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Wildlife-domestic animals interface: “g-local”
changes and risk for human health.

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Summary – English

In Europe, climate change and policies in management of land and natural environments are transforming the interface between wildlife, domestic animals and humans, leading to an increased circulation of pathogens through it. In this scenario, ticks and tick-borne pathogens are of particular concern, as they are, respectively, key vectors and agents of emerging infectious diseases. Shared spaces such as hiking trails may represent a relevant transmission point. The rising incidence of tick-borne zoonoses and the complexity of their circulation highlights the need for comprehensive epidemiological research on wildlife-associated and vector-borne diseases, which has, in recent years, gathered increasing attention from the scientific community.

An integrated surveillance approach should account for multiple host species (wild and domestic animals, humans, including the environment) and employ diverse data collection methodologies. Citizen science projects, for instance, have been proved valuable in monitoring the distribution of wildlife and tick populations, providing broad-scale ecological and epidemiological data that can integrate the ones produced by the scientific community. Meanwhile, camera traps offer high-resolution insights into wildlife dynamics, generating detailed data crucial for epidemiological modelling and assessing the host-pathogen relationship. Monitoring the temporal occupancy of wild ungulates (deer species and wild boar) and mesocarnivores, these datasets can improve predictions of tick abundance as well as the prevalence of tick-borne pathogens such as Piroplasmids, Anaplasmataceae, *Borrelia burgdorferi* or Rickettsiae of the Spotted Fever Group, especially when targeting single tick species and life stages like *Ixodes ricinus* nymphs, recognized as posing the highest transmission risk to humans. Also, they highlight the different impact that host species and external factors such as seasonality and the environment can have on ticks' abundance and their pathogens' prevalence.

Despite an increasing awareness of the risk of exposure, individuals engaged in outdoor recreational or professional activities experience high rates of tick-borne infections, with prevalence values often comparable across different host groups such as game animals, hunting dogs, questing ticks and hunters. Given this widespread pathogen circulation, a holistic surveillance framework is necessary to integrate ecological, epidemiological, and citizen science data. This interdisciplinary approach will enhance predictive models, refine risk assessments, and support targeted public health interventions.

Summary – Italian

In Europa il cambiamento climatico e le politiche di gestione del territorio e degli ambienti naturali stanno trasformando l'interfaccia tra fauna selvatica, animali domestici e esseri umani, favorendo una maggiore circolazione di patogeni. In questo contesto, le zecche e i patogeni trasmessi da zecche rappresentano una particolare preoccupazione, in quanto, rispettivamente, vettori chiave e agenti di malattie infettive emergenti. Spazi condivisi, come i sentieri escursionistici, possono costituire punti di trasmissione rilevanti. L'aumento dell'incidenza delle zoonosi trasmesse da zecche e la complessità del loro ciclo di trasmissione evidenziano la necessità di una ricerca epidemiologica approfondita sulle malattie associate alla fauna selvatica e a vettori, un ambito che negli ultimi anni ha ricevuto crescente attenzione dalla comunità scientifica.

Un approccio di sorveglianza integrato dovrebbe considerare più specie ospiti (animali selvatici e domestici, esseri umani, oltre all'ambiente) ed impiegare metodologie di raccolta dati diversificate. I progetti di citizen science, ad esempio, si sono dimostrati strumenti preziosi per il monitoraggio della distribuzione della fauna selvatica e delle popolazioni di zecche, fornendo dati ecologici ed epidemiologici su larga scala che possono integrare quelli prodotti dalla comunità scientifica. Parallelamente, l'uso di fototrappole consente di ottenere informazioni ad alta risoluzione sulla dinamica della fauna selvatica, generando dati dettagliati fondamentali per la modellizzazione epidemiologica e la valutazione della relazione ospite-patogeno. Monitorando l'occupazione temporale di ungulati selvatici (come cervidi e cinghiali) e mesocarnivori, questi dataset possono migliorare le previsioni sull'abbondanza di zecche e sulla prevalenza di patogeni trasmessi da zecche, come Piroplasmidi, Anaplasmataceae, *Borrelia burgdorferi* o *Rickettsia* del gruppo causante febbre bottonosa. Ciò è particolarmente rilevante quando l'analisi si concentra su determinate specie di zecche e fasi del loro ciclo vitale, come le ninfe di *Ixodes ricinus*, che rappresentano una rilevante minaccia dal punto di vista zoonotico. Inoltre, tali modelli permettono di evidenziare il differente ruolo che le specie ospiti e fattori esterni, come la stagionalità e le caratteristiche ambientali, possono avere sull'abbondanza delle zecche e sulla prevalenza dei patogeni che veicolano.

Nonostante una crescente consapevolezza del rischio di esposizione, gli individui coinvolti in attività ricreative o professionali all'aperto continuano a registrare elevati tassi di infezioni da patogeni trasmessi da zecche, con valori di prevalenza spesso comparabili tra diversi gruppi ospiti, tra cui ungulati selvatici, cani da caccia, zecche in cerca di ospite e cacciatori. Considerata l'ampia circolazione di questi patogeni, è necessario adottare un quadro di sorveglianza olistico che integri dati ecologici, epidemiologici e di citizen science. Questo approccio interdisciplinare permetterà di migliorare i modelli predittivi, affinare le valutazioni del rischio e supportare interventi mirati di sanità pubblica.

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1. Introduction

1.1. The wildlife-human interface and shared diseases

1.1.1. Expansion and characterization of the interface

In the past century, the European continent has registered a human population growth of 30%, with almost 76% residing in urban areas (<https://www.worldometers.info/world-population/europe-population/>). This process has resulted to an increase in livestock and pet animals numbers (Vicente et al., 2021) and has impacted the natural areas. Indeed, in the past decades the deforestation trend has reverted, the contribution of Europe to the global agriculture added value has been reduced (FAO, 2015) and the forest surface has grown in most, if not all, EU countries (Fuchs et al., 2015), with soil coverage of mature forests of deciduous trees progressively increasing (Vicente et al., 2021). Consequently, despite the biodiversity loss due to human-mediated habitat change, biodiversity remains significantly high, particularly in areas such as the Mediterranean basin or the Alpine area (Vicente et al., 2021). Fragmented landscapes, with an increase in suburban areas, are already boosting the overlap of the presence of wild, domestic animals and humans (Pătru-Stupariu et al., 2024).

Factors causing disease emergence at the wildlife-livestock interface involve both global and local factors (Martin et al., 2011). Global factors are related to the polarization of agriculture between intensification and extensive breeding, climatic and environmental changes, the latter including resource exploitation, habitat fragmentation and consequent species overabundance and changes in biodiversity, human population growth and change in distribution (urbanization) and, finally, microbial adaptations consequence of vaccine and antibiotics generalized use (Gortázar et al., 2016; Martin et al., 2011). Local factors instead involve the population dynamics of species, farming practices, hunting management, which is also facing the ageing of hunters demographic and the increasing trend in introducing artificial feeding, and the publics' behaviour towards wild spaces (Gortázar et al., 2016; Martin et al., 2011). In more detail, anthropogenic factors generally belong to two different categories: human encroachment into wildlife habitat, and increasing interest in wildlife, which generates socio-political conflicts.

Increased livestock production has also coincided with a growing consumer demand for high-quality and more animal-friendly open-air production. To match such requests, extensive breeding exposes animals to direct or indirect contact of animals. Bovine tuberculosis (caused by *Mycobacterium bovis*) is in certain parts of the world a disease transmitted that heavily involves wildlife for its maintenance and transmission. Species such as badgers (*Meles meles*) or wild ungulates are the main source of infection for cattle (Palmer et al., 2023). However, pathogens such as Classical and African Swine Fever

Viruses (CSFV, ASFV), or *Brucella* spp., pose a concrete threat to high-biosecurity farms as well (Bellini et al., 2021).

Ninety per cent of the European continent can host from one to five species of wild native ungulate species (ENETWILD-consortium et al., 2021). Rural abandonment, agriculture intensification (which can lead to increased availability of alimentary resources for wildlife) and increasing forested habitats, are favouring the population growth of wild boar (*Sus scrofa*) and several wild ruminants (Massei et al., 2015; Milner et al., 2006). Climatic change may have also contributed, through the improvement of the food supply, to the increased abundance of wild ungulates (Jedrzejewska et al., 1997). As a consequence, this may produce damages which extend from economic losses to sanitary risks (Gortázar et al., 2006). The richness of ungulate species is high in Central Europe, including the Alps. Native wild boar, roe deer and red deer are widely distributed across Europe, while mountain ungulates (i.e. *Rupicapra rupicapra*, *Capra ibex*) add diversity in constrained mountainous areas. Apart from fallow deer and mouflon, which are relatively widespread, most introduced species instead tend to be more locally distributed (ENETWILD-consortium et al., 2021). At a European scale, roe deer (*Capreolus capreolus*) is the most abundant wild cervid, while red deer (*Cervus elaphus*) is mainly locally abundant and generally widespread. On the other side, wild boar is expanding its geographical range and numbers throughout Europe, generating concern regarding disease maintenance and emergence (Massei et al., 2015). Additionally, being the most abundant large game species in all of Europe, wild boar can be considered a sentinel species for several diseases (A. C. Abrantes & Vieira-Pinto, 2023). Besides this, in Europe we also assisted in the decline of small game species such as the European hare (*Lepus europaeus*) (Báldi & Faragó, 2007) and ground-nesting birds, and the recovery of large predators such as the wolf (*Canis lupus*), which around the Alpine territory was subject to conservation projects such as the Life Wolf Alps EU (<https://www.lifewolfalps.eu/en/>, accessed on 15th October 2024).

At an administrative level, overlapping of ungulate and livestock is more intense for cattle and pigs in Central Europe and other local areas such as Spain (for the extensive breed pigs, while). For small ruminant livestock, it is more intense in the Mediterranean and Balkans region. Factors such as livestock husbandry, landscape and land use, as well as wildlife management practices, can locally influence the interface. In some cases, the distribution is linked to protected areas, which are crucial points for the study of the interface, of shared diseases and control options (ENETWILD-consortium et al., 2021).

1.1.2. Shared emerging diseases at the interface

Deer species can act as relevant hosts for shared infections and zoonoses, including also foodborne zoonoses (toxoplasmosis or salmonellosis) and vector-borne diseases (anaplasmosis, piroplasmosis, bluetongue) (Abrantes & Vieira-Pinto, 2023). Wild bovids, such as the Alpine ibex (*Capra ibex*), may be also relevant sources of infection at a local

scale, as it has happened during a *Brucella melitensis* spill-over outbreak from Alpine ibex to cattle (Mick et al., 2014). On the other side, wild boar is an important reservoir of pathogens such as Hepatitis E, Bovine tuberculosis, CSF or ASF (Abrantes & Vieira-Pinto, 2023; Vicente et al., 2021) and the economic and sanitary relevance of such diseases is the reason for the concern at the expansion trends of this species (Massei et al., 2015).

Peridomestic mice and rats play a crucial role as bridge hosts for zoonotic bacterial pathogens like *Salmonella* and *Leptospira* (Himsworth et al., 2013; Strand & Lundkvist, 2019), as well as serving as intermediate hosts for *Toxoplasma gondii* and *Neospora caninum* (e.g. Krücken et al., 2017; Muradian et al., 2012), which have significant implications for human health, in the former, and livestock health and management, in the latter. Small rodents also act as reservoirs for emerging tick-borne pathogens such as *Borrelia burgdorferi* or tick-borne encephalitis virus (TBEv). As mentioned, European badgers are recognized as important maintenance hosts for *Mycobacterium bovis*, the primary agent of animal tuberculosis, complicating efforts to eradicate this zoonotic disease in livestock (Palmer et al., 2023). Canids such as the widespread red fox (*Vulpes vulpes*) and the expanding wolf (*Canis lupus*) are involved in the transmission cycles of various viral, bacterial, and parasitic infections, including *Leishmania infantum* (Millán et al., 2014), a zoonotic vector-borne disease expanding due to climate change, and hydatidosis (*Echinococcus granulosus* in wolves and *E. multilocularis* in foxes) (Dakkak, 2010). Recently, lagomorphs such as hares (*Lepus* spp.) have also been shown to serve as maintenance hosts for *L. infantum* (Millán et al., 2014; Ruiz-Fons et al., 2013).

Wildlife can also play a dual role in vector-borne diseases, as hosts for the pathogen and for the vector, and, additionally, contribute to the disease spread allowing co-feeding transmission. The mentioned phenomena of environmental changes in their broadest concept are driving certain vector species of animal and human health relevance to spread both geographically and in density (Gortázar et al., 2016). This expansion involves several species and pathogens, such as sandflies and leishmaniasis, mosquitoes and driven infections and ticks and tick-borne diseases, including Lyme disease, TBE and Crimean-Congo Haemorrhagic Fever (CCHF) (Adepoju et al., 2023; Semenza & Suk, 2018; Swei et al., 2020). Geographical expansion has also been registered for pathogens such as *E. multilocularis*, which is progressively found in new countries in Europe. Changes in competent definitive hosts, such as natural movement and increasing densities of foxes, movement of domestic dogs and the introduction of the raccoon dog, have contributed to this expansion (Vicente et al., 2021).

According to past studies, the most relevant pathogens at the wildlife-livestock-human interface affecting wild ruminants in Europe include *Salmonella*, *Coxiella burnetii*, foot-and-mouth disease virus, *M. bovis*, bluetongue virus, and European tick-borne encephalitis virus (Ciliberti et al., 2015). In swine, the primary pathogens of concern are *Salmonella*, *Campylobacter*, *Escherichia coli*, *Leptospira* spp., hepatitis E virus, swine

influenza virus, and Japanese encephalitis virus (Ruiz-Fons, 2017). A review from 2016 (Gortázar et al., 2016) ranked the relevance of 46 disease agents at this interface based on their relevance to human and animal health, as well as the role of wild hosts in sustaining infections. Of these, 10 were vector-borne diseases (including tick-borne diseases and West Nile fever (WNV), which were defined as having high relevance, and leishmaniasis and bluetongue, with a medium relevance), 27 were zoonotic, and 13 were food-borne diseases, highlighting the complexity and breadth of health risks at this interface. The One Health working group of the European Food Safety Authority (EFSA) identified 10 priority zoonotic pathogens for integrated surveillance from a list of 50 emerging diseases (ENETWILD-consortium et al., 2022b). Such pathogens include the CCHFv, *E. granulosus/multilocularis*, Hepatitis E virus, Highly Pathogenic Avian Influenza virus (HPAIV), *B. burgdorferi sensu lato*, *C. burnetii*, Rift Valley Fever virus (RVFV), Swine Influenza A virus, TBEv and WNV.

Dealing with pathogens at this interface requires also an extensive consideration of stakeholders. The decline of hunters Europe-wide can pose a problem in the control of some species, especially wild boar (Frank et al., 2015; Massei et al., 2015). However, in rural societies in particular, the increasing popularity of game meat products, which have also become more accessible, expanded the interface for the transmission of foodborne pathogens (Navarro-Gonzalez et al., 2016). The rural view of animals that was diffused in the past century has been converted into a more conservationist and animalist approach, which requires a more careful approach to decision-making (Gortázar et al., 2016; Vicente et al., 2021). In general, a One Health or integrated approach is required to plan effective surveillance strategies and control interventions at the interface.

1.2. Wildlife integrated monitoring

The prevalence of a pathogen is among the most basic parameters that are used to assess and manage infectious diseases. Prevalence is numerically composed of two parts, the *numerator data*, the number of infected individuals, and the *denominator data*, the size of the target population. However, information is often lacking on one side or the other (Cardoso et al., 2022; Gortázar et al., 2016). Most current wildlife disease surveillance plans lack appropriate population size monitoring (also called *The Missing Denominator*; Stallknecht, 2007), which hinders a proper spatiotemporal assessment of trends and the effectiveness of interventions (Cardoso et al., 2022; Gortázar et al., 2016).

Early and accurate detection of diseases, as well as precise estimations of prevalence and temporal trends are fundamental to generating good quality numerator data. This would require a combination of passive and active surveillance (Gortázar et al., 2016). Passive disease surveillance will improve the likelihood of early detection of emerging diseases, while active surveillance and population monitoring are required to assess epidemiological dynamics and the outcome of interventions on endemic ones. However, long-term and large-scale surveillance data on wildlife disease prevalence are strikingly

scarce. In consequence, there is no clear indication that wildlife health is improving or worsening over time (Cardoso et al., 2022).

