Seasonal dynamics of adult *Dermacentor reticulatus* in a peri-urban park in southern Europe

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Abstract

Studies on the human-biting pathogen vectors *Dermacentor reticulatus* and *Ixodes ricinus* have been scarce in southern Europe. The aims of the present study were to determine the abundance of these ticks in a peri-urban park in northern Italy, describe the seasonal activity of *D. reticulatus* and examine the correlation between tick occurrence and environmental factors. Ticks were collected monthly from April 2015 to May 2016 using both dragging and flagging techniques. Various climatic variables (mean temperature, relative humidity and evapotranspiration for the collection dates; and 30-day moving averages preceding each collection date were calculated for rainfall, temperature, relative humidity and saturation deficit) also were recorded. Overall, 444 adults of *D. reticulatus* and 10 adults of *I. ricinus* were collected. Males of *D. reticulatus* appeared earlier in the year than females, but overall females were collected more frequently than males (1:1.25). Statistical analysis showed significant differences in the density of *D. reticulatus* among sampling transects and among months. The seasonal dynamic of *D. reticulatus* was characterized by a single peak of activity in the early spring. Tick density was associated with climatic variables: the 30-day moving average saturation deficit was particularly significant as most ticks (83%) were collected at values below 5.2 mmHg. At the level of individual sampling transects, seasonal dynamics could be influenced by habitat type and host availability. We found *D. reticulatus* to be most abundant in mixed forests dominated by oaks and rich in ponds.As to *I. ricinus,* though found in a small number, its presence can be confirmed by our investigation. In light of the results of this study, the risk for encounters with *D. reticulatus* and *I. ricinus* may be higher than previously thought in northern Italy.

Key words: Questing ticks, *Dermacentor reticulatus*, *Ixodes ricinus*, seasonal dynamics, peri-urban park, Italy

1. Introduction

The emergence of ticks in urban and peri-urban areas is a cause for public health concern given the risk for increased exposure of humans and domestic animals to tick-borne pathogens. In the last decades, some tick species, e.g. *Dermacentor reticulatus* and *Ixodes ricinus*, appear to have expanded their habitats, including invading peri-urban biotopes (Biernat et al., 2014; Hornok et al., 2014; Mancini et al., 2014; Rizzoli et al., 2014; Mehlhorn et al., 2015; Paul et al., 2016).

Dermacentor reticulatus is one of the most important vectors of tick-borne pathogens in Europe (Bullová et al., 2009). Apart from being the main vector of *Babesia canis*, the causative agent of babesial infection in dogs, and of other pathogens such as *Babesia caballi*, *Theileria equi*, *Rickettsia raoultii* and *R. slovaca*, it can also transmit tick-borne encephalitis virus (Gray et al., 2009; Földvári et al., 2013; Karbowiak, 2014; Jongejan et al., 2015). Moreover, natural infections with *Anaplasma phagocytophilum*, *Bartonella* spp., *Coxiella burnetii* and *Francisella tularensis* have been recorded for *D. reticulatus* (Földvári et al., 2016). This tick occurs in the Eurasian temperate climate zone, ranging from the Iberian Peninsula (West) to the basin of the Yenisei River in Siberia (East). The known upper boundary is located in England at 53.72° N for western and central Europe whilst in Latvia at 56.68° N for Eastern Europe (Rubel et al., 2016); the southernmost collection location for *D. reticulatus* is currently in Spain at 41.20° N (Estrada-Peña et al., 2013). The tick has recently spread into new areas, presumably linked to climatic change including increasing minimum temperatures and shortening of the winter period (Beugnet and Marié, 2009). Despite no previous evidence of its presence to the south of the Alps (Rubel et al., 2016), *D. reticulatus* was recently recorded from north-eastern Italy and associated with *B. canis* infection in dogs living in this area (Olivieri et al., 2016).

Over the last decades, studies on the distribution and seasonal dynamics of *D. reticulatus* have shown a clustered distribution in areas characterized by higher humidity, along forest paths, shrubby pasture, meadows and forests located near stagnant waters, marshes, rivers and lakes

(Estrada-Peña et al., 2004a; Karbowiak, 2014). However, in recent years, a progressive expansion into drier habitats was shown (Rubel et al., 2016). *Dermacentor reticulatus* poses substantial risk for biting humans due to its spread in parks and fallow lands in urban and sub-urban areas (Gilot et al., 1973; Dautel et al., 2006; Széll et al., 2006; Rubel et al., 2016). Generally, adults of *D. reticulatus* are active in spring, from March to May, and again in autumn, from September to November (Nosek, 1972; Martinod and Gilot 1991; Földvári and Farkas 2005; Földvári et al., 2016; Földvári et al., 2007).

