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Tick exposure and risk of tick-borne pathogens infection in hunters and hunting dogs: a citizen science approach

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Abstract

Citizen science may be described as a research involving communities and individuals, other than scientists. Following this approach, along with the evidence of a high prevalence of *Rickettsia* spp. in *Dermacentor marginatus* from wild boars in hunting areas of southern Italy, this study aimed to assess the occurrence of tick-borne pathogens (TBPs) in ticks collected from hunters and their hunting dogs. From October 2020 to May 2021, ticks were collected from wild boar hunters (n = 347) and their dogs (n = 422) in regions of southern Italy (i.e., Apulia, Basilicata, Calabria, Campania and Sicily). All ticks were morphologically identified, classified according to gender, feeding status, host, geographic origin, and molecularly screened for zoonotic bacteria. Adult ticks (n = 411) were collected from hunters (i.e., n = 29; 8.4%; mean of 1.6 ticks for person) and dogs (i.e., n = 200; 47.4%; mean of 1.8 ticks for animal) and identified at species level as *D. marginatus* (n = 240, 58.4%), *Rhipicephalus sanguineus* sensu lato (n = 135, 32.8%), *Rhipicephalus turanicus* (n = 27, 6.6%) and *Ixodes ricinus* (n = 9, 2.2%). Overall, 45 ticks (i.e., 10.9%, 95% CI: 8.3-14.3) tested positive for at least one tick-borne agent, being *Rickettsia slovaca* the most frequent species (n = 37, 9.0%), followed by *Rickettsia raoultii*, *Rickettsia aeschlimannii*, *Rickettsia monacensis*, *Coxiella burnetii*, *Borrelia lusitaniae* and *Candidatus* Midichloria mitochondrii (n = 2, 0.5% each). Data herein presented demonstrate a relevant risk of exposure to TBPs for hunters and hunting dogs during the hunting activities. Therefore, the role of hunters to monitor the circulation of ticks in rural areas may be considered an effective example of the citizen science approach, supporting the cooperation toward private and public health stakeholders.

