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Detection of tick-borne pathogens in ticks collected in the suburban area of Monte Romano, Lazio Region, Central Italy

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Abstract

Background. A study on tick species characterization and tick borne pathogens detection was performed by a survey conducted during 2012 and 2013 in the Viterbo province (Lazio Region, Central Italy). Seven sites were selected for the study investigation, including two farms and a military zone.

Methods. A total of 255 ticks, *Rhipicephalus (Boophilus) annulatus* (n = 215), *Rhipicephalus bursa* (n = 28), and *Hyalomma marginatum* (n = 12) were screened individually by molecular methods for the tick borne bacterial agents: *Borrelia burgdorferi* sensu lato group, *Bartonella* spp., *Coxiella burnetii, Ehrlichia* spp., *Francisella* spp., and *Rickettsia* spp.

Results and conclusion. Overall, 182 ticks (71%) were infected with at least one pathogen; among these co-infections were found in 94 ticks. Tick borne pathogens identified were *C. burnetii, B. burgdorferi* s.l.., *Bartonella* spp., *Rickettsia* spp., *Francisella* spp., and *Ehrlichia* spp. In *R. bursa* and *H. marginatum*, the presence of *B. burgdorferi* s.l. was positively correlated with that of *C. burnetii*, *Rickettsia* spp., and *Bartonella* spp. and their coinfection probabilities were 29.8%, 22.7% and 11.7%, respectively; the probability of coinfection for *Francisella* spp. and *Rickettsia spp.* and for *Francisella* spp. and *Bartonella* spp. was 14.9% and 17.9%, respectively. In *R. (Boophilus) annulatus*, the probability of coinfection between *C. burnetii* and *B. burgdorferi* s.l. was 11.3%, while those between *C. burnetii* and *Bartonella* spp. and between *B. burgdorferi* s.l. and *Bartonella* spp. were 0.8%. Further studies are needed in order to assess the risk associated with these unusual tick-borne pathogens in Central Italy.

Key words

- ticks
- *Borrelia burgdorferi* sensu lato
- *Bartonella* spp.
- *Ehrlichia* spp.
- *Coxiella burnetii*
- *Francisella* spp.
- *Rickettsia* spp.
- co-infection
- Italy

INTRODUCTION

Ticks can transmit a great variety of pathogenic agents to animals and humans. Different factors such as global warming, dynamics of ticks, human population density, animal fauna composition in urban and peri-urban environments, or socio-demographic elements (urban, suburban, and rural) may influence and modulate the interactions of the vectors with hosts and pathogens. All mentioned aspects expose susceptible hosts to infections with tick-borne pathogens' [1, 2].

Among pathogens of veterinary and medical importance transmitted by hard ticks, we can include *Borrelia burgdorferi* s.l. complex (Lyme disease), *Rickettsia* spp. (rickettsiosis, including Mediterranean spotted fever), and *Ehrlichia* spp. However, *Bartonella* spp. (cat starch disease), *Francisella* spp. (tularemia) and *Coxiella burnetii* (Q fever) have also been detected in these arthropods but so far, they are only suspected for the disease transmission [3]. Urban areas with recreational zones and peri-urban habitats with their natural sites can produce a particular gradient of adaptation involving wildlife, ticks and related pathogens defining a complex ecological system [4, 5].

This ecological modification became of particular im-

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portance because humans and pets can encounter potentially infected ticks from outskirt environment [2]. In Italy, the prevalence of human tick-borne diseases is realistically underestimated because the surveillance system is fragmented and not well supported. As far as ticks and tick-borne pathogens are concerned, very limited studies have been performed in Central Italy, and only seroepidemiological surveys have been described in healthy and professional people for infection with *B. burgdorferi* and the tick-borne encephalitis [6-9].

To better understand the circulation of tick bacterial zoonosis in Lazio Region (Central Italy), and after several reports about the high density of ticks and tick-bites from soldiers operating in a military shooting area within the municipality of Monte Romano (province of Viterbo, Lazio Region, Central Italy), we planned to investigate the presence of tick borne bacterial agents in tick collections. In relation to potential risk factors for tick-borne infections, arthropods were collected in seven representative sites of the suburban environment of Monte Romano municipality, including two farms and the military area [10].

MATERIALS AND METHODS

Study site and tick collection

This study was carried out in the suburban area of Monte Romano (42°16'05"N 11°53'55"E) in the Viterbo Province (Lazio Region), with typical Mediterranean climate, flora and fauna well characterized [11, 12]. Seven sites of this area were chosen for the tick-borne pathogens investigation, including two farms and a military zone *(Figure 1)*. The study was conducted from June to September 2012 and from March to October 2013, and ticks were obtained by dragging or directly picked from cattle. Ticks were identified morphologically at species level [10].

