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Prevalence of tick-borne pathogens in an urban park in Rome, Italy

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Abstract

Introduction. Limited information is available about the presence of tick-borne pathogens in urban parks in Italy. To fill this gap, ticks were collected in a public park in Rome over a 1-year period and screened by molecular methods for tick-borne pathogens.

Results and conclusion. The most abundant tick species were *Rhipicephalus turanicus* and *Ixodes ricinus*. The predominant pathogens detected were *Borrelia. burgdorferi* sensu lato (36%), *Rickettsia* spp. (36%), and *Coxiella burnetii* (22%). Among less frequently detected pathogens, *Babesia microti* was detected for the first time in Italy, with a prevalence of 4%. Neither *Bartonella* spp. nor *Francisella tularensis* were detected. With regard to co-infections, the most frequent double and triple infections involved *Rickettsia* spp., *B. burgdorferi* sl., and *C. burnetii*.. A positive correlation was detected between pathogens and *I. ricinus*. Further studies are needed in order to assess risk associated with tick-borne pathogens in urban areas.

Key words

ticks, urban park, *Rickettsia* SFG group, *Ehrlichia* spp., *Coxiella burnetii, Borrelia burgdorferi* sensu lato, *Babesia microti,* co-infection

INTRODUCTION

Ticks feed on a wide range of vertebrates, including mammals, birds and reptiles with a very low host specificity. These blood-sucking arthropods are vectors of pathogens for both humans and animals. In particular, they are considered important competent reservoirs for infectious agents, playing an essential role in the eco-epidemiology of diseases, such as Lyme borreliosis, rickettsiosis, babesiosis, ehrlichiosis, Q fever or tularaemia [1]. Besides *Borrelia burgdorferi* sensu lato and the tick-borne encephalitis virus, other pathogens, e.g. *Babesia spp*., Spotted Fever Group (SFG) *Rickettsia* spp., *Anaplasma phagocytophilum*, and *Bartonella spp.,* are of increasing public health interest. Monitoring tick distribution and prevalence of tick-transmitted pathogens is therefore important for describing and understanding the risk of tick-borne disease involving the predominant tick species.

Urban areas and public parks seem to differ from wooded areas regarding the occurrence of tick-borne pathogens [2]. In spite of this, there are very few epidemiological studies on simultaneous detection of various tick-borne pathogens in European urban areas [3, 4]. In Italy, several investigations have been conducted in wild habitats [5, 6, 7, 8, 9], but only one survey is documented in public parks located in urban and periurban areas of northern Italy [10]. In the presented study, the bacterial agents found were *Bartonella* spp., *B. burgdorferi s.l,*. and *Rickettsia* spp. In particular, a high prevalence of *B. henselae,* the agent of human cat scratch disease, was observed, suggesting an interface between urban and wild milieu and the presence of *R. monacensis* and. *R. helvetica*, species rarely found in Italy, and never recognized in public parks [11].

In order to provide a contribution in this poorly investigated field, the presence was analyzed of the microbial pathogens in ticks collected in an urban park of Rome during an entomological survey conducted in 2011 [12].

OBJECTIVE

The aim of the study was:

1) to investigate the occurrence of *Rickettsia* spp., *B. burgdorferi s.l.*, *Bartonella* spp., a *Ehrlichia* spp., *Coxiella burnetii, Francisella tularensis,* and *Babesia microti* in ticks collected in a public park of Rome, highly frequented daily by visitors and used for recreational activities;
2) to evaluate possible co-infections in the same ticks.

MATERIALS AND METHODS

Study area and tick collections. Localized in the north-western part of Rome, the Insugherata Natural Reserve is characterized by an extraordinary biodiversity, and its various environments are connected to the green zones outside the urban area. The park represents an important area for human recreational activities, and crops and pasture for flocks of sheep are also present.

Ticks were collected from January – December 2011, by dragging a 1 m^2 woollen blanket through the vegetation, at 3 different sites in the park characterized by wheat fields and pasture for flocks of sheep, deciduous mixed wood, and areas with sporadic trees and small lawns. Ticks were identified according to morphological characters [13], and stored at –80 °C.