KEYWORDS
citizen science, dogs, hunting, Italy, tick-borne pathogen, zoonosis

1 | INTRODUCTION

Hard ticks (order Acarina, family Ixodidae) represent one of the main public health issues worldwide, being vectors of a wide range of tick-borne pathogens (TBPs) including viruses, bacteria and parasites of human and animal concern (Dantas-Torres et al., 2012). Although predictive spatio-temporal models on tick abundance may be important to reduce the incidence of tick-borne diseases (TBDs) (Hartemink et al., 2015), the full understanding of factors involved, such as climatic conditions, vegetation type and wildlife density is not completely outlined (Estrada-Peña & de la Fuente, 2016; Garcia-Marti et al., 2017). Globally, the occurrence of TBDs is increasing worldwide (Jaenson et al., 2009; Kuehn, 2019) and the most common in Europe is Lyme disease caused by bacteria belonging to *Borrelia burgdorferi* sensu lato complex, being *Borrelia afzelii*, *Borrelia garinii* and *B. burgdorferi* sensu stricto the main zoonotic genospecies (Rauter & Hartung, 2005; Rizzoli et al., 2014). Other relevant TBPs include *Anaplasma phagocytophilum*, *Coxiella burnetii* and *Rickettsia* spp., which can be transmitted to a wide range of wild and domestic mammals, as well as humans (Bowser & Anderson, 2018; Cutler et al., 2019; Estrada-Peña et al., 2017; Krause, 2019; Parola et al., 2013; Sgroi et al., 2021; Sprong et al., 2018). The increased urbanization of European areas contributes to a higher circulation of synanthropic wildlife, and consequently may expose people to TBPs and other zoonotic agents since these animals could harbour a plethora of tick vectors (Baneth, 2014; Rizzoli et al., 2014; Sgroi et al., 2020). In Italy, little data are available on ticks collected from citizens, as well as on the prevalence of TBPs in humans due to the paucity in clinical case notifications (Beltrame et al., 2018; Otranto et al., 2014). However, considering the great vocation for tourism and several outdoor recreational activities (e.g., sports, hiking, hunting) of the Italian peninsula (Otranto et al., 2014; Sandifer et al., 2015), rural areas are at high risk of exposure to tick bites, especially in southern regions where there is an amazing number of tick species (Dantas-Torres & Otranto, 2013). Consequently, a high seroprevalence for *A. phagocythophilum* (5.7%), *B. burgdorferi* s.l. (7.5%) and spotted fever group rickettsiae (SFGR) (8.0%) in forestry workers, farmers and livestock breeders of central-southern Italy was reported (Mendoza-Roldan et al., 2021; Santino et al., 2004), suggesting the high risk of exposure to tick bite (Toepp et al., 2018) due to their frequent exhibitions in rural areas. Similarly, hunters and hunting dogs are highly exposed to arthropods and pathogens they transmit owing to their field activities in forest areas (Mahachi et al., 2020). Accordingly, seropositivity to *Rickettsia* spp. has been ascertained in hunters with prevalence ranging from 9.1% (Germany; Jansen et al., 2008) to 14.7% (Brazil; Kmetiuk et al., 2019). Therefore, hunting dogs may act as sentinels and potential reservoirs of TBPs and consequently, hunters are more likely exposed to tick bites and tick-borne infections than hikers or forest guards (Hornok et al., 2013; Mahachi et al., 2020; Toepp et al., 2018). In this scenario, hunters may themselves play a role in monitoring the presence of ticks in a given area, cooperating with the sanitary stakeholders (i.e., citizen science approach) to increase knowledge on the spread of arthropods and their transmitted pathogens (Garcia-Marti et al., 2017; Hamer et al., 2018; Tanner Porter et al., 2019). Therefore, the present survey aimed to assess the tick bite exposure and the risk of infection by TBPs in hunters and their dogs in an area where a high prevalence of wild boars (i.e., 18.3%) harboured *Dermacentor marginatus* ticks.

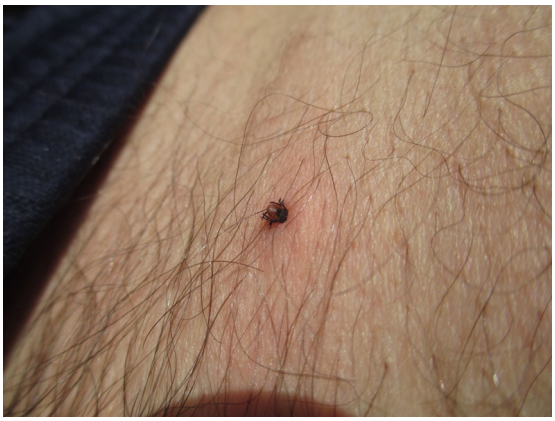

FIGURE 1 *Ixodes ricinus* female specimen during blood feeding on a hunter's limb (Courtesy Dr. Antonio Zotti)

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in regions of southern Italy (i.e., Apulia, Basilicata, Calabria, Campania and Sicily), which face on the Ionian, Mediterranean and Tyrrhenian seas. The area has a total surface of 123.417 km^2 characterized by a typical Mediterranean temperate climate with progressively continental features in inland and mountainous landscapes.

2.2 | Sampling

From October 2020 to May 2021, hunters ($n = 347$) divided in 17 hunting teams (i.e., groups on average of 20 hunters) were enrolled to collect ticks from themselves (Figure 1) and their dogs ($n = 422$). To evaluate the human tick exposure, hunters were classified in two different groups: hunters who used to hunt with one or more dogs (group 1) or without any dog (group 2). Ticks were collected at the end of the day, after the hunting session. All participants were trained on the proper removal technique of ticks from themselves and their dogs, following the guidelines of the Center for Disease Control and Prevention (CDC, 2019). Participants were provided with vials, tweezers, ethanol (70%) and a specific form reporting: (i) body site where ticks were collected and their number; (ii) environmental features of the hunting area (i.e., wooded or grassland), along with anamnestic data (i.e., age and gender) of hunters and dogs. Each tick recovered was stored in numbered plastic vials containing 70% ethanol until the morphological identification. All samples were delivered to the Unit of Parasitology at the Department of Veterinary Medicine, University of Bari Aldo Moro (Italy).