Pathogens detection by molecular analyses

From a total of 518 ticks collected in our previous study [10], 255 samples were available for molecular analyses. Genomic DNA was extracted from each homogenized tick, using Dneasy blood and tissue kit (Qiagen, Hilden, Germany) according to manufacturing protocol.

Molecular detection of *Rickettsia* spp. and *Ehrlichia* spp. was performed by classical PCR amplification, as previously described [13, 14]. PCR products were resolved by electrophoresis on a 1.5% agarose gel, and stained with ethidium bromide.

The real time PCR was employed to identify *Borrelia burgdorferi* sensu lato group, *Bartonella* spp., *Coxiella burnetii* and *Francisella* spp. All real time PCRs were performed into glass capillary tubes (Roche Diagnostics GmbH, Mannheim, Germany) and carried out in a LightCycler instrument (Roche Diagnostics), with primers/probes and protocols as previously described [15-18].

Statistical analysis

Molecular results for all pathogens screened were used as the binary response variables (pathogen detected/not detected by the PCR) for the statistical analyses. The presence of each pathogen was evaluated based on several characteristics of the collected ticks land cover type, season in which the ticks were collected, collection site and state of maturity of the tick (nymph or adult). The association was evaluated through a multivariable regression analysis. In particular, first logis-

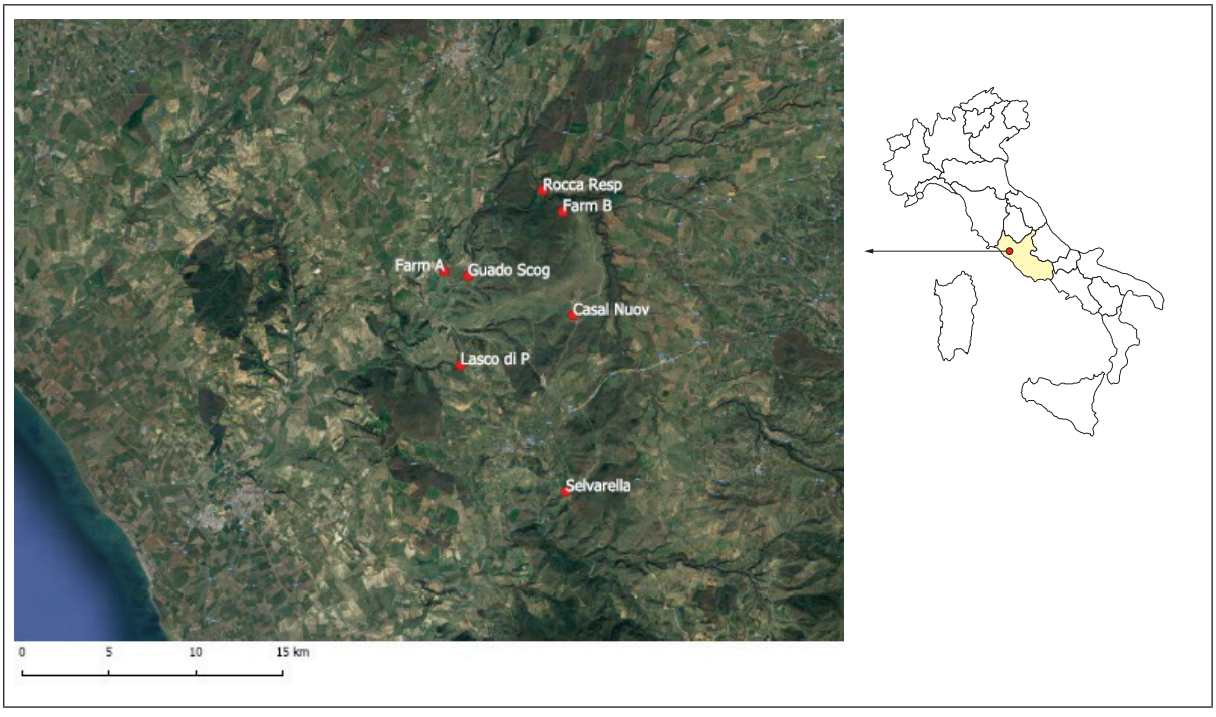

Figure 1
Tick collection sites, suburban area of Monte Romano, Lazio Region, Central Italy

tic regressions were carried out and evaluated within a frequentist framework to get some insight on what could be appropriate value for the parameters and to test significance of the covariate parameters. Then a Bayesian model was determined based on the parameter estimate obtained from the frequentist models. The multi-response approach was used to model response variables simultaneously. A multi-response hierarchical logistic regression model with conditional autoregressive (CAR) spatial random effects was carried out [19]. For each pathogen, PCR results for the other pathogens were included in the model as covariates. Neighborhoods were defined based on the distance between the area centroids. Errors at the individual level were modeled as multivariate normal random variables to estimate correlations among pathogens. The other terms in the equation were estimated as univariate normal random variables. We fitted a model for *R. (Boophilus) annulatus* and a model including *R. bursa* and *H. marginatum*. These two species were analyzed together due to the small numbers and because of previous analysis, in which the two tick species were analyzed separately yielded similar estimates for *R. bursa* and *H. marginatum*. Model parameters were estimated by drawing 10 000 samples from their joint posterior distributions using the Markov Chain Monte Carlo (MCMC) algorithm implemented in WinBugs [20, 21].

