

Ticks Infesting Humans in North-Western Italy and Associated Pathogens: A Cross-Sectional Study in a Three-Year Period (2017-2019) in North-Western Italy.

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Abstract

Background. Tick-borne diseases are widespread in many European Countries and high incidence has been reported in the past few years. Ticks are able to transmit several pathogens to the host while feeding and are considered the most important vectors of infectious agents worldwide together with mosquitos. As tick-bite events has remarkably increased in North-Western Italy and information about the prevalence of tick-borne pathogens in ticks removed from humans in Italy are scarce, this study aimed to identify the species of ticks biting humans and the tick-borne pathogens they can transmit.

Methods. Ticks collected from bitten humans during 2017-2019 were morphologically identified and screened by biomolecular essays. A total of 1,290 ticks were analysed. Most of the ticks belonged to the *Ixodes* genus: 1,009 ticks (78.0%) were classified as *Ixodes ricinus*. Some *Ixodes* ticks (n = 158; 12.3%) were identified only at genus level due to lack of morphological features. Overall, 500 ticks were PCR tested for the presence of *Rickettsia* spp., *Borrelia* spp., and *Anaplasma* spp. in at-risk population (elderly and children). Pathogen identity was confirmed by Sanger sequencing.

Results. The overall prevalence was 22.8%; (n = 114; 95%CI 19.19-26.73%), meaning that at least one pathogen was detected. Molecular analysis was carried out on a subset of 500 ticks showing the presence of *R. helvetica* (n=31), *R. monacensis* (n=35), *R. slovacica* (n=3), *R. aeschlimannii* (n=1), *Rickettsia* spp. (n=6); *B. afzelii* (n=11), *B. burgdorferi sensu stricto* (n=1), *B. garinii* (n=3), *B. lusitaniae* (n=4), and *B. valaisiana* (n=2), *Borrellia* spp. (n=11); *Anaplasma phagocytophilum*(n=6).

Conclusions. These data highlight the importance of surveillance to assess the epidemiology of TBDs that pose a threat to human health by the implementation of control strategies for both tick infestations and their associated pathogens.

Background

Ticks are amongst the most important vectors of diseases in temperate climates (Heyman et al. 2010) and display a worldwide distribution being adapted to different environments, climate and host species (Danta-Torres et al. 2012). Their medical and veterinary relevance is mostly due to their great capacity of transmitting viral, bacterial, protozoan and helminthic infections, which may cause a diverse range of affections, commonly referred to as tick-borne diseases (TBDs) (Sonenshine et al. 2018). Many TBDs are zoonosis, such as Rickettsiosis, Lyme Borreliosis, Anaplasmosis (Alciati et al. 2001) and TBE (tick-borne encephalitis). These diseases can evolve as asymptomatic or manifest themselves symptomatically with the involvement of the central nervous system, leading to death the patient, but also of skin system, vascular system. People more at risk are children, elderly and immunosuppressed subjects.

Rickettsiosis is a bacterial disease; it is caused by an obligate intracellular α -proteobacteria, gram negative, pleomorphic bacillus of the genus *Rickettsia*. In Italy, the regions most affected are Sicily, Sardinia, Lazio and Calabria. Until 2002, *Rickettsia conorii conorii* was the only pathogenic *Rickettsia* recorded in Italy as causal agent of Mediterranean Botton Fever (FBM). Today, molecular techniques have

allowed the identification of other *Rickettsia* species. Actually, a growing number of new *Rickettsiae* were described and recognized as causative agents of human diseases diagnosed in Europe during the last decades. Twenty-six *Rickettsia* species with validated and published names are currently reported (Parola et al. 2013).

Borrelia burgdorferi is the etiological agent of Lyme borreliosis. Lyme disease typically presents with an erythema migrans rash and non-specific symptoms such as fatigue, fever, headache and muscle and joint pains and, if left untreated, it can become a multisystem disease. Lyme disease is rarely fatal, but deaths linked to Lyme carditis have recently been reported (Seltzer et al. 2000, Kugeler et al. 2011). In Italy, the most affected regions are Friuli-Venezia Giulia, Liguria, Veneto, Emilia-Romagna, Trentino Alto Adige (Autonomous Province of Trento), while *B. burgdorferi* is reported sporadically in the southern central regions and islands (website Epicentro, ISS).