TABLE 1 Tick-borne pathogens investigated in this study with target genes, primers nucleotide sequences and fragment length

Tick-borne pathogens	Target gene	Primers	Sequence (5′–3′)	Fragment length (bp†)	References
Anaplasma/Ehrlichia spp.	16S rRNA	EHR-16SD	GGTACCYACAGAAGAAGTCC	345	Martin et al. (2005)
	Flagellin	EHR-16SR	TAGCACTCATCGTTTACAGC	482	
Borrelia burgdorferi		FLA1	AGAGCAACTTACAGACGAAATTAAT		Wójcik-Fatla et al. (2009)
	IS1111a	FLA2	CAAGTCTATTTTGGAAAGCACCTAA	687	
sensu lato complex		Trans-1	TATGTATCCACCGTAGCCAGT		Berri et al. (2000)
	*glt*A	Trans-2	CCCAACAACACCTCCTTATTC	401	
Coxiella burnetii		CS-78F	GCAAGTATCGGTGAGGATGTAAT		Labruna et al. (2004)
	*omp*A	CS-323R	GCTTCCTTAAAATTCAATAAATCAGGAT	632	
Rickettsia spp.		Rr190.70F	ATGGCGAATATTTCTCCAAAA		Regnery et al. (1991)
		Rr190.701R	GTTCCGTTAATGGCAGCATCT		
Spotted fever group rickettsiae					

†bp = base pairs.

2.3 | Morphological identification of ticks

All ticks were classified according to gender (i.e., male and female), developmental stage (i.e., larval, nymph, adult) and feeding status (i.e., fed and unfed). Tick species were identified by stereomicroscopy (Leica MS5—Leica Microsystems Ltd. Heerbrugg, Germany), using the morphological keys available in the literature (Estrada-Peña et al., 2004, 2017; Manilla & Iori, 1992).

2.4 | DNA extraction, PCR protocols and sequencing

DNA was extracted from individual tick using a commercial kit (QIAampDNA Blood & Tissue, Qiagen, Hilden, Germany), according to the manufacturer's instructions and analysed for the detection of different TBPs. Details regarding PCR protocols are reported in Table 1. All PCR products were examined on 2% agarose gel stained with GelRed (VWR International PBI, Milan, Italy) and visualised on a GelLogic 100 gel documentation system (Kodak, New York, USA). Amplicons were then purified and sequenced in both directions using the same primers as for PCRs, by the Big Dye Terminator version 3.1 chemistry in a 3130 Genetic Analyzer (Applied Bio-systems, Foster City, CA, USA). Sequences were edited and analysed by the Geneious software version 9.0 (Biomatters Ltd., Auckland, New Zealand) (Kearse et al., 2012) and compared with those available in the GenBank database by the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.5 | Statistical analysis

Exact binomial 95% confidence intervals (CIs) were established for proportions found in the present work, using five alternative calculation methods as described in Brown et al. (2001). A Chi-squared test was used to assess any statistical difference of tick infestation on humans and dogs, as well as the prevalence of different tick-borne agents among gender, feeding status and geographical origin of ticks. A p value less than .05 was considered significant. Odds ratios (ORs) were calculated to assess the tick infestation risk according to several variables of hunters and dogs. Chi-squared, 95% CIs, p values and ORs were calculated by using the software Epitools—Epidemiological Calculators (Sergeant, 2018). The number of hunters and hunting dogs enrolled, along with the circulation of different tick species in the administrative regional boundaries of the study area, was obtained with ArcGIS (version 10.3, ESRI, Redlands, CA, USA).