RESULTS

Tick species and tick-borne pathogens detection

The species composition of the 255 ticks were morphologically identified as follows: *Rhipicephalus (Boophilus) annulatus* (n = 215; 84%), *Rhipicephalus bursa* (n = 28; 11%), and *Hyalomma marginatum* (n = 12; 5%). All *R. bursa* and *H. marginatum* were collected by dragging while *R. (Boophilus) annulatus* were picked from animals.

From the totality of ticks examined by PCR methods, 182 (71%) samples were positive for tick-borne pathogen DNAs. As shown in *Table 1*, pathogens were found in all *H. marginatum* (12/12), in 69% of *R. (Boophilus) annulatus* (148/215), and in 79% of *R. bursa* (22/28).

In particular, *C. burnetii, B. burgdorferi* s.l.., *Bartonella* spp., *Rickettsia* spp., *Francisella* spp., and *Ehrlichia* spp. were detected in 83, 79, 48, 47, 32 and 27 ticks, respectively.

The prevalence of infection in *R. (Boophilus) annulatus*, *R. bursa* and *H. marginatum* species were reported in *Table 1*.

Co-infection analysis

Concerning the 182 positive ticks, 48% (88/182) showed one infectious agent, whereas 32% (59/182), 17% (30/182) and 3% (5/182) were co-infected with two, three and four pathogens, respectively *(Table 2)*.

The most frequent infection due to only one agent was observed with *C. burnetii* (16%), *Rickettsia* spp. (11%), and *B. burgdorferi* sl. (8%). The recurrent double and triple infection involved *C. burnetii / B. burgdorferi* sl. (8%) and *Bartonella spp. / C. burnetii / B. burgdorferi* sl. (5%), respectively. Only few cases of co-infections with four pathogens were detected *(Table 2)*.

A high proportion of multiple infections was found in *R. bursa* and *H. marginatum (Figure 2)*, with the exception of the coinfection between *C. burnetii* and *Bartonella spp.* which was more frequent in *R. (Boophilus) annulatus (see Table 3)*.

As shown in *Table 3*, in *R. bursa* and *H. marginatum*, *B. burgdorferi* s.l. was positively correlated with *C. burnetii* (ρ: 0.502), *Rickettsia* spp. (ρ: 0.323), and *Bartonella* spp. (ρ: 0.240).

Their joint probabilities ranged between 29.8% for *C. burnetii and* 11.7% for *Bartonella* spp and were significantly higher than the product of the corresponding marginal probabilities.

Similarly, *Francisella* spp. was positively correlated with *Rickettsia* spp. (ρ: 0.467) and with *Bartonella* spp. (ρ: 0.307) with joint probabilities respectively of 17.9% and 14.9%. Also in this case, the joint probabilities were significantly higher than the product of the corresponding marginal probabilities.

Table 1
Prevalence of pathogens detected in ticks

		Pathogens n. (%)					
Tick species (n.)	**Positive ticks n. (%)**	***Rickettsia* spp**	***Ehrlichia* spp**	***C. burnetii***	***Francisella* spp.**	***Bartonella* spp.**	***B. burgdorferi* s.l.**
H. marginatum (12)	12 (100)	8(66)	2 (16)	4 (33)	8 (66)	3 (25)	6 (50)
R. (Boophilus) annulatus (215)	148 (69)	36(16)	17 (7)	70 (32)	18 (8)	40 (18)	58 (26)
R. bursa (28)	22 (79)	3(10)	8 (28)	9 (32)	6 (21)	5 (17)	15 (53)
Total (255)	182	47	27	83	32	48	79
Prevalence *R. (Boophilus) annulatus*		0.178	0.089	0.329	0.089	0.186	0.265
(95% CI)		(0.131; 0.231)	(0.057; 0.132)	(0.269; 0.391)	(0.060; 0.126)	(0.143; 0.236)	(0.213; 0.324)
Prevalence *R. bursa* and *H. marginatum*		0.270	0.274	0.316	0.367	0.174	0.524
(95% CI)		(0.002; 0.411)	(0.002; 0.415)	(0.001; 0.454)	(0.001; 0.505)	(0.001; 0.287)	(0.001; 0.659)

