Bull. Soc. R. Liege, 2023, 92(1), 53 - 70 *doi: 10.255118/0037-9565.11396*

Diversity of hard tick (Acari: Ixodidae) infesting small ruminants in some breeding farms in Tizi-Ouzou area (Northern Algeria)

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Manuscrit reçu le 6 septembre 2023 et accepté le 16 octobre 2023

Abstract

Ticks are hematophagous arthropods and obligate ectoparasites with a cosmopolitan distribution. They are of great medical and veterinary importance. Studying the composition of the tick fauna is a crucial step in establishing vector control programs. In this context, an inventory of hard ticks infesting small ruminants in the Tizi-Ouzou area was carried out over a one-year period, from December 2020 to November 2021. Using the direct collection technique on the host animal, a total of 663 animals examined, 170 were infested and a total of 1318 adult tick's specimens were collected in four sampling sites. The result shows a tick diversity comprising six different species. The most abundant species was *Rhipicephalus bursa* (68.66%), followed by *Rhipicephalus sanguineus* (29.74%) and *Ixodes ricinus* (1.36%). Other species were less abundant, each representing only 0,08% of the total abundance. The highest specific richness was recorded in the Ain El Hammam site located at high altitude. A positive correlation was observed between the farms' altitude and the tick diversity. The result of FCA analysis showed that distribution of ticks is related to altitude and habitat of tick's species.

Keywords: diversity, Ixodidae, small ruminants, Tizi-Ouzou, Algeria

Résumé

Diversité des tiques dures (Acari : Ixodidae) infestant les petits ruminants dans quelques fermes d'élevage dans la région de Tizi-Ouzou (Nord d'Algérie). Les tiques dures sont des arthropodes hématophages et des ectoparasites obligatoires d'une répartition cosmopolite. Elles ont une grande importance d'intérêt médical et vétérinaire. L'étude de la composition de la faune des tiques est une étape cruciale pour établir des programmes de lutte antivectorielle. Dans ce contexte, un inventaire des tiques dures infestant les petits ruminants dans la région de Tizi-Ouzou a été réalisé sur une période d'une année, allant du mois de décembre 2020 au mois de novembre 2021. D'un total de 663 animaux examinés, 170 ont été infestés et un nombre de 1318 spécimens de tiques adultes a été collecté dans quatre sites échantillonnés, en utilisant la technique de la collecte directe sur hôte animal. Le résultat obtenu exprime une richesse de six espèces de tiques. L'espèce la plus abondante a été *Rhipicephalus bursa* (68,66%), suivie de *Rhipicephalus sanguineus* (29,74%) et *Ixodes ricinus* (1,36%). Les autres espèces ont été moins abondantes, elles présentent chacune une abondance de 0,08% de l'abondance totale. La richesse spécifique la plus élevée a été enregistrée dans le site d'Ain El Hammam, situé à haute altitude. Une corrélation positive a été observée entre l'altitude des fermes et la diversité des tiques. Le résultat de l'analyse AFC a montré que la distribution des tiques est liée à l'altitude et à l'habitat des espèces de tiques.

Mots-clés : diversité, Ixodidae, petits ruminants, Tizi-Ouzou, Algérie

1. Introduction

Ticks are arthropods that belong to the arachnid class, acarid group and metastigmata order (Tissot Dupont & Raoult, 1993). They are characterized by a wide diversity, containing around 900 different species (Boulanger et *al*., 2019) divided into three major families (Tissot Dupont & Raoult, 1993; Klompen et *al*., 1996; D.McCog & Boulanger, 2015, Boulanger et *al*., 2019), the Argasidae family with 193 species (Boulanger et *al*., 2019), the Ixodidae family with 700 species and only one species representing the Nuttalliellidae family (D.McCog & Boulanger, 2015; Boulanger et *al*., 2019). Ticks are obligate ectoparasites of terrestrial vertebrates (Klompen et *al*., 1996; Wall & Shearer, 2001). They are hematophagous at all stages of development (Klompen et *al*., 1996; Guiguen & Degeilh, 2001; Boulanger et *al*., 2019). The blood meal is essential for their life cycles (Peter & Brossard, 1998; Boulanger et *al*., 2019). Ticks play an important role in the transmission of bacteria, viruses, protozoa, and helminths that cause both human and animal disease (krčmar, 2019). These mites have an extremely varied global distribution, ranging from icy zones to the most inhospitable desert areas, and from lowland to high-altitude regions (Perez-Eid & Gilot, 1998; Boulanger et *al*., 2019). Currently, with global warming, environmental conditions are changing, which can have an impact on the distribution patterns and vector potential of ticks (Rehman et *al*., 2017).