TABLE 2 Tick bite exposure of hunters and hunting dogs, as numbers and percentage of ticks collected according to different body localization

Body localization	Hunters	Hunting dogs	Total (%)
Neck	15 (31.2%)	38 (10.5%)	53 (12.9%)
Limbs	5 (10.4%)	38 (10.5%)	43 (10.5%)
Head	19 (39.6%)	117 (32.2%)	136 (33.1%)
Ears	9 (18.7%)	170 (46.8%)	179 (43.5%)
Total (%)	48 (11.7%)	363 (88.3%)	411 (100%)

3 | RESULTS

A total of 411 adult ticks (i.e., 179 males, 109 unfed females, 123 fed females) were collected, being *D. marginatus* the most representative species (n = 240, 58.4%), followed by *Rhipicephalus sanguineus* sensu lato (n = 135, 32.8%), *Rhipicephalus turanicus* (n = 27, 6.6%) and *Ixodes ricinus* (n = 9, 2.2%). The total number of ticks collected from different body sites of hunters and hunting dogs was 48 (i.e., 11.7%) and 363 (i.e., 88.3%) (Table 2), with 9 (18.7%) and 114 (31.4%) fed specimens, respectively. Out of 347 hunters and 422 dogs enrolled and distributed according to the geographical regions (Figure 2), 29 (i.e., 8.4%) and 200 (i.e., 47.4%) were infested by at least one tick spec-

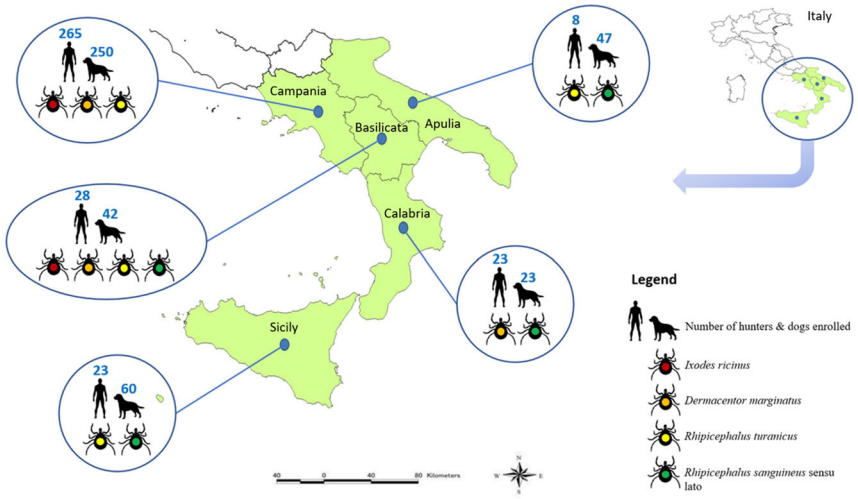

FIGURE 2 Map showing the number of hunters and hunting dogs enrolled, along with the circulation of different tick species, in different regions of the study area

imen with a mean intensity value (i.e., average amount of ticks for each positive hunter/dog) of 1.6 and 1.8 for hunters and dogs, respectively. For hunters, a statistically significant association was found in the tick exposure frequency with the hunting practice along with dogs (χ^2 = 25.73, p < .001, OR = 8.1) and the hunting practice in wooded areas (χ^2 = 6.76, p = .009, OR = 2.8), whereas no statistical relationship was found according to age (χ^2 = 0.46, p = .796). A single case of coinfestation by *D. marginatus* and *R. sanguineus* s.l. was detected on a hunter. For hunting dogs, a statistically significant association was found in tick exposure frequency with the age group of 1–5 years (χ^2 = 27.37, p < .001) and the hunting practice in wooded areas (χ^2 = 10.82, p = .001, OR = 1.9); no statistical relationship was found according to gender (χ^2 = 0.52, p = .471). Data on the tick bite exposure of hunters and hunting dogs, according to their different body districts, are reported in Table 2.