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DNA extraction. Ticks were individually dissected and homogenized under sterile conditions. Genomic DNA was extracted using Dneasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturing protocol. DNA samples were stored at –20°C and later used as templates for the PCR amplification.

Molecular pathogen detection. Specific oligonucleotides (primers and probes) used in this study are listed in Table 1. Detection of *Rickettsia* spp. was performed with primers RpCS.877p–RpCS.1258n of the *glt*A gene [14], while for the discrimination between the spotted typhus group (SFG) and typhus group (TG) two different sets of primers of the *gro*EL gene were used as previously described [15]. A classical PCR amplification was also performed for *Ehrlichia* spp. and *Babesia. microti* using primers and PCR cycling conditions, as previously described [16, 17]. PCR products were resolved by electrophoresis on a 1.5% agarose gel, then stained with ethidium bromide.

The presence of *Borrelia burgdorferi* sensu lato group, *Bartonella* spp., *Coxiella burnetii*, *Francisella tularensis tularensis* (type A) and *Francisella tularensis holoarctica* (type B) in tick DNA extracts was tested by real time PCR, using specific primers and probes for each pathogen (Tab. 1). All real time PCRs were performed in 20 µl (final volume) into glass capillary tubes (Roche Diagnostics GmbH, Mannheim, Germany) and carried out in a LightCycler instrument (Roche Diagnostics), with protocols and PCR parameters as previously described [18, 19, 20, 21].

The following pathogen genomic DNAs were used as positive controls in specific PCR analyses: *R. conorii*, *R. typhi*, *Anaplasma phagocytophilum, B. burgdorferi* B31, *B. microti, B. henselae* Houston-1, *C. burnetii*, and *F. tularensis* subsp. *tularensis*.

Statistical analysis. Data were checked for outliers, duplicate records, distribution of the variables, and missing values. The association between pathogens and tick species was investigated by logistic regression. OR, prevalence of pathogens and 95%CI were reported. Co-infection was tested by the χ^2 test. Statistical analyses were performed using Stata 11.

Table 1. Primers and probes used for detection of pathogens in ticks

Organism	Gene target	Primer/Probe sequence (5′ → 3′)	Reference
Rickettsia spp	*glt*A	GGGGACCTGCTCACGGCGG ATTGCAAAAAGTACAGTGAACA	[14]
Rickettsia (TG and SFG)	*gro*EL	GATAGAAGAAAAGCAATGATG CAGCTATTTGAGATTTAATTTG	[15]
Rickettsia (TG)	*gro*EL	GGTGAAGCACTTGCGACG AGGAGCTTTTACTGCTGC	[15]
Ehrlichia spp	16S *rRNA*	GGTACCYACAGAAGAAGT TAGCACTCATCGTTTACA	[16]
Babesia microti	*SS-rDNA*	GTCTTAGTATAAGCTTTTATACAGCG ATAGGTCAGAAACTTGAATGATACATCG	[17]
Borrelia burgdorferi sensu lato	*rec*A	GTGGATCTATTGTATTAGATGAGGCTCTCG GCCAAAGTTCTGCAACAT TAACACCTAAAG	[18]
Bartonella spp	*gltA*	GGGGACCAGCTCATGGTGG AATGCAAAAAGAACAGTAAACA GCAAAAGATAAAAATGATTCTTTCCG- Fluorescin LC640-CTTATGGGTTTTGGTCATCGAGT-Phosphate	[19]
Coxiella burnetii	*icd*	CGTTATTTTACGGGTGTGCCA CAGAATTTTCGCGGAAAATCA Fam-CATATTCACCTTTTCAGGCGTTTTGACCGT-Tamra-T	[20]
Francisella tularensis tularensis (type A)	*pdp*D	GAGACATCAATTAAAAGAAGCAATACCTT CCAAGAGTACTATTTCCGGTTGGT Fam-AAAATTCTGCTCAGCAGGATTTTGATTTGG TT- Tamra	[21]
Francisella tularensis holoarctica (type B)	*ISFtu2*	CTTGTACTTTTATTTGGCTACTGAGAAACT CTTGCTTGGTTTGTAAATATAGTGGAA Fam-ACCTAGTTCAACCTCAAGACTTTTAGTAATGG GAA TGTCA-Tamra	[21]