Numerous studies have been carried out on small ruminant ticks around the Mediterranean: in north-central Spain (Estrada-Pena et *al*., 2004), Turkey (Ozubek & Aktas, 2017), Lebanon (Dabaja et *al*., 2017), Iraq (Omer et *al*., 2007) and Tunisia (Elati et *al*., 2018). In Algeria, cattle ticks have been the primary subject of the majority of studies carried on farm animal ticks (Yousfi- Monod & Aeschlimann, 1986; Boulkaboul, 2003; Benchikh Elfegoun et *al*., 2007; Benchikh Elfegoun et *al*., 2013; Benchikh Elfegoun et *al*., 2019; Derradj & Kohil, 2020). Only a few research on small ruminant ticks have been done in the Adrar region (Bouhous et *al*., 2011), El Tarf (Leulmi et *al*., 2016), Sidi Bel Abbes and Saida (Abdelkadir et *al*., 2019), and Constantine (Foughali et *al*., 2021). In the Tizi-Ouzou area, the only studies conducted are those by Abdul Hussain et *al*. (2004) on cattle ticks and free-stage ticks on vegetation and Bedouhene et *al*. (2022) on cow ticks during their study in the western Djurdjura. This region lacks an inventory of the ixodid fauna of small ruminants, in this sense the current study was conducted.

The goal of this preliminary work is to identify the different tick species infesting small ruminants in some farms in the Tizi-Ouzou area. This will serve as groundwork for further research in this field.

2. Materials and methods

2.1. Study area

Tizi-Ouzou is a department in northern Algeria, it is limited by the Mediterranean Sea from the north, Bouira department from the south, Boumerdes department from the west and Bejaia department from the east. The area has a Mediterranean subhumid climate. Our work was carried out in four sites in the Tizi-Ouzou area, on various mixed goat and sheep farms. Their geographical characteristics are shown in table 1. The geographical location of the study sites is shown in figure 1.

Table 1: Geographical characteristics of study sites.

Sites	Code of sites	Geographical coordinates of sites	Altitude (m)
Ain El Hammam	AEH	36°28'55"N 4°17'44"E	1019
Irdjen	IR	36°38'37"N 4°09'23"E	507
Tizi Rached	TR	36°41'46"N 4°13'02"E	117
Freha	FR	36°43'57"N 4°16'00"E	132

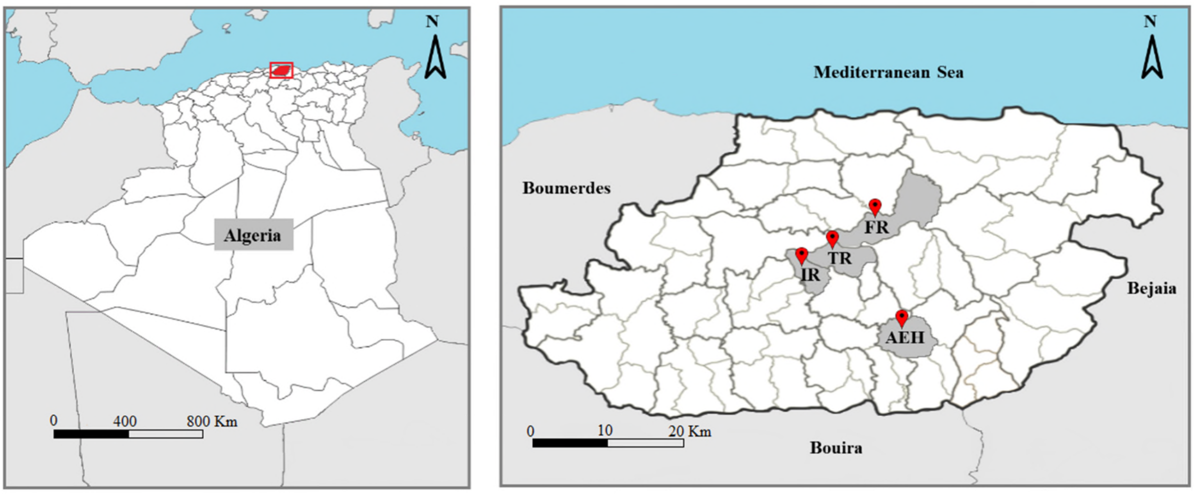

Figure 1: Geographical location of sites sampled in the Tizi-Ouzou department. (For abbreviation see table 1).

2.2. Tick collection and preservation

Ticks were collected from small ruminants (goats and sheep) twice a month from December 2020 to November 2021. A random sampling method was used to choose animals on which the presence of ticks can be inspected. Then ticks were carefully removed with forceps and transferred to dry tubes containing a 70% ethanol solution to preserve their morphological characteristics. Each tube is labeled with the study site, collection date and host type.

2.3. Tick identification

Collected specimens were transferred to the Laboratory of Ecology and Biology of Terrestrial Ecosystems LEBIOT of Mouloud Mammeri University, Tizi-Ouzou (Algeria), for morphological identification of genera and species. This procedure was completed using an OPTIKA stereomicroscope (WF 10X/21), with reference to the taxonomic key of Walker et *al*. (2003). Our identification was verified at the Parasitic Eco-epidemiology and population genetics laboratory of the Pasteur Institute of Algeria.