Anaplasma phagocytophilum is responsible for granulocytic anaplasmosis. Clinically, people can have asymptomatic *A. phagocytophilum* infections, but most frequently have non-specific symptoms (e.g. fever, headache and muscle aches). The fatality rate is less than 1% (Dumler et al. 2005, Biggs et al. 2016).

These zoonotic TBDs may be associated with both domestic and wild animals, with a high risk of acquiring infections for humans frequenting tick-infested areas, such as forests, meadow habitats and grasslands (Danta-Torres et al. 2012, Jaensen et al. 2009, Medlock et al. 2013). TBDs are constantly expanding worldwide and their incidence has increased over the past few years (Danta-Torres et al. 2012). This increase is closely related to survival and spread of vectors that depend on different climatic and environmental factors. Local climatic factors (macro- and micro-climate) can facilitate the appearance or reappearance of vector-borne diseases in a given area. (Jongejan et al. 2004). Seasonality, distribution, and prevalence of TBDs are influenced significantly by climate factors, primarily high and low temperature extremes and precipitation patterns. Climate changes can result in modified weather patterns and an increase in extreme events that can affect disease outbreaks by altering biological variables such as vector population size and density, vector survival rates, the relative abundance of disease-carrying animal, reservoir hosts, and pathogen reproduction rates. (Gage, K. L et al. 2008). Collectively, these changes may contribute to an increase in the risk of pathogens being carried to humans. Mediterranean regions are areas with considerable geographical and wildlife diversity, with high environmental variability due to the influence of altitude and distance from the sea. The variability of environmental characteristics favours the formation of tick populations. With about 40 species (Manilla et al. 1998), tick fauna in Italy is one of the most diverse across Europe, with more species than countries such as Portugal (Santos-Silva et al. 2011) and UK (Scharlemann et al. 2008, Smith et al. 2011). Italy, in particular, extends its territory for about 10 parallels presenting a high wealth of different habitats; moreover, given its geographical location, it represents an important bridge in the Mediterranean for the passage of new pathogens from the African to the European continent.

The incidence of human TBDs in Italy is likely underestimated because of poor surveillance and the limited number of available studies. Since 2011, the Istituto Zooprofilattico Sperimentale of Piedmont, Liguria and Valle d'Aosta (IZS PLVA), a public health institute which operates in North-Western Italian regions, is actively involved in TBD surveillance on the territory. The Institute offers an important service to support the general practitioners in the clinical diagnosis of TBDs, by analysing ticks collected from humans. Ticks are identified on morphological basis at the species level and subjected to biomolecular investigations (PCR) for the detection of *Rickettsia* spp., *Borrelia* spp., and *Anaplasma phagocytophilum*. Aim of this study is to report the results of the surveillance carried out during years 2017–2019 in North-Western Italy.

Methods

Tick collection and identification

Ticks collected from humans between 2017 and 2019 (n = 1,290) were included in the study. Most samples came from areas of IZS PLVA's competence (n = 1,254), some samples from people who had travelled abroad (n = 12), some from neighbouring areas (n = 14), some were of unknown origin (10). All specimens were kept in 70% undenatured alcohol or frozen at -20 °C and submitted to the laboratories of IZS PLVA. Samples through the doctor or the ASL veterinary service arrived at the laboratory in tubes accompanied by a short medical record on which the patient's data was reported as well as place, date, location biting. Species identification was performed using appropriate taxonomic keys (Estrada-Pena et al., 2017) for each developmental stage (i.e. larvae, nymphs, adult female - male).

DNA extraction, PCR and DNA sequence analysis

A subset of ticks (samples collected from people under 18 and over 70-years-old) were tested for the presence of pathogens of the genera *Rickettsia*, *Borrellia*, *Anaplasma*. Ticks were processed as briefly described hereafter.

DNA extraction was performed from individual adults, nymphs or larvae. Each microtube with a tick was filled with sterile PBS solution (pH 7.2) using a volume of 350 µl for larvae and nymphs, and 600 µl for adults. Ticks were homogenized using the FastPrep FP120 instrument (ThermoSavant) for 45 sec. at maximum speed. After homogenization, microtubes were centrifuged (MIRKO 22R, Hettich zentrifugen) for 10 min. at 14,000 rpm and 150 µL of the supernatant were used for total DNA extraction employing the QIAamp DNA Mini kit (Qiagen) with an automated QIAcube protocol.