Out of 411 ticks collected, 45 (i.e., 10.9%, 95% CI: 8.3–14.3) tested positive for at least one tick-borne bacteria, being *Rickettsia slovaca* the most prevalent (n = 37, 9.0%), followed by *Rickettsia raoultii* (n = 2, 0.5%), *Rickettsia aeschlimannii* (n = 2, 0.5%), *Rickettsia monacensis* (n = 2, 0.5%), *C. burnetii* (n = 2, 0.5%), *Borrelia lusitaniae* (n = 2, 0.5%) and *Candidatus* Midichloria mitochondrii (hereafter *M. mitochondrii*) (n = 2, 0.5%). Out of 45 positive ticks, 9 (i.e., 20%) were collected from hunters and 36 (i.e., 80%) from dogs. Four cases (1.0%) of coinfections by different tick-borne agents in tick specimens were also identified (Table 3). Details regarding the exposure of hunters and hunting dogs to tick species positive for at least one tick-borne microorganism are reported in Table 4; data on ticks positive to tick-borne agents according to their gender, feeding status and administrative regions are detailed in Table 5. Representative sequences of all bacteria herein detected displayed 99–100% query coverage and nucleotide identity with those available in the literature and were submitted to GenBank under the accession numbers: MZ964142 for *R. slovaca*, MZ964139 for *R. raoultii*, MZ964141 for *R. aeschlimanni*, MZ964140 for *R. monacensis*, MZ964137 for *C. burnetii*, MZ964138 for *B. lusitaniae* and MZ954838 for *M. mitochondrii*.

TABLE 3 Number and species of ticks positive to different tick-borne agents (n = 45) from hunters and dogs

Tick-borne agents	Tick species
Single infections	*D. marginatus* (6, - 26, D), *I. ricinus* (1,) *R. sanguineus* s.l. (1,)*D. marginatus* (2,)*D. marginatus* (1,), *R. sanguineus* s. l. (1,)*D. marginatus* (2,)*I. ricinus* (1,)
R. slovaca	
R. raoultii	
R. aeschlimanni	
R. monacensis	
M. mitochondrii	
Coinfections	*I. ricinus* (1,)*D. marginatus* (2,)*I. ricinus* (1,)
R. slovaca—B. lusitaniae	
R. slovaca—C. burnetii	
M. mitochondrii—B. lusitaniae	

†H = Hunters.
‡D = Dogs.

4 | DISCUSSION

Based on the results presented, the citizen science approach showed to be an effective and low-cost tool for monitoring tick and related transmitted pathogen circulation in hunters and their dogs. This approach has been for the first time applied to hunters as a specific class of citizens, differently from previous experiences conducted in Canada (Lewis et al., 2018), USA (Nieto et al., 2018; Tanner Porter et al., 2019), Netherlands (Garcia-Marti et al., 2017, 2018) and Belgium (Lernout et al., 2019). All ticks collected were adults, suggesting that even if an accurate body inspection is conducted, smaller tick stages may have been unnoticed, thus, representing a limitation for such kind of surveys. On the other hand, studies where physicians performed the body inspection and collection of ticks, larvae and nymphs were also identified from human patients in different countries (e.g., Italy—Audino et al., 2021; Otranto et al., 2014; USA— Nieto et al., 2018; Belgium—Lernout et al., 2019 and Serbia—Banović et al., 2021). *Dermacenor marginatus* was the most abundant tick species on hunters and hunting dogs as well as on wild boars, in the Mediterranean area (Spain—Ortuño et al., 2006; Corsica—Grech-Angelini et al., 2016 and Italy—

TABLE 4 Tick bite exposure of hunters and hunting dogs with number and percentage of ticks positive for at least one tick-borne agent