Ixodes ricinus, the most common tick in Europe, is the main vector of *Borrelia burgdorferi* sensu lato (s.l.), the causative agents of Lyme borreliosis, as well as various other pathogens (Gern et al., 2010). It has a wide geographical distribution and feeds on a large variety of hosts, including mammals, birds and reptiles (Estrada-Peña et al., 2004a; Estrada-Peña, 2011; Estrada-Peña et al., 2013; Medlock et al., 2013). The phenology of *I. ricinus* can vary considerably both within the same country and among years (Gray, 1991; Randolph et al., 2002; Perret et al., 2004). The seasonal dynamics are influenced by various abiotic and biotic factors (Estrada-Penã et al., 2006; Medlock et al., 2008; Randolph, 2008, 2009; Daniel et al., 2009; Gray et al., 2009; Danielová et al., 2010; Gilbert, 2010; Tagliapietra et al., 2011). Generally in Central and Southern Europe, this tick occurs at altitudes between 500 and 1500 m above sea level; in the last decades, though, it has shown an expansion both toward higher altitudes and at sea level (Kirby et al., 2004; Danielová et al., 2006; Materna et al., 2008; Jore et al., 2011; Jaenson et al., 2012; Léger et al., 2012; Medlock et al., 2013; Martello et al., 2014).

To improve our knowledge about the ecology of the southernmost European populations of *D. reticulatus*, located south of the Alps, we conducted an acarological study in a peri-urban park located in northern Italy. The study aimed at: i) investigating which tick species were present and how commonly they occurred; ii) describing the seasonal activity of *D. reticulatus* to clarify the peak period of the year with risk for tick bites; and iii) examining the association among local environmental factors and the occurrence and relative density of questing *D. reticulatus*.

2. Material and methods

2.1 Study area

The study was carried out in the Groane Regional Park, (northeastern Italy; 45°34'37.87''N, 9°5'3.96''E, 152-262 m.a.s.l.), located in the peri-urban area of two provinces (Milan and Monza Brianza) of Lombardy region (Figure 1). The Park includes 17 municipalities with a human population consisting of 316,000 residents and a human density of 2.3/km^2. The environment is characterized by clay loam favouring the presence of several rivers and ponds close to a mixed forest interspersed with roads, housing and agricultural areas. The dominant species of trees include oaks (*Quercus robur*, *Quercus petraea*, hybrid oak *Quercus petraea* x *Quercus robur* and *Quercus rubra*), pine (*Pinus sylvestris*), birch (*Betula pendula*) and black locust (*Robinia pseudoacacia*). Common shrubs include *Molinia arundinacea, Pteridium aquilinum, Vinca minor, Polygonatum multiflorum* and *Hedera helix*. Suitable hosts (e.g., dogs, horses, cattle, wild ungulates and voles) for ticks are widespread in the Park (Ente Parco delle Groane, 2008).

The average annual temperature of the study area is 12.4 °C, and the average annual rainfall is 1,000 mm (ARPA, http://www.arpalombardia.it/arpa_splash/splash.asp).

2.2 Tick collection and identification

From April 2015 to May 2016, tick collection was performed once a month in five permanent transects selected from the typical habitat suitable for *D. reticulatus* (Table 1, Figure 2). Sampling was performed during daytime, between 10:30AM and 05:00PM, and was carried out only in the absence of precipitation and strong wind. Ticks were collected with a woolen blanket (100 × 90 cm) using both dragging and flagging techniques on the leaf litter and the vegetation, respectively. Each sampling session was performed over 100 m^2 for each transect, with both sides of the cloth examined every 2 meters. Attached ticks were collected using tweezers and preserved in vials containing 70% ethanol. Then, they were counted and morphologically identified by taxonomic keys (Pomerantzev, 1959; Arthur, 1962; Estrada-Peña et al., 2004a, Estrada- Peña et al., 2014) .

. Further, they were categorized by site of collection, stage and sex. Questing tick density was expressed as the number of ticks collected per 100 m^2.

2.3 Climatic data

Climatic variables were obtained from the meteorological stations, located inside the Groane Regional Park, closest to the sampling site (data provided online by ARPA, http://www.arpalombardia.it/arpa_splash/splash.asp, and the Ministry of Agricultural, Food and Forestry Policies https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/202). For each date of sampling, mean temperature (°C), mean relative humidity (RH, %) and evapotranspiration (mm), a measure of the water extracted from land surfaces due to evaporation and transpiration and hence a measure of the hydric stress conditions to which ticks are exposed (Ruiz-Fons et al., 2012), were recorded. Rainfall (mm), temperature (°C) and relative humidity (RH, %) also were calculated as 30-day moving averages preceding each collection, as well as the saturation deficit (SD), a measure of the drying power of the air, that was calculated using the following formula: $SD = (1 - RH/100) \times 4.9463 \times e(0.0621T)$, (Randolph and Storey, 1999; Perret et al., 2000).