Table 2
Bacterial pathogen infections and co-infections in ticks

	Sample		Pathogen	
	n.	(%)	n.	(%)
Bartonella spp.	9	(5)	47	(15)
C. burnetii	29	(16)	27	(9)
Ehrlichia spp.	8	(4)	83	(26)
Rickettsia spp.	21	(11)	32	(10)
Francisella spp.	6	(3)	48	(15)
B. burgdorferi s.l.	15	(8)	79	(25)
Bartonella spp.+*C. burnetii*	6	(3)		
Bartonella spp.+ *Ehrlichia* spp.	2	(1)		
Bartonella spp + *Rickettsia* spp.	0	(0)		
Bartonella spp. +*B. burgdorferi s.l.*	7	(4)		
Bartonella spp.+*Francisella* spp.	3	(2)		
C. burnetii + *Ehrlichia* spp.	3	(2)		
C. burnetii + *Rickettsia* spp.	7	(4)		
C. burnetii+ *B. burgdorferi s.l.*	14	(8)		
C. burnetii + *Francisella* spp.	1	(0)		
Ehrlichia spp.+*Rickettsia* spp.	2	(1)		
Ehrlichia spp.+ *B. burgdorferi s.l.*	4	(2)		
Ehrlichia spp.+ *Francisella* spp.	0	(0)		
Rickettsia spp.+ *B. burgdorferi s.l*	4	(2)		
Rickettsia spp.+ *Francisella* spp.	2	(1)		
B. burgdorferi s.l. + *Francisella* spp.	4	(2)		
Bartonella spp. + *C. burnetii* + *Ehrlichia* spp.	0	(0)		
Bartonella spp .+ *C. burnetii* + *Rickettsia* spp.	0	(0)		
Bartonella spp. + *C. burnetii* + *B. burgdorferi s.l.*	9	(5)		
Bartonella spp. + *C. burnetii* + *Francisella* spp.	2	(1)		
Bartonella spp.+ *Ehrlichia* spp. + *Rickettsia* spp.	0	(0)		
Bartonella spp.+ *Ehrlichia* spp. + *B. burgdorferi s.l.*	2	(1)		
Bartonella spp.+ *Ehrlichia* spp. + *Francisella* spp.	0	(0)		
Bartonella spp.+ *Rickettsia* spp. + *B. burgdorferi s.l.*	0	(0)		
Bartonella spp.+ *Rickettsia* spp. + *Francisella* spp.	1	(0)		
Bartonella spp. + *B. burgdorferi s.l.* + *Francisella* spp.	4	(2)		
C. burnetii + *Ehrlichia* spp. + *Rickettsia* spp.	0	(0)		
C. burnetii + *Ehrlichia* spp. + *B. burgdorferi s.l.*	3	(2)		
C. burnetii + *Ehrlichia* spp. + *Francisella* spp.	0	(0)		
C. burnetii + *Rickettsia* spp. + *B. burgdorferi s.l.*	2	(1)		
C. burnetii + *Rickettsia* spp. + *Francisella* spp.	1	(0)		
C. burnetii + *B. burgdorferi s.l.* + *Francisella* spp.	2	(1)		
Ehrlichia spp. + *Rickettsia* spp. + *B. burgdorferi s.l.*	0	(0)		
Ehrlichia spp. + *Rickettsia* spp. + *Francisella* spp.	0	(0)		
Ehrlichia spp. + *B. burgdorferi s.l.* + *Francisella* spp.	1	(0)		
Rickettsia spp. + *B. burgdorferi s.l.* + *Francisella* spp.	3	(2)		
Bartonella spp. + *C. burnetii* + *Ehrlichia* spp. + *B. burgdorferi s.l.*	1	(0)		
Bartonella spp.+ *Rickettsia* spp. + *B. burgdorferi s.l.* + *Francisella* spp.	1	(0)		
Bartonella spp. + *C. burnetii* + *Rickettsia* spp.+ *B. burgdorferi s.l.*	1	(0)		
Rickettsia spp. + *C. burnetii* + *B. burgdorferi s.l.* +*Francisella* spp.	1	(0)		
Rickettsia spp. + *Ehrlichia* spp. + *C. burnetii* + *B. burgdorferi s.l.*	1	(0)		
Negative	73	(29)		