Tick species	Hunters† Pos/Tot (%)	Hunting dogs† Pos/Tot (%)	Total (%)
D. marginatus	6/36 (16.7%)	33/204 (16.2%)	39/240 (16.2%)
R. sanguineus s. l.	1/7 (28.6%)	1/128 (0.8%)	2/135 (2.2%)
R. turanicus	0/0	0/27	0/27
I. ricinus	2/5 (40%)	2/4 (50%)	4/9 (44.4%)
Total (%)	9/48 (16.7%)	36/363 (9.6%)	45/411 (10.9%)

†Pos/Tot (%) = number of ticks positive to at least one agent on the total number of ticks from hunters and hunting dogs.

TABLE 5 Ticks tested positive for at least one tick-borne agent according to gender, feeding status and administrative regions in this study (95% CI = Confidence Interval 95%)

Variables	†Pos/Tot (%)	95% CI
Gender		
Male	21/179 (11.7%)	7.8–17.3
Female	24/232 (10.3%)	7.0–14.9
Chi-squared; *p*-value	$\chi^2 = 0.20$; $p = .655$	
Feeding status (females *n* = 232)		
Fed	16/123 (13.0%)	8.2–20.1
Unfed	8/109 (7.4%)	3.8–13.8
Chi-squared; *p*-value	$\chi^2 = 2.00$; $p = .157$	
Region		
Apulia	0/50 (-)	–
Basilicata	1/21 (4.8%)	0.8–22.7
Calabria	6/33 (18.2%)	8.6–34.4
Campania	38/228 (16.7%)	12.4–22.0
Sicily	0/79 (–)	–
Total	45/411 (10.9%)	8.3–14.3

†Pos/Tot (%) = number of ticks positive to at least one agent on the total number of ticks collected.

Sgroi et al., 2020). Indeed, these ungulates are involved in the maintenance of this tick species in the environment (Selmi et al., 2017; Sgroi et al., 2020) and, therefore, representing a potential risk for hunters and hunting dogs in the area where wild boars thrive. The higher frequency of *D. marginatus* infestation in hunters frequenting woods with their dogs than those practicing hunting without them, combined with the presence of this tick species on the animals, may suggest a likely tick exposure of people in close contact with hunting dogs (Audino et al., 2021; Toepp et al., 2018). This could also indicate a role of these animals as sentinels for the human tick exposure (Bowser & Anderson, 2018; Hornok et al., 2013) considering the high tick infestation rate on hunting dogs herein found (i.e., 42.5%). The above-mentioned scenario has been previously confirmed in Spain, where hunting dogs were more exposed to TBPs than other classes of owned dogs (Miró et al., 2015). Particularly, the high frequency of infestation for hunters and hunting dogs seems to be associated with wooded areas compared to grassland (Audino et al., 2021; Lernou et al., 2018), confirming the role of the environment as a major driver for the perpetuation of the biological life cycle of ticks. Also, the larger percentage of tick infestation in dogs aging from 1 to 5 years old may be associated with the fact that animals younger than 1 year and older than 5 years are less employed in hunting activities (Orr et al., 2019). The presence of ticks on hunters and dogs from all the studied regions confirms that both mainland and insular areas of southern Italy are suitable for tick development and circulation due to the optimal climatic and environmental conditions present (Dantas-Torres & Otranto, 2013). In addition, the overall prevalence of zoonotic agents herein detected (i.e., 10.9%) highlights a risk for human and dog infections mainly by SFGR. Similarly, high serological exposure to *Rickettsia* spp. in hunters (i.e., 9.1%, Germany—Jansen et al., 2008; 14.7%, Brazil—Kmetiuk et al., 2019) and hunting dogs (53%, Spain—Ortuño et al., 2009; 14.1%, Nicaragua —Fiorello et al., 2017) has been reported in several countries. The higher occurrence of *R. slovaca* (i.e., 9.0%), compared to other SFGR detected, is of relevance considering its pathogenicity to humans (Li et al., 2018; Parola et al., 2009). The recent finding of *R. slovaca* in *D. marginatus* collected on park rangers from the same study area (Mendoza-Roldan et al., 2021), further confirms the circulation of this zoonotic bacterium in rural areas of southern Italy. Moreover, the detection of *R. raoultii*, *R. aeschlimannii* and *R. monacensis* in ticks collected from dogs suggests their role as indicators of potential human exposure to pathogens in hunting areas (Guggione et al., 2021; Madeddu et al., 2012; Tosoni et al., 2016), and their increased occurrence in southern part of the Italian peninsula (Gomez-Barroso et al., 2019). The occurrence of *C. burnetii* in *D. marginatus* collected on hunting dogs is of public health concern, since the prevalence of this bacterium in hard ticks is significantly higher in Mediterranean area than in other European countries (Körner et al., 2021). Although the vectorial competence of *D. marginatus* for *C. burnetii* (via faecal excretion—Körner et al., 2020) has been ascertained, the role of this tick species in the epidemiology of Q fever appears negligible compared to *Hyalomma lusitanicum*, in which the prevalence of this pathogen (i.e., 18%) is higher than in *D. marginatus* (i.e., 1.4%) around Europe (Körner et al., 2021). The finding of *B. lusitaniae* in *I. ricinus* infesting hunters and hunting dogs confirms the presence of this zoonotic genospecies in rural areas of southern Italy, as previously established in lizards (Mendoza-Roldan et al., 2019) and foxes (Sgroi et al., 2021). Despite the pathogenic role of *B. lusitaniae* in humans has not been completely clarified, the occurrence of long-lasting skin lesions in the site of tick bites in patients affected by this pathogen, underlines its clinical relevance