Pioneer programs in addressing such issues are the EU4Health One Health Surveillance Grants and the ENETWILD project (<https://enetwild.com/>, accessed on 15th October 2024), a consortium of scientists working with wildlife across Europe funded by EFSA, to enhance European capabilities in monitoring wildlife health under the One Health approach.

1.2.1. Disease surveillance involving wildlife

With many diseases being transboundary and with wildlife being implicated in most of them, integrated disease surveillance under the One Health approach is a fundamental step for effective disease surveillance and control. However, knowledge of wildlife health is often scarce, incomplete, isolated and not comparable at geographical and temporal scales (Barroso et al., 2021).

Globally speaking, the oldest surveillance system for animal diseases was created by the World Organization for Animal Health (WOAH), which expanded to include wild animals at the beginning of this century. Virtuous examples of extensive disease surveillance in wildlife are also present in Australia (<https://www.wildlifehealthaustralia.com.au/>), and Canada (<http://www.ccwhc.ca/>).

In Europe, there are at least 360 surveillance programs operating under the One Health framework, each targeting a specific cross-border pathogen across most Member States. However, several constraints hinder the effective implementation of integrated programs, spanning multiple aspects (ENETWILD-consortium et al., 2022b). Despite progress in the animal health sector, which has shown the most openness to cross-institutional collaboration, further integration across sectors and enhanced international cooperation remain essential (as discussed in Gortázar et al., 2016), as widely distributed species require a common transboundary approach over Europe. In addition to fostering collaboration, the development of appropriate legislation is also crucial. Another critical gap in the current surveillance efforts is the lack of regular evaluation procedures, which are necessary to ensure the effectiveness and adaptability of these programs (ENETWILD-consortium et al., 2022b).

Ideally, an optimal surveillance strategy would combine both passive and active surveillance strategies; however, this is applied in fewer than one-third of cases, with passive surveillance being disproportionately favoured (ENETWILD-consortium et al., 2022b). For example, ASF in wild boar will go undetected without a careful investigation of sick animals and carcasses, requiring a scanning surveillance approach, while tuberculosis surveillance would benefit from a systematic monitoring of prevalence in a targeted surveillance (Gortázar et al., 2016). Passive surveillance, though potentially subject to bias depending on the source of reports or samples, is particularly well-suited

for integrating data from samples collected for other purposes, aligning with a One Health approach. Active surveillance, while requiring additional resources for both development and data interpretation, can provide more timely and comprehensive information. Notably, programs involving cross-sector collaboration tend to adopt a higher proportion of combined surveillance strategies, emphasizing the added value of cooperation for more robust surveillance systems (ENETWILD-consortium et al., 2022b). Coordinated efforts are also evident in programs aimed at evaluating control or eradication strategies, further illustrating the importance of collaboration in achieving more effective outcomes (ENETWILD-consortium et al., 2022b).

Assessing the One Health surveillance systems established under the EU4Health framework, which included a total of 246 activities, it was highlighted that the most represented target was the environment, showing great interest of member states in non-invasive disease surveillance strategies, which have great potential in terms of cost-effectiveness and early detection. While domestic ruminants remain the most frequent animal target, the inclusion of wild species in surveillance programs, from carnivores to ungulates, shows potential to expand passive surveillance to new targets (ENETWILD-consortium et al., 2024b). While the number of pathogens included per single surveillance program is normally low, and multiple pathogen taxa are often not much represented, the inclusion of species in the view of a multi-host and multi-pathogens surveillance would indeed be beneficial for effective surveillance (ENETWILD-consortium et al., 2022b).

In the view of exhaustive data collection, new data sources should be considered. As a (virtuous) example, citizen science projects for data collection have been in some cases also extended to disease surveillance. For instance, they have been implemented to collect data on ticks and Lyme disease, showing moderate correspondence with direct surveillance (Tran et al., 2021), or for avian flu detection, pointing out mortality events in contemporary or in some cases even earlier than the outbreaks recorded by official sources (Saavedra et al., 2023). Citizen science tools for monitoring wildlife presence, such as the Mammalnet (<https://mammalnet.com/>, accessed on 16th October 2024) project and its app iMammalia, allowing public sharing of geographically localized photos of dead or alive animals have been used for wild boar carcass signalling in Serbia (Palencia et al., 2023a). The benefits of such an approach for integrating data collection are various, and can involve not only the scientific community, but also the communication with and the education of the general public, with an increased engagement and a broader source of data. Biases in reporting and the need to correctly address the public to extend the use of citizen science tools are limits that should be considered in data analysis (Lawson et al., 2015; Tran et al., 2021).

Additionally, algorithms such as epidemic intelligence (EI) and syndromic surveillance are being perfected. EI encompasses all activities related to the early identification of

potential health hazards, and their verification, assessment and investigation so that appropriate public health control measures can be recommended (definition by Paquet et al., 2006). EI integrates both an indicator-based and an event-based component. 'Indicator-based component' refers to structured data collected through routine surveillance systems. 'Event-based component' refers to unstructured data gathered from sources of intelligence of any nature (Paquet et al., 2006). A Europe-wide survey has, for instance, detected 17 examples of epidemic intelligence activities monitoring HPAI in animals, 7 for WNV in animals, and 7 of leptospirosis and tularaemia, while comparable numbers (e.g. 9 activities for WNV, or 10 for seasonal influenza) are obtained for activities targeting humans (Dub et al., 2023). In this optic, platforms such as the Epidemic Intelligence from Open Sources (EIOS), an initiative from the World Health Organization, can be used to integrate open source (digital newspaper articles for instance) data to implement disease surveillance (<https://www.who.int/initiatives/eios>, accessed on 16th October 2024).

1.2.2. Harmonization of population data

As mentioned, proper homogeneous and constant population monitoring is as fundamental for effective surveillance as it is lacking in several countries (Gortázar et al., 2016).

Several methods are available to estimate wildlife population size. An appropriate method should produce accurate and reliable data, have the potential to be used for cross-validation or return results comparable with other areas, and present good sustainability. Generally speaking, while several methods can be implemented on a local scale, factors such as habitat (including geographical, vegetational and land use conditions), weather conditions, species biology and habits and other ecological factors must be considered to select the best method to estimate animal density (ENETWILD-consortium et al., 2018, 2020a, 2020b). Camera trapping (CT) has potentials to be the most effective method, showing a good cost-effort balance, high accuracy and precision also in low densities, and applicability in most conditions (ENETWILD-consortium et al., 2018, 2020a, 2020b). Additionally, algorithms which do not require individual recognition such as the Random Encounter Method (REM) (Rowcliffe et al., 2008) can make this methodology even more effective. The European Observatory of Wildlife (EOW) has created a standardized methodology to acquire wildlife density data through a network of study areas across Europe, implementing the REM approach showing reliable estimations for wild ungulate (ENETWILD-consortium et al., 2022c, 2023b). In the European context, camera traps were implemented to study mesocarnivore species such as the pine marten (*Martes martes*) in Northern Ireland (Tosh & Twining, 2017) and the red fox in Spain (Barroso & Palencia, 2024). Depending on the species and the habitat type, other methods may be implemented. For example, in open areas, for the most robust outcomes, estimation of wild ruminants density should be addressed through direct detection of

animals. Also, for wild carnivores, a combination of methods, usually trapping, marking, telemetry and CT, seems to be the best-suggested approach.

Hunting statistics, including raw hunting bag data, are useful to study large-scale phenomena such as long-term trends or large-scale patterns. Limitations for their use include the availability of biological, ecological and, mostly, anthropogenic factors (first of all, the hunting effort) that can affect such statistics. However, standardized hunting data have the potential to be used as relative abundance or for predictive spatial modelling at a large scale (ENETWILD-consortium et al., 2019b, 2024a), because of having the highest availability across broad regions (even the whole continent) and, therefore, comparability potential. Additionally, hunting statistics alone may not be enough, as calibration with more accurate density estimation methods is often required at the local scale. Comparing it with occurrence data models, hunting bag statistics seem to be suitable for roe deer as a proxy of abundance, but not for red deer. Such difference may be linked to the impact of other predictors: for instance, environmental predictors seem to explain better roe deer pattern, rather than management or anthropic factors, which could be more relevant for red deer. Additionally, bioregions seem to be another element heavily impacting model fit (ENETWILD-consortium et al., 2022d).

To properly fit models, the definition of true absence data is another fundamental next step, as this could also help point out areas of potential expansion of the species according to environmental characteristics. A further challenge in this direction regards the quantity, quality and distribution of data. Despite lacking funding in some areas to set up solid and detailed data collection, especially when dealing with transboundary diseases, there is a need to compile and validating abundance data at an international level, harmonizing data collection and standardizing information (ENETWILD-consortium et al., 2022b, 2024b).

1.2.3. Case example: vector-borne surveillance

With their multi-host and often also multi-pathogen dynamic, vector-borne diseases are particularly fit for an integrated surveillance system. Indeed, more than half the prioritized diseases by the One Health EFSA working group are vector-borne (ENETWILD-consortium et al., 2022b), and the environment, which includes also vector surveillance, is the most frequently addressed target. Human cases of Lyme disease, CCHF and TBE are also monitored by the European Centre for Disease Prevention and Control (ECDC). Even if mandatory (especially for those three diseases), the surveillance effort is heterogeneous in many countries, counting on spontaneous reporting in some areas and requiring obligations in others (Garcia-Vozmediano et al., 2022). Likewise, multidisciplinary is not always implemented in the surveillance systems across the continent (Garcia-Vozmediano et al., 2022). Because many aspects of their complicated transmission cycle are under strong influence of environmental conditions (including weather), strategies for their surveillance and further steps may demand approaches

other than or in addition to those developed for other diseases, as indicators not only relate to the occurrence of human disease, but also on the abundance of pathogen and/or vector (Braks et al., 2011). Globalisation and environmental changes, sociodemographic changes and health system capacity are three interacting drivers that can set the stage for novel vector-borne disease scenarios and that should be accounted for (Semenza & Zeller, 2014).

Several types of data can be integrated into such surveillance. The use of proper methods and sampling strategies may contribute to the preparedness of health systems (Garcia-Vozmediano et al., 2022). From a human side point of view, data about pathogen presence, serological, clinical data and syndromic surveillance can be collected (Semenza & Zeller, 2014). This can be done by integrating epidemiological surveillance, reports, literature analysis, and specific surveys, but also with the implementation of sentinel clinics, the exploration of administrative claims data (e.g. private medical insurance), and the mining of lab test data (Clow et al., 2019; Hai et al., 2014; Johnson et al., 2022). Animals can be addressed similarly, but they can be also used as sentinels for vector collection, especially ticks (Clow et al., 2019; Hai et al., 2014). Vector surveillance should be the basis for an integrated approach; the entomological side would include the assessment of vector distribution and pathogen circulation (Garcia-Vozmediano et al., 2022; Hai et al., 2014; Johnson et al., 2022; Semenza & Zeller, 2014). Finally, modelling would be the ultimate approach to implementing surveillance with forecasts (Hai et al., 2014)

Risk data would include the presence/absence and abundance of vectors, environmental factors (climatic elements, landscape design, wildlife presence) and behavioural risk factors (Braks et al., 2011; Semenza & Zeller, 2014). As a general remark, the use of standards or the harmonization of data collection across different approaches and regions allows proper integration of datasets. To be credible, standards should be internationally developed and agreed on (Braks et al., 2011; Clow et al., 2019) and surveillance strategies as international as possible, as arbitrary borders, which do not apply to vectors, can create biases in the results (Clow et al., 2019). Undergoing a comprehensive approach, it should be remarked the importance of raising awareness and increasing knowledge among the general public, public health practitioners, and policymakers about disease vectors and their relationship with infectious diseases (Semenza & Zeller, 2014).

As a final remark, several systems have highlighted the importance of citizen science and stakeholders' involvement in the surveillance. In addition to the advantage of extensive data collection with low effort required from scientists and authorities, returning feedback helps in educating the public and keep engagement (Clow et al., 2019; Garcia-Vozmediano et al., 2022; Johnson et al., 2022). Also, it helps practitioners to correctly perform diagnosis and reporting (Clow et al., 2019). National coordination and an integrated surveillance approach provide evidence-based support for policy and

advocacy to international organization, and highlight community needs, priorities, and capacities (Johnson et al., 2022).

1.3. Tick-borne pathogens as cause emerging diseases

1.3.1. Geographical expansion and increasing abundance of vectors

The latitudinal and altitudinal expansion of ticks in the European continent is already a broadly demonstrated phenomenon in different countries. As for *I. ricinus*, the UK Tick Surveillance Scheme detected an increased coverage from 7.8% in 2013 to 11.2% in 2018 (Gandy et al., 2023). A questionnaire in Sweden detected a northern expansion in 30 years (1980s to 2008), from the areas close to the Baltic Sea to the Northern regions of the country (Jaenson et al., 2012). An increased altitudinal range in the past decades was detected across the whole of Europe, from Norway (De Pelsmaeker et al., 2021) to the Czech Republic and Slovakia (Bullová et al., 2008; Daniel et al., 2003) to the alpine and Mediterranean region (Garcia-Vozmediano et al., 2020; Martello et al., 2014).

Changes in altitude are mainly linked to increased seasonal temperatures, such as mild winters and extended spring and autumn seasons, which extend the period allowing for tick activity (Medlock et al., 2013; Semenza & Suk, 2018). Figure 1 shows how the suitable area for *I. ricinus* across Europe may change in future years according to the different climatic scenarios, and remarking an expansion towards northern areas. As altitude acts as a determinant of climate, thresholds vary across Europe, from 700m above the sea level in the UK to above 1800 m a.s.l. (Garcia-Vozmediano et al., 2020). However, in drier climate conditions such as Greece, no tick was detected below 600m a.s.l. (Papa et al., 2008), remarking humidity being a controlling factor for ticks activity. Despite the decrease in presence, altitude does however impact negatively eggs survival and hatching (Materna et al., 2008), which decreases questing ticks' density. An additional aspect of the altitude effect is that slope orientation can affect tick abundance differently (Burri et al., 2007; Gern et al., 2008).

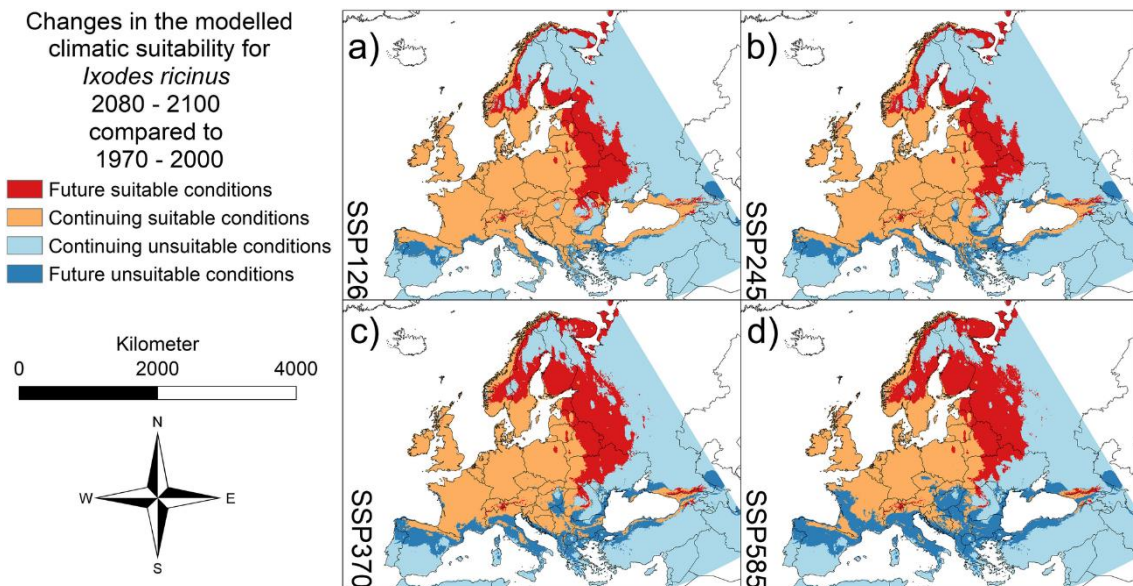


Figure 1. Projected changes in climatic suitability for *I. ricinus* according to the four scenarios derived from the Shared Socioeconomic Pathways. From Cunze et al., 2022.