2.4 Statistical analysis

The *Dermacentor* tick density distribution was checked for normality with Shapiro-Wilk test. Since the data were aggregated, statistical models were performed with a negative binomial distribution and log link-function. The variables entered in the full models were selected based on biological criteria; collinearity was tested and when it occurred, only the most significant variables were included.

A generalized linear model (GLM) was performed to investigate the effect of the collection period and location on tick density. A Generalized Linear Mixed Model (GLMM) was used to assess the influence of climatic variables on tick density; daily mean temperature, daily mean humidity, SD, evapotranspiration and rainfall were considered. Location and month of sampling were entered in the model as nested random effects.

Both GLM and GLMM final models were developed with backward elimination considering the goodness of fit with the Akaike information criterion (AIC) and Akaike information criterion corrected (AICC), respectively. Statistical analysis was performed with SPSS (version 20.0; SPSS, Chicago, IL).

3. Results

3.1 Ticks collected

A total of 454 questing adults of *D. reticulatus* and *I. ricinus* were collected in five locations (Table 2). We checked the collected *Ixodes* ticks whether or not they are *I. inopinatus,* a new species recently described; all *Ixodes* were confirmed belonging to *I. ricinus.*Immature stages were not collected for either of these tick species. *D. reticulatus* was the more abundant species with 444 collected specimens. This included 247 females and 197 males, for a male/female ratio of 1:1.25. (Table 2, Table 3). *D. reticulatus* was found in all transects, with the highest densities in transects 1, 2 and 3 (Table 2); GLM analysis showed significant differences in *D. reticulatus* density among transects (Table 4). *Ixodes ricinus* (nine females and one male) was collected sporadically in transects 1 and 2.

3.2 Description of seasonal trend

The density of questing *D. reticulatus* varied considerably over the fourteen months of sampling and the highest tick densities were recorded in the second year of sampling (Table 3). Particularly, *D. reticulatus* was collected from April to June 2015 and from January to May 2016. Males appeared earlier in the year and were more abundant than females from January to March 2016. Females dominated over males only in May 2015 and April 2016 (Table 3, Figure 3). Ticks were most abundant in the spring months, particularly in March (n = 64, 14.41%) and April 2016 (n = 302, 68.02%); starting from May, tick density decreased (n = 11, 2.48% in 2015 and n = 36, 8.11% in 2016). The questing season of ticks showed a peak in April 2016, while in 2015 it was not very clear (Table 3). In the GLM analysis, the difference of tick density among months was statistically significant (df = 7, $\chi^2 = 38.366$, $p < 0.0001$) (Table 4). The risk of tick infestation was higher during April 2016 and March 2016 when compared to the reference month (January 2016).

Further, significant differences in dynamics were found among transects (df = 4, $\chi^2 = 28.187$, $p < 0.0001$) (Table 4). In transect 1, the first tick appeared in January 2016. Then, in April 2016, tick density showed a peak, equal to 88 ticks /100 m^2, to decrease gradually, though in May 2016 some ticks were still collected (27 ticks/100 m^2). In transect 2, the first tick was collected in February 2016. In March (41 ticks/100 m^2) and April (141 ticks/100 m^2) 2016 their highest numbers occurred; then, in May, the density of *D. reticulatus* decreased rapidly (3 ticks/100 m^2). In transect 3, in April (73 ticks/100 m^2) and May (6 ticks/100 m^2) 2016 the highest tick densities were registered. In transects 4 and 5, only one tick each was collected, in April and June of 2015, respectively.

In the case of *I. ricinus,* most of the ticks were collected in May 2015 and 2016; specifically, in transect 1 (1 female in 2015; 2 females and 1 male in 2016) and in transect 2 (3 females in 2015 and 1 in 2016).

3.3 Effects of meteorological variables on tick densities

Throughout the collection period, the daily mean temperature varied between 3.8°C (February) and 28.0°C (August), and the daily mean relative humidity reached 53% (August) and 92% (January). The 30-day moving average SD ranged from 0.5 mm/Hg (January) to 13.1 mm/Hg (August), increasing when the climate was drier and warmer within the period from May to October. The 30-day moving average rainfall was around 0.08 mm in December/January and reached the highest level in March (6.37 mm). The daily mean evapotranspiration varied between 10.5 (December) and 152.8 mm (July).