Total DNA was used for PCR amplification of target genes for the detection of *Rickettsia* spp., *Borrelia burgdorferi* sensu lato, and *Anaplasma phagocytophilum*. PCR reactions were performed in a total volume of 25 µl according to previously published protocols (Choi et al 2005; Skotarczak et al. 2002; Massung et al. 2003). Target genes, primer sequences and expected amplicon sizes are summarized in Table 1.

Table 1

molecular detection of tick-borne pathogens: target genes, primers nucleotide sequences, amplicon size (bp).

Species	Target gene	Nucleotide sequence (5'-3')	Amplicon size (bp)	Reference
Rickettsia spp.	ompB	GTAACCCGGAAGTAATCGTTTTCGTAA (f) GCTTTATAACCAGCTAAACCACC (r)	511	Choi et al 2005
Borrelia burgdorferi s.l.	p4Flagellin	AGAGCAACTTACAGACGAAATTAAT(f) CAAGTCTATTTTGGAAGCACCTAA (r)	482	Skotarczak et al. 2002
Anaplasma phagocytophilum	MSP2	CCAGCGTTTAGCAAGATAAGAG (f) GMCCAGTAACAACATCATAAGC (r)	334	Massung et al. 2003

PCR products were run on 2% agarose gel, submitted to electrophoresis, and visualized using Gel Red staining under UV-light.

Amplicons were purified using the QIAquick Gel Extraction kit (Qiagen). The cycle sequencing reaction was subsequently prepared following the protocol of the BrilliantDye Terminator v.3.1-Nimagen kit (1 ml BrilliantDye v3.1, 3.5 ml 5x Sequencing Buffer, 1 ml Template, 1 ml primer 5pMol, 13.5 ml Water) using the PCR primers for sequencing. Cycle-sequencing reactions were purified with the AutoSeq G-50 Dye Terminator Removal kit (GE Healthcare). Sequencing was carried out by capillary electrophoresis using a 3130XL Genetic Analyzer (Applied Biosystems).

Obtained sequences were compared, using the Basic Local Alignment Search tool (Blast) search, with sequence records available in GenBank for confirmation of pathogen identification and species assignment.

All statistical analysis were performed using Stata 15.1 (StataCorp. 2007. Stata Statistical Software: Release 15.1. College Station, TX: StataCorp LP).

Results

During 2017–2019, a total of 1,290 ticks were collected from humans (Table 2).

Table 2
number of ticks collected from
humans in 2017–2019.

Year	Samples (n)	%
2017	239	18.5
2018	624	48.4
2019	427	33.1
Total	1,290	100

People involved reported biting on limbs in 43% of cases, on trunk in 21%, and on head or neck (especially children) in 12% of cases. Almost all of the patients reported being bitten during a walk in the woods (40%) or in a domestic contest such as in the garden, lawn, park (46%); the remaining 14% were bitten in other locations such as beach, train, and other.

Ticks were taxonomically assigned to 11 species belonging to the genera *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus* (Table 3).

Table 3
species of ticks examined; nd = not determined.

Species	Samples (n)	%
<i>Amblyomma parvum</i>	1	0.08
<i>Dermacentor marginatus</i>	6	0.47
<i>Haemaphysalis punctata</i>	3	0.23
<i>Hyalomma marginatum</i>	1	0.08
<i>Ixodes acuminatus</i>	1	0.08
<i>Ixodes frontalis</i>	1	0.08
<i>Ixodes hexagonus</i>	13	1.01
<i>Ixodes ricinus</i>	1,009	78.22
<i>Ixodes spp.</i>	158	12.25
<i>Rhipicephalus sanguineus</i>	2	0.16
<i>Rhipicephalus spp.</i>	6	0.47
Nd	89	6.90
Total	1,290	100

Most ticks were identified as *Ixodes ricinus* (78.0%). Some ticks (12.3%), belonging to the genus *Ixodes*, resulted to have been damaged during collection (e.g. the rostrum was absent) making it impossible to classify them at the species level). These ticks came from North-West of Italy. We identified one *Amblyomma parvum*, belonging to a man travelling abroad and bitten in Brazil, and one *Hyalomma marginatus* belonged to a man bitten in Greece. The other ticks coming from mans who travelled abroad were identified as *Ixodes ricinus*.