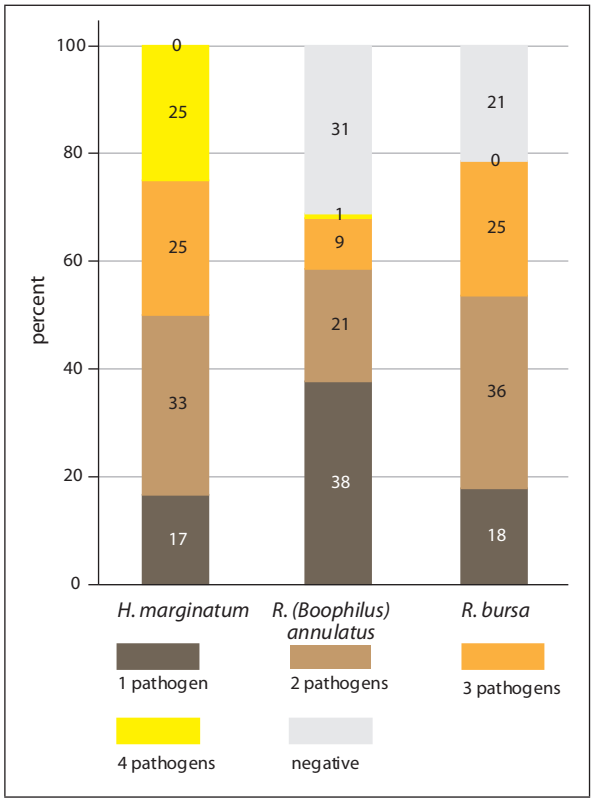

Figure 2
Infections and co-infections in tick species.

B. burgdorferi s.l. and *Francisella* spp. had a probability of coinfection of 17.6% but their correlation was of small magnitude (ρ: 0.086). In the same way, *Ehrlichia* spp. and *C. burnetii* had a prevalence of coinfection of 11.8% (p = 0.171) and a positive correlation of 0.118, whereas, *Ehrlichia* spp. and *Bartonella* spp. had a prevalence of coinfection of 0.8% (p = 0.091) and a small negative correlation of -0.043.

In *R. (Boophilus) annulatus*, *C. burnetii, B. burgdorferi* s.l. and *Bartonella* spp. were positively correlated. The probability of coinfection between *C. burnetii* and *B. burgdorferi* s.l. was 11.3% (p = 0.014) and those between *C. burnetii* and *Bartonella* and between *B. burgdorferi* s.l. and *Bartonella* were 0.8%. These were significantly higher than the product of their marginal probabilities (*C. burnetii* and *Bartonella*: p = 0.035; *B. burgdorferi* s.l. and *Bartonella*: p < 0.001). *B. burgdorferi* s.l. and *Francisella* spp. (ρ: 0.239), *Bartonella* spp. and *Francisella* spp. (ρ: 0.496) and *Ehrlichia* spp. and *B. burgdorferi* s.l. (ρ: 0.358) had a probability of coinfection of about 0.3%, and their joint probabilities were significantly higher than the product of their marginal probabilities with p values of 0.037, 0.007 and 0.085, respectively.

DISCUSSION AND CONCLUSION

Tick-borne diseases represent an increasing threat worldwide for human and animal health. Several aspects contributing to the global changes of our planet directly influence the spread of the vector borne diseases. Arthropods and microbes are revealing a remarkable adaptation to the globalization, migration, wildlife modifications, deforestation, new socio-demographic factors, climate changes and global warming [2]. Gardens, public parks and green areas between urban and peri-urban zones potentially expand tick populations and act as suitable places for the exposure of humans and animals, including pets, to tick bites, favoring the diffusion of zoonotic pathogens [4, 5, 12]. In these sites, there is a preponderance of generalist tick species capable to adapt to different host vertebrate species such as wildlife, rodents, birds or companion animals. The potential transmission of tick-borne agents to humans and the maintenance of the vector reservoir are related to the interaction between ticks and hosts [22-24].

This investigation, started after several tick-bite reports from soldiers of the military area, was focused on the presence of tick-borne bacterial agents in tick species collected in selected suburban environments, including the military shooting range. We screened ticks with the aim to improve and recognize the potential risk transmission of these pathogens. *R. (Boophilus) annulatus* was the most abundant collected species and being closely associated with the cattle on which it feeds during its life cycle [22], it has been almost exclusively picked up on these animals. *R. bursa* rarely bites humans and is generally found in environments like bushy glades and lawns. As expected, these ticks were collected from June to August, while *H. marginatum*, that could be very common in this Region, was found in early summer according to the wide range of the phenology of the species [22-24]. Besides all pathogens recognized, we found interesting the coinfection results acquired in around half of the positive ticks. In fact, the direct and the simultaneous blood transmission of more pathogens from a single tick may influence the disease progression in term of correct diagnosis and treatment.

In this study, *C. burnetii* / *B. burgdorferi* sl. / *Bartonella* spp. coinfection were positively correlated in *R. (Boophilus) annulatus*. All these microbial agents and diseases are unusual in Lazio Region and generally in Central Italy. Lyme disease is present in North Italy while Q fever is a notifiable disease but with marginal impact in the public health of our Country, and the risk is significantly associated with direct occupational exposure [25-27]. Bartonellae are emerging pathogens distributed worldwide and strictly related to mammalian hosts, vectors and favorable environment [28]. Roaming animals, pets and ticks may act as reservoir in the urban area, and their potential role in the maintenance of the bacterium may be important, notably due to the intracellular persistence of the pathogen [28]. Even if the tick species reported in this study are not considered in letterature as main vectors of the pathogens here investigated, they are known to be able to participate to their circulation. *H. marginatum* is a tick species known to participate to the circulation of Q fever in Italy, according to other studies, reporting the isolation of *C. burnetii* in this species [22, 29, 30]. In Sicily, *H. marginatum* resulted infected with *Rickettsia* spp. (n = 3/67; 4%). *R. bursa* is a vector and reservoir for *C. burnetii* in Bulgaria, Spain and in Crimea, where

