



Short Communication

Survey on tick-borne pathogens in ticks removed from humans in Northwestern Italy



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ABSTRACT

Ticks are able to transmit several pathogens to the host while feeding, and thus are considered the most important vectors of infectious agents together with mosquitos. The global incidence of tick-borne diseases (TBDs) is rising, due to increased interactions between pathogens, hosts and vectors, linked to global changes. Given that information about the prevalence of tick-borne pathogens in ticks removed from humans in Italy are scarce, the aim of the present study was to identify the species of ticks biting humans in Northwestern Italy and tick-borne pathogens they harbour. An overall number of 128 ticks from 92 patients were collected from April to October 2018, almost 98% of which belonging to the *Ixodes ricinus* species. Molecular analysis showed the presence of *Babesia* spp. in 29 out of 93 analysed tick pools, with a Minimum Infection Rate (MIR) of 31.18% (29/93; CI95% 22.67–41.19%), while 1 out of 93 pools tested positive for SFG *Rickettsiae* (MIR = 1.08%; CI95% 0.19–5.84%). No samples tested positive for *A. phagocytophilum* and *Borrelia* spp. Sequencing revealed the presence of *Babesia venatorum* (28 pools), *Theileria buffeli/orientalis* complex (1 pool) and *Rickettsia monacensis*. Among these, *B. venatorum* and *R. monacensis* are zoonotic pathogens able to cause from moderate to severe infections in humans. These data highlight the importance of passive surveillance to assess the epidemiology of TBDs that pose a threat to human health.

1. Introduction

Ticks of the Ixodidae family are, together with mosquitoes, the most important vectors of pathogens with medical and veterinary importance worldwide (Dantas-Torres and Otranto, 2016). They are able to transmit several pathogens such as viruses, bacteria and protozoa while feeding on the host, e.g. Crimean-Congo haemorrhagic fever virus, *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and *Babesia* spp. (Jongejan and Uilenberg, 2004). Moreover, tick bites have been recently associated to the onset of red meat allergy in humans (Van Nunen et al., 2009). In the last decades, due to several factors as climate and habitat changes, human encroachment into natural areas and wildlife population increase, the incidence of tick-borne diseases (TBDs) is globally rising (Colwell et al., 2011). In Italy, several papers have been published on the distribution of ticks and TBDs in animals and environment (Ceballos et al., 2014; Maurelli et al., 2018), while information about ticks collected from humans are limited to few regions (Otranto et al., 2014; Beltrame et al., 2018). As a consequence, no

data are present for Piedmont, one of the largest regions of Italy hosting more than 4 million inhabitants. The present study aims to identify, through a passive surveillance system, the species of ticks biting humans in this area and the tick-borne pathogens they harbour.

2. Materials and methods

The survey was conducted from January to December 2018 in 6 healthcare facilities in the western part of the region, in the Metropolitan area of Turin. Following the patient's informed consent, ticks were collected from humans reaching the healthcare facilities for a tick bite and stored in ethanol 70% until further processing. Moreover, some information about the age, gender, jobs and frequented environments were collected using a questionnaire. Up to 3 months after the tick collection, patients were interviewed by phone about the presence of signs and symptoms related to the tick bite. Ticks were identified to the species or complex level by using the standard morphological keys (Estrada-Peña et al., 2004) and DNA was extracted by using the

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TRIreagent (Sigma-Aldrich, St. Louis, MO, USA) following manufacturer's instructions. For pathogen detection, ticks from each patient were pooled based on the species and the developmental stage. The presence of piroplasmids, *A. phagocytophilum*, SFG *Rickettsiae* and *Borrelia* spp. was investigated with protocol described elsewhere (Roux et al., 1996; Liebisch et al., 1998; Brown et al., 2001; Zanet et al., 2014). Positive amplicons were sequenced (Macrogen Europe, Spain; LGC Genomics, Germany) and obtained sequences were compared with those deposited in GenBank®. Logistic regression was performed by using R software (3.5.1) (R Development Core Team, 2018), to investigate possible risk factors for TBD infection.

3. Results and discussions

An overall number of 92 patients (55 men and 37 women) were enrolled in this study, with a median age of 34 (range 2–81). The majority (62%, 57/92) of the patients reported the tick bite during outdoor activities (hiking, mushroom picking), followed by leisure activities as gardening (28.3%, 26/92) and job activities (forestry workers) (3.3%, 3/92). Remaining patients did not specify anything in the questionnaire. All the tick bites occurred in the geographical area of the study, except one patient that reach the healthcare facilities after a travel in Sardinia region (Italy).