The identification process was carried out in the following steps:

✓ First, the ticks were sorted out to determine their stage of development and gender. The adult stage of the ticks was determined on the basis of the presence of four pairs of legs, porose areas and a genital groove. Gender determination was carried out by observing the dorsal surface of the ticks. The male differs from the female by the presence of a chitinous plate, the scutum, on the entire dorsal surface, whereas in the female it covers only part of the back.

✓ The genus was then identified on the basis of the morphological characteristics of certain parts of the tick's body:

● The position of the anal groove in front of the anus has made it possible to differentiate the genus *Ixodes* from other tick genera.

● The length of palp articles 2 is greater than the length of the palp articles 1 and 3 in the genus *Hyalomma*.

● The hexagonal shape of the entire basis of the capitulum and the presence of the festoons on the posterior edge of the idiosoma have enabled the genus *Rhipicephalus* to be identified.

● The rectangular shape of the basis of the capitulum, the broad shape of the palp articles 2 and the morphology of the coxae of the legs 1 confirmed the genus *Haemaphysalis*.

✓ Finally, species identification was based on certain morphological details. Here are the main morphological characteristics that helped to distinguish between the three predominant species in the study:

● Genital groove between coxae 4 and the internal spur on coxa 1 is long in the female of *Ixodes ricinus*.

● The shape of the eyes, scutum and spiraclular plates distinguish between females of both *Rhipicephalus bursa* and *Rhipicephalus sanguineus*.

● Adanal plates shape is narrow and trapezoid in male of *Rhipicephalus sanguineus,* broad and curved in male of *Rhipicephalus bursa*.

2.4. Data analysis

2.4.1 Ecological indices

The ixodidae population collected from the different sites of the study area was examined using several indices:

Specific richness (S) is one of the essential factors characterizing a population. The total richness of a biocenosis corresponds to all the species that make it up (Ramade, 1984).

Relative abundance (RA%) is the percentage of the number of individuals of a species (ni) in relation to the total number of individuals (N) (Faurie et *al*., 1980).

RA%=ni x100/N

ni: number of individuals of a species i taken into consideration
N: total number of all individuals

Frequency occurrence (FO) of each study site is calculated as the ratio of the number of records containing the study species (Pi) to the total number of records carried out (P), expressed as a percentage (Dajoz, 1975).

FO (%) = Pi x 100/P

Pi: is the number of records containing the species
P: is the total number of records

Based on FO values, we distinguish three classes of constancy. When FO>50% species are considered constant, as accessory when 25%<FO<50% and accidental when FO<25%.

Shannon diversity index (H') provides information on the effective diversity of a community and varies according to the number of species present (Ramade, 2003). It is calculated using the following formula:

$$H' = -\sum_{i=1}^{s} Pi\ log^2\ (Pi)$$

Where pi= ni/N
H': Shannon diversity index expressed in binary units
ni: is the number of each species i
N: is the total of the ni of all species combined
Log^2: logarithm - base 2.

Equitability index (E) gives the theoretical maximum diversity of a community, taking into account its specific richness (Ramade, 2003). It is calculated using the following formula:

$$E = H' / H' \max$$

With H' max = $\log^2 S$
H' max: is expressed in bits
S: specific richness.

2.4.2 Statistical analysis

Statistical analysis was carried out using R software, version 4.1.2. The Pearson correlation coefficient (Millot, 2018) was applied on the parameters to demonstrate the impact of altitude on species diversity. The factorial correspondence analysis (FCA) is used to summarize and visualize the information contained in the contingency table formed by two qualitative variables (Carricano et *al*., 2010). FCA is used to demonstrate the relationship between the altitudinal gradient and the distribution of species in the study area.

3. Results

From a total of 663 animals examined, 170 were infested and a number of 1318 adult tick's specimens were collected, of which 690 were males and 628 were females. All of the ticks were found on the ears.

3.1. Specific richness

During this study, six tick species were recorded on sheep and goats. These species are represented in table 2 and illustrated in figure 2. The Ain El Hammam site (AEH: located at 1019 m) is the richest in terms of species, with six species recorded (*Rhipicephalus bursa*, *Rhipicephalus sanguineus*, *Ixodes ricinus*, *Rhipicephalus turanicus*, *Hyalomma marginatum marginatum*, *Haemaphysalis punctata*), followed by the Irdjen (IR: located at 507 m) and Tizi Rached (TR: 117 m) sites with two species each, namely *Rhipicephalus bursa* and *Rhipicephalus sanguineus*. On the other hand, the Freha site (FR: located at 132 m) is the least diversified, with just one species (*Rhipicephalus sanguineus*). Figure 3 displays the specific richness of hard ticks found at each location. As can be observed, altitude has an impact on the values of species richness. The Pearson test performed on the data revealed a positive correlation (r: 0,858) between altitude and tick diversity in the study area (Figure 4).