Table 3
Probabilities of coinfection for *R. (Boophilus) annulatus* and *R. bursa* and *H. marginatum*

	R. (Boophilus) annulatus					
	***Rickettsia* Spp.**	***Ehrlichia* Spp.**	***C. burnetii***	***B. burgdorferi* s.l.**	***Bartonella* spp.**	***Francisella* spp.**
Rickettsia spp.		0.005 (0.001; 0.012) P = 0.996	0.033 (0.018; 0.055) P = 0.998	0.022 (0.010; 0.039) P = 0.999	0.004 (0.001; 0.011) P = 1.000	0.018 (0.007; 0.033) P = 0.390
Ehrlichia spp.	0.005 (0.001; 0.012) P = 0.996		0.009 (0.004; 0.019) P = 1.000	0.035 (0.017; 0.063) P = 0.085	0.009 (0.003; 0.019) P = 0.962	0.004 (0.001; 0.011) P = 0.933
C. burnetii	0.033 (0.018; 0.055) P = 0.998	0.009 (0.004; 0.019) P = 1.000		0.113 (0.079; 0.152) P = 0.014	0.078 (0.052; 0.110) P = 0.035	0.015 (0.007; 0.027) P = 0.997
B. burgdorferi s.l.	0.022 (0.010; 0.039) P = 0.999	0.035 (0.017; 0.063) P = 0.085	0.113 (0.079; 0.152) P = 0.014		0.082 (0.055; 0.114) P < 0.001	0.034 (0.019; 0.053) P = 0.037
Bartonella spp.	0.004 (0.001; 0.011) P = 1.000	0.009 (0.003; 0.019) P = 0.962	0.078 (0.052; 0.110) P = 0.035	0.082 (0.055; 0.114) P < 0.001		0.030 (0.015; 0.052) P = 0.007
Francisella spp.	0.018 (0.007; 0.033) P = 0.390	0.004 (0.001; 0.011) P = 0.933	0.015 (0.007; 0.027) P = 0.997	0.034 (0.019; 0.053) P = 0.037	0.030 (0.015; 0.052) P = 0.007	

	R. bursa* and *H. marginatum					
	***Rickettsia* Spp.**	***Ehrlichia* Spp.**	***C. burnetii***	***B. burgdorferi* s.l.**	***Bartonella* spp.**	***Francisella* spp.**
Rickettsia spp.		0.059 (0.016; 0.135) P = 0.741	0.077 (0.027; 0.160) P = 0.665	0.227 (0.110; 0.370) P = 0.035	0.027 (0.007; 0.067) P = 0.930	0.149 (0.068; 0.260) P = 0.074
Ehrlichia spp.	0.059 (0.016; 0.135) P = 0.741		0.118 (0.045; 0.223) P = 0.171	0.072 (0.023; 0.161) P = 0.972	0.080 (0.025; 0.159) P = 0.091	0.008 (0.001; 0.028) P = 1.000
C. burnetii	0.077 (0.027; 0.160) P = 0.665	0.118 (0.045; 0.223) P = 0.171		0.298 (0.166; 0.447) P = 0.005	0.014 (0.002; 0.040) P = 0.998	0.059 (0.019; 0.128) P = 0.977
B. burgdorferi s.l.	0.227 (0.110; 0.370) P = 0.035	0.072 (0.023; 0.161) P = 0.972	0.298 (0.166; 0.447) P = 0.005		0.117 (0.058; 0.205) P = 0.070	0.176 (0.090; 0.290) P = 0.661
Bartonella spp.	0.027 0.007; 0.067) P = 0.930	0.080 (0.025; 0.159) P = 0.091	0.014 (0.002; 0.040) P = 0.998	0.117 (0.058; 0.205) P = 0.070		0.179 (0.079; 0.305) P < 0.001
Francisella spp.	0.149 (0.068; 0.260) P = 0.074	0.008 (0.001; 0.028) P = 1.000	0.059 (0.019; 0.128) P = 0.977	0.176 (0.090; 0.290) P = 0.661	0.179 (0.079; 0.305) P < 0.001	

P = P (pathogen1) P (pathogen2) ≤ P (pathogen1, pathogen2); (95% CI) are reported in brackets.

is able to maintain the circulation of this infectious agent [22-24]. Recently, the presence of *C. burnetii* (n = 2/83; 20%) and *Bartonella sp*. (n = 1/22; 4%) has been recently detected in this species for the first time in Sardinia [30]. *R. annulatus*, reported as the main vector of haemoparasites, had not been reported before in the transmission of these infections. However, this arthropod may act as secondary vector in the maintenance of rickettsiae and coxiellae, considering that *R. annulatus* was found positive to *C. burnetii* (n = 2/83; 2%) in Sardinia, confirming a previous study carried out in Senegal (n = 1/5; 20%) [30, 31]. Another similar case was found in Israel where a *R. annulatus* tick picked-up from a Mesopotamian fallow deer resulted positive for *R. sibirica mongolitimonae* [32].