Together with climatic conditions, the broader coverage of mature forests, and consequent re-population of some wild species such as roe deer, can also influence the change in the distribution of ticks, *I. ricinus* in particular. The recent reporting of such tick species on mammals such as chamois (*Rupicapra rupicapra*), that inhabit traditionally unfavourable conditions for ticks, may suggest the possibility for alternative tick-host interaction cycles under the influence of a more suitable climate (Medlock et al., 2013). Likewise, in certain parts of Germany with stable roe deer populations, large increases in wild boar abundance have been linked with the increase in *I. ricinus* abundance (Schwarz et al., 2009). European policies go towards agri-environmental schemes encouraging habitat defragmentation with the creation of corridors (and urban green corridors in urban areas), thus enhancing connectivity and promoting wild animal movements. As a consequence, this increases the probability of a successful establishment of ticks in new areas. Since factors such as leaf litter, sward height, presence of shrubs and compartmentalization appear to favour tick abundance, woodlands management can also play a fundamental role (Medlock et al., 2013).

Figure 2 shows the complexity of the pattern of factors that affect ticks biology, allowing for their expansion in the geographic range and abundance. While some of them are clearly anthropogenic, the majority of the others, such as climate, habitat structure or host presence, are still affected by changes as consequences of human activity and policies.

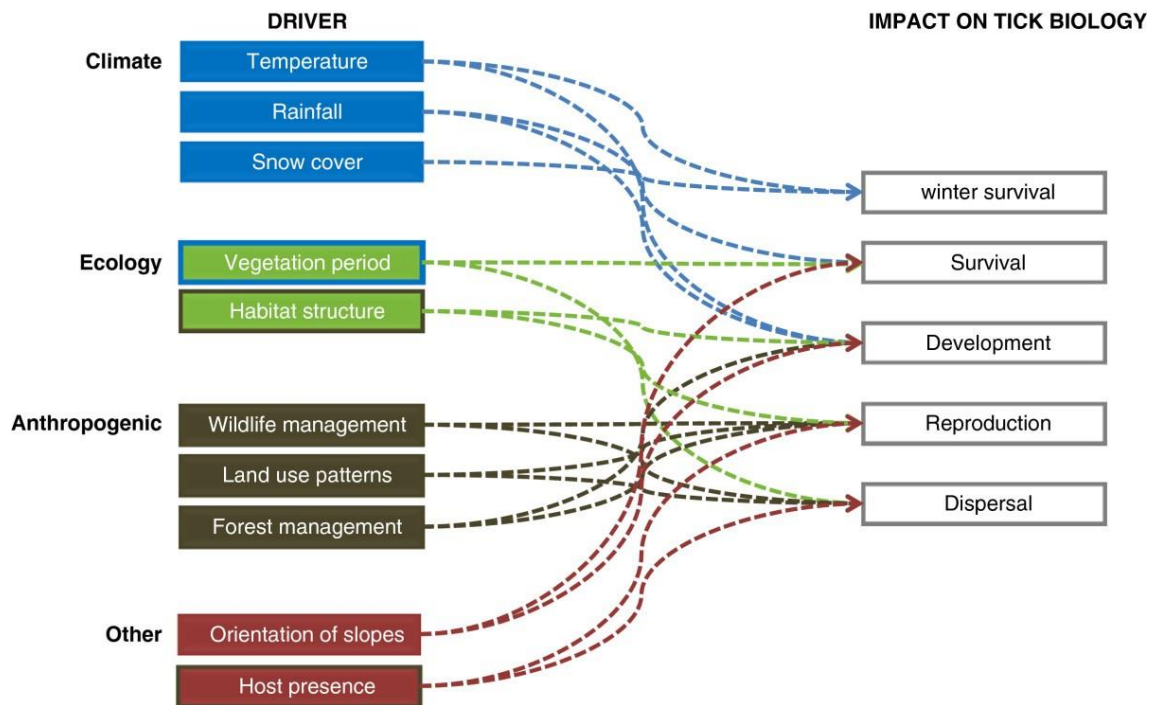


Figure 2. Conceptual framework of drivers for change in geographical distribution of *I. ricinus*. From Medlock et al., 2013.

While those considerations were mostly addressed to the expansion of *I. ricinus*, the most ubiquitous tick species in Europe, similar factors are leading also to the expansion of other species, such as *Dermacentor reticulatus* (Cunze et al., 2022; Földvári et al., 2016; Karbowski, 2014). Large areas of north-western and central Europe, such as Germany, Poland, Hungary, Slovakia, but also the Netherlands and Belgium, formerly thought not suitable for *D. reticulatus* survival and reproduction due to low temperatures, have experienced a remarkable spread of this tick species. Likewise, ticks of *Hyalomma* genera prefer warmer and drier habitats, thus being favoured in the southern part of the continent by the climatic changes and expanding their range, which is now limited to 50° North latitude (Shahhosseini et al., 2021; <https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/tick-maps>, accessed on 21st October 2024).

1.3.2. Emerging tick-borne zoonoses

With the increasing abundance and expanding geographic range of their vectors, tick-borne zoonoses (TBZ) have become some of the most significant vector-borne diseases in Europe (Hartemink & Takken, 2016).

B. burgdorferi s.l. and TBEv are best known because of their relatively high and widespread prevalence and high public health impact (Rochlin & Toledo, 2020). Indeed, the economic burden of Lyme disease includes a cost of tens of millions of euros in several European countries (Mac et al., 2019) and a total burden of 10.55 disability-adjusted life years (DALY) per 100 000 population (van den Wijngaard et al., 2015). Increasing

incidence of this pathogen in the human population up to 2018 was detected in most European countries, up to over 20% (Vandekerckhove et al., 2021). In Europe, several genospecies of the *B. burgdorferi* s.l. complex are pathogenic to humans, such as *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, *B. bavariensis* and *B. spielmanii*, (Hartemink & Takken, 2016). Additionally, an increase in severe forms, the insurgence of new endemic areas and the identification of new possible reservoirs characterize this disease as emerging (Hai et al., 2014). Regarding TBE, the European Surveillance System (TESSy) detected an increasing trend in the continent from 2015 (1857 notified cases) to 2020 (3604 notified cases) (Van Heuverswyn et al., 2023). TBE is extending its burden by extending its areas of circulation (Hai et al., 2014).

Human babesiosis has been reported in almost all European regions with an increasing prevalence. The causative pathogen is not exclusively the well-known *B. divergens*, but other species have been recently detected causing babesiosis cases, such as *B. microti* and *B. venatorum* (Bajer et al., 2022). Human granulocytic anaplasmosis has a lower incidence (estimated below 300 cases in Europe), but it is widespread across the continent, with seroprevalences from around 1% to above 30% depending on the country and the target group (Matei et al., 2019). Human cases are increasing and *Anaplasma phagocytophilum* is emerging in new forms (e.g. congenital infection) and fatal cases (Hai et al., 2014). On the other side, the Mediterranean Spotted Fever, caused by *Rickettsiae* belonging to the spotted fever group, is decreasing the number of cases in European countries, despite being still widespread in the continent (ECDC, 2013). However, newly identified locations in endemic regions, and a northward expansion make it still an emerging issue (Hai et al., 2014). Other zoonotic tick-borne bacteria of interest are *Borrelia miyamotoi*, *Bartonella henselae*, *Candidatus N. mikurensis*, *Coxiella burnetii* and *Francisella tularensis*. Regarding viruses, in addition to TBE, also CCHFv and Louping ill virus are relevant for human health. With 6 new cases in 2024 (6 cases were recorded in Europe, 4 in Spain, 1 in Portugal and 1 in Bulgaria, where it is reported as endemic), CCHFv is emerging due to more favourable conditions of its vector in the southern part of the continent, but still scarcely present (Eslava et al., 2024). On the other side, the Louping ill virus is endemic in some countries such as the UK (Hartemink & Takken, 2016).

With the growing popularity of outdoor activities (Fernandez et al., 2021), the risk of exposure to tick bites is increasing for people dedicated to leisure or professional activities in the natural environment, ranging from hunting activities (Panczuk et al., 2019) to outdoor sport (Blasko-Markic & Socan, 2012; Jore et al., 2020) and even dog walking (Nygren et al., 2022). However, factors related to how the activity is practised (frequency, choice of clothing, use of protective measures, type of habitat crossed and speed) may affect the level of exposure and, therefore, the risk (Velay et al., 2024). However, risk perception of people may vary depending on the target, and, with it, implementation of protective measures (Hassett et al., 2022; Slunge et al., 2019).

1.3.3. The wildlife side: host-tick-pathogen competence

The transmission cycle of tick-borne zoonoses is influenced by transmission competence at three critical levels: the tick's competence as a vector, and the wild vertebrate host's competence both as a reservoir and as a source of pathogen transmission back to the tick, as well as its role as a suitable tick host. Since this is a broad and complex topic, with many aspects still requiring further investigation, the following discussion will focus on the tick and wildlife species, as well as the pathogens, specifically examined in the present study.

I. ricinus is the most widespread and abundant tick species in the natural environment across Europe (ECDC & EFSA, 2023; Salman & Tarrés-Call, 2013). Immature stages typically parasitize small mammals, such as rodents, while adult ticks, and occasionally nymphs, prefer larger hosts like deer, but in general exhibit a broad host range (Kahl & Gray, 2022; Mihalca & Sándor, 2013; Mysterud et al., 2021). Notably, roe deer have been observed to support all life stages of *I. ricinus* (Carpi et al., 2008; Mysterud et al., 2014). In contrast, *Dermacentor marginatus* and *Haemaphysalis* spp. are still widespread but less abundant (ECDC & EFSA, 2023), and exhibit more specialized host preferences. Immature stages of *D. marginatus* are likely nidicolous (Sands et al., 2021). Adults primarily feed on wild boar, but also on wild ruminants, hares, and hedgehogs. *Haemaphysalis* spp. are typically associated with wild ruminants, while immature stages may occasionally parasitize hares, hedgehogs, and rodents (Salman & Tarrés-Call, 2013).

A. phagocytophilum, agent of the human granulocytic anaplasmosis, is the most relevant zoonotic species of the genera *Anaplasma* spp. Ticks of genera *Ixodes* spp. and *Ha. punctata* are associated with its transmission (Salman & Tarrés-Call, 2013). Wild ungulate species and red foxes are considered competent hosts for this pathogen and contribute to its transmission and maintenance in the environment (Cafiso et al., 2021a; Ebani et al., 2011, 2022; Nahayo et al., 2014). Among other Rickettsiales, the SFG Rickettsiae present great relevance around the Mediterranean basin. Despite most species being primarily transmitted by *Rhipicephalus* and *Dermacentor* spp. (e.g. *R. conorii*, *R. slovaca*, *R. aeschlimannii*), *Ha. punctata* can be vector of *R. slovaca* and *R. helvetica*, while *I. ricinus* can transmit *R. monacensis* (M. Salman & Tarrés-Call, 2013). Wild ungulates and mesocarnivores are not recognized as common hosts for such pathogens, despite investigations is proceeding due to the high prevalence of these pathogens in ticks removed from wild boar especially (Castillo-Contreras et al., 2022; Skarphedinsson et al., 2005b).

Ixodes spp. is the main genus transmitting *B. burgdorferi* s.l., and ticks usually acquire the pathogen from insectivores, rodents or hares, or by cofeeding on larger animals (Salman & Tarrés-Call, 2013). Competence for transmission was not identified in deer species (Jaenson & Tälleklint, 1992; Pato et al., 2013), but it was hypothesized for wild boar and red fox (Heidrich et al., 1999; Kurtenbach et al., 1998b).

Piroplasmids include a wide range of both zoonotic and non-zoonotic species, with *I. ricinus* serving as the primary vector. *Babesia divergens* and *B. divergens*-like is recognized as the principal zoonotic Piroplasmid in Europe (Bajer et al., 2022), with wild ruminants, particularly deer species, acting as the primary reservoir (Ebani et al., 2016; Hrazdilová et al., 2020; Razanske et al., 2019; Yabsley & Shock, 2013). *B. capreoli* is likely maintained in the environment by roe deer (Kauffmann et al., 2017; Silaghi et al., 2020; Tampieri et al., 2008). Deer also serve as the wild reservoir for *B. venatorum* (formerly *Babesia* sp. EU1) (Hrazdilová et al., 2020; Remesar et al., 2019; Yabsley & Shock, 2013; Zanet et al., 2014), a zoonotic species with an increasing number of reported cases (Bajer et al., 2022). Eventually, *B. microti* and *B. microti*-like species represent the final relevant group of zoonotic Piroplasmids. The wild reservoirs for *B. microti* typically include small mammals (Hersh et al., 2012; Karshima et al., 2021), while the red fox is a reservoir for *B. vulpes*, which was recently classified within the *B. microti*-like group (Goethert, 2021; Mierzejewska et al., 2021).

Additionally, it is important to remember that host competence for pathogen transmission or as a reservoir may not coincide with anecdotal findings of pathogen DNA in the species, such has been the case of wild boar for several of the abovementioned pathogens (Castillo-Contreras et al., 2022; Kurtenbach et al., 1998b; Sgroi et al., 2023).

2. Aims of the work

To summarize, three key elements define the scope of this study:

- The expanding human-wildlife interface, with increasing exposure to tick-borne pathogens, which are responsible for emerging diseases
- The necessity for integrated disease surveillance
- Availability of tools to monitor wildlife population with detail and good effectiveness

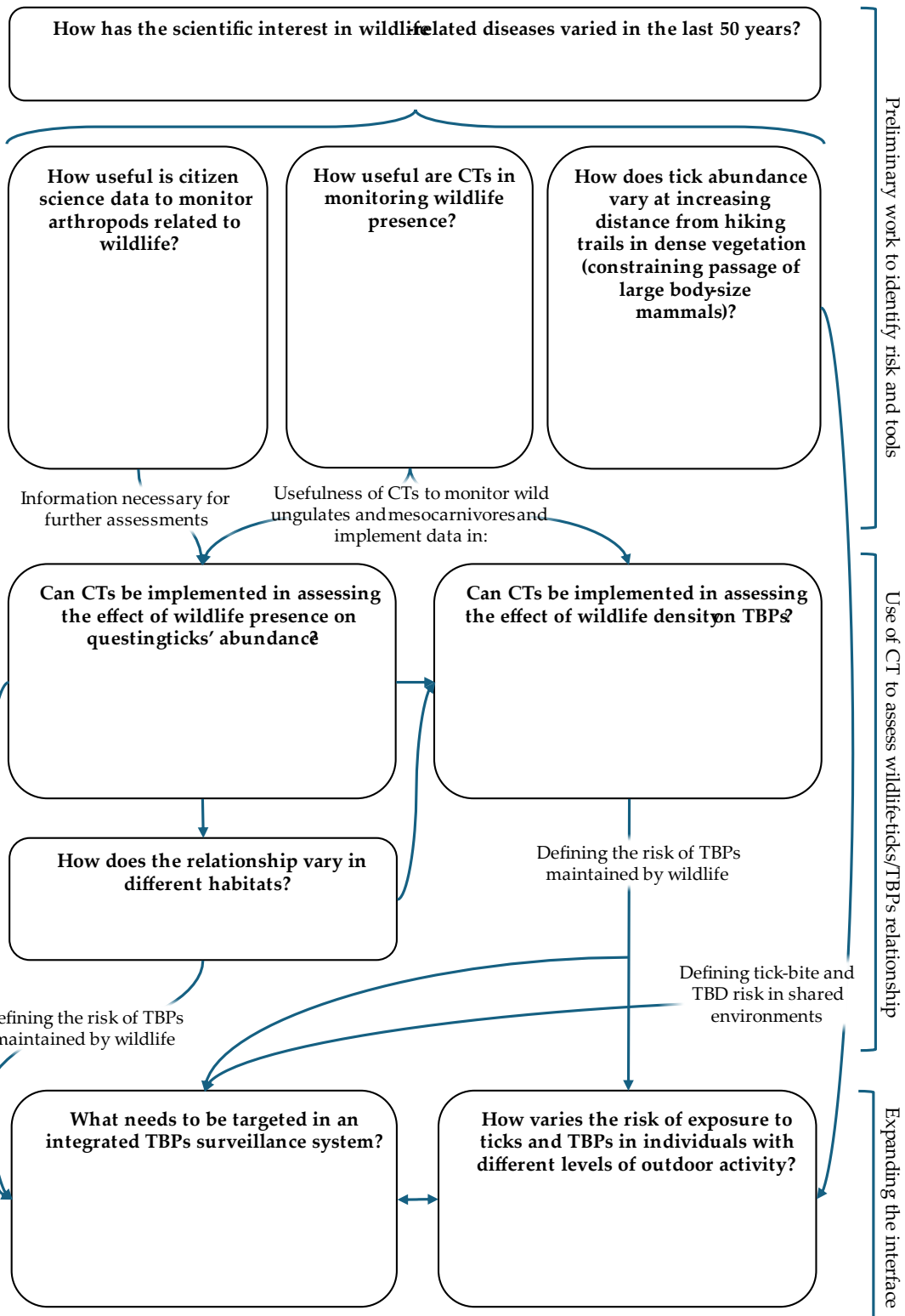
Within this framework, the objective of the present work is to detail the risk of human exposure to tick-borne diseases in natural environments, with a focus on how wildlife and the habitat influence pathogen circulation, also through influencing vector presence.