Table 2: Species richness of hard ticks recorded during this study.

Familly	Genus	Species
Ixodidae	*Rhipicephalus*	*Rhipicephalus sanguineus* (Latreille, 1806)
		Rhipiciphalus bursa Canestrini & Fanzago, 1878
		Rhipicephalus turanicus Pomerantsev, 1936
	Hyalomma	*Hyalomma marginatum marginatum* Koch, 1844
	Ixodes	*Ixodes ricinus* (Linnaeus, 1758)
	Haemaphysalis	*Haemaphysalis punctata* Canestrini & Fanzago, 1878

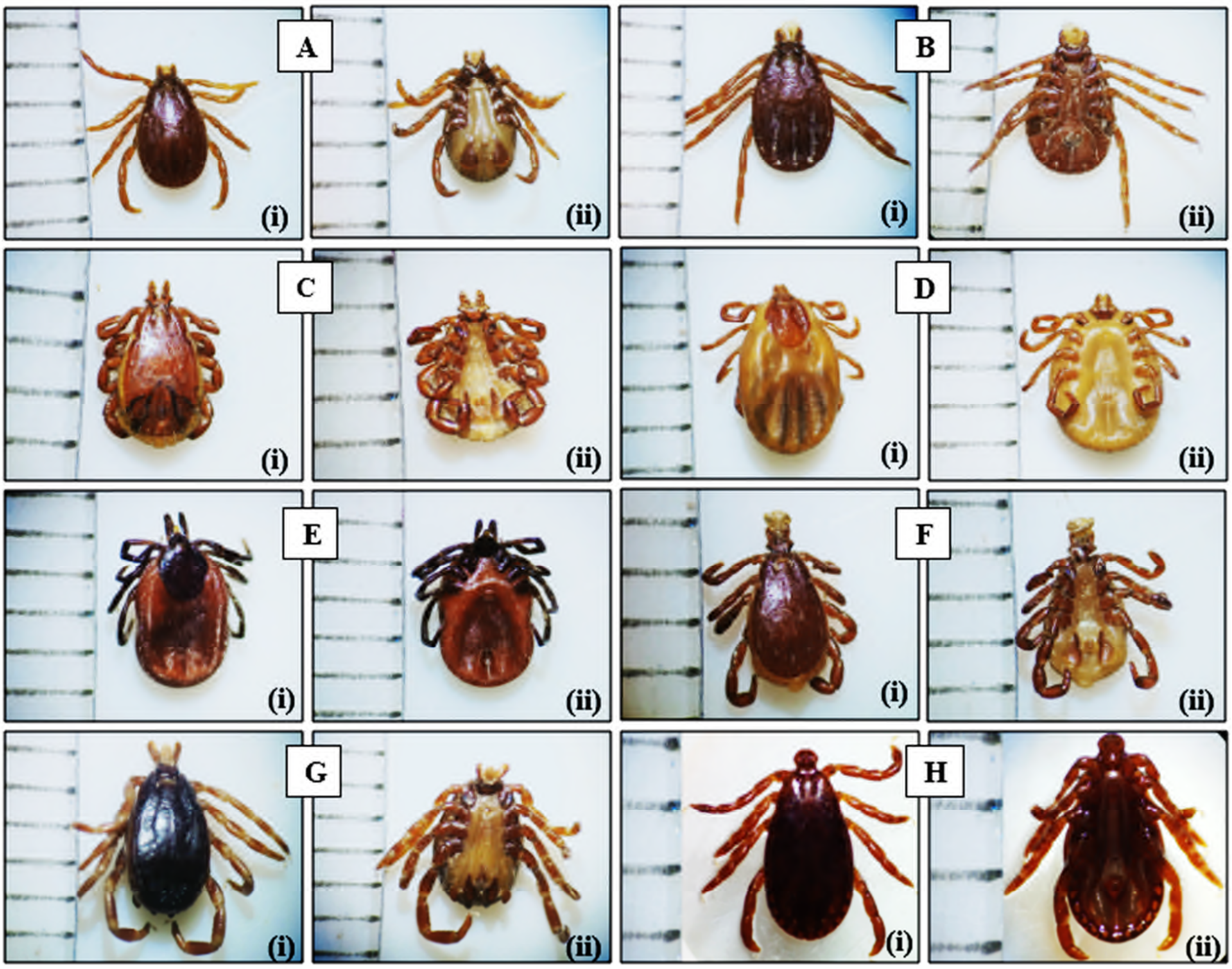

Figure 2: **A**. *Rh. bursa* male, (i) dorsal, (ii) ventral; **B.** *Rh. bursa* female, (i) dorsal, (ii) ventral; **C.** *Rh. sanguineus* male, (i) dorsal, (ii) ventral; **D.** *Rh. sanguineus* female, (i) dorsal, (ii) ventral; **E.** *I. ricinus* female, (i) dorsal, (ii) ventral; **F.** *Rh. turanicus* male, (i) dorsal, (ii) ventral; **G.** *Hy. marginatum marginatum* male, (i) dorsal, (ii) ventral. **H.** *H. punctata* male, (i) dorsal, (ii) ventral. Enlargement: A, B, C, D, E, F, G **x20** and H **x40**. Scale bar: **1 mm**.