In conclusion, this study provides new information on the circulation of ticks and tick borne pathogens in Lazio Region (Central Italy). The aim of our study was the direct detection of pathogens in tick samples and potentially characterize the molecular prevalence of active infection(s), differing to serological studies that revealing past exposure or a not active infection.

The recognition of uncommon and potentially pathogenic agents in ticks from urban and suburban areas, may implement the surveillance screening of tick borne diseases. Further studies are required to determine the role of arthropod-vectors as carriers of these bacteria in the Mediterranean ecosystem.

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REFERENCES

1. Randolph SE. Tick-borne disease systems emerge from the shadows: the beauty lies in molecular detail, the message in epidemiology. Parasitology. 2009;136:1403-13. doi: 10.1017/S0031182009005782
2. Kilpatrick AM, Randolph SE. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet. 2012;380:1946-55. doi: 10.1016/S0140-6736(12)61151-9
3. Socolovschi C, Mediannikov O, Raoult D, Parola P. Update on tick-borne bacterial diseases in Europe. Parasite. 2009;16:259-73. doi: 10.1051/parasite/2009164259
4. Di Luca M, Toma L, Bianchi R, Quarchioni E, Marini L, Mancini F, Ciervo A, Khoury C. Seasonal dynamics of tick species in an urban park of Rome. Ticks Tick Borne Dis. 2013;4:513-7. doi: 10.1016/j.ttbdis.2013.06.008
5. Mancini F, Di Luca M, Toma L, Vescio F, Bianchi R, Khoury C, Marini L, Rezza G, Ciervo A. Prevalence of tick-borne pathogens in an urban park in Rome, Italy. Ann Agric Environ Med. 2014;21:725-9. doi: 10.5604/12321966.1129922
6. Verani P, Balducci M, Lopes MC, Alemanno A, Saccà G. Survey for antibodies against arthropod-borne viruses in man and animals in Italy. Serologic status of human beings in a central Italian region (Fondi province). Am J Trop Med Hyg. 1967;16:203-10.
7. Santino I, Cammarata E, Franco S, Galdiero F, Oliva B, Sessa R, Cipriani P, Tempera G, Del Piano M. Multicentric study of seroprevalence of *Borrelia burgdorferi* and *Anaplasma phagocytophila* in high-risk groups in regions of central and southern Italy. Int J Immunopathol Pharmacol. 2004;17:219-23.
8. Tomao P, Ciceroni L, D'Ovidio MC, De Rosa M, Vonesch N, Iavicoli S, Signorini S, Ciarrocchi S, Ciufolini MG, Fiorentini C, Papaleo B. Prevalence and incidence of antibodies to *Borrelia burgdorferi* and to tick-borne encephalitis virus in agricultural and forestry workers from Tuscany, Italy. Eur J Clin Microbiol Infect Dis. 2005;24:457-63 DOI 10.1007/s10096-005-1348-0
9. Di Renzi S, Martini A, Binazzi A, Marinaccio A, Vonesch N, D'Amico W, Moro T, Fiorentini C, Ciufolini MG, Visca P, Tomao P. Risk of acquiring tick-borne infections in forestry workers from Lazio, Italy. Eur J Clin Microbiol Infect Dis. 2010;29:1579-81. DOI 10.1007/s10096-010-1028-6
10. Toma L, Di Luca M, Mancini F, Severini F, Mariano C, Nicolai G, Laghezza Masci V, Ciervo A, Fausto AM, Cacciò SM. Molecular characterization of *Babesia* and *Theileria* species in ticks collected in the outskirt of Monte Romano, Lazio Region, Central Italy. Ann Ist Super Sanità. 2017;53:30-4. doi: 10.4415/ANN_17_01_07
11. Blasi C. L'Ambiente nella Tuscia laziale. Aree protette e di interesse naturalistico della provincia di Viterbo. In: *Lineamenti della vegetazione dell'Alto Lazio*. Viterbo: Università della Tuscia di Viterbo; 1992.
12. Celletti S, Papi R. Fauna vertebrata terrestre della provincia di Viterbo. In: *Seconda Relazione sullo Stato dell'Ambiente*. Viterbo: Provincia di Viterbo Assessorato Ambiente e Pianificazione del Territorio; 2003.
13. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol. 1991;173:1576-89.
14. Parola P, Roux V, Camicas JL, Baradji I, Brouqui P, Raout D. Detection of Ehrlichiae in African ticks by polymerase chain reaction. Trans R Soc Trop Med Hyg. 2000;94:707-8.
15. Pietila¨ J, He Q, Oksi J, Viljanen MK. Rapid differentiation of *Borrelia garinii* from *Borrelia afzelii* and *Borrelia burgdorferi* sensu stricto by LightCycler fluorescence melting curve analysis of a PCR product of the *rec*A gene. J Clin Microbiol. 2000;38:2756-9.
16. Ciervo A, Ciceroni L. Rapid detection and differentiation of *Bartonella* spp. by a single-run real-time PCR. Mol Cell Probes. 2004;18:307-12. doi: 10.1016/j.mcp.2004.04.004
17. Klee SR, Tyczka J, Ellerbrok H, Franz T, Linke S, Baljer G, Appel B. Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. BMC Microbiol. 2006;6:2. doi: 10.1186/1471-2180-6-2
18. Byström M, Böcher S, Magnusson A, Prag J, Johansson A. Tularemia in Denmark: identification of a *Francisella tularensis* subsp. *holarctica* strain by real-time PCR and high-resolution typing by multiple-locus variable-number tandem repeat analysis. J Clin Microbiol. 2005;43:5355-8. doi:1 0.1128/JCM.43.10.5355-5358.2005
19. Besag J, Kooperberg C. On conditional and intrinsic autoregression. Biometrika. 1995:733-46.
20. Spiegelhalter D J, Thomas A, Best NG. WinBUGS Version 1.2 User Manual. MRC Biostatistics Unit; 1999.
21. Thomas A. BUGS: a statistical modelling package. RTA/BCS Modular Languages Newsletter. 1994;2:36-8.
22. Manilla G. Acari Ixodida. Fauna d'Italia. 36. Bologna: Calderini; 1986.
23. Iori A, Di Giulio A, De Felici S. Zecche d'Italia, parte III. In: Cringoli G (Ed). Mappe parassitologiche (6) (Zecche). Napoli: 2005.
24. Randolph SE, Rogers DJ. Ecology of tick-borne disease and the role of climate. In: Ergonul, O, Whitehouse C (Eds), Crimean-Congo hemorrhagic fever. A global perspective. The Netherlands: Springer; 2003.
25. Mantelli B, Pecchioli E, Hauffe HC, Rosa R, Rizzoli A. Prevalence of *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum* in the wood tick Ixodes ricinus in the Province of Trento, Italy. Eur J Clin Microbiol Infect Dis.

