ivan Matevski:** Resources, Investigation, Formal analysis. **Jane Vlahov:** Resources, Investigation, Formal analysis. **Ivana Shikoska:** Resources, Investigation, Formal analysis. **Betim Xhekaj:**

Resources, Investigation, Formal analysis. **Jovana Stefanovska**: Project administration. **Aleksandar Cvetkovikj**: Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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