This will be addressed through a two-step approach:

- Utilizing camera traps to collect wildlife data and relating such data to ticks' abundance and tick-borne zoonotic pathogens' presence and prevalence
- Expanding the scope of research along the interface by assessing the presence of tick-borne zoonotic pathogens in humans

In addition, preliminary work was conducted to explore the relevance of wildlife diseases in the scientific community, the utility of camera traps in monitoring wildlife and of citizen science to gather data on vectors related to wildlife and the dispersal patterns of ticks along hiking trails.

In the following scheme, the main research question for each study presented in this thesis is interconnected with the others, forming a cohesive framework that defines the underlying logic of the research. This structured approach ensures that each individual study contributes to a broader understanding of the overarching research theme, creating a logical progression that strengthens the thesis. While the preliminary work focused on identifying the issue (the relevance of wildlife-related diseases in the scientific community), the available tools to integrate and improve epidemiological studies, such as citizen science data and camera trap-based wildlife monitoring, and the risk of tick bites in shared environments like hiking trails, the core publications centred on developing methods to estimate the relationship between wildlife presence and ticks/tick-borne pathogens and comprehensively assessing the risk of tick-borne diseases when facing the interface with humans.



TBPs = tick-borne pathogens; CTs = camera traps

3. Preparatory work

3.1. Fifty years of wildlife diseases in Europe: a citation database meta-analysis.

Vada, R., Zanet, S., & Ferroglio, E. (2022). Fifty Years of Wildlife Diseases in Europe: A Citation Database Meta-Analysis. *Veterinary Sciences*, 9(11), 629.

For original formatting and the complete version of the article, see:

<https://doi.org/10.3390/vetsci9110629>

Fifty Years of Wildlife Diseases in Europe: A Citation Database Meta-Analysis

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Simple Summary: In the past decades, wildlife has been progressively recognized as relevant for the circulation of pathogens affecting not only wild species but also humans and domestic animals. Currently, there is no clear picture regarding which pathogens have been the subject of research over the years and how the investigation has evolved over time. Such information would be useful to guide future steps for wildlife disease management. In this paper, we aimed at answering this question by going through the outputs of a citation database, Web of Science, in terms of the number of wildlife disease publications, and the frequency of disease related MeSHs. Overall, the scientific interest increased over time, with a peak in the last 20 years. The focus of research changed over time and shifted to contemporary hot topics, such as zoonoses, conservation-related diseases and emergent diseases. The increasing complexity of diseases related to wildlife is an aspect that should be kept in mind when drafting surveillance and control plans.

Abstract. Although wildlife has progressively been recognized as a booster for the spillover of pathogens to humans and other wild and domestic animals, the interest of scientists in this topic has not been constant over time and uniform in its targets. Epidemiological outbreaks and technological development have contributed to this. Through the analysis of the number of publications from a citation database, we aimed to obtain an indicator of the scientific community's interest towards wildlife diseases over the years. Using Web of Science, bibliographic searches were performed by adding to the basic search string "Wildlife AND Disease" terms detailing topics such as aspect investigated, pathogen type, aetiologic group and species group. For each host species group, the 100 most frequent Medical Subject Headings (MeSHs) related to specific diseases in each decade were extracted. The scientific production regarding wildlife diseases has increased 3.7 times the relative proportion of publications on diseases during the last 50 years, focusing mainly on zoonotic or epizootic pathogens, and with a sharp growth in contemporary hot topics. Wildlife disease scenarios are complex and challenging to approach. Knowing the trends in the scientific interest in the past decades may pose a guide to direct future steps and actions in several fields, from public health to ecosystem management.

Keywords: zoonoses; wild animals; scientific databases; trend analysis; publication trends

1. Introduction

With the progressive consolidation of the One Health concept, the role of wildlife as a reservoir and spillover denominator has gathered increasing attention (Messmer, 2020). In shared ecosystems, the health of humans, animals and the environment becomes interconnected, and pathogens coevolve with populations (McLellan et al., 2014). For millennia, pathogens jumped the species barrier from animals to humans and vice versa. In the past 20 years, 60.3% of emerging infectious diseases affecting humans originated from animals, and the majority of these (71.8%) came from wildlife (Jones et al., 2008). Wildlife disease surveillance systems and control programs play a central role in preventing and controlling the spillover of pathogens at the human–livestock–wildlife interface (Gortázar et al., 2007). One of the first examples is the rabies vaccine campaign in wild canids (Robardet et al., 2016; Rosatte, 2013).

Wildlife is without any doubt a key factor for the circulation of zoonotic or epizootic pathogens of great relevance, such as *Echinococcus multilocularis* (Hegglin et al., 2015) or the avian influenza virus (Bárbara et al., 2017). Additionally, wildlife can boost the circulation of vectors: *Hyalomma* ticks were introduced to Spain through the migratory routes of wild birds from Africa and, in recent years, have been responsible for introducing the Crimea–Congo hemorrhagic fever virus to the Iberian peninsula (Palomar et al., 2016). Likewise, global warming and climatic changes have favored vectors and diseases to expand to previously unsuitable areas (Carvalho et al., 2017): *Leishmania infantum* has spread northwards in the past decades and its expansion is bound to continue (Biglino et al., 2010). Recently, interest in wildlife diseases has also included pathogens that pose a risk to the conservation of biodiversity (De Castro & Bolker, 2005).

Despite certain limits of accuracy, publication and citation counts can be considered valuable indicators of scientific research interest and its fluctuations over time (Gortázar et al., 2010). By describing and quantifying the scientific research carried out on wildlife diseases, important information can be obtained to define future research topics and research tools.

The purpose of the study was to analyze the trend of publications on wildlife diseases in Europe in the past 50 years, by defining the main research targets and how they changed over time in terms of pathogens and target host species. This analysis compared the relative importance attributed to diseases and host species by various stakeholders within the public health, veterinary and environmental sectors.

2. Materials and methods

The number of articles published about wildlife diseases makes a systematic review of each paper impractical and uninformative.

A more general approach was adopted. We explored the query outputs using the specific analysis feature provided by the citation database (https://support.clarivate.com/ScientificandAcademicResearch/s/article/Web-of-Science-Steps-to-analyze-results?language=en_US, accessed on 14 September 2022). The query addressed all databases and collections included in Web of Science and each specific search string searched pertinent papers by correspondence between the search string itself and the “Topic” of each paper (which in Web of Science includes title, abstract and key words). The bibliographic search was performed between November and October 2021. The first query algorithm was: “wildlife” AND “disease”. We ran the most general and simple search string to ensure a solid research baseline. Exploratory trials with more specific search strings showed no difference in temporal trends (data not shown). The query was then refined for countries of the European continent (thus including all papers whose authors are affiliated to an organism or institution belonging to countries of the European continent). No temporal limitations were applied to publication year. Subsequent queries added terms one at a time to the algorithm with the “AND” operator. Additional terms belonged to four clusters: (i) disease aspect investigated, (ii) disease type, (iii) disease agent type, (iv) target species group and (v) publication type.

Terms are listed in Table 1 and the query links are reported in Supplementary Table S1.

For each query, we retrieved the number of articles published per year and grouped them by decade for subsequent analysis. We performed a linear regression between the years and the logarithm of the number of publications to obtain the regression coefficient as an indicator for the increment in the number of papers over time. The regression’s R^2 pointed out how much of the increase was attributable to the progress of time. Moreover, the cumulative H index was extracted for each search string and normalized over the total number of publications. This parameter was also used as an indicator of interest on a specific topic.

Table 1. Scheme of the additional term clusters.

Disease Aspect Investigated	
Epidemiology	Therapy
Diagnostic	Surveillance
Identification	Economic impact
Disease Type	
Vector-borne	Livestock interest
Zoonotic	Biodiversity conservation
Emerging disease	Infection
Disease Agent Type	
Bacteria	Parasite
Virus	Fungi
Prions	
Target Species Group	
Carnivores	Lagomorphs
Wild ruminants	Chiroptera
Wild boar	Insectivores
Rodents	Birds
Publication Type	
Article	Letter/editorial
Review	Conference
Book	

Finally, for each group of species (the full list is reported in Table S1), we downloaded the first 100 MeSHs (Medical Subject Headings) ranked by frequency for each decade. After an exploratory overview to spot any recurrent patterns, terms referring to a pathogen or disease were extracted. The frequencies of those referring to the same disease were merged. Tick-Borne Diseases were also merged. Consequently, a classification of the most frequent MeSHs related to a specific disease was obtained, divided per species group and decade. Eventually, the classification was graphically represented and analyzed.

3. Results

Since 1940, a total of 63,170 publications concerning wildlife diseases have been produced globally. Of these, 21,254 (33.65%) are by Europe-based authors, with the first papers published in 1972. Thus, the last 50 years (1972–2021) was the analyzed period. The results for all queries are reported in Supplementary Table S2. Comparing with publications about diseases in general, “wildlife” extracted 0.154% of publications in 1972 and 0.574% in 2021, increasing the proportion by 3.7 times. The country operativity in wildlife disease research was greater (43%) in Northern European countries (according to the United Nations Geoscheme, www.unstats.un.org, accessed on 20 September 2022). All additional terms used in this study returned a positive regression coefficient. The R^2 showed a strong link between the year and the number of publications, fluctuating between

0.85 and 0.92. The seven exceptions are the outputs of the searches with: vector-borne ($R^2 = 0.54$), prion ($R^2 = 0.79$), rodents ($R^2 = 0.65$), insectivores ($R^2 = 0.44$), bat ($R^2 = 0.70$), conference ($R^2 = 0.55$) and books ($R^2 = 0.40$). In those cases, the increase in the number of publications is only partially explicable by the progression of the years.

In the “aspect of disease investigated” cluster, the highest number of publications was returned by the term “epidemiology” (6400 publications), followed by “therapy” (2518) and “diagnostic” (2317). However, the higher coefficients of “surveillance” (0.051) and “economic impact” (0.047) reflect a sharper increase for related publications in the last decades. On the contrary, economic impact collected the highest relative H index. The results are presented in Figure 1.

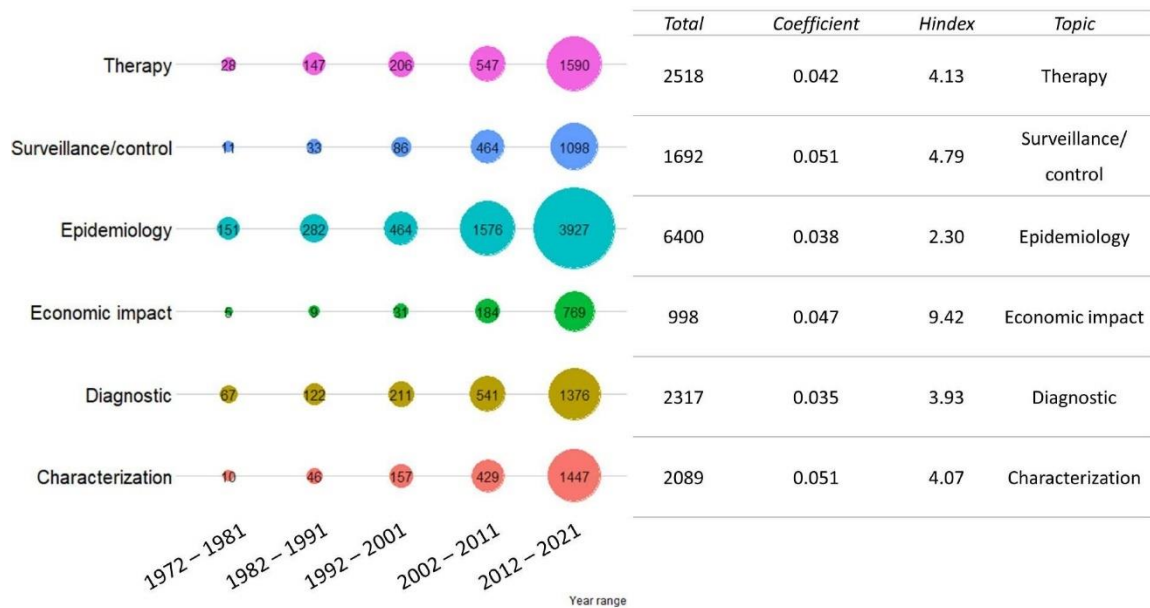


Figure 1. Publication trend for “aspect of disease investigated” cluster. The total number of publications was grouped by ten-year period. For each disease aspect investigated, the total number of publications in the last 50 years, regression coefficient (as growth rate) and cumulative H index divided by the number of publications are shown on the right. Colors are not informative.

Although “zoonotic” and “livestock” are the terms that returned the highest numbers of publications (1712 and 1835, respectively), the outputs of “biodiversity conservation” and “emerging diseases” presented a strong increase in the last decades, with two of the highest regression coefficients (0.050 and 0.054, respectively). “Vector-borne” returned a more constant growth (0.024), while “conservation” returned the highest relative H index. The results are presented in Figure 2.

Parasites are the etiological group which returned the highest number of publications, double the number of publications on viruses and bacteria (1250 total publications). Despite this, viruses and prions returned the highest regression coefficient (0.040 and 0.047, respectively). The H index was not produced for parasites, due to the number of

publications (Web of Science returns such values when the number of outputs is less than 10,000); therefore, comparison of this parameter was not possible. The results are presented in the Figure 3.

Among the queries for species groups, “carnivores” was the term that returned the highest number of publications (2763) and “birds” the second highest (2453). The principal increase in publication numbers regarded “wild boar” and “wild ruminants” (0.045 and 0.041, respectively), while “lagomorphs” and “rodents” returned some of the lowest (0.025 for both), maintaining a more constant publication rate over the years. The results are presented in the Figure 4.

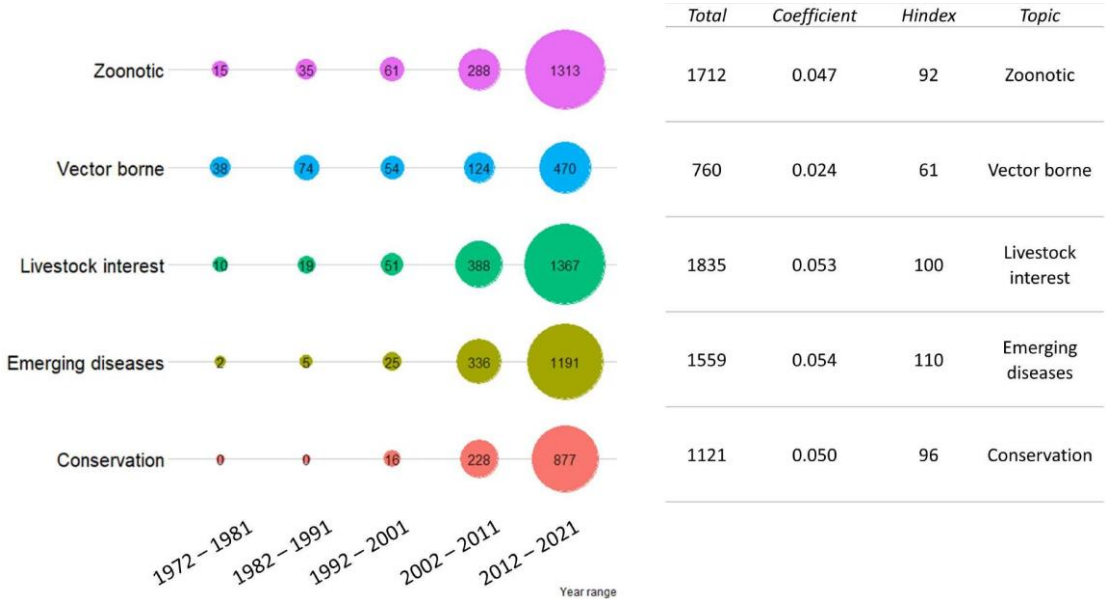


Figure 2. Publication trend for the “disease type” cluster. The total number of publications was grouped by ten-year period. For each disease type, the total number of publications in the last 50 years, regression coefficient (as growth rate) and cumulative H index divided by the number of publications are shown on the right. Colors are not informative.