2006;25:737-9. doi: 10.1007/s10096-006-0208-x

26. Piccolin G, Benedetti G, Doglioni C, Lorenzato C, Mancuso S, Papa N, Pitton L, Ramon MC, Zasio C, Bertiato G. A study of the presence of *B. burgdorferi, Anaplasma* (previously *Ehrlichia*) *phagocytophilum*, *Rickettsia*, and *Babesia* in *Ixodes ricinus* collected within the territory of Belluno, Italy. Vector Borne Zoonotic Dis. 2006;6:24-31. doi: 10.1089/vbz.2006.6.24
27. European Centre for Disease Prevention and Control. *Technical report. Risk assessment on Q fever*. Solna, Svezia: ECDC; 2010: Available from: http://ecdc.europa.eu/en/publications.Publications1005_TER_Risk_Assessment_Qfever.pdf.
28. Breitschwerdt E. Bartonellosis: one-health perspectives for an emerging infectious disease. ILAR J. 2014;55:4658. doi: 10.1093/ilar/ilu015
29. Rocchigiani G, Ebani V, Nardoni S, Bertelloni F, Bascherini A, Leoni A, Mancianti A, Poli A. Molecular survey on the occurrence of arthropod-borne pathogens in wild brown hares (*Lepus europaeus*) from Central Italy. Infect Genet Evol. 2018;59:142-7. doi: 10.1016/j.meegid.2018.02.005
30. Chisu V, Foxi C, Mannu R, Satta G, Masala G. A five-year survey of tick species and identification of tick-borne bacteria in Sardinia, Italy. Ticks and Tick-borne Dis. 2018;9:678-81. doi: 10.1016/j.ttbdis.2018.02.008
31. Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, Molez JF, Sokhna C, Trape JF, Raolut D. *Coxiella burnetii* in Humans and Ticks in Rural Senegal. PLoS Negl Trop Dis. 2010;4(4):e654. doi: 10.1371/journal.pntd.0000654
32. Waner T, Keysary A, Eremeeva ME, Beth Din A, Mumcuoglu KY, King R, Atiya-Nasagi Y. *Rickettsia africae* and Candidatus *Rickettsia barbariae* in Ticks in Israel. Am J Trop Med Hyg. 2014:90;920-2. doi: 10.4269/ajtmh.13-0697