During the sampling period, no questing *D. reticulatus* were collected when the daily mean temperature was below 10°C or exceeded 26°C. Ticks were found when temperatures ranged between 13°C and 18°C, and daily mean RH varied between 45% and 60% (Figure 4a and 4b). Ticks were only occasionally collected when RH reached its highest levels (Figure 4b). Considering the 30-day moving averages, 85% (n=379) of the ticks were collected when the 30-day average temperature was below 10°C. The first tick appeared at 5°C and with 30-day average temperature and relative humidity reaching 96%. A high percentage of ticks (81.76%; n=363) was collected when 30-day average relative humidity was under 70%. When considering rainfall, the peak of *D. reticulatus* in April 2016 was preceded by a sudden increase of monthly precipitation (Figure 4c). Moreover, 90.5% of *D. reticulatus* was collected at values of evapotranspiration below 50 mm (Figure 5a).

In the GLMM final multivariate model, only saturation deficit resulted significantly associated with variation in adult density of *D. reticulatus* ($p < 0.001$; Coefficient = 0.473, Standard error = 0.016). Particularly, most of the ticks (82.66%) were collected with values of saturation deficit below 5.2 mmHg (Figure 5b). Although not statistically associated with density of *D. reticulatus*, the other climatic variables did show characteristic trends when linked to tick density.

4. Discussion

Dermacentor reticulatus was the dominant tick species in the study area. The density of this tick was lower as compared with reports from other countries, i.e. Poland (Bartosik et al., 2011), the Czech Republic (Široký et al., 2011), Slovakia (Bullová et al., 2009) and the Baltic countries (Paulauskas et al., 2015), where *D. reticulatus* is the second most abundant tick species. Other studies on *D. reticulatus* (Dobec et al., 2009; Mihaljica et al., 2012; Bonnet et al., 2013, Schaarschmidt et al., 2013) did not report tick density.

In the study area, *D. reticulatus* showed a single peak of activity mainly restricted to early spring. This unimodal pattern of activity is different from what is described in other European countries, where the tick has a bimodal pattern with an activity peak that can be either in spring (Szymanski, 1987; Martinod and Gilot, 1991; Földvári and Farkas, 2005; Földvári et al., 2007; Cochez et al., 2012) or in autumn (Szell et al., 2006; Hornok, 2009). In the past, a single peak reported in Romania (Feider, 1965), but a more recent study showed an all-year round tick activity (Chitimia-Dobler, 2015).

In agreement with other surveys, the sex ratio favoured questing females (Siroky et al., 2011; Buczek et al., 2013); it may be due to their need to find a host for mating and for egg development (Buczek et al., 2013). The female dominance was recorded only in April, a period with the highest tick activity under the most favourable conditions, whereas questing males appeared more abundant in previous months. As suggested by Buczek et al., 2014, the different temporal activity of males and females being influenced by temperature and humidity changes could be related to their morphological and physiological differences, determinant in the maintenance of an advantageous water balance and, consequently, in their survival.

Variations in tick activity and abundance among transects were also observed, presumably influenced by habitat type and host availability. The environmental features of the areas harbouring the highest numbers of *D. reticulatus* included numerous ponds. The presence of these ponds is due to a peculiarity of the soil made of compact and impermeable ground clay with limited absorption capacity and high ability to retain water, which allows stagnation of rain water with consequent formation of wetlands, even of a temporary nature. Another important factor influencing the presence of both ticks and small mammal hosts is the existence of an urban mixed forest dominated by oaks combined with bushes, abundant shrubs and uncut lower vegetation; the many leaves accumulating on the ground contribute to establish a microhabitat suitable for tick rehydratation. In addition, the presence of a large recreational area together with an adjacent housing estate supports a high access to the park by people walking dogs *s*.

Unfavourable conditions to the tick life cycle occurred in areas with absolute absence of dogs, the presence of only one pond that cannot guarantee a high level of humidity to the site ground and the presence of a small lake instead of the typical ponds. Also the existence of pine forests may limit the abundance of small mammals, suitable hosts for the immature stages of ticks.

In the study area, the conditions required for the establishment of foci of *D. reticulatus* are dependent on the availability of suitable hosts for ticks. Our data confirmed that *D. reticulatus* species prefers typical biotopes mostly characterized by wetlands in mixed forests with oaks as dominant trees and rich in ponds with stagnating water. Weather conditions can greatly influence the density of questing ticks. The results showed that the threshold of 30-day moving average of temperature at which *D. reticulatus* starts its activity is low, and in agreement with other studies ticks proved to be active at a very wide range of temperatures (10°-26°C) (Bartosik et al., 2011; Karbowiak, 2014).