RESULTS

Ticks collection. A total of 325 ticks were collected. About 70% of them were collected in pastures and cultivated areas. In order to obtain a representative sample of each collection, 129 ticks were processed for infectious agents analyses. *Rhipicephalus turanicus* was the most abundant species (66%), with 29 males and 56 females, followed by *Ixodes ricinus* (26%) – 11 males and 22 females, *Dermacentor marginatus* (5%) – 1 male and 6 females, and *Haemaphysalis punctata* (3%) – 1 male and 3 females.

R. turanicus showed a mono-modal seasonal pattern from spring to early summer, while *I. ricinus* and *D. marginatus* exhibited a similar seasonal dynamic, active from October – May and from October – April, respectively. *H. punctata* was rare, with a seasonal activity in autumn-winter [12].

Molecular pathogen detection. Of the 129 ticks screened by molecular methods, 84 (65%) samples were positive namely, 85% *I. ricinus* (28/33), 60% *R. turanicus* (51/85), 43% *D. marginatus* (3/7), and 50% *H. punctata* (2/4) were positive for the presence of tick-borne pathogen DNA (Tab. 2).

Table 2. Prevalence of tick-borne pathogens detected in ticks

		Pathogens n°(%)						
Tick species (n°)	Positive ticks n°(%)	*Rickettsia* SFG	*Ehrlichia* spp	*C. burnetii*	*B. burgdorferi s.l*	*B. microti*	*Francisella* spp.	*Bartonella* spp.
R. turanicus (85)	51 (60)	22 (26)	0 (0)	23 (27)	34 (40)	1 (1)	0 (0)	0 (0)
I. ricinus (33)	28 (85)	23 (70)	2 (6)	3 (9)	9 (27)	4 (12)	0 (0)	0 (0)
D. marginatus (7)	3 (43)	1 (14)	1 (14)	1 (14)	3 (43)	0 (0)	0 (0)	0 (0)
H. punctata (4)	2 (50)	0 (0)	1 (25)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
Total	**84 (65)**	**46**	**4**	**28**	**46**	**5**	**0**	**0**
Prevalence (95%CI)		**0.36 (0.27, 0.45)**	**0.03 (0.01, 0.08)**	**0.22 (0.15, 0.30)**	**0.36 (0.27, 0.45)**	**0.04 (0.01, 0.09)**		

Rickettsia spp., *B. burgdorferi* s.l.., *C. burnetii, Ehrlichia* spp. and *B. microti.* were detected in 46, 46, 28, 4 and 5 ticks, respectively (Tab. 2), while genomic DNA of *Bartonella* and *Francisella* was never detected. The same prevalence of 36% was found for *Rickettsia* spp. and *B. burgdorferi* s.l.. while a prevalence of 22% was obtained for *C. burnetii*. The prevalence of *Ehrlichia* spp. and *B. microti* was low, – 3% and 4%, respectively. All 46 rickettsiae detected belonged to the spotted fever group (SFG).

Rickettsia SFG was found in *I. ricinus* (n=23; 70%), *R. turanicus* (n=22; 26%), and *D. marginatus* (n=1; 14%); *B. burgdorferi* s.l.was detected in *R. turanicus* (n=34; 40%), *I. ricinus* (n=9; 27%), and *D. marginatus* (n=3; 43%); *Coxiella burnetii,* was recognized in *R. turanicus* (n=23; 27%), *I. ricinus* (n=3; 9%), in *H. punctata* (n=1; 25%) and *D. marginatus* (n=1; 14%); *Ehrlichia* spp. was observed in *I. ricinus* (n=2; 6%), in *H. punctata* (n=1; 25%) and *D. marginatus* (n=1; 14%). *B. microti* was found in 4 specimens of *I. ricinus* (12%) and in 1 of *R. turanicus* (1%) (Tab. 2; Fig. 1).