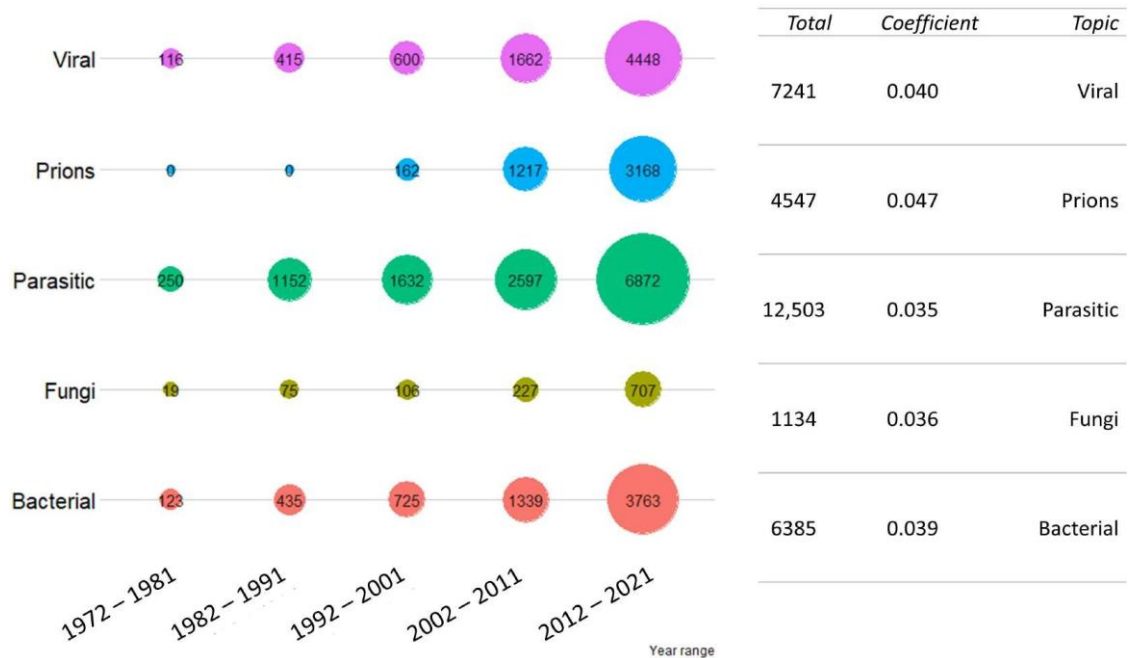


Figure 3. Publication trend for the “disease agent type” cluster. The total number of publications was grouped by ten-year period. For each disease agent type investigated, the total number of publications in the last 50 years and regression coefficient (as growth rate) are shown on the right. Colors are not informative.

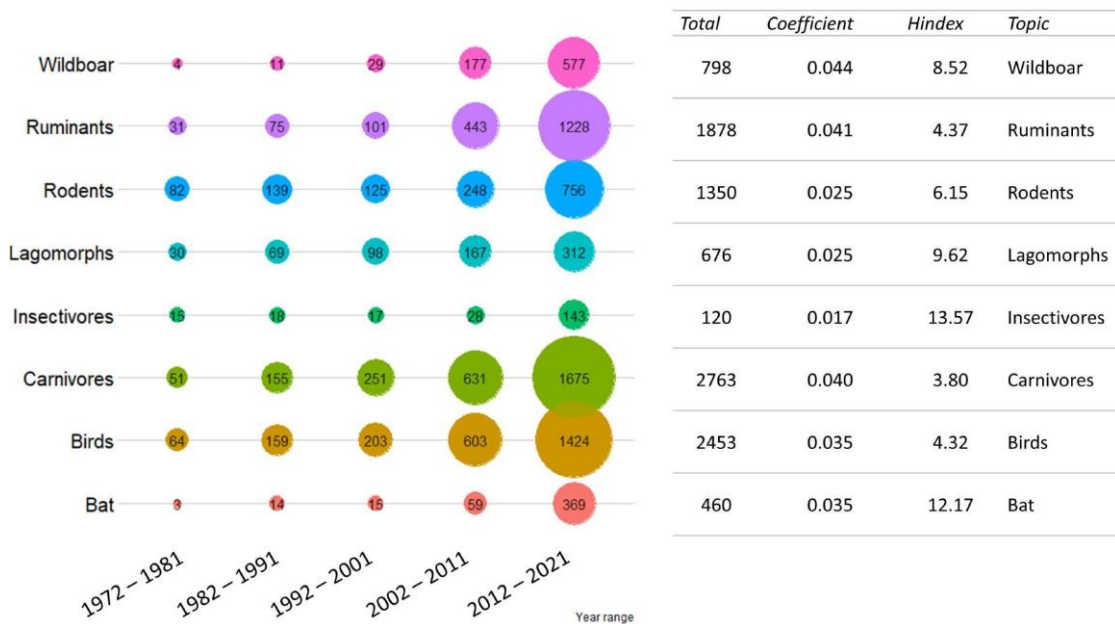


Figure 4. Publication trend for the target species group investigated. The total number of publications was grouped by ten-year period. For each target species group investigated, the total number of publications in the last 50 years, regression coefficient (as growth rate) and cumulative H index divided by the number of publications are shown on the right. Colors are not informative.

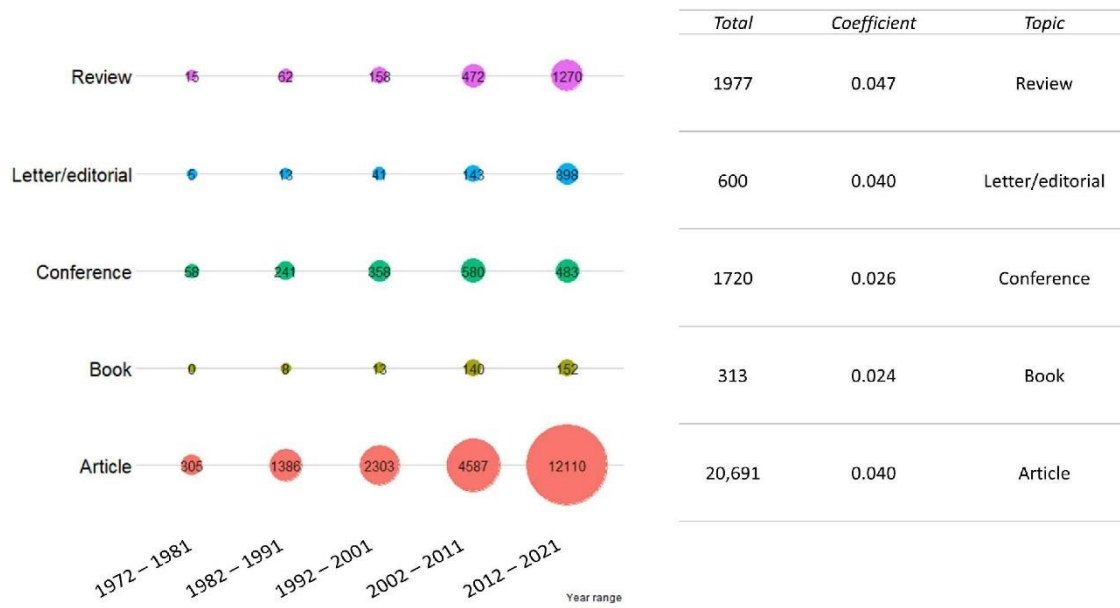


Figure 5. Publication trend for the “publication type” cluster. The total number of publications was grouped by ten-year period. For each publication type investigated, the total number of publications in the last 50 years and regression coefficient (as growth rate) are shown on the right. Colors are not informative.

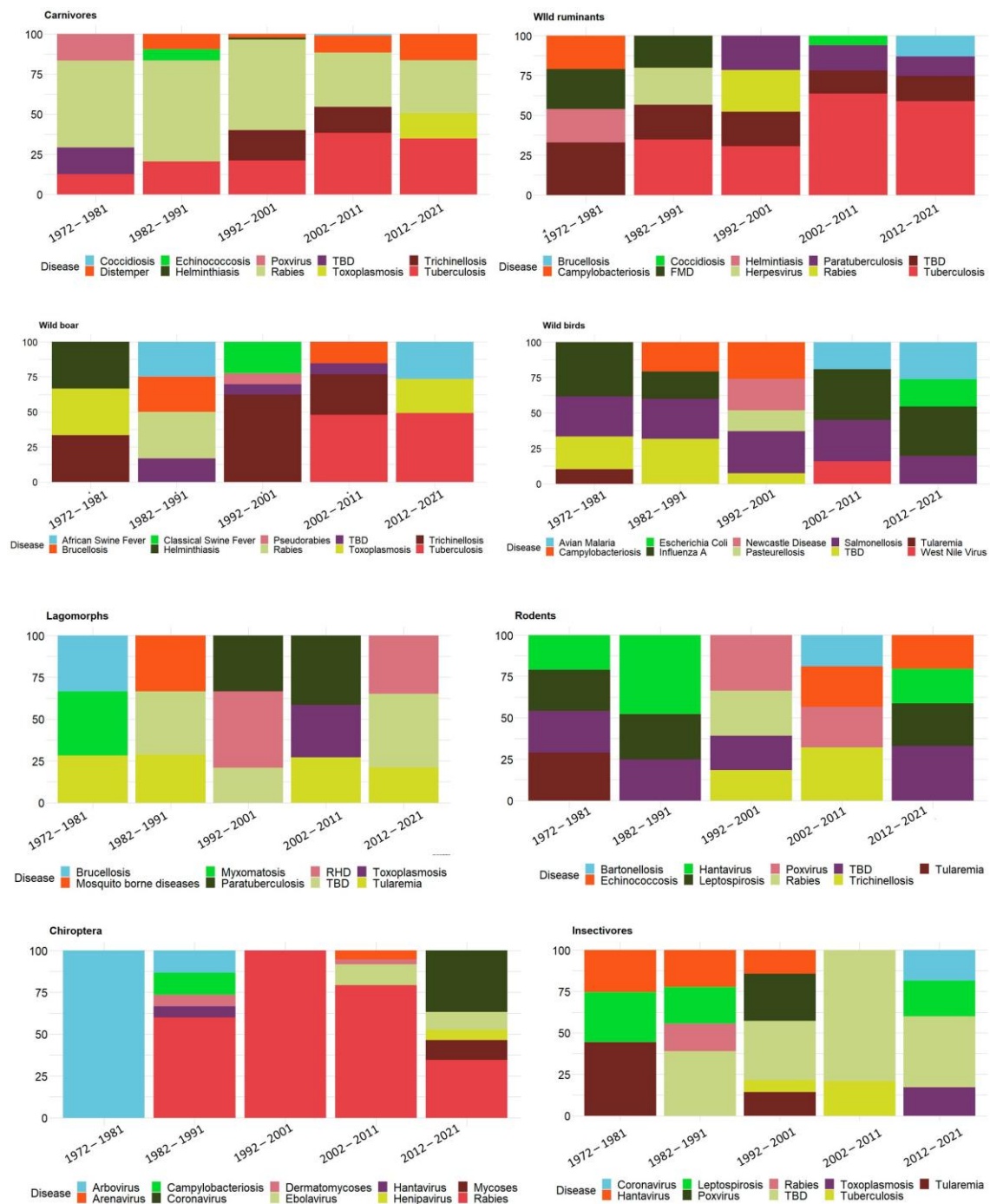


Figure 6. For each species group and decade, the first 10 most frequent (per species group, considering all decades) disease-related MeSH terms are represented with their relative frequency to the decade. Colors vary according to the species group (legend below each group). X axis: decade; Y axis: percentage of relative frequency. TBD = tick-borne disease. FMD = foot and mouth disease. RHD = rabbit haemorrhagic fever.

Articles are the most common type of publication (20,691). Despite this, reviews have had the sharpest increase in the last decades (coefficient 0.047), while book production

remained almost constant (0.023). Conferences were the second most common type of publication in the 1970s (58), although the scenario changed in the following decades. The results are presented in Figure 5.

The complete results of the MeSH analysis are presented in Supplementary Table S3, while the graph in Figure 6 presents the first 10 MeSH terms per species group. Tick-borne diseases present high frequencies in all mammals, while tuberculosis increased in susceptible species. A similar trend can be spotted for zoonoses such as trichinellosis, echinococcosis and toxoplasmosis. While wild ruminants present a polarization towards fewer disease related MeSHs, the wild boar search showed an increased complexity in the number of pathogens. In carnivores, rabies decreased its frequency over the decades. Lagomorphs presented a high variety of different diseases, with tularemia being among the most frequently reported since the 1970s and rabbit hemorrhagic disease since the 1990s. In rodents, high frequencies of hantaviruses and leptospirosis are present in all decades. Chiropterans showed a predominance for viral diseases. As a constant over time, avian influenza dominated the research interest in wild birds, followed by salmonellosis and campylobacteriosis and, with lower frequencies, by the West Nile virus and malaria. Finally, the high complexity of pathogens presented by insectivores could be biased by the small number of publications, which leads almost all MeSH terms to enter the first 100.

4. Discussion

The scientific production regarding wildlife diseases has undergone a transversal increase on all the main research topics during the last 50 years, with a predominant interest in epidemiological studies and a more recent sharp growth in fields that are also contemporary hot topics, such as emerging diseases, pathogens that threaten biodiversity or prion-borne diseases. The fluctuations in terms of the number of publications for each species group and disease reflect the changes through the last decades in the health concerns regarding wild and domestic animals.

The increase in scientific activity is a trait that accompanies all aspects of research, and this trend affects wildlife disease publications as well. About a third of worldwide papers published on the topic comes from European countries, with a major proportion from northern countries. This scenario may reflect the idea that the interest in research, and in one topic rather than another, is dependent on economic wealth (King, 1987). We can therefore suppose that wildlife is still not a priority research field worldwide. However, the increase in the number of publications, especially in the last two decades, may be an indicator of the rising awareness of the crucial role that wild species play in the circulation of pathogens of zoonotic or economic interest (Messmer, 2020). Moreover, it must be kept in consideration that the attribution of a paper to a country by the Web Of Science platform is based on the authors' affiliations, rather than on the geographic setting of the study. Therefore, it would be more accurate to speak of country operativity rather than of research

activity in the country.

Epidemiology has always been recognized as a crucial part of the control efforts against transmissible diseases, and papers that involve this aspect have dominated the scene in the last decades. However, the sharp increase in publications including surveillance and control may be considered an indicator that countries are starting to value the importance of wildlife within a comprehensive investigation approach for disease management (King, 2004). Parallely, the economic impact of a spillover from wildlife is also being recognized as having potentially important outcomes, as it has been remarked upon in the literature (Artois et al., 2009). Pathogens that affect human or livestock health have always received interest, as it emerged in both the temporal trends and MeSH frequency of the related query outputs. In recent years, the peak in publications that include diseases threatening biodiversity broadens the spectrum of One Health even more; its focus has come to include not only public health and livestock, but also ecosystem conservation, in light of the role of pathogens as a threat to the survival of endangered species (Knobel et al., 2005) and that of biodiversity as a determinant factor for the reduction in disease rates (Rohr et al., 2020). Climate change and global warming effects were reflected in publication trends; the emerging diseases search term returns an increasing number of publications in Europe, and several studies have already detected the spread of related pathogens to new territories (Biglino et al., 2010).