Rainfall and high relative humidity values resulted the most effective in stimulating *D. reticulatus* activation after the diapause. Despite this, the highest tick activity was recorded at low values of 30-day relative humidity (>70%) and at low range of daily mean relative humidity (45-60%). These results are not consistent with the distribution pattern described in other European countries where ticks are spread, and the boundary of daily relative humidity for tick activity was 60-100% (Meyer-Konig et al., 2001; Buczek et al., 2013). The peak of tick activity was observed at low level of evapotranspiration, confirming the inverse correlation of this variable on the tick hydric balance as demonstrated in other studies (Ruiz-Fons et al., 2012; Dantas-Torres and Otranto, 2013).

In April 2016, *D. reticulatus* questing activity showed an increase compared to April 2015, which could be due to differences concerning parametres of the climatic variables; in particular, 30-day moving average of temperature and evapotranspiration resulted lower in 2016, thus confirming the preference of ticks in cooler areas. In these areas, saturation deficit represents the best variable to describe the climatic effects on tick density, showing an adverse effect on tick questing behavior in agreement with other studies on tick population dynamics (Perret et al., 2000; Randolph et al., 2002; Estrada Pena et al., 2004b; Estrada Pena et al., 2013). In particular, the saturation deficit registered in the Groane Park reached high values during summer; as a consequence, tick population increased when saturation deficit decreased, showing the negative effect of drought on questing activity.

Evidence of *I. ricinus* in the study area is the first record of this tick species there.Although *I. ricinus* specimens are scarce, they can confirm the presence of *I. ricinus* in the Po plain where the Groane Regional Park is located. This tick species had been previously recorded in humans and in dogs from the study area (Manfredi unpublished data, 1997) and subsequently in some hosts (*Lacerta bilineata*) as well as in the environment of the nearby park of the Ticino river valley (Scali et al., 2001; Pistone et al., 2010). Notably, this is a park where Lyme borreliosis spirochetes were found in ticks (Pistone et al., 2010). In the Groane Regional Park, the presence of hosts considered the main reservoirs of this pathogen in Europe (*Myodes glareolus, Apodemus sylvaticus* and *Apodemus flavicollis*) (Humair et al., 1993) together with appropriate environmental conditions for the vector *I. ricinus* could pose at risk for Lyme disease. Meanwhile, the absence of proper hosts for the tick adult stage (ruminants, horses) increases the risk of tick attachment for humans and domesticated animals.

Finally, this acarological study confirms that *I. ricinus* is spreading at low altitude, being the study area located between 220 m.a.s.l. and 240 m.a.s.l.

5. Conclusions

Though confined to a single peri-urban park in northern Italy, our study revealed the presence of two tick vector species, *D. reticulatus* and *I. ricinus,* in a highly urbanized area previously considered to present minimal or low risk for tick-bites. Additional investigations are needed on the pathogens occurring in these tick species in the study area. Even if, it seems that adult *D. reticulatus* do not very frequently bite humans (as *I. ricinus* does however) and the immatures never do so,in light of our findings, monitoring peri-urban green areas should be considered crucial to implement strategic plans focused on preventing the risk of tick-bites and the consequent transmission of pathogens causing tick-borne diseases in humans and animals.

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Conflict of interest

The authors have no conflict of interest.

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Table 1: Location and main characteristics of the sampling transects in the peri-urban park in southern Europe where the ticks were collected.

Transect	Site Coordinates		Typology of the transect	Presence of water reservoir		Type of vegetation	Host availability for adult ticks	
	Latitude	Longitude		Type	Number		Type	Frequency
1	45.637643	9.092137	Forest paths for pedestrians and bikers with picnic sites, close to human inhabited area	Pond	5	Mixed forest with dominance of oak forest	Dog	High
							Human	High
2	45.630066	9.100153	Forest paths for pedestrians and bikers with recreational sites, close to industrial area	Pond	4	Mainly bushes with sparse trees	Dog	High
							Human	High
3	45.622075	9.094086	Forest paths exclusively used for dog training	Pond	3	Mixed forest with dominance of oak forest	Dog	High
							Human (exclusively dog trainer)	Low
4	45.585234	9.094161	Open meadow, ex dog-training camp close to cultivated areas (public access forbidden)	Pond	1	Meadows and bushes	Dog	None
							Human	None
5	45.587103	9.087535	Forest paths for pedestrians and bikers	Small lake	1	Mixed forest with dominance of pine forest	Dog	Low
							Human	Low

Table 2: Number of adult ticks of *Dermacentor reticulatus* and *Ixodes ricinus* collected in the five transects of the surveyed peri-urban park in southern Europe during the study period (April 2015-May 2016).