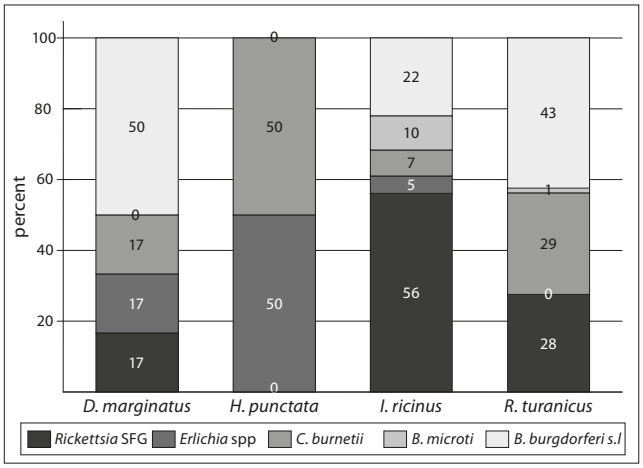

Figure 1. Pathogens distribution in tick species

I. ricinus was about 4 times more likely to be infected by any pathogen (OR= 3.73; 95% CI [1.31–10.66]), and about 7 times more likely to be infected by *Rickettsia* SFG (OR= 6.59; 95% CI [2.71–15.99]) than *R.. turanicus.* Finally, *D. marginatus,* and *H. punctata* had ORs similar to that of *R. turanicus.*

Co-infection analysis. Among the 84 positive ticks, 58% (49/84) were infected with 1 agent, 30% (25/84) were co-infected with 2 pathogens, and 12% (10/84) carried 3 pathogens (Tab. 3).

Recurring infection was observed with *Rickettsia* SFG or *B. burgdorferi* sl. The most frequent double infection and 90% (9/10) of triple infection involved *Rickettsia* SFG, *B. burgdorferi* sl. and *C. burnetii.* However, few cases of co-infections between *Rickettsia* SFG-*B. microti*, *B. burgdorferi sl-B. microti*, *Ehrlichia* spp.-*B. burgdorferi* s.l and *Rickettsia* SFG- *Ehrlichia* spp.-*B. burgdorferi* s.l were detected. Moreover, a positive statistically significant correlation was recorded only between *C. burnetii* and *B. burgdorferi* sl. (p=0.007) (Tab. 3).

There was no correlation between single or multiple pathogen infections in *R. turanicus,* or *D. marginatus* or *H. punctata.,* although the percentage of infection and co-infection varied substantially among tick species. A high proportion (> 50%) of double or triple co-infections were found in *I. ricinus* and *R. turanicus* (Fig. 2).

Table 3. Bacterial pathogen infections and co-infections in ticks

	Sample		Pathogen	
	N	%	N	%
Rickettsia SFG	21	(16)	46	(36)
Ehrlichia spp.	1	(1)	4	(3)
C. burnetii	7	(5)	28	(22)
B. burgdorferi s.l	18	(14)	46	(36)
B. microti	2	(2)	5	(4)
Rickettsia SFG+ *Ehrlichia* spp.	0	(0)		
Rickettsia SFG+*C. burnetii*	5	(4)		
Rickettsia SFG+*B. microti*	2	(2)		
Rickettsia SFG+*B. burgdorferi s.l*	8	(6)		
Ehrlichia spp.+*C. burnetii*	0	(0)		
Ehrlichia spp.+*B. microti*	0	(0)		
Ehrlichia spp.+*B. burgdorferi s.l*	2	(2)		
C. burnetii+*B. microti*	0	(0)		
C. burnetii+*B. burgdorferi s.l*	7	(5)		
B. microti+*B. burgdorferi s.l*	1	(1)		
Rickettsia SFG+ *Ehrlichia* spp.+*C. burnetii*	0	(0)		
Rickettsia SFG+ *Ehrlichia* spp.+*B. microti*	0	(0)		
Rickettsia SFG+ *Ehrlichia* spp.+*B. burgdorferi s.l*	1	(1)		
Rickettsia SFG+*C. burnetii*+*B. microti*	0	(0)		
Rickettsia SFG+*C. burnetii*+*B. burgdorferi s.l*	9	(7)		
Rickettsia SFG+*B. microti B. burgdorferi s.l*	0	(0)		
negative	45	(35)		

