

Preliminary investigation on tick fauna in the neighborhood of Tarquinia, Lazio, Italy

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Abstract

Introduction. This study represents a preliminary inquiry on tick fauna composition carried out in Maremma, Central Italy, where very few data are available.

Materials and methods. The study area was selected in the limestone hills surrounding Tarquinia town, on the base of suitable elements directly affecting the tick occurrence and the possible circulation of tick-borne pathogens, such as wild cattle rearing, kind of vegetation and human activities. Since a proper understanding of tick ecology is critical in predicting the risk of tick-borne pathogen transmission in a given area, a series of surveys was planned in such area in 2011.

Results. One hundred fifty-four ticks belonging to 4 genera and 6 species, namely *Ixodes ricinus* (n. = 109; 70%), *Rhipicephalus bursa* (n. = 18; 12%), *Rhipicephalus turanicus* (n. = 14; 9%), *Rhipicephalus sanguineus* (n. = 6; 4%), *Hyalomma marginatum* (n. = 4; 3%) and *Dermacentor marginatus* (n. = 3; 2%) were identified and reported for the area.

Discussion. The results of this acarological research represent a significant contribution to the knowledge of the tick fauna of rural areas in Northern Lazio Region, as first step toward a future molecular investigation on pathogen circulation.

Key words

- tick occurrence
- Maremma, Italy

INTRODUCTION

Although many investigations have been carried out in wild environments in several areas of Central Italy, Rome city and surrounding included [1-5], no data are available about tick occurrence in Maremma, with the exception of a study carried out in 2008 in the Tolfa Mountains [6], limiting the more southern border of this region. Maremma is a historical and physical region extending along the Tyrrhenian Sea, from Southern Tuscany to Northern Lazio Regions and inland to the Apennine foothills. In the last decades so many environmental changes have occurred but the northern part of Lazio region has maintained its dominant features in terms of land use and rearing of wild cattle representing favorable conditions for the spread of tick populations and the rise of tick-borne diseases. The area is characterized by a Mediterranean climate with a yearly average temperature of 14.5 °C and a large variety of biotopes.

For this purpose, in the limestone hills surrounding Tarquinia town, an interesting naturalistic area, four sites were selected for their high potentiality of tick-human contact, due to several outdoor recreational or working activities. Since a proper understanding of tick ecology is critical in predicting the risk of tick-borne pathogen transmission in a given area, a series of acarological surveys were planned in different rural ecosystems in the neighborhood of Tarquinia, during 2011, in collaboration with Università degli Studi della Tuscia di Viterbo and Università Agraria di Tarquinia.

MATERIAL AND METHODS

Study area

The study area includes two localities, named Ancarano and Le Fornacette, in the northern and northeastern outskirts of Tarquinia, an ancient town in the Viterbo

Province and separated by the Marta River. The hilly territory harbors oak species and pinewoods with both stone pine and maritime pine [7]. Woods and maquis alternated to pastures and cultivated areas host the typical wild fauna of Viterbo province of which representative elements are boars, roe deer, foxes, martens, hares and small mammals like rodents and insectivorous [8]. Based on different ecological and environmental conditions of the area, 4 sites were selected, one in Ancarano (site 1) and three in Le Fornacette (sites 2, 3 and 4) areas. Site 1 (42°16.344'N 11°49.869'E; 300 m² approximately extended) is characterized by grazing and scattered scrubs with oaks; site 2 (42°18.645'N 11°45.218'E; about 600 m²) named Roccaccia's wood, is a pinewood with both stone pine and maritime pine, broken by sporadic glades where many tracks and trails of boars were frequently observed. Site 3 (42°20.390'N 11°44.917'E; about 400 m²), located in the territory of the farm of Università Agraria di Tarquinia, is a pinewood usually used to restrict horses, donkeys and cattle. Site 4 (42°20.066'N 11°44.948'E; about 600 m²), also included in the territory belonging to the Università Agraria di Tarquinia, is an ecotonal area characterized by bushy glades and uncultivated lawns and limited by the marginal pines; the area is usually used as wild cattle pastures.

Tick collection

Tick collections were performed monthly in the four selected sites from May 2011 to April 2012, along a total of 16 fixed transects covered per survey by two investigators between 8:00 and 12:00 in the morning. Ticks were collected by dragging by using of a 1 m² wolen blanket through the bushes and on the ground. Because collection efficiency may vary individually, the collectors were systematically changed in each site. The number of ticks on the cloth was counted and picked up every 10 m and tick abundance was calculated as the number of ticks collected per 100 m². During each sampling, temperature (T) and relative humidity (RH) were recorded using a battery powered thermo-hygrometer; rainy event occurred before the survey were also noted. The collected specimens were identified according to morphological characters [9] and stored at -80 °C for further molecular analysis.

Statistical analysis

Chi-square test was used to assess the association between tick species distribution and the capture site, the temperature and the humidity level. A p-value of < or = 0.05 was considered as statistically significant.