An analysis focusing only on long-term studies on wildlife diseases (Barroso et al., 2021) has shown similar results to the current work regarding the top-ranking target diseases. On the contrary, the work by Barroso and colleagues (Barroso et al., 2021) arrived at different conclusions when grouping pathogens into viruses, bacteria and parasites, the latter being only the third most popular topic. In the current work, which is not limited to long-term research projects, papers involving parasitic diseases are the most frequently published. Despite the highest frequency of parasitology-targeting papers, MeSHs showed a predominance of bacterial and viral diseases. It is legitimate to suppose that, despite the high number of papers, the parasitic world is extremely complex and only a few singular parasitic diseases appeared in top frequencies of MeSH terms. For example, the choice to group all tick-borne diseases has allowed us to highlight the importance of ticks as pathogen vectors.

An OIE wildlife health survey report published in 2021 (OIE, 2021) listed the main wild species that should be targeted by surveillance programs involving wildlife, according to the veterinary authorities of the OIE. The priority pattern emerging from the OIE survey places birds as the top-ranking taxa, while our meta-analysis ranked carnivores as the most frequent species group, followed by birds. The avian influenza A virus is most likely the cause behind this inconsistency between the two analytical strategies. Avian influenza is the principal pathogen that appears in

publications regarding wild birds, with a constant effort over time. Moreover, in the same OIE report, it was selected as the principal disease on which wildlife surveillance was needed, undoubtedly because of the recent outbreaks and their consequences (Napp et al., 2018), which are of particular relevance considering the OIE mission and scopes. This could be an indicator of the fact that, despite being carnivore carriers of significant pathogens that can affect livestock and humans as well (i.e., the increasing publication trend on tuberculosis and echinococcosis), their role is resized, and priority is given to other diseases. Moreover, the original interest towards carnivores was mainly focused on rabies, until the vaccination campaigns reduced its worldwide burden. The intense and recently increased investigation on the distemper virus is also noteworthy, confirming the trend of interest in pathogens that do not concern human or livestock health, but have significant impacts on conservation (Knobel et al., 2005).

The importance of wild ungulates is commonly recognized and involves diseases of great concern worldwide, such as tuberculosis or brucellosis (Gortázar et al., 2007). Moreover, the explosion of prion-related publications was strictly related to the outbreaks of bovine spongiform encephalopathy and the recent emergence of chronic wasting disease (Pirisinu et al., 2018). The MeSH “population density” was also quite frequent for wild ruminants. This topic has been in the spotlight in recent years, as many wildlife diseases are strictly dependent on density, and the latter is a determinant factor for disease control (Gortázar et al., 2006). In the case of wild boar, it is remarkable how the number of publications targeting this single species are equal to one third of the publications of the entire carnivore group and to half of those of all wild ruminants together. Considering that the complexity of pathogens investigated is increasing alongside the number of studies, it is clear how this species was progressively recognized as an important host of several relevant diseases. These diseases related to wild boar have changed over time. Considering the variety of diseases for which the wild boar is epidemiologically relevant (ENETWILD-consortium et al., 2019a), our data confirm this species as one of the main species of research interest in Europe. This relevance is also attributable to its extensive use as wild game meat. According to FAO data, wild boar is the most frequently consumed wild ungulate across Europe, and the implications in terms of public health make this species of particular interest for food-borne diseases (Thomas et al., 2020).

As for chiropterans, the high importance given by the OIE and the relatively low number of publications may be due to the fact that the discovery of such species as carriers of high-impact zoonotic pathogens in Europe was mostly recent (Kohl & Kurth, 2014). The diseases identified by our MeSH search are also identified by OIE members as emergent diseases with a high risk of spillover from wildlife to humans and that should be targeted with surveillance programs (OIE, 2021). Current research on

bats targets diseases with high visibility, such as hantaviruses, ebolaviruses or coronaviruses. On the other hand, rodents, which are long-known as carriers of zoonotic pathogens (Meerburg et al., 2009), have generally had a constant trend in publications. The diseases related to rodents are numerous and varied, as shown in the present study, and are of great interest for human health. Outbreaks of rabbit hemorrhagic fever, myxomatosis or tularemia were already well-known in lagomorphs at the beginning of our study period (Abrantes et al., 2012), and interest towards this group of species was steadier compared to others. A decline in the number of publications on myxomatosis mirrored a rise in the interest toward the rabbit hemorrhagic disease virus, a pathogen with significant implications for conservation (Abrantes et al., 2012). Being carriers of zoonotic and conservation-related diseases, and recently discovered as carriers of emerging pathogens such as *L. infantum* (Ruiz-Fons et al., 2013), it is surprising how lagomorphs are still a marginal focus in European wildlife disease research. Insectivores, as well as rodents, are typical wildlife that inhabits peri-urban (or even urban) environments and thus are a reservoir of tick-borne diseases, as already suggested (Skuballa et al., 2010). They can represent a potentially important source of infection because of the geographical proximity to humans and the high burden of feeding ticks (Skuballa et al., 2010).

5. Conclusions

As wildlife is an essential component in the epidemiology of many, if not most, zoonoses, it should be considered in risk analysis frameworks and in disease surveillance systems. The scientific activity does not always match the priorities identified by stakeholders, and the wildlife disease scenario is becoming complex rather than polarized, a situation that should be well considered when addressing surveillance plans. This should also be recognized in relation to target host species, as neglected ones are of interest as important reservoirs of relevant diseases. Following the recent trend recognizing the importance of a One Health approach, we may expect increasing interest in wildlife disease surveillance and emergent pathogens. Likewise, zoonoses will maintain relevance, as food-borne pathogens for game animals, and viruses for other species. We may also expect an increase in complexity, both in terms of pathogens and in terms of target species.

The geographic extent (limited to Europe) and the methodologies of the work make these results subject to improvement. Of course, this analysis left aside many groups of hosts (reptiles, amphibians, fish and marine mammals) that would be worth considering. A transboundary characterization of disease epidemiology is fundamental in a globalized world, where pathogens move together with animals and people. A similar analysis carried out on a global scale and focused on different macro-regions may provide information useful for a One Health approach to disease control.

3.2. Abundance trends of immature stages of ticks at different distances from hiking trails from a natural park in north-western Italy.

Vada, R., Zanet, S., Battisti, E., & Ferroglio, E. (2024). Abundance Trends of Immature Stages of Ticks at Different Distances from Hiking Trails from a Natural Park in North-Western Italy. *Veterinary Sciences*, 11(10), 508.

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Abundance Trends of Immature Stages of Ticks at Different Distances from Hiking Trails from a Natural Park in North-Western Italy

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Simple Summary: Hiking trails in a natural park may increase the risk of tick exposure for people and pets, as ticks dropped by wildlife can easily latch onto individuals walking nearby. Understanding the range of movement of ticks dropped by wildlife hosts on trails, along with the acarological risks linked to park infrastructures, is crucial for guiding actions to reduce exposure to ticks and tick-borne pathogens. This study examines how tick abundance changes with increasing distance from hiking trails, focusing on a range of 0 to 4 m. We observed a decreasing trend in tick abundance that varies by developmental stage: nymphs show a steady decline in abundance as distance increases, while larvae exhibit a peak near the trail, followed by a sharp drop at greater distances. These findings highlight how the immediate proximity of a hiking trail may serve as a potential source of tick bites for both humans and pets.

Abstract. Hiking trails may act as hotspots at the wildlife–human interface, posing an acarological risk for people and their pets. Ticks that are maintained in the environment by wild animals may quest on people walking along the trails. Assessing the risk of tick bites for people involved in outdoor activities is a further step in mitigating the risk of tick-borne diseases. This work describes the variation of tick abundance along a gradient of distances from hiking trails, where wildlife passage is favored by higher accessibility. Hiking trails with dense vegetation on the sides were sampled for ticks along a 100 m dragging transect, located in a natural park in North-Western Italy. Additional transects were replicated at 1, 2 and 4 m away from the trail on both sides. After morphological identification, descriptive statistics and modeling were applied to determine the abundance patterns across distances. Larvae were most abundant near the trail, peaking at 1 m and dropping sharply at further distances. Nymphs showed a more gradual and consistent decrease at progressing distance from the trail. Few adults were collected, preventing the identification of a clear trend. With higher tick abundance, the immediate vicinity of hiking trails may represent a source of acarological risk for humans and pets.

Keywords: tick abundance; tick dispersal; wildlife–human interface; acarological risk; landscape management

1. Introduction

Peri-urban parks are characterized by a high frequency of direct and indirect contact among wildlife, domestic animals and humans, which can boost the risk of transmission of zoonotic or epizootic pathogens (Mackenstedt et al., 2015). Hiking trails, especially

at nighttime, are frequently used by several wild mammals (Kays et al., 2017); prey species (from ungulates to small mammals) seem in some cases to be attracted by busier trails, presumably using humans as a deterrent against predators activity (Muhly et al., 2011), whereas mesocarnivores can use trails for more efficient hunting and traveling (Doherty et al., 2015). However, the extent of such a behavior may vary across species and the avoidance of human tracks has been registered, especially for deer species (Erb et al., 2012; Mols et al., 2022) and some rodent species (Shorb et al., 2020). As shared environments, these trails may serve as preferential sites for the transmission of parasites and zoonotic pathogens, including tick-borne zoonoses through the risk of tick bites.

Several studies have tried to characterize how recreational areas in natural parks expose people and their pets to acarological risk. This risk may be associated with landscape and trail characteristics (Anderson et al., 2021; Dobson et al., 2011), but also with visitors' behavior (Hassett et al., 2022), in addition to elements such as vegetation and host presence (Iijima et al., 2022; Janzén et al., 2023). Hiking trails have also been described as hotspots for tick passage from wild to human hosts (Janzén et al., 2023; Rasi et al., 2018; Van Gestel et al., 2021). Footpaths in protected areas of Northern Italy have been sampled, confirming both the presence of the acarological risk and the zoonotic risk associated with tick-borne diseases (Aureli et al., 2015; Corrain et al., 2012; Millet et al., 2019).

When not on the host, ticks are subject to a certain range of dispersion. A study in Poland and Slovakia suggested that ticks tend to be more abundant on animal trails than at a range of 5 m distance from the trail (Rasi et al., 2018), but there is limited literature available on the topic. While many studies focus on the abiotic factors that influence *Ixodes ricinus* loco- motor activity, such as temperature, humidity, electromagnetic fields and light/darkness hours (Alekseev et al., 2000; Perret et al., 2003; Vargová et al., 2022), dispersal models have been provided for *Ixodes scapularis* (Madhav et al., 2004) and have been studied in other species such as *Dermacentor reticulatus* (Buczek et al., 2017). For *I. ricinus*, the maximum distance of attraction with carbon dioxide traps has been estimated to be up to 3.5 m for adult females and 1 m for nymphs (Gray, 1985). Information on dispersion behavior would not only broaden the knowledge about ticks' ecology but would also represent a significant factor to be considered in risk assessments for the transmission of vector-borne diseases (i.e., Lyme disease, babesiosis and tick-borne encephalitis). Additionally, it may inform infrastructure design and management strategies in peri-urban parks in order to reduce the risk.

This study aims to determine the gradient of environmental ticks' distribution at different distances from pathways, an issue that has still been little explored despite its relevance to addressing public recommendations to reduce human exposure to ticks.

2. Materials and Methods

Study Area

The study was performed in La Mandria Regional Park in Piemonte, Italy. It is a fenced, protected area of 6571 Ha at an average altitude of 386 m above sea level. Due to the absence of an altitude gradient and a small area, the vegetation type is homogeneous in the whole area, with a total of 51.4% of the park covered in deciduous forest (made mainly of oak, hornbeam and ashwood) and 30.2% covered in grassland for mowing fields (Corine Land Cover data, downloaded from <https://geoportale.igr.piemonte.it/cms/>, accessed on 30 August 2024). The annual precipitation in 2020 was 898.2 mm and it registered an maximum temperature of 32 °C and a minimum temperature of -4 °C). It welcomes an average of 2000 visitors every day. Ungulate culling for population control is constant throughout the year. Few horse farms are present, but no livestock is kept in the park and dogs are not allowed, except for the ones owned by farmers. Regarding the wildlife population, ungulates are present at high densities (ENETWILD-consortium et al., 2023b). Ticks and tick-borne pathogens that may spill over to humans have been recorded with a high prevalence in the study area; for instance, a prevalence of almost 45% of *Babesia* spp. has been recorded in red deer by Zanet and colleagues (Zanet et al., 2014). The mammal species of larger sizes that are most abundant in the park include wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*), as well as wolves (*Canis lupus*) and mesocarnivores such as foxes (*Vulpes vulpes*) and badgers (*Meles meles*) (ENETWILD-consortium et al., 2022c, 2023b). The abundance of other mustelids such as the European pine marten (*Martes martes*) and the beech marten (*Martes foina*) is comparatively low.

Sampling Design

We selected three sampling sites, constituting a main trail with dense and homogeneous grass or undergrowth (20–30 cm height) on the sides. Such vegetation would constrain the passage of larger size mammals (wild ungulates, wolves, foxes and badgers) to the trail, while rodents, other mustelids and reptiles would have easier access to the vegetation, in addition to different behaviors in relation to trail use. To maximize the possibility of collecting ticks, all sampling sites were identified in deciduous forest habitats. Additionally, no sign of passage of mentioned wild species, such as scats, tracks or vegetation damage (Keeping & Pelletier, 2014) was detected outside of the trail, while the trails presented signs of passage. At each sampling site, we conducted dragging/flagging linear transects of 100 m in length, parallel to the trail. These transects were performed at perpendicular distances of 0 m, 1 m, 2 m and 4 m from the trail on both sides (Figure 1). A 1 square meter white cloth was used for sampling and was checked every 20 m. To account for seasonal differences in tick abundance and biases related to sampling events, dragging/flagging was repeated at each transect every two weeks from May to August 2021, for a total of 9 repetitions. All of the sites were sampled on the same day, avoiding the central hours during summer. Days with significant rain events, as well as the 48 h immediately following these, were excluded. Ticks were

separately collected for each transect and stored in 70% ethanol for subsequent morphological identification (Estrada-Peña et al., 2018; Manilla & Iori, 1992, 1993).

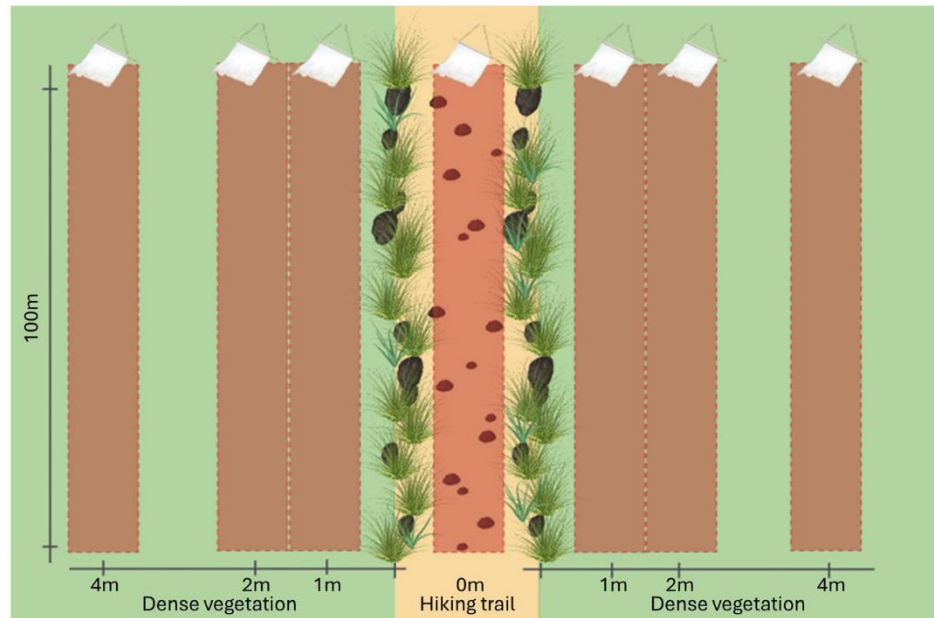


Figure 1. Transect deployment at each sampling site. Differentiating the hiking trail (light yellow) from the dense vegetation (light green), the dragging transects are plotted on the map (the brown rectangles represent a surface of 1 m width and 100 m length). Their distances are the following: one transect is on the trail (0 m) there is one in the vegetation for each side at 1 m, 2 m and 4 m.