The life stages most frequently collected were nymphs (59.8%) and adult female (28.9%) followed by larvae (2.3%) and males (0.7%) (Table 4).

Table 4
ticks life-stage.

Life stage	number	Prevalence (%)
Female adult	373	28.91 (95%CI 26.45–31.47)
Male adult	9	0.70 (95%CI 0.32–1.32)
Nynphs	771	59.77 (95%CI 57.03–62.46)
Larvae	30	2.33 (95%CI 1.57–3.30)
N.D.	107	8.29 (95%CI 6.85–9.94)
Total	1,290	100

Table 5 and Fig. 1 show a map with the geographic distribution (%) of ticks collected in North-Western Italy.

Table 5
geographic distribution (%) of ticks collected.

Collection site	Prevalence of total ticks collected (%)	n
Piedmont provinces		
VCO	68.91 (95%CI 66.31–71.43)	889
TO	7.36 (95%CI 6-8.93)	95
VC	12.33 (95%CI 10.58–14.24)	159
AT	0.08 (95%CI 0.002–0.43)	1
AL	0.16 (95%CI 0.02–0.56)	2
CN	2.40 (95%CI 1.64–3.40)	31
NO	1.78 (95%CI 1.13–2.66)	23
BI	2.48 (95%CI 1.70–3.48)	32
Valle D'Aosta		
AO	0.08 (95%CI 0.002–0.43)	1
Liguria provinces		
GE	0.16 (95%CI 0.02–0.56)	2
SP	0.16 (95%CI 0.02–0.56)	2
SV	1.32 (95%CI 0.77–2.10)	17
Italy (out of territory of IZSPLVA competence)	1.01 (95%CI 0.59–1.81)	14
Abroad	0.93 (95%CI 0.48–1.62)	12
N/A	0.78 (95%CI 0.37–1.42)	10

Legend for province

Piedmont

AL: Alessandria, AT: Asti, BI: Biella, CN: Cuneo, NO: Novara, TO: Turin, VC: Vercelli, VCO: Verbano-Cusio-Ossola

Liguria

SV: Savona, LV: Livorno, GE: Genova, SP: La Spezia

Valle D'Aosta

AO: Aosta

A total of 500 tick samples were subjected to biomolecular investigations for pathogen detection. Ticks were analysed as single sample or pooled; pools were constructed when ticks belonged to the same species and were collected from the same individual. Overall, 114 ticks (22.8%; 95%CI 19.19–26.73%) were positive to one or more pathogen. Seventy-six samples were positive for *Rickettsia* sp. (15%; 95%CI 12.17–18.65%) and sequencing analysis identified the following species: *R. helvetica* (n = 31; *I. ricinus*: n = 3 larvae; n = 16 nymphs; n = 7 adult females. *Ix. sp.*: n = 4 nymphs: n = 1 adult female), *R. monacensis* (n = 35; *Ix. ricinus*: n = 23 nymphs and n = 11 adult females; *Ixodes* sp. n = 1 adult female), *R. slovaca* (n = 3. *Ix. ricinus*: n = 1 nymph; n = 1 adult female. *Dermacentor marginatus*: n = 1 adult female), *R. aeschlimannii* (n = 1 *Rh. sanguineus* adult male) (Table 6).