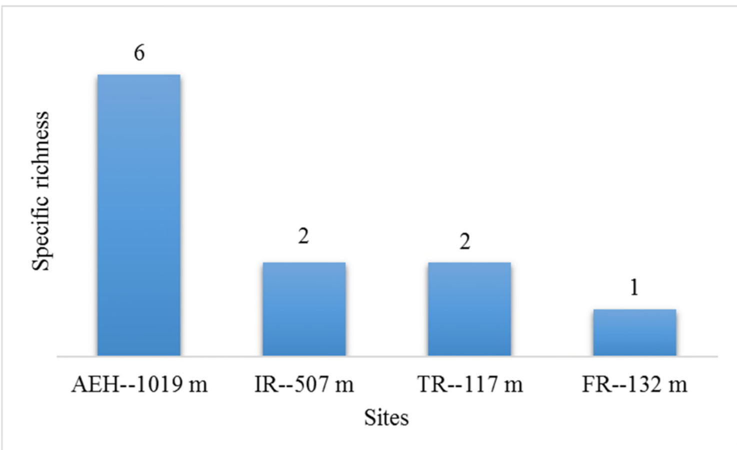

Figure 3: Specific richness of hard tick at the various study sites.

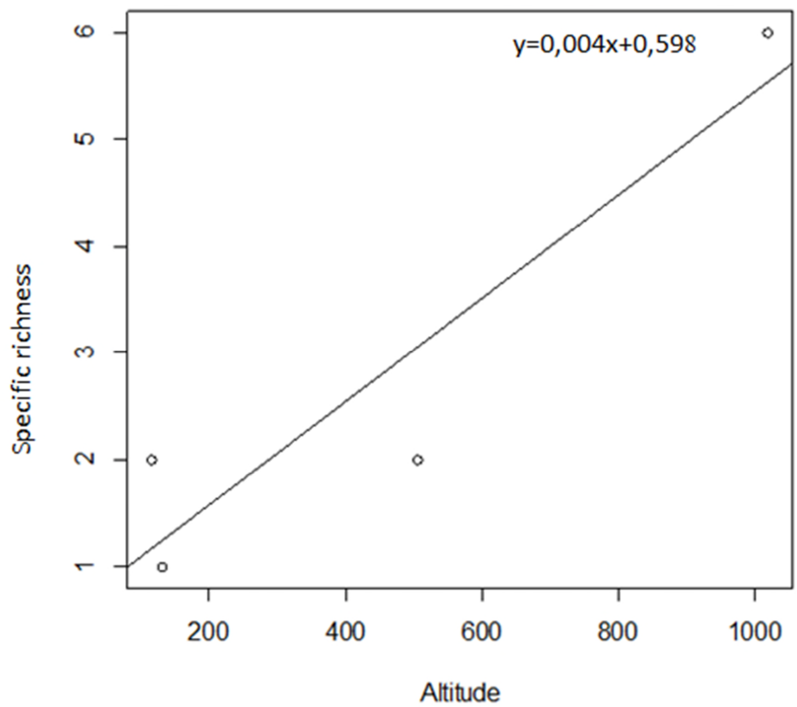

Figure 4: Pearson test showing the relationship between altitude and tick species richness recorded at each study site.

3.2. Relative abundance and frequency occurrence

The most abundant species in this study was *Rh. bursa* at 68.66%, followed by *Rh. sanguineus* at 29.74% and *I. ricinus* at 1.36%. The other species were very low, with an abundance of 0.08% each (Figure 5).

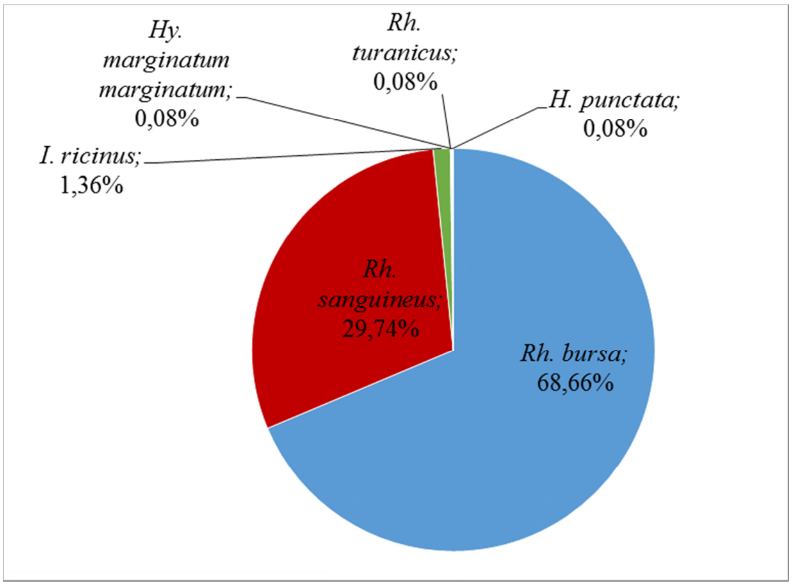

Figure 5: Relative abundances of the different tick's species collected during this study.