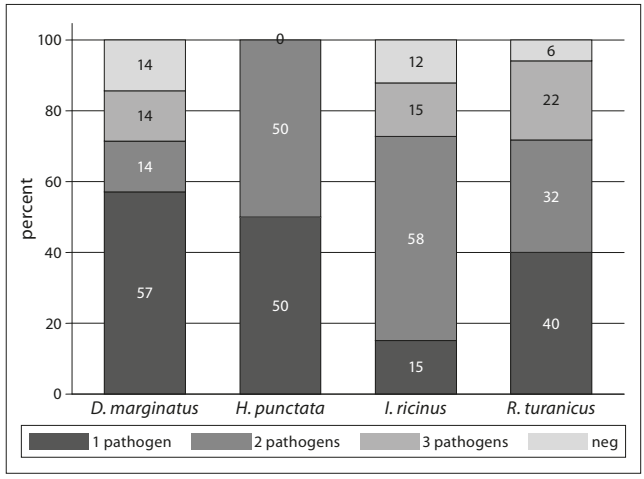

Figure 2. Infections and co-infections in tick species

DISCUSSION

Worldwide, ticks are important vectors of human and animal pathogens, and a variety of tick-borne infections are of medical interest. Several European studies conducted in ticks revealed that the prevalence of *Borrelia* species ranges from 6.2% – 51% [22], the frequency of *Rickettsia* SFG ranges from about 3% – 15% [23], while the prevalence rate for *F. tularensis* and C. *burnetii* does not seem to exceed 1.6% and 2.6%, respectively [24]. *Bartonella* species in ticks can vary as much as from 3.7% – 40% [22], whereas *Ehrlichia* spp. was detected in from 5 – 25% of tick exposed people [25].

In addition to these bacterial agents, human babebiosis can also be transmitted by tick bite. In previous investigations in several European countries, the species *B. divergens*, *B. microti* and *Babesia* sp. EU1 have been detected and the prevalence in ticks ranged from 0.6 – 51.04% [26].

Tick-borne pathogens can occur not only in natural woodlands, but also in recreational urban areas; however, epidemiological investigations in urban sites are uncommon, but have reported a large variety of pathogens and different infection rates [3, 4]. In spite of this consideration, only one investigation is documented in public parks of Italy; in that study, *Bartonella* spp., *B. burgdorferi* s.l. and *Rickettsia* spp. were found [10].

In view of this fact, a 1-year survey was conducted to investigate the presence of tick-borne pathogens in the Insugherata Natural Reserve of Rome. The main tick species found were *R. turanicus* and *I. ricinus* [12], well-known vectors of different animal and human pathogens recognized in Italy, such as *B. burgdorferi* s.l., *Rickettsia* SFG, *Babesia* spp., *Anaplasma* spp., *C. burnetii*, and the TBE virus [8, 9, 11, 27].

The results of the presented study in questing ticks demonstrated the expected occurrence of *B. burgdorferi* s.l., *Rickettsia* SFG and *Ehrlichia* spp., an unusual frequency of *C. burnetii,* a noteworthy finding of *B. microti*, and a predictable absence of *F. tularensis* and *Bartonella* spp.

Ehrlichia species are widely distributed in Italian regions, whereas *B. burgdorferi* s.l. and *Rickettsia* SFG are prominent in north-central Italy and central-south Italy, respectively [5, 6, 7, 8, 9. 28].