Table 6
number of positivity for *Rickettsia* sp./province of north-western Italy

Species	BI	CN	NO	TO	VC	VCO	ND	SV	LV	Total
<i>R. helvetica</i>	2	2	1	1	4	19	2			31
<i>R. monacensis</i>	2	2	1	4	5	20		1		35
<i>R. slovaca</i>		1				2				3
<i>R. aeschlimannii</i>									1	1
Spp.		1		1	1	3				6
Total	4	6	2	6	10	44	2	1	1	76

As regards *Borrelia burgdorferi* s.l., 32 samples were positive for *Borrelia* spp. (6.4%; 95%CI 4.44–8.95%) Sequencing and Blast analysis identified the following genospecies: *B. afzelii* (n = 11. *Ix ricinus*: n = 6 nymphs; n = 3 adult females. *Ixodes* Sp.: n = 2 adult females), *B. burgdorferi sensu stricto* (n = 1 *Ix. ricinus* nymphs), *B. garinii* (n = 3 *Ix. ricinus* nymphs), *B. lusitaniae* (n = 4 *Ix. ricinus*: n = 1 nymphs; n = 3 adult females), and *B. valaisiana* (n = 2 adult females). Table 7 shows the distribution of positive samples by province of Piedmont region.

Table 7
number of positivity for *Borrelia* sp./province

Species	TO	VC	VCO	SP	ND	Total
<i>B. afzelii</i>	6		5			11
<i>B. burgdorferi</i>			1			1
<i>B. garinii</i>		1	2			3
<i>B. lusitaniae</i>			4			4
<i>B. valaisiana</i>			2			2
Spp.			9	1	1	11
Total	6	1	25			32

Finally, six ticks were positive for *Anaplasma phagocytophilum* (n = 6 *Ix. ricinus* nymphs; 1.2%; 95%CI 0.44–2.59%). Table 8 shows the distribution of positive samples by province.

Table 8
number of positivity for *Anaplasma* sp./province

Species	CN	VC	VCO	Total
<i>A. phagocytophilum</i>	1	1	4	6
Total	1	1	4	6

With regard to Italy, the province is a local authority with jurisdiction over a group of municipalities, not necessarily contiguous, and at the same time a peripheral constituency of state offices.

In 4 cases two pathogens were detected (**co-infections**): *A. phagocytophilum* + *R. monacensis*; *B. afzelii* + *R. Helvetica*; *B. burgdorferi* s.s + *R. monacensis*; *B. lusitaniae* + *R. monacensis*.

Table 9 shows positivity in relation between life stage and specie of ticks

Table 9
Positive samples: life stage vs species of ticks

Life stage	<i>Ixodes ricinus</i>	<i>Ixodes</i> spp.	<i>Rhipicephalus sanguineus</i>	<i>Dermacentor marginatud</i>
Female adult	37	6	1	1
Nymphs	57	9	0	0
Larvae	2	1	0	0
Total	96	16	1	1

Table 10 and Fig. 2 show the number and prevalence of conferred positive ticks / month.

Table 10
prevalence of ticks positive conferred / month

Month	Average number of ticks per month
January	1
March	3
April	13
May	38
June	30
July	12
August	5
September	6
October	5
November	1

Discussion

This study describes a 3-year survey of ticks and tick-borne pathogens circulating in different areas of North-Western Italy. The results revealed a wide distribution of tick-borne pathogens in this area. *Ixodes ricinus*, one of the most abundant ticks in Italy, confirms to play an important role as a source of infection: this species was the most frequently detected and also the most common species found on humans. This finding is in accordance with a previous study reporting that *I. ricinus* was widespread in woodland areas of North-Western Italy, where *Ixodes* ticks find optimal conditions in terms of temperature (i.e. 20–23 °C) and relative humidity (i.e. 85–98%) for its development (Tagliapietra et al. 2011). Due to of their small size, the majority of ticks collected from people in the present study were nymphs (60%), which might be easily overlooked (Wilhelmsson et al. 2013).

Rickettsia was the most frequently pathogenic genus detected, with 76 out of 500 positive ticks (prevalence 15%; 95%CI 12.17–18.65%). Sequencing showed the presence of three *Rickettsia* species: *R. helvetica*, *R. monacensis*, and *R. slovacica*.

These *Rickettsiae* are part of the Spotted Fever Group; *R. monacensis* was first isolated in Germany and it is widespread throughout Europe in *I. ricinus* tick vectors. Its prevalence in ticks reaches 34.6% in some European countries (Oteo and Portillo 2012). To date, it has been identified in human cases with symptoms similar to MSF (Mediterranean Spotted Fever-like), in Spain (Jado et al. 2007) and in Sardinia island (Italy), where the disease is now considered endemic (Madeddu et al. 2012). In the past, *R.*

helvetica has been associated with patients with perimyocarditis (Nilsson et al. 1999), fever and skin rash (Nilsson 2009) and, more recently, with subacute meningitis (Nilsson et al. 2010). Its main carrier is *I. ricinus* and its prevalence varies in European countries (Oteo and Portillo 2012).