In the proximity of each sampling site, we placed a sensor (Temperature and Humidity Data Logger PRO-USB-2, RS, Corby, UK) set to register the temperature and humidity hourly, 15 cm above the ground. The saturation deficit was calculated per day (Tagliapietra et al., 2011; Zajac et al., 2021) and an average value for the period between the repetitions was retrieved.

Statistical Analysis

Statistical analysis was performed in R studio (R Core Team, 2023). We implemented descriptive statistics to highlight tick distribution at different distances from the trail. We plotted the average abundance of ticks per transect, encompassing all developmental stages and species and larvae and nymphs separately (adults were disregarded due to poor representation). To explore different behaviors among species, we also plotted the two most abundant genera recorded in our study, namely *Ixodes* and *Haemaphysalis*. Both species are of interest as vectors of zoonotic diseases (Salman & Tarrés-Call, 2013).

After explorative analysis, we described trends in tick abundance by structuring Generalized Additive Models (Wood, 2017), an implementation of General Linear Models that allows us to describe non-linear relationships with the explanatory variables (called smooth terms). This aspect is particularly fitted for ecological modeling and, in

our case, to describe the trends in ticks' abundance, which we expected to not be linear. We generated models for the total number of ticks, nymphs and larvae, implementing date and average saturation deficit as linear predictors and the distance from the hiking trail as the smooth parameter. The sampling site was tested as a weight factor. The models were selected based on the deviance explained, the significance of coefficients and the residual analysis.

3. Results

A total of 181 ticks were collected, for an average abundance of 0.01 tick/m²; these ticks comprised 132 larvae, 46 nymphs and 3 adults (Table 1).

Table 1. Total count of ticks for species and developmental stage.

	Adults	Nymphs	Larvae	Total
<i>Dermacentor</i> sp.			1	1
<i>Haemaphysalis concinna</i>		3		3
<i>Haemaphysalis punctata</i>	1	2	60	63
<i>Ixodes ricinus</i>	2	41	66	109
<i>Rhipicephalus sanguineus</i> complex			5	5
Total	3	46	132	181

The majority (109) were identified as *I. ricinus*, followed by *Haemaphysalis punctata* (63), *Rhipicephalus sanguineus* complex (5), *Haemaphysalis concinna* (3) and *Dermacentor* sp. (1). Tick abundance is shown to increase over time, except for the months of June and August (Table 2).

Table 2. Average number of collected ticks for each species by month.

	<i>I. ricinus</i>	<i>H. punctata</i>	<i>H. concinna</i>	<i>Dermacentor</i> sp.	<i>Rhipicephalus</i> <i>sanguineus</i> Complex	Total
May	1.06	0.11	0.06	0.00	0.00	1.23
June	0.21	0.08	0.04	0.00	0.00	0.33
July	1.67	1.04	0.00	0.00	0.13	2.84
August	1.08	1.33	0.00	0.04	0.08	2.53

Tick abundance was not constant throughout all the repetitions for the three sampling points, with a general increasing trend culminating in peaks during summer and an additional peak in May for sampling site B. However, nymphs alone present a peak earlier in time (end of the spring), while larvae present a peak during summer (Figure 2).

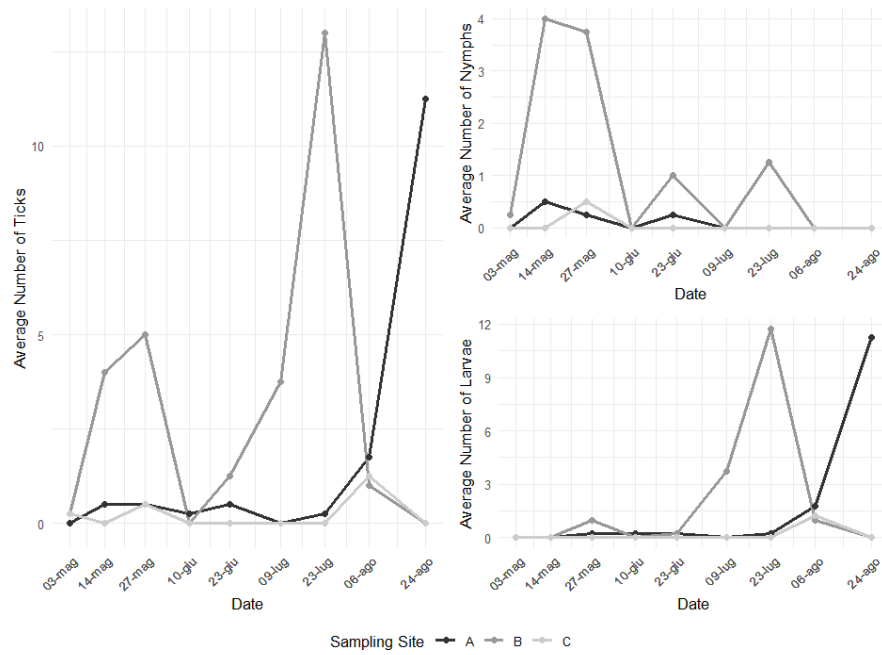


Figure 2. Fluctuation of tick abundance across the 9 repetitions in the three sampling sites.

The trend in tick abundance at a progressive distance from trails differed by developmental stage (Figure 3). Most larvae were collected within 1 m, with the average abundance increasing by 59.2% from 0 m to 1 m. We registered the lowest average abundance at 2 m (decrease of 72.2% from 1 m to 2 m) and a final increase at 4 m (69.6% from 2 m to 4 m). The average abundance at 4 m was still 26.2% lower than at 1 m. This trend was also visible for the total number of ticks and *Haemaphysalis* spp.

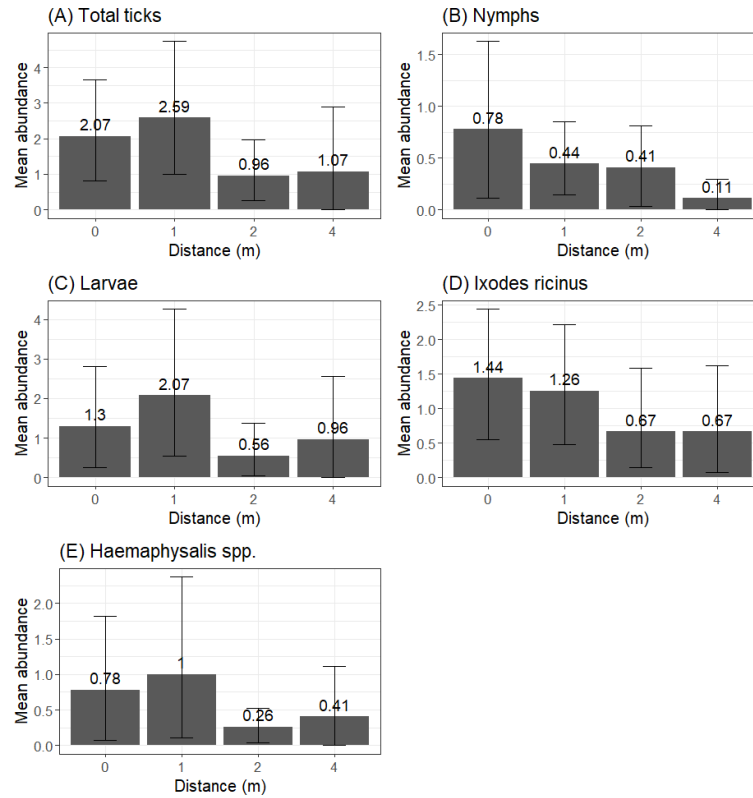


Figure 3. Plot illustrating the distribution of the average number of ticks at different distances (0 m, 1 m, 2 m and 4 m) from human trails for the following: (A) total amount of ticks, (B) nymphs, (C) larvae, (D) *Ixodes ricinus* species and (E) *Haemaphysalis* spp. species.

The average abundance of nymphs decreased when becoming further from the trail: –43.6% from 0 m to 1 m, –6.8% from 1 m to 2 m and –73.1% from 2 m to 4 m and showing a very limited presence at 4 m. This trend was also identified for *I. ricinus*.

Due to the limited number of specimens collected, we did not identify abundance trends for adults. Despite the difference in average abundance, a similar trend was shown at all sampling sites (Table 3).

Table 3. Average number of collected ticks (considering together all species and developmental stages) by sampling site at each distance.

	Sampling Site A	Sampling Site B	Sampling Site C
0 m	2.89	3.11	0.22
1 m	2.89	4.22	0.67
2 m	0.56	2.33	0.00
4 m	0.33	2.89	0.00
Total	1.67	3.14	0.22

Based on the deviance explained, the significance of coefficients and residuals performance, we identified three models describing, respectively, the abundance of the total number of ticks (deviance explained 22.5%, Poisson distribution), larval abundance (deviance explained 35.9%, Poisson distribution) and nymphal abundance (deviance explained 35.9%, negative binomial distribution). All models introduced the date and saturation deficit as linear predictors and the distance from the trail as a smooth term. Date has a significant positive coefficient ($p < 0.05$) in the general and the larval model and a significant negative coefficient in the nymphal model, while saturation deficit has a significant positive coefficient ($p < 0.05$) in all models (Table 4).

Table 4. Parametric coefficients and approximate significance of smooth terms for each model.

General			
Parametric Coefficients	Estimate	Std. Error	Pr ($> z$)
(Intercept)	-2.50	0.12	$<2 \times 10^{-16}$
Date	0.07	0.01	1.43×10^{-9}
Saturation deficit	0.7	0.03	$<2 \times 10^{-16}$
Smooth terms	edf	Ref.df	<i>p</i>-value
s(distance)	3	3	$<2 \times 10^{-16}$
Larvae			
Parametric coefficients	Estimate	Std. Error	Pr ($> z$)
(Intercept)	-4.42	0.15	$<2 \times 10^{-16}$
Date	0.34	0.02	$<2 \times 10^{-16}$
Saturation deficit	0.65	0.03	$<2 \times 10^{-16}$
Smooth terms	edf	Ref.df	<i>p</i>-value
s(distance)	3	3	$<2 \times 10^{-16}$
Nymphs			
Parametric coefficients	Estimate	Std. Error	Pr ($> z$)
(Intercept)	-3.45	0.51	1.95×10^{-11}
Date	-0.62	0.07	$<2 \times 10^{-16}$
Saturation deficit	1.41	0.19	1.34×10^{-13}
Smooth terms	edf	Ref.df	<i>p</i>-value
s(distance)	2.06	2.4	2.25×10^{-7}

Smooth terms are significant ($p < 0.05$) in all three models, showing trends that remark the ones highlighted by descriptive statistics (Figure 4), with a peak at 1 m for the general model and the larval model and a progressive decrease for the nymphs model.

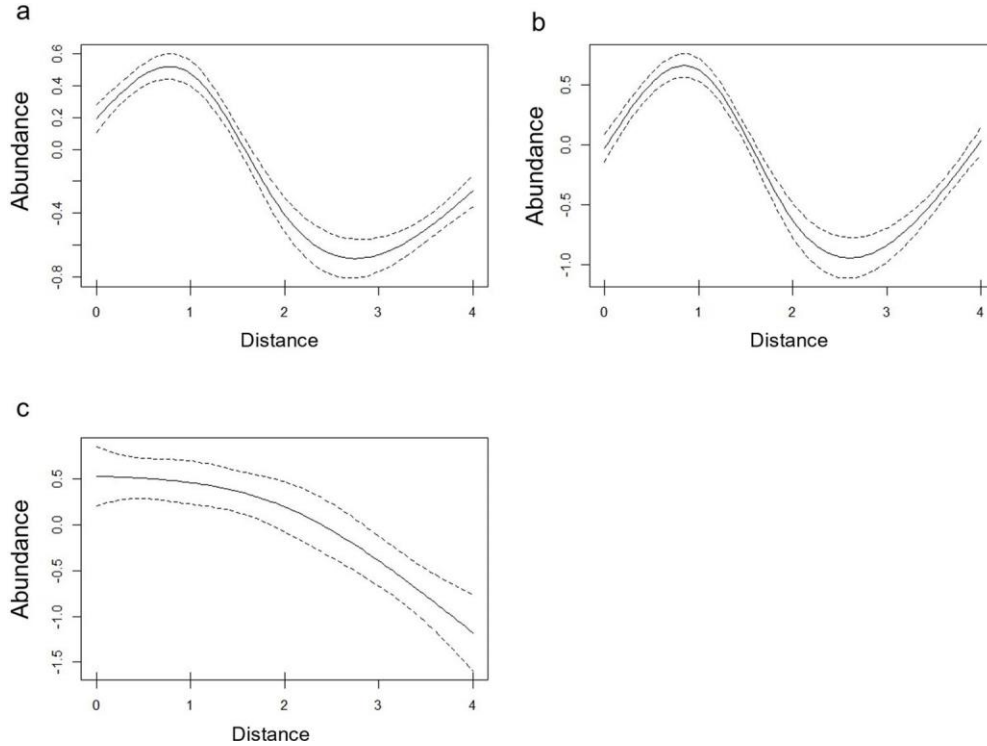


Figure 4. Plot of smooth term (distance from hiking trail, x axis) effects on tick abundance (y axis). Effects are plotted for the general model (a), the larval model (b) and the nymphal model (c).

4. Discussion

Hiking trails can be appealing for wildlife movement, particularly when dense vegetation along the sides obstructs passage, making the trails the easiest route to navigate. Our data showed a higher abundance of ticks at 0 m and 1 m distance with a heavy drop at 2 m, which is in line with the results previously obtained (Rasi et al., 2018), suggesting that ticks' abundance decreases when moving away from the path of animals or humans. The proximity of a pathway is a convenient spot for questing ticks to find a host, as the probability of passage is higher, and the adjacent vegetation creates a more suitable microhabitat for the tick's activity and survival.

The average ticks' abundance observed in our study was lower than those reported in other studies conducted at higher latitudes. For example, Van Gestel and colleagues (Van Gestel et al., 2021) recorded a density of nymphs of 0.46 per square meter, while Mols and colleagues a density of 0.75 per square meter (Mols et al., 2022). However, studies conducted in nearby areas reported a lower density, though this was still higher than what we observed (Millet et al., 2019). Differences in habitat characteristics, such as temperature, humidity and vegetation type, do play a role in

influencing ticks' abundance. Additionally, Hasset and colleagues (Hassett et al., 2022) noted that tick density was greater in areas with less human presence (Iijima et al., 2022). This could also be observed when referring to a study (Ferroglio et al., 2024b) undertaken concurrently with the present one in the forest and grassland parcels of the park, where the registered abundance was higher, but sampling was also performed deeper in the forest/meadows.

While the total abundance of ticks per sampling site and repetition varied significantly between sites, this variation did not interfere with identifying a general trend. Factors such as the movement rates of different species and the types of vegetation present may account for these differences. A lower presence of wild ungulates and mesocarnivores was indeed correlated with a lower ticks abundance in the park (Ezio Ferroglio et al., 2024b).

Nymphs showed a decreasing pattern when collections were gradually taken further away from the trail and they were rarely collected at a 4 m distance, corroborating the thesis of a limited dispersion previously hypothesized with carbon dioxide traps (Gray, 1985). Similarly, larvae were also concentrated along the proximity of the trail. Besides dispersion behavior, and consistent with the hypothesis that larvae have limited or no movement in the environment (Daniels & Fish, 1990), the accessibility of the host to the area on the sides of the trail likely played a role in shaping the observed patterns. Small mammals, which contribute to the presence of questing nymphs in the environment, have better access through dense vegetation, and some species may also show avoidance behaviors towards trails (Shorb et al., 2020), thus maintaining nymphal abundance more consistently even further away.

The pattern shown by different species was biased by the composition in terms of developmental stages: *Haemaphysalis* spp., 91% of which was constituted by larvae, followed the larval pattern, while *I. ricinus*, with a more even ratio of larvae–nymphs (56–41%, respectively), followed a more uniform decrescent pattern. Similar considerations can be addressed to the trend for the total average abundance of ticks. Therefore, our results could not allow us to draw conclusions about different dispersal behavior among tick species. Trends were identified through the Generalized Additive Models: the complexity of tick ecology may be the reason for the relatively low deviance explained, but the pattern described with the smooth terms clearly represents the trend observed with descriptive statistics.