The relative abundances of each species collected per site are presented in table 3 and illustrated in figure 6. *Rh. bursa* is the most abundant species in all the study sites (AEH: 70.36%, IR: 69.89% and TR: 54.55%), except for the FR site which is represented by only one species (*Rh. sanguineus*). The coefficient of occurrence of each species was calculated in the different sites, revealing two classes of constancy in the study area (Table 3). The frequencies of occurrence calculated for the AEH site, show that the two species found (*Rh. bursa* and *Rh. sanguineus*) are qualified as accessory species, whereas the other species are accidental. The two species sampled at the IR site are classified as accessory species, while those found at the TR site are considered accidental. Only one accidental species was found at the FR site.

Table 3: Relative abundance (%) and Frequency occurrence of Ixodidae species collected at the various sampling sites.

Species	AEH	IR	TR	FR
Rh. sanguineus	27,29% (A)	30,11% (A)	45,45% (a)	100 % (a)
Rh. bursa	70,36% (A)	69,89% (A)	54,55% (a)	-
Rh. turanicus	0,11% (a)	-	-	-
Hy. marginatum marginatum	0,11% (a)	-	-	-
I. ricinus	2,01% (a)	-	-	-
H. punctata	0,11% (a)	-	-	-

(A): Accessory; (a): accidental.

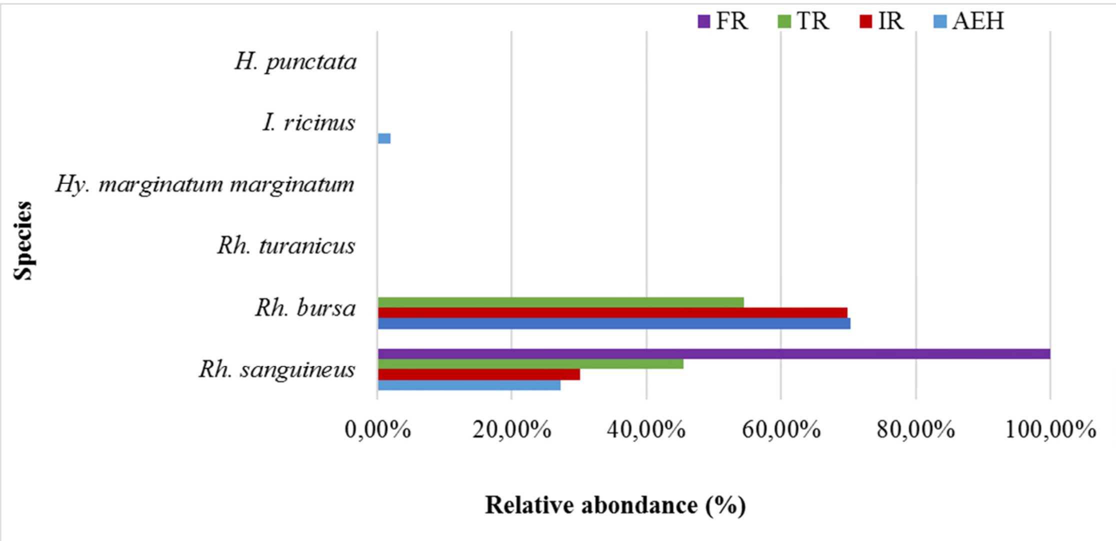

Figure 6: Relative abundance of each species collected per site.

3.3. Shannon diversity and equitability index

The Shannon diversity and equitability index calculated for the four studied sites were shown in table 4. The values of these parameters fluctuated between 0 and 1,01 bits for the Shannon index, and between 0 and 0,39 for equitability in the different sites. The maximum values of the Shannon and equitability index were recorded in the site of AEH, which are located at high altitude. While, the low value of these two parameters were recorded in the site of FR, as only one species of tick was found.

Table 4: Shannon diversity and equitability index.

Site	AEH	IR	TR	FR
H' (bits)	1,01	0,88	0,99	0
H' max (bits)	2,58	1	1	0
E	0,39	0,88	0,99	0

3.4. Factorial correspondence analysis (FCA)

The factorial correspondence analysis was performed on the various species found at the four study sites (Figure 7). It has revealed that the two factorial axes (Dim1xDim2 plane) are responsible for the greatest contributions, accounting for 80,70% and 19,30% of the cloud inertia, which corresponds to a cumulative total of 100%. Three groupings appear clearly in the Dim1xDim2 plane. These groupings mainly concern the different types of distribution of the tick species collected. The first one corresponds to the omnipresent species (*Rh. bursa*) in the three study sites AEH, IR and TR. The second one group corresponds to the one species (*Rh. sanguineus*) captured at FR at low altitude. As for the third one, it corresponds to the species recorded only at AEH at high altitude (*Rh. turanicus*, *I. ricinus*, *Hy. marginatum marginatum* and *H. punctata*).