Transect	Sex	*Dermacentor reticulatus*		*Ixodes ricinus*		Total	Average tick density/100m^2
		n of ticks	%	n of ticks	%	n of ticks	(± SD[a])
1	Male	73	44.	1	20	74	5.3 (8.6)
	Female	92	56	4	80	96	6.9 (15.5)
	Total	165	100	5	100	**170**	**12.2 (23.8**)
2	Male	84	43	0	0	84	6.0 (15.0)
	Female	112	57	5	100	117	8.3 (23.5)
	Total	196	100	5	100	**201**	**14.3 (38.3)**
3	Male	40	49	0	0	40	2.9 (9.6)
	Female	41	51	0	0	41	3.0 (9.8)
	Total	81	100	0	0	**81**	**5.9 (19.4)**
4	Male	0	0	0	0	0	-
	Female	1	100	0	0	1	0.1 (0.3)
	Total	1	100	0	0	**1**	**0.1 (0.3)**
5	Male	0	0	0	0	0	-
	Female	1	100	0	0	1	0.1 (0.3)
	Total	1	100	0	0	**1**	**0.1 (0.3)**
Total	**Male**	**197**	**44**	**1**	**10**	**198**	**2.8 (2.5)**
	Female	**247**	**56**	**9**	**90**	**256**	**3.7 (3.4)**
	Total	**444**	**100**	**10**	**100**	**454**	**6.5 (5.9)**

[a] Standard Deviation

Table 3: Dermacentor reticulatus adult ticks collected in the investigated peri-urban park in southern Europe stratified by sampling year, months and gender.

Year	Month	Sex						
		Overall		Males		Females		Gender ratio (F:M)
		n of ticks	%	n of ticks	%	n of ticks	%	
2015	April	14	3.2	7	1.6	7	1.6	1 : 1
	May	11	2.5	5	1.2	6	1.3	1.20 : 1
	June	4	0.9	2	0.5	2	0.4	1 : 1
	July	0	0.0	0	0.0	0	0.0	0
	August	0	0.0	0	0.0	0	0.0	0
	September	0	0.0	0	0.0	0	0.0	0
	October	0	0.0	0	0.0	0	0.0	0
	November	0	0.0	0	0.0	0	0.0	0
	December	0	0.0	0	0.0	0	0.0	0
2016	January	1	0.2	1	0.2	0	0.0	0
	February	12	2.7	8	1.8	4	0.9	0.50 : 1
	March	64	14.4	36	8.1	28	6.3	0.78 : 1
	April	302	68.0	120	27.0	182	41.0	1.52 : 1
	May	36	8.1	18	4.1	18	4.0	1 : 1
	Total	444	100.0	197	44.5	247	55.5	1.25 : 1

Table 4: Comparison of *Dermacentor reticulatus* densities among sampling months and transects. Results from generalized linear model (GLM). Only significant months are shown.

Variables	Category	*p*- value	Parameter estimate	95% CI[a] min-max
Transects	1	<0.001	4.861	2.183-7.539
	2	0.0001	4.356	1.708-7.005
	3	0.022	3.122	0.454-5.789
	4	0.913	-0.194	-3.666-3.278
	5 (reference)		1	.
Months	April 2015	0.042	2.841	0.101-5.581
	March 2016	0.003	4.010	1.408-6.612
	April 2016	<0.0001	5.853	3.280-8.426
	May 2016	0.006	3.651	1.037-6.264
	January 2016 (reference)		1	

[a] Confidence Interval

Figure 1: Geographical location of the peri-urban park (red dot) where ticks were collected, in Lombardy (blue line indicates regional boundaries), Italy.