(Collares-Pereira et al., 2004). The detection of *M. mitochondrii* endosymbiont DNA in two out of nine (i.e., 22.2%) *I. ricinus* deserves further attention regarding the interaction between *M. mitochondrii* and its hosts, and its involvement in the transmission pathway of *B. burgdorferi* s.l. by *I. ricinus*, considering that the prevalence of this microorganism is higher in humans infested by ticks than in those not infested (Mariconti et al., 2012), as well in patients affected by Lyme disease compared to those not affected (Serra et al., 2019). This proteobacterium has also been previously detected in dogs, sheep and horses from southern Italy (Bazzocchi et al., 2013) and in roe deer, *Capreolus capreolus*, from France (Serra et al., 2018). Finally, the finding of coinfected ixodids suggests that multiple agents may develop in the same tick being potentially transmitted to the same host (Audino et al., 2021; Otranto et al., 2014).

Data herein presented demonstrated the high circulation of ticks and related zoonotic pathogens in wild boar hunting areas of southern Italy suggesting a high risk of infestation and zoonotic pathogen transmission to hunters and their dogs. This survey also reveals that the active collection of ticks by hunters may be an additional resource for monitoring ticks in rural areas, being an effective example of the citizen science approach. Lastly, a one health perspective, involving physicians, veterinarians and other collaborating stakeholders (e.g., hunters) is advocated to control and prevent the spread of ticks and related pathogens.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

Giovanni Sgroi: Conceptualization, investigation, writing original draft. Roberta Iatta: Conceptualization, writing-reviewing and editing, supervision. Riccardo Paolo Lia: Methodology, data curation. Ettore Napoli: Acquisition of data, formal analysis. Francesco Buono: Acquisition of data. Marcos Antonio Bezerra-Santos: Writing-review and editing. Vincenzo Veneziano: Acquisition of data, formal analysis. Domenico Otranto: Validation, supervision, Writing: review and editing.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to the European Directive 2010/63/EU, in accordance with the rules of the Istituto Zooprofilattico Sperimentale del Mezzogiorno (Portici, Italy) and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. High standards of veterinary care and client consent have been employed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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