C. burnetii, responsible for Q fever, was the third most prevalent pathogen found in all tick species, but predominantly recovered in *R. turanicus*. Humans become infected mainly by inhalation of contaminated aerosol, but the role of ticks in bacterial transmission in wild and peridomestic cycles is clearly recognized [29]. In addition, *C. burnetii* infects several tick species with transovarial and transtadial transmission [29]. Its high incidence in this study suggests that ticks may act as reservoir in an urban area, and their potential role in the maintenance of the bacterium may be important.

The vector-borne transmission of *F. tularensis* has never been detected in Italy, and the lack of specific DNA in questing ticks is not a surprise. Contrary to the results of another investigation conducted in the public parks in Italy [10], no *Bartonella* spp. DNA was identified. *Bartonella* spp. infection in ticks has been reported from all over the world, including Europe [30]. However, despite several reports of indirect data, there is little evidence that *Bartonella* spp. can replicate in ticks, and the role of the bacterium transmission by arthropods to vertebrate host has not been demonstrated [30].

It has been already documented that *I. ricinus* may harbour Babesia EU1, *B. divergens* and *B. Microti,* and all species are responsible for human babebiosis infection in Europe. Contrary to the well-documented data on the occurrence of several *Babesia* species in wild and periurban areas, the prevalence of *B. microti* in such habitats is still low [26]. In Italy, Babesia EU1 and *B. divergens* were also detected in ticks, while *B. microti* has been never found [31]. The presented study is the first to report the occurrence of *B. microti* in *I. ricinus* and *R. turanicus* in Italy, in particular in a peri-urban area. Unlike Babesia EU1 and *B. divergens*, *B. microti* is not vertically transmitted in ticks by transovarial or transtadial routes, but the parasite is acquired from a previously infected host. Moreover, it has been reported that *B. microti* found in foxes and cats is a possible link among wild, rural, and resident environments [32]. The detection of *B. microti* in actively questing ticks in recreational areas has considerable public health implications since human babesiosis occurs almost exclusively in splenectomised or immunocompromised people [33]. In addition, the unselective feeding pattern of *I. ricinus* and *R. turanicus* tick species, combined with global warming, could contribute to the maintenance of this agent between wild and domestic animals, and may enhance the risk of infection for people living in an urban context.

Moreover, the nonspecific feeding habits with a wide involvement of a wide variety of vertebrates, potential reservoirs for several tick-borne pathogens, focuses the interest in health risk due to multiple infections. In a context of public health and the clinical implications, tick-borne polymicrobial infections may be crucial for planning prophylactic measures and for increasing the probability of misdiagnosis.

In this study, dual and triple infections were recognized in 30% and 12% of cases, respectively. The most common co-infections involved *Rickettsia* SFG, *B. burgdorferi sl*., and *C. burnetii*. The high levels of co-infection rates found in the current study may be influenced by different environmental variables, such as grassy areas, anthropogenic behaviour, circulation of pathogens between the vector and its hosts, and the abundance of tick species and host availability.

These findings, representing the first evidence of co-infection with multiple pathogens in ticks from an Italian urban park, contribute to a better understanding of the potential risk of multiple infections from a single tick bite.

However, caution is needed on the interpretation of the epidemiological significance of these results, because the presence of a pathogen in ticks does not necessarily mean transmission to susceptible hosts. In spite of this, the presented study may be important and helpful for further epidemiological studies of tick-borne pathogens in urban areas in Italy, and for the prevention of tick-borne pathogens transmission to humans and animals. Moreover, the list of pathogens studied is not complete, and other tick-borne agents including viruses, bacteria and parasite should be studied in the future.

CONCLUSION

In conclusion, several bacterial species in ticks collected in urban areas. *B. microti* were identified for the first time in Italy. Moreover, several co-infections were detected. These findings underline the potential risk of transmission of tick-borne human pathogens in urban areas and necessitate appropriate interventions.

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