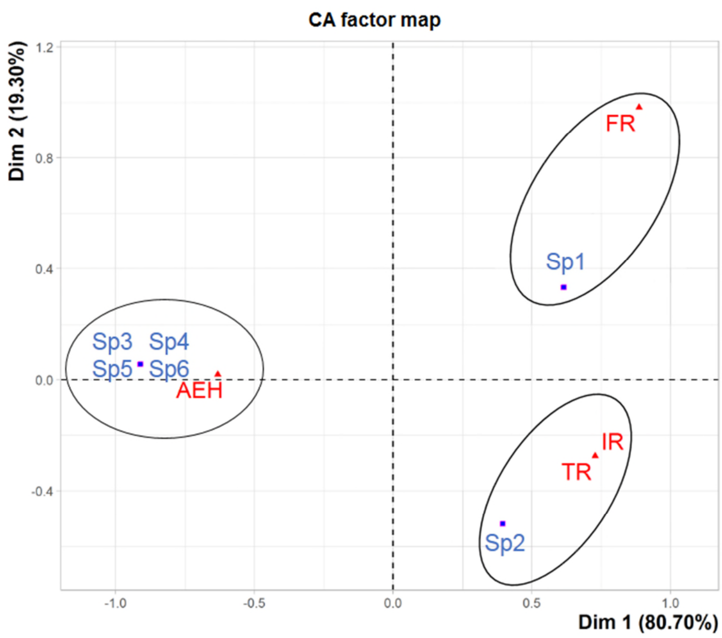

Figure 7: Factorial correspondence analysis of the ixodid distribution in the four study sites. Sp1: *Rh. sanguineus*, Sp2: *Rh. bursa,* Sp3: *Rh. turanicus,* Sp4: *I. ricinus,* Sp5: *Hy. marginatum marginatum*, Sp6*: H. punctata.*

4. Discussion

In the present study, the species richness found is significant, especially in the forest site of AEH located at high altitude (1019 m) recorded with six species. However, the FR site has only one species as it is an agricultural site where acaricide treatments are often applied. Despite the application of these treatments, the presence of the *Rh. sanguineus* species is due to the presence of dogs in the farm. According to Walker et *al*. (2003), the preferred host for all stages of *Rh. sanguineus* species is dog, while other hosts are generally infested only when dogs are present to maintain a tick population.

The Pearson test demonstrates that species richness rises with altitude. In fact, a significant change in altitude can cause significant climatic changes (Zeroual et *al*., 2014). This variability in climatic characteristics combined with adequate vegetation and host overpopulation provide suitable habitat and biotic conditions for several tick species (Estrada-Pena et *al*., 2004; Zeroual et *al*., 2014).

In other studies, carried out on small ruminant ticks, Estrada-Pena et *al*. (2004) found nine species, while Bouhous et *al.* (2011); Arnaudov et *al*. (2014); Dabaja et *al*. (2017) and Chaligiannis et *al*. (2018) reported seven species. Five species were reported by Ozubek & Aktas in 2017, while Leulmi et *al*. (2016) and Foughali et *al*. (2021) mentioned four species. Only three species were reported by Abdelkadir et *al*. (2019) and two species by Elati et *al*. (2018).

The *Rh. bursa* species was very abundant in our study with almost 70%. Indeed, according to Walker et *al*. (2003), *Rh. bursa* is one of the common *Rhipicephalus* species of livestock in North Africa. In Algeria, similar results have been found in several studies (Abdelkadir et *al*., 2019; Benchikh Elfegoun et *al*., 2019; Foughali et *al*., 2021; Bedouhene et *al*., 2022). While other studies have recorded low abundances of *Rh. bursa*, only 91/659 individuals were sampled by Leulmi et *al*. (2016) in the El Tarf region in north-eastern Algeria. In a previous study conducted in the Tizi-Ouzou region on cattle ticks and free-stage ticks on vegetation, *Boophilus annulatus* was the predominant species with 70.57% (Abdul Hussain et *al*., 2004). The latter, however, was not present during the investigation since it is a species that is often associated with cattle but can also occur with sheep and goats (Walker et *al*., 2003).