RESULTS

The surveys allowed to collect 154 ticks belonging to 4 genera and 6 species, namely *Ixodes ricinus* Linnaeus, 1758 (n. = 109; 70%), *Rhipicephalus bursa* Canestrini & Fanzago, 1878 (n. = 18; 12%), *Rhipicephalus turanicus* Pomerantsev 1936 (n. = 14; 9%), *Rhipicephalus sanguineus* Latreille, 1806 (n. = 6; 4%), *Hyalomma marginatum* Koch, 1844 (n. = 4; 3%), *Dermacentor marginatus* (Sultzter, 1776) (n. = 3; 2%).

Out of 68 adult specimens, 34% was identified as *I. ricinus*, with 13 females, 10 males; 26% as *R. bursa*, with

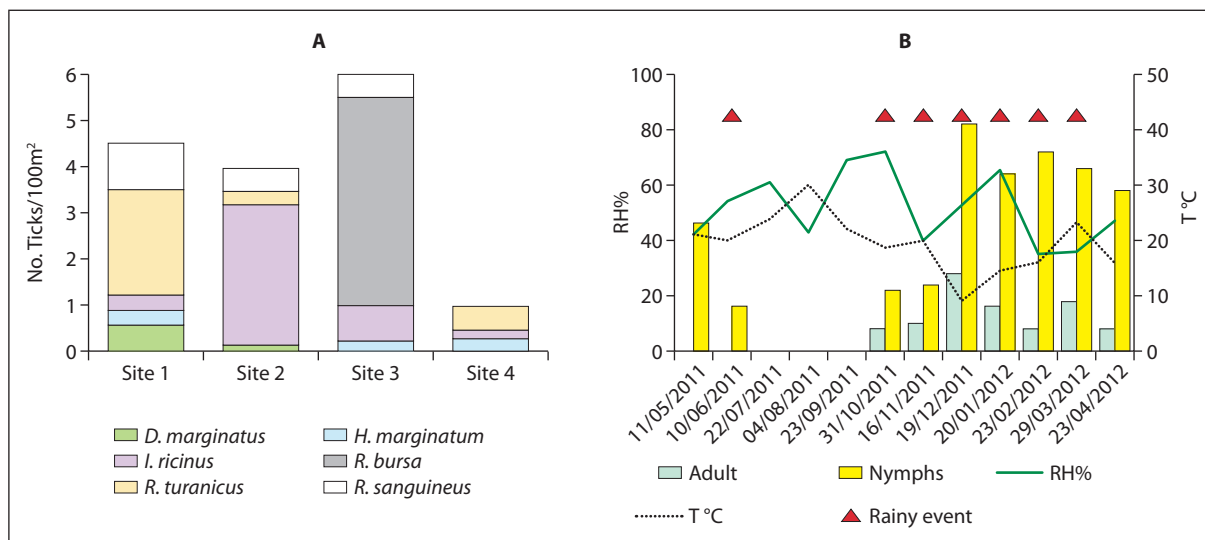
9 females and 9 males; 21% as *R. turanicus*, with 5 females and 9 males, 9% as *R. sanguineus*, with 2 females and 4 male, 6% as *H. marginatum*, with 4 females and 4% as *D. marginatus*, with 2 female and 1 male. Moreover 86 nymphs were also collected during the study period and 100% of sample belonged to *I. ricinus*. This species was the prevalent one recorded in the whole collection activity (23 adults and 86 nymphs), occurring in all the four sites studied (Figure 1A). No tick specimens were found between August and September 2011.

The seasonal trend of *I. ricinus* nymph exhibited an occurrence from October 2011 to June 2012, with peaks of abundance in December, February and April, while the season of adults ended before (in April), showing a peak of abundance in December (Figure 1B). *R. bursa* occurred in July with a single, but high presence in site 3 (Figure 1A). In our field collection *R. turanicus* was the third species in percentage of presence and it was recorded in all the sites in May 2011 and at the end of March 2012 (Figure 1B). Only six specimens of *R. sanguineus* were collected in the sites 1 and 2, in June 2011 and during the two surveys in March-April 2012 (Figure 1A). The rarest species were *H. marginatum* and *D. marginatus*. The first one was found in sites 1, 3 and 4 in May and in March 2012: the second one was found in sites 1 and 2, in May 2011 and in January and March 2012.

A statistical association between the tick species distribution and the capture site (p = 0.05), temperature (p = 0.05) and relative humidity (p = 0.05) was found.