One hundred and twenty-eight ticks were collected, most of them belonging to the genus *Ixodes ricinus* (97.7%, 125/128), while three specimens were identified as *Rhipicephalus sanguineus* s.l. (2.3%, 3/128). Nymphs were most frequently collected (78.9%, 101/128), followed by adults (13.3%, 17/128) and larvae (7.8%, 10/128). The three *R. sanguineus* s.l. specimens were a nymph and two adults. Among adults, 5 females were fully engorged at the time of removal. Ticks were collected from April to October, with a peak of frequency in June, from 4 out of the 6 healthcare facilities enrolled in the study. No ticks were collected during cooler months (from November to March).

Overall, 93 pools consisting of all the collected ticks were processed for pathogen identification. Among these, 30 pools (2 larva pools, 26 nymph pools and 2 adult pools all consisting of *I. ricinus*) tested positive for at least one pathogen, with a Minimum Infection Rate (MIR) of 32.26% (CI95% 23.62–42.30%). In particular, *Babesia* spp. showed a MIR of 31.18% (29/93; CI95% 22.67–41.19%), while 1 out of 93 pools tested positive for SFG *Rickettsiae* (MIR = 1.08%; CI95% 0.19–5.84%). No samples tested positive for *A. phagocytophilum* and *Borrelia* spp.

Nine selected positive amplicons for *Babesia* spp. and the positive sample for SFG *Rickettsiae* sequenced, and results revealed the presence of *Babesia venatorum* in 8 out of 9 positive amplicons (MIR = 88.9%; CI95% 56.50–98.01%), with sequences showing 100% similarity to those reported from ticks from the Czech Republic [GenBank: KX857480]. The remaining amplicon was positive for *Theileria buffeli/orientalis* complex (MIR = 11.1%; CI95% 1.99–43.50%), showing 100% similarity to those reported from Asia [e.g. KX965722], while the SFG *Rickettsiae* positive amplicon was identified as *R. monacensis*.

Regarding symptoms, 2 patients (2.15%) reported a local rash in the weeks after the tick bite that heal spontaneously without treatment. Ticks collected from these patients tested negative for all the analysed pathogens. No other patients reported any signs or symptoms that could have indicated a tick-borne infection.

Statistical analysis did not show association between tick positivity for any of the pathogens and possible risk factors as age, gender, frequented environments and activities at the time of tick bite.

Even if the incidence of TBDs is rising (Colwell et al., 2011), few data on ticks and TBDs in humans are available. In Italy, previous studies were carried out in the Southern and Eastern parts of the nation (Otranto et al., 2014; Beltrame et al., 2018). The greater prevalence of *I. ricinus* in comparison to *R. sanguineus* s.l. found in the present study can be explained by the activities carried out by patients at the time of the tick bite and the frequented environments. In fact, *I. ricinus* is the most widespread tick species in Northern Italy that prefer natural areas

characterized by deciduous and coniferous woodland, forests, urban and peri-urban park. For this reason, human encroachment into natural habitats for hiking, gardening or hunting may expose people to contacts with this tick species. In contrast, *R. sanguineus* s.l. is an endophilic tick species preferring animal burrows and nests, and mainly associated to dog presence and warmer climates.

Regarding pathogens, almost a third of all the analysed pools were infected. It is noteworthy that *B. venatorum*, the most prevalent reported species, and *R. monacensis* are zoonotic species able to cause from moderate to severe infections in humans. In particular, *B. venatorum* was recorded as the etiological agent of babesiosis in asplenic and immunocompromised patients in Europe (Yabsley and Shock, 2013), and has the roe deer as reservoir host. In Northwestern Italy, previous findings of this species were reported in wild ungulates (Zanet et al., 2014), ticks collected from owned dogs (Ferroglio et al., in prep.) and questing ticks (Ferroglio et al., in prep.), highlighting the circulation of this parasite at the sylvatic-domestic interface. Moreover, human cases of babesiosis are increasingly reported in Europe, likely due to both an increase in the actual incidence as well as increased awareness of the disease (Homer et al., 2000). In contrast, *T. buffeli/orientalis* complex consist of several isolates with uncertain phylogeny and no zoonotic potential, even though is responsible for benign bovine babesiosis (Gubbels et al., 2000). *R. monacensis* is a recently described SFG *Rickettsia* causing a Mediterranean spotted fever-like disease (Madeddu et al., 2012) and its presence has been observed in ticks collected from several urban parks across Europe (Simser et al., 2002; Szekeres et al., 2016). With nearly 85,000 cases of Lyme borreliosis per year in Europe (ECDC, 2011), *Borrelia burgdorferi* s.l. is an important threat for human health. Although no ticks tested positive in our study, up to 7% of ticks collected from humans were infected with this pathogen in Northern Italy (Beltrame et al., 2018). Together, these data highlight the importance of passive surveillance to assess the epidemiology of TBDs that pose a threat to human health.

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Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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