The three models also allow us to remark on the effect of the progression of the season on ticks abundance, which is additive for larval stages and detractive for nymphs, whose population peak is usually registered between the end of spring and the beginning of summer (Gray, 2008; Kahl & Gray, 2022). The saturation deficit showed a significant impact on ticks activity; although a positive effect on ticks activity was registered only by

Tagliapietra and colleagues (Tagliapietra et al., 2011), on most occasions, it has been detected as a limiting factor (Zajac et al., 2021).

The composition, in terms of species and developmental stages, of the sampled ticks' population reflected the literature data (Salman & Tarrés-Call, 2013), with a higher abundance of *I. ricinus* and larvae, and a decreasing proportion of, in order, nymphs and adults. The sampling season may primarily account for the low number of adults collected (Gray, 2008), in addition to them being generally less abundant than in immature stages. Nymphs are consistently less abundant than larvae at all distances, despite questing on the upper stratum of the vegetation (Mejlon & Jaenson, 1997), which makes them more easily captured by the dragging/flagging cloth, suggesting that our sampling strategy was effective in also detecting the stages that usually quest closer to the ground.

Several studies in the European context have related park infrastructures to the risk of tick bite (Janzén et al., 2023; Van Gestel et al., 2021); despite remarking that tick abundance is higher in the forest interior or in areas where fewer people have access, acarological risk is still relevant along trails and, with that, the risk for tick-borne zoonoses (Anderson et al., 2021; Millet et al., 2019; Rasi et al., 2018; Van Gestel et al., 2021). Animal presence, in addition to vegetation characteristics, is the fundamental driver for such exposure (Iijima et al., 2022; Mols et al., 2022). Additionally, visitors' behavior contributes to defining the risk for people engaging in outdoor activities (Hassett et al., 2022; Janzén et al., 2023). For instance, humans and their pets are more likely to spend more time in low vegetation spaces or along trails than in more remote areas. A study in the USA showed that, despite knowing of the risk of a tick bite, people believed they had a minimal risk of tick encounters and consequently tended not to conduct tick checks (Hassett et al., 2022).

5. Conclusions

Analyzing the risk of tick-borne pathogen transmission in a park demands a focused consideration of infrastructures such as hiking trails. Developing integrated management strategies of infrastructures necessitates evaluating not only their impact on wildlife welfare and behavior but also their potential for drawing ticks closer to people. Our findings show that hiking trails exhibit higher tick abundance, increasing the acarological risk to both passing visitors and their pets.

3.3. Not just pictures: utility of camera trapping in the context of African swine fever and wild boar management.

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Not Just Pictures: Utility of Camera Trapping in the Context of African Swine Fever and Wild Boar Management

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Abstract. African swine fever (ASF) is a highly contagious disease affecting all suids and wild boar (*Sus scrofa*). Since 2007, ASF has spread to more than 30 countries in Europe and Asian regions, and the most recent outbreak has been in mainland Italy (reported on January 2022). When the genotype II of the ASF virus infects a population, a mortality rate close to 90% is usually reported. This drop in wild boar abundance produces a cascade effect in the entire ecosystem. In this context, effective monitoring tools for deriving management parameters are a priority aspect, and the utility of camera trapping could have been overlooked. Here, sampling the infected area in north Italy, we showed the utility of camera traps in the context of ASF infection. Specifically, we used 43 camera traps randomly distributed to (i) estimate movement parameters and population density of wild boar, roe deer (*Capreolus capreolus*), and wolf (*Canis lupus*); (ii) quantify wild boar recruitment; and (iii) assess whether the human restriction rules are being met. On the first spring after the outbreak detection, our results for wild boar indicated a density of $0.27 \text{ ind} \cdot \text{km}^{-2} \pm 0.11$ (standard error, SE), a daily activity level of 0.49 ± 0.07 (i.e., $11.76 \text{ h} \cdot \text{day}^{-1}$), a daily distance travelled of $9.07 \pm 1.80 \text{ km} \cdot \text{day}^{-1}$, a litter size of $1.72 \text{ piglets} \cdot \text{group}^{-1}$, and a 72% of pregnant females. Despite human outdoor activities being restricted in the infected zone, we recorded human presence in 19 camera traps. The wide range of parameters estimated from the camera trap data, together with some intrinsic and practical advantages of this tool, allows us to conclude that camera traps are well positioned to be a reference approach to monitor populations affected by ASF. The population-specific parameters are of prime importance for optimizing ASF control efforts.

1. Introduction

The African swine fever (ASF) epidemic is caused by a lethal viral hemorrhagic disease of swine (ASFV) that infects both wild and domestic suids and causes a mortality rate of about 90% within 4 to 15 days postinfection (Gallardo et al., 2015). ASF is affecting Eurasia, and it is transmitted by direct contact between infected animals and by ingestion of infected pork or other contaminated materials, been blood contact (carcass-based) the most efficient means of transmission (Dixon et al., 2020; Pepin et al., 2020). In Eurasia, suitable soft ticks are either absent or not relevant for ASF transmission, but the role of other mechanical transmissions such as clothing, transport trucks, or feed supplies has been discussed (Dixon et al., 2020). Since 2007, when the virus was first observed in Georgia, the ASFV has spread to more than 30 countries in the European and Asian regions (Sauter-Louis et al., 2021). In brief, the virus spread quickly to neighbouring countries, and ASFV genotype II has been notified in Belarus, Belgium, Bulgaria, Czechia, Estonia, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Moldova, North Macedonia, Poland, Romania, Russia, Serbia, Slovakia, and Ukraine (Sauter-Louis et al., 2021). Belgium became free from ASF in 2020, Czechia in 2019, and the Baltic states showed a declining trend in polymerase chain reaction- positive wild boar carcasses (EFSA et al., 2022; Sauter-Louis et al., 2021). The ongoing ASF pandemic is unprecedented in its geographical spread and impact on the global pork industry, and nowadays its management is a priority aspect at a global level (Sauter-Louis et al., 2021) and wild suid populations (Morelle et al., 2020). In many ASF-affected countries of Eurasia, wild boar (*Sus scrofa*) is a relevant wildlife host for the ASF virus, contributing to infection, maintenance, and spread, representing a notable challenge for disease control (EFSA et al., 2022).

Wild boar is a widespread native Palearctic ungulate whose population has sharply increased in the last decades (Massei et al., 2015). Because of both natural expansion and human introductions, the wild boar now occurs in all continents except Antarctica (Melletti & Meijaard, 2017). This species is involved in diverse and increasing conflicts, such as those related to damage to agriculture, collisions with vehicles, environmental effects, and the transmission of shared infections (Carpio et al., 2021). Moreover, the wild boar populations have also expanded spatially, overlapping with areas of livestock production (Foster et al., 2014). In outdoor pig farms, wild boar-domestic pig interactions usually occur and thus the risk of maintenance and spread of ASF (Jiménez-Ruiz et al., 2022).

In this context, monitoring wild boar populations for effective ASF management is a priority aspect. Wild boar is an elusive species with nocturnal activity patterns. These behavioural characteristics limited the utility of most traditional wildlife monitoring methods that are usually based on the direct observation of the animals (Acevedo et al., 2007). However, during the last decades, the use of remotely activated cameras (i.e., camera traps) has overcome most of these limitations and has been frequently described as a reliable and cost-efficient monitoring method (Wearn & Glover-Kapfer, 2019). Regarding the utility of camera traps in the context of ASF pandemic, three studies

have been already published. Cadenas-Fernandez et al. (Cadenas-Fernández et al., 2019) examined the interaction between free-ranging pigs and wild boar in an ASF-endemic area of Sardinia; Morelle et al. (Morelle et al., 2020) evaluated the disease-induced mortality due to ASF in Poland; and Bollen et al. (Bollen et al., 2021) assessed the spatiotemporal variability in wild boar occupancy in infected and noninfected zones in Belgium. To the best of our knowledge, these are the only three studies that have used camera traps in the context of wild boar and the ASF pandemic.

Other relevant applications of camera trapping in the ASF context are still underexplored. First, camera trapping is a noninvasive methodology, and thus a minimum disturbance is caused in the sampled population, which is a priority aspect when an outbreak is reported in a population (EFSA et al., 2020). Camera traps can provide useful data regarding the spread, persistence, and impact of the virus. Regarding the spread, it is well described that the vast majority of ASF outbreaks are mediated by human movements, for instance, by pork-contaminated products and/or transporting the virus by clothes (Iscaro et al., 2022; Woźniakowski et al., 2021). Thus, human restriction laws and prohibiting outdoor activities are habitually implemented. Camera traps can provide evidence of the violation of the laws in areas difficult to control by other methods. Camera traps can be also applied to estimate movement parameters such as activity patterns and daily distances travelled (Palencia et al., 2021a; Rowcliffe et al., 2014, 2016). Regarding disease persistence, it has been described that spring recruitment (expressed as the proportion of females giving birth to piglets) has a strong effect on ASF persistence (Gervasi & Guberti, 2021). Newly born wild boar is susceptible to infection and virus transmission (Gervasi & Guberti, 2021). Camera traps can provide reliable data on piglet presence and litter size (Barasona et al., 2020).

Finally, regarding ASF impacts, recently described multi-species methodologies could be useful to derive population parameters such as abundance, not only of wild boar but also of other mammals indirectly affected (and involved) by ASF outbreaks (Palencia et al., 2022).

In this context, we aimed to provide further insights into the utility of camera traps to monitor wild boar populations affected by an ASF outbreak. Especially, we targeted (i) to estimate movement parameters such as the proportion of the day that the population spend in movement and the average daily distances movement by the individuals; (ii) to provide robust and reliable population density estimates of not only wild boar but also other mammal's species with a relevant role under a scenario of ASF outbreak (e.g., roe deer (*Capreolus capreolus*) and wolf (*Canis lupus*)); (iii) to quantify spring recruitment; and (iv) to provide empirical evidence of compliance with the rules of human movement restriction. For that, we considered a case study of the recently infected area in north Italy (Iscaro et al., 2022), where not essential outdoor activities were restricted (Italian Ministry of Health, 2022).

2. Materials and Methods

2.1. Study Area. The study was carried out in the Natural Park Capanne di Marcarolo National Park, a territory of 8288 ha located in north Italy (Alessandria province, the Piedmont region) (Figure 1). It is a mountainous area, with altitudes ranging from 335 to 1170 m.a.s.l (average 700 m.a.s.l.). The vegetation is characterized by sparse wood of oaks (*Quercus petraea*), beeches (*Fagus sylvatica*) and pines (*Pinus pinaster* and *Pinus silvestris*), and dry grass-lands. A total checklist of 955 plant species with 3 strict endemic ones (*Aquilegia ophiolithica*, *Cerastium utriense*, and *Viola bertolonii*) has been described (Barberis et al., 2004). The climate is continental-temperate with marked seasons and annual precipitation ca. 1500 mm. The management team of the natural park authorized and supported the study.

2.2. Field Methods. From March to August 2022, 43 camera traps (Browning Strike Force HD X Pro-model BTC- 5HDPX) were randomly deployed covering the study area (Figure 1). Cameras were deployed facing north, 50 cm above the ground, with the sensor angled parallel to the slope, and fixed to the tree with a steel cable for security reasons. Realised sampling locations deviated from the design by as much as 100 m to mount camera traps on trees and avoided very unfavourable conditions (e.g., high slopes in front of the camera). Only one camera was pointed out at a human trail. Cameras were set to be operative all day, to record a burst of eight consecutive pictures (rapid fire settings) at each activation, with the minimum time lapse (0.22 sec) between consecutive activations. Nocturnal pictures were illuminated with infrared flash (low glow). A table describing camera trap settings can be found in Appendix S1. Neither baits nor attractants were used. The date and time of each capture were automatically stamped onto each picture. Once a month, we checked the battery and memory status of the cameras. After each fieldwork session, boots, clothes, and materials were disinfected with a Virkon S solution.

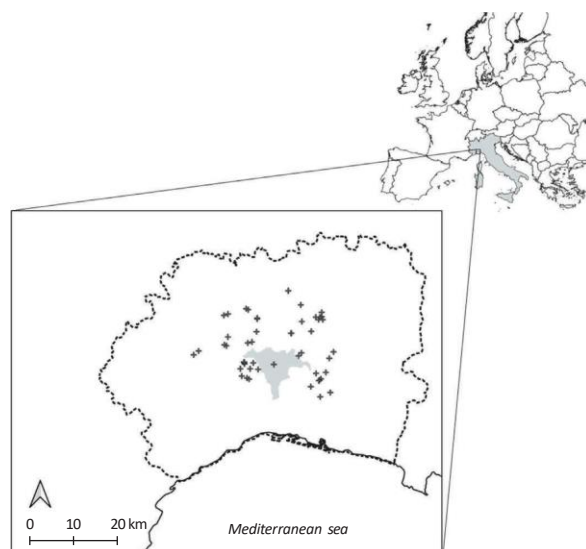


Figure 1. Location of the study area in northern Italy. Crosses represent positive African swine fever (ASF) wild boar reported; the dashed line represents the zone II of restriction according to administrative zonation due to ASF emergence; and the grey polygon in the bottom panel represents the area surveyed in this study placed in the core of the infected area.

To lastly estimate the location of the photo-captured animals in the field of view of the camera (see details below), we used a photogrammetry method (Wearn et al., 2022). Thus, on each deployment (43 field stations), we recorded 20 pictures of a 1 m length pole with marks at 20 cm intervals. These 20 pole locations were randomly distributed within the camera field of view, covering the entire viewing angle (55° in our devices) and up to a maximum distance of 15 m from the camera. The pole was held perpendicular to the camera's passive infrared sensor. These 20 photos were taken before and after each camera was checked to evaluate battery and memory status. Additionally, to apply this photogrammetry method, it is necessary to calibrate the camera trap model. The camera model was calibrated once in a flat area without dense vegetation. We used the same 1m pole used on the field to calibrate the deployments. In this case, the pole was set at known distances. Specially, we defined four distances (2.5, 5, 7.5, and 10 m) from the camera, and we placed 6 times on each distance the pole, covering the viewing angle. Thus, we recorded 24 pictures of the pole at known distances to the camera.

2.3. Analysis

2.3.1. Movement Patterns. Wild boar but also wolves' movements have been described as the potential drivers of ASF spread by faces and/or mechanical transportation of the virus with mud on the paws (Kemenszky et al., 2022; Szewczyk et al., 2021). Camera trap-based methodologies have been described to estimate (i) activity level (i.e., the proportion of the day that the population spend in movement) and daily distance travelled (i.e., day range) of wildlife. From camera trap data, the activity level is estimated by fitting circular kernel distribution to the time in which the animals were recorded (Rowcliffe et al., 2014). The day range is estimated by multiplying the activity level by the average speed of movement (Rowcliffe et al., 2016) and accounting for different movement behaviours (Palencia et al., 2021a). Movement speed was estimated by dividing the distance travelled by each individual photo- captured by the time elapsed. Individuals reacting to the camera were discarded from the analysis as their natural behaviour was modified by our sampling method. Animal positions to derive speed density were estimated by the photogrammetry methods and using CTtracking tools (Wearn et al., 2022). In brief, we tracked the pixel position of each individual in each picture. Then, using the calibration imagery, we converted each pixel position into radial and angular distances from the camera. These distances were trigonometrically transformed to estimate the distance moved across each encounter and then estimate the speed of movement by accounting for the time elapsed (see Wearn et al., 2022 for a more in detail explanation). The presence of different movement behaviours in the sampled population was explored by fitting log-normal mixture models using the R package trapping motion and following the procedure described by (Palencia et al., 2021a).

2.3.2. Mammal's Population Density. The random encounter model (REM) (Rowcliffe et al., 2008) was applied to estimate the population density of the target species (i.e., wild boar, roe deer, and wolf). The REM has been validated as a reliable method to estimate population density when individual identification is not possible (Palencia et al., 2022) and has been applied as a reference method to monitor wild boar density (ENETWILD-

consortium et al. 2019b, 2022a; Palencia et al., 2021b) . It has also been evidenced the utility of the REM to estimate the population density