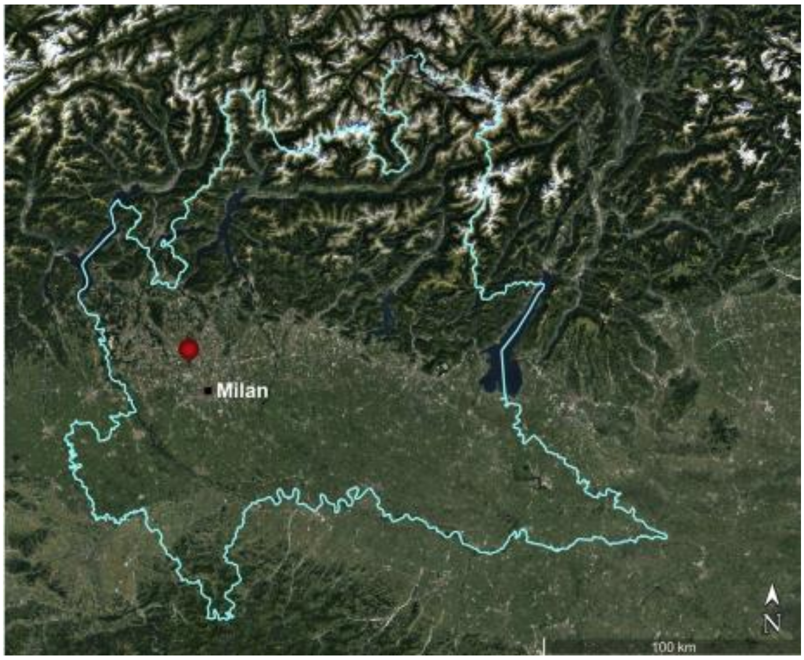

Figure 2: Study area with locations of the sampling transects: a) map of peri-urban park in southern Europe with georeferenced transects; b) transect 1, c) transect 2 d) transect 3 e) transect 4, f) transect 5. Green line corresponds to the length of the sampling transect.

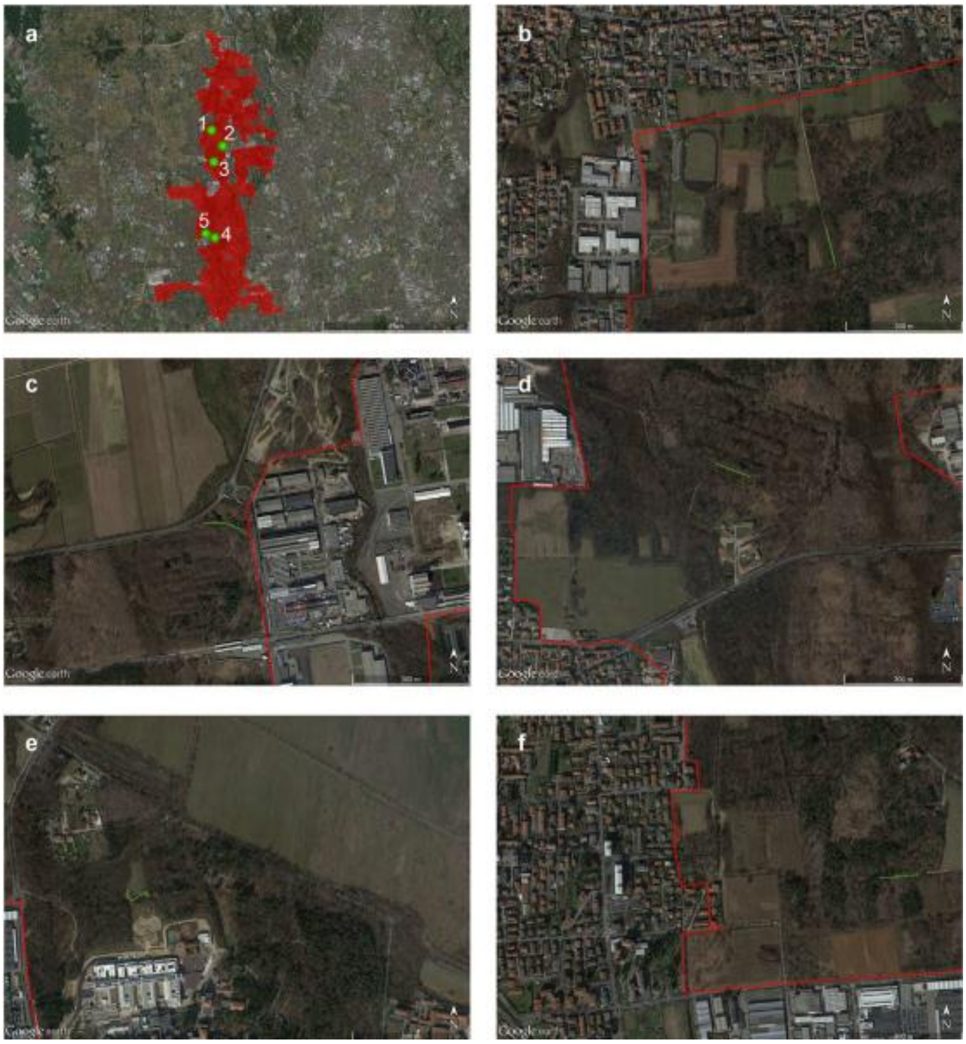

Figure 3: Monthly distribution of females (white) and males (grey) of *Dermacentor reticulatus* collected in the peri-urban park in southern Europe. The rectangle shows the number of monthly collected ticks, the black segment inside the rectangle shows the median; the whisker shows the minimum and maximum.