Rh. bursa has also been very prevalent in numerous studies all over the world. In Iraq, 90% of ticks collected from sheep and goats were *Rh. bursa* (Omer et *al*., 2007). In Parvomai, southern Bulgaria, the same species was invasive and predominant in both animals (Arnaudov et *al*., 2014). This species was the most frequent on both hosts in Turkey with a rate of 63.4% (Ozubek & Aktas, 2017). However, *Rh. bursa* was less abundant in Lebanon and Greece (Dabaja et *al*., 2017; Chaligiannis et *al*., 2018). This species is implicated in the transmission of different pathogens, particularly *Theileria* (Aktas et *al*., 2006; Garcia-Sanmartin et *al*., 2008; M'ghirbi et *al*., 2010), *Babesia* (Altay et *al*., 2008; Garcia-Sanmartin et *al*., 2008; Aydin et *al*., 2015), *Anaplasma* (de la Fuente et *al*., 2004) and *Rickettsia* (Leulmi et *al*., 2016; Dib et *al*., 2019).

Rh. sanguineus was the second most abundant species in the study which goes in tune with Leulmi et *al*. (2016). While other studies show that it is less abundant (Estradaa-Pena et *al*., 2004; Arnaudov et *al*., 2014). *Rh. sanguineus* is known to be the vector of *Rickettsia conorii conorii*, the causal agent of Mediterranean spotted fever (Sarih et *al*., 2008; Bitam et *al*., 2009) and *Rickettsia massiliae* (Bitam et *al*., 2009).

I. ricinus was the third most abundant species in the study. Only one individual of it was collected from sheep in El Tarf in north-eastern Algeria by Leulmi et *al*. (2016). This species was among those inventoried on sheep in the region of Aragon (north-central Spain) (Estrada-Pena et *al*., 2004), and was also found on sheep and goats in Parvomai, southern Bulgaria (Arnaudov et *al*., 2014). *I. ricinus* transmits *Borrelia garinii* (Benredjem et *al*., 2014) and *Rickettsia* spp. (Dib et *al*., 2009; Benredjem et *al*., 2014; Dib et *al*., 2019).

As for the other species, they were very sparse. They were limited to a single individual recorded for each species: *Rh. turanicus*, *Hy. marginatum marginatum* and *H. punctata*. According to Bitam et *al*. (2009), *Hy. marginatum marginatum* is implicated in the transmission of *Rickettsia aeschlimannii* and *Rh. turanicus* transmits *Rickettsia massiliae*. The species *H. punctata* is a vector of *Rickettsia slovaca* (Leulmi et *al*., 2016).

The results obtained for the coefficient of occurrence of each species in the different sites reveal that four species (*Rh. turanicus*, *I. ricinus*, *Hy. marginatum marginatum* and *H. punctata*) were identified as accidental species in the region. This is probably due to the fact that these species are much more frequently sampled on cattle in the Tizi-Ouzou area (Abdul Hussain et *al*., 2004; Bedouhene et *al*., 2022).

The preferred attachment site for ticks in this study was the ears. Several studies have found similar results. For instance, in Iraq, 85% of sheep and goat ticks (mainly *Rh. bursa*) were collected on the ears, 9.8% on the mammal and 5.1% on the hind legs and around the eyes (Omer et *al*., 2007). According to Elati et *al*. (2018), the common attachment site for *Rhipicephalus* ticks was near the ear tag, whereas *Hyalomma* ticks were mainly collected on the sternum.

The results obtained for the Shannon index show that there is no significant diversity in the studied sites. The value of equitability at AEH site is 0.39, which shows that there is a dominant species (*Rh. bursa*) in relation to the other species. Whilst, at the TR and IR sites, the species tend to be in equilibrium, since equitability is close to 1.

The factorial correspondence analysis shows that the spatial organization of the ticks sampled in the farms of the four sites in the study area, is directly related to altitude and the habitat of the species. In fact, vegetation indirectly impacts the tick's reproductive cycle due to the microclimate that plants generate, which controls both the humidity and temperature in which a tick grows. Some plant species can serve as indicators of the presence of ticks, but not directly through their ability to prevent them from desiccation; rather, they do so indirectly through the attraction they exert on specific hosts on which certain tick species feed (D.McCoy & Boulanger, 2015).

5. Conclusion

In the light of our results, we conclude that small ruminants (goats and sheep) are infested by six hard tick species in the Tizi-Ouzou area. The current data provide an important baseline for future epidemiological studies. As vectors of numerous diseases and arthropods that cause serious damage to small ruminants, particular attention should be paid to tick control and prevention. In the future, further studies should be carried out on ticks to examine their possible role in the transmission of various pathogens in our study area.

6. Acknowledgments

The corresponding author would like to thank all animal's farmers of study sites of Tizi-Ouzou who accepted to carry out our sampling on their animals. We also thank Dr. Massinissa Faci for his valuable advice and comments on improving the manuscript.

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T.D.: Conceptualization, Investigation, Methodology, Formal analysis, Validation, Writing - original draft, Writing - review & editing. **S.C.:** Formal analysis, Writing-review & editing. **A.B.:** Methodology. **N.E.:** Writing - review & editing, Validation. **K.B.:** Conceptualization, Supervision, Validation, Writing-review & editing. All authors read and approved the final manuscript.

8. Conflicts of interest

The authors declare no conflict of interest

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