DISCUSSION

The present study describes the ixodidic fauna in the southern part of Maremma, in Lazio region, a poorly investigated area from this point of view. We compared our results with those reported in a previous study, the only carried out in a nearby area, in Tolfa Mountains [6]. Spite of differences in the sample collected due to the greater number of selected sites, some considerations can be made on the aspects that are common and on the ones that differentiate the two surveys. First of all, also in Tolfa areas *I. ricinus* was the most abundant species (48.3%) and resulted prevalent in the woodland (95.0%). Moreover, the other species occurring in both surveys, *R. bursa*, *R. sanguineus* and *H. marginatus*, were present in the sample in a similar relative percentage (24.1%, 2.5% and 2.3%, respectively). About the discrepancies, *Rhipicephalus (Boophilus) annulatus* (Say, 1821) was found in 2008 but only in association with the cattle and not in the field collections. On the contrary only in our survey *R. turanicus* and *D. marginatum* were found only in our survey, maybe because of the occurrence of flocks and wild boars. In Tarquinia area the species composition of ticks collected reflected the difference in environments and in the host occurrence of collection sites. Moreover, as expected and confirmed by statistical analysis, the seasonal trend of tick species appeared to be directly influenced by temperature and relative humidity [9-12]. In fact, the most abundant collections were concentrated between May and July 2011 and December 2011 and April 2012. During the summer (from August to September) few or none specimens were collected in

**Figure 1**

A. Tick species composition by site in the study area. **B.** Seasonal activity of *I. ricinus*, adults and nymphs, in the study area respect to climatic factors, mean temperature (T °C) and relative humidity (RH%), recorded during each survey.

presence of high temperatures but low humidity and lack of rainfall (Figure 1B). *I. ricinus*, the main vector of *Borrelia burgdorferi* sensu lato and of the Tick-borne encephalites virus in Italy [1, 10, 11], was the predominant tick recorded in the study area. The species showed a trend with a main peak of adults in winter, a weaker in spring, and a more evident occurrence of nymphs from winter to spring, disappearing from our collections during the summer, as previously reported in other cases in Central Italy [5]. Although *I. ricinus* occurred in all of the four sites, the site 2 and 3, characterized by dense pinewood, exhibited the highest abundances of the species. This observation, supported by statistical analysis, confirms what is already well known in literature, i.e. the close association between the wood tick and its environment [9, 12, 13]. *R. bursa* is considered the main vector of *Babesia* spp. for small ruminants in Mediterranean countries but rarely biting humans [14]. Moreover the species show the higher activity between June and August in areas characterized by Xerophytes, pinewoods and oakwoods, and the typical hosts are domestic as well as wild ungulates equines, swine and cattle [12]. Our findings confirm this phenology showing the only peak of occurrence in July, concentrating in the site 3, characterized by sparse pinewood and usually frequented by equine and bovine. *R. turanicus*, widespread in central and southern Italy [12] and proven vector of different animal and human pathogens like *Rickettsia conorii*, *Babesia* spp., *Anaplasma* spp., *Theileria ovis*, *Coxiella burnetii*, and Crimean-Congo Haemorrhagic Fever Virus (CCHFV) [15], was the third prevalent species in our study. This species was collected mainly in the site 1, frequented by ovine flocks, while a minority of the sample was found in site 2 and in the site 4. The species showed peaks in March and May even if it is known to be thermophilic, with a great affinity for Mediterranean habitats [16, 17]. *R. sanguineus*, a very common tick in Italy, constituted a small part of the sample but our collections concentrated in early

summer (in 2011) and in spring (in 2012), confirmed the seasonality of feeding activity of this species, mainly feeding on Canidae. A few specimens of *H. marginatum* were found in three sites in early summer and in spring according to the wide range of the phenology of the species [12]. It is important to highlight that this widespread species and its subspecies play an important potential role in transmission of tick-borne diseases for animals and humans, including CCHFV [15]. The smallest number of *D. marginatus*, collected in two sites in spring and winter, is in agreement with the phenology reported in literature [12]. This species able to bite humans is vector of some animal and human pathogens [17]. However, even if ungulates, its favourite hosts were present in the area, the scarcity of the sample does not allow any kind of speculation.

It is worthy to note that, the whole study area is highly frequented by people for recreational activities (hunters and trekkers) or for work (rangers, cowboys, shepherd, foresters and farm workers), especially in occasion of events, such as the annual “Merca fair” in April-May that rises concern about the potential risk of tick-borne diseases. In fact, every year in the site that hosts this event there is a high concentration of people and livestock coming from many places, also so far, with a consequently-significant increase of risk of contact among ticks, animals and humans. In addition, after this event, the departure of livestock, owners and visitors could promote not only the spread of ticks, but also of pathogens carried by them into other areas of the country.

CONCLUSIONS

The present inquiry pointed out a rather various ixodidic fauna composed by typical species of ecotonal environments. Even if the number of specimens was not so abundant to completely define the seasonal trends of several species, tick composition seems to reflect the

presence of wild animals and cattle in the study area, also at the light of previous data. However the study shows how an evaluation of tick occurrence in areas where human activities are ordinary carried out is crucial for understanding the entity of tick bite risk and tick-borne disease transmission; in fact this knowledge constitutes the base to plan aimed control strategies. The results of this acarological research represent a significant contribution to the knowledge of the tick fauna of rural areas in Northern Lazio Region, as first step toward a future molecular investigation on pathogen circulation.

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Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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