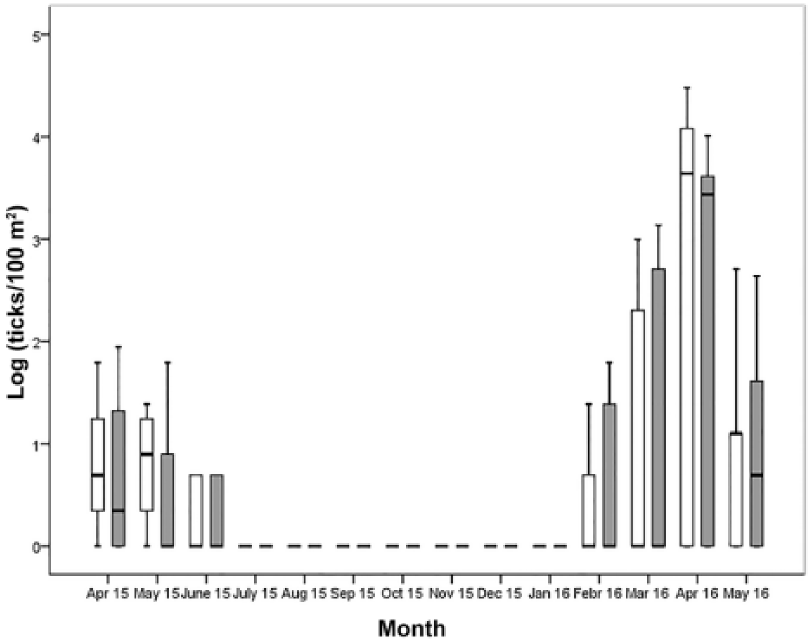

Figure 4: *Dermacentor reticulatus* collected in the peri-urban park in southern Europe: tick density (number of ticks per 100m^2) **a**) and 30-day moving average temperature (30-T°) and mean daily temperature (T°) **b**) and 30-day moving average relative humidity (30-RH) and mean daily relative humidity (RH) in % **c**) and 30-day moving average rainfall (30-R) in mm.

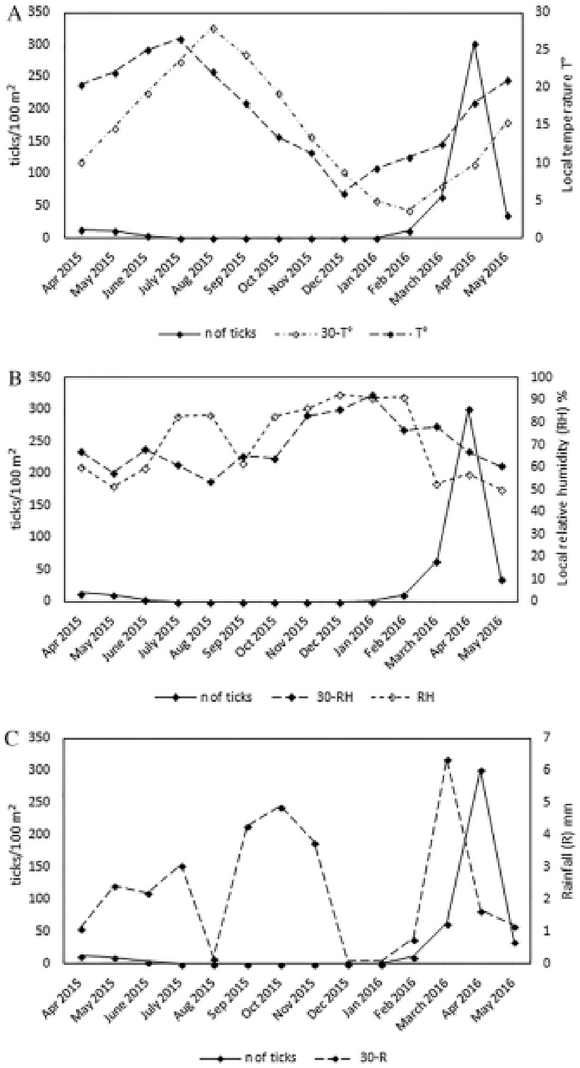

Figure 5: *Dermacentor reticulatus* collected in the peri-urban park in southern Europe: tick density (number of ticks per $100 m^2$) **a**) and evapotranspiration (ETa) in mm **b**) and 30-day moving average saturation deficit (30-SD) in mmHg.