Regarding *Borrelia burgdorferi* s.l., our study confirmed the presence of the complex in ticks from Piedmont even though the number of positive ticks was lower (32 out of 500) with a prevalence of 6,4% (95%CI 4.42–8.91%). Sequencing allowed to identify five genomic groups of *B. burgdorferi* s.l. complex: *B. afzelii*, *B. burgdorferi* s.s., *B. garinii*, *B. lusitaniae*, and *B. valaisiana*. Among these species, three are considered pathogenic to humans, in particular *B. afzelii* is mainly associated with skin manifestations, *B. burgdorferi* s.s. is the agent of Lyme arthritis, while *B. garinii* is the only species linked to neuroborreliosis (Rauter and Hartung 2005). *B. lusitaniae* and *B. valaisiana* are instead of uncertain pathogenicity. Our study adds new knowledge about the distribution of the genospecies of the *B. burgdorferi* complex, which is not only of ecological and epidemiological interest, but also of clinical relevance.

Ecological and climatic conditions of North-Western Italy are favourable to the persistence and spread of ticks and this has certainly favoured the growing exposure of the population to the bite of these parasites. In our study, the number of ticks collected yearly was variable, ranging from 239 to 624 samples, and this likely reflect climatic conditions, considering the fact that sampling has not been systematic in the three years. In fact, while 2017 was quite a dry year, the period between spring and summer 2018 was characterized by abundant rainfall, so much to rank May 2018 as the seventh wettest month since 1958 (source ARPA Piedmont - Forecast Systems). The hot temperatures recorded in the subsequent summer, which however remained average (source ARPA Piedmont - Natural Risks), thus created the ideal conditions for an escalation of the parasitic environmental pressure. As a consequence, there was a considerable increase in episodes of tick bites in humans in Piedmont between May and July 2018. Also in 2019, the highest number of ticks was registered in May and June.

Most of the patients in our study were bitten during a walk in the woods, which represents the ideal habitat of *I. ricinus*, also named “wood tick”. However, high tick presence, even in non-wooded environments, emerged from the anamnestic data collected from physicians who conferred ticks for analysis. Overall, the finding of pathogens in 24% of the analyzed ticks underlines the importance of surveillance, prevention, and correct diagnosis in the human sector. Increase in tick-borne disease prevalence and transmission are important public health issues. The geographic spread of tick species caused by micro- and macroclimate changes, human behaviour, land use, vector population growth, and many other factors has allowed tick-borne bacterial diseases to follow in their wake. As we continue to discover new species of bacteria, it is important to monitor the emergence of both new and existing pathogens. Tick surveillance and tracking enhance our understanding of tick spread and ecology helping to identify areas of risk for disease transmission.

It is important to raise awareness of the population; personal protective strategies can help in the prevention of tick-borne diseases. Exposure to ticks may also result from exposure to domestic and

companion animals that bring ticks into the house. Tick prevention in pets using repellents and tick checks after domestic animal exposure may assist in prevention. As tick-transmitted pathogens are discovered and emerge in new geographic regions, our ability to detect, describe, and understand the growing public health threat must also grow to meet the challenge.

Conclusions

The risk of tick-borne diseases in humans is associated with local tick abundance, infection prevalence, density of vertebrate reservoir hosts, climate changes.

Moreover, important is the local information campaign. Our results show that humans bitten by ticks in North-Western Italy are at risk of infection by different pathogens in agreement with data by Otranto et al. (2014). Co-infected ixodids were detected thus indicating that more than one pathogen may be transmitted by the same tick with potential for multiple infections in humans. Further analysis of these factors may help in assessing risks and to guide the implementation of public health policies against tick-borne diseases.

Abbreviations

TBDs

tick-borne diseases

FBM

Mediterranean Botton Fever

TBE

tick-borne encephalitis

IZS PLVA

Istituto Zooprofilattico Sperimentale of Piedmont, Liguria and Valle d'Aosta

PCR

polymerase chain reaction

MSF

(Mediterranean Spotted Fever-like)

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

T.A. and A.P. analyzed data and wrote the manuscript. V.B. carried out DNA extraction. A.F., F.V. performed molecular investigations on the parasites. V.C., C.G., B.I. carried out tick identification. M.P., M. B., G.C. collected and submitted ticks from humans. S.P. performed Sanger sequencing for PCR confirmation. V.B., A.T. performed molecular analysis for confirmation at species level of pathogens. L.T., M.C., C.C. critically revised the manuscript. R.D. carried out the statistical analysis and supervised the study. All Authors read and approved the final version of the manuscript.

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Figures

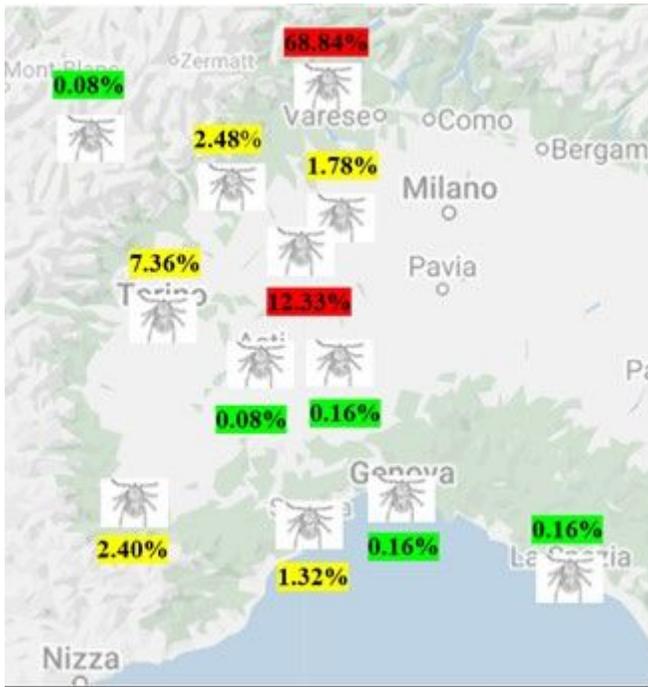


Figure 1

Map showing the geographic distribution (%) of ticks collected in North-Western Italy.

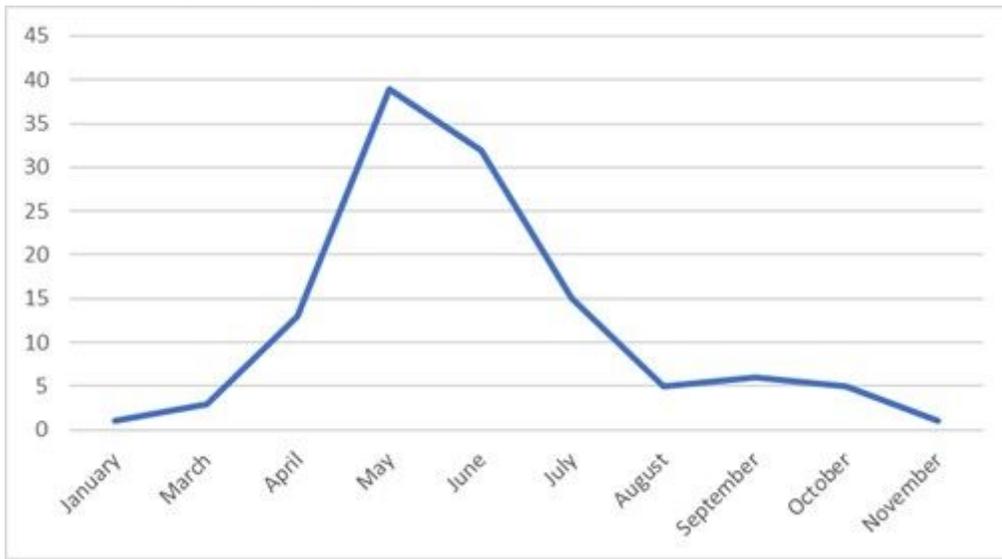


Figure 2

Prevalence of positive ticks conferred / month.

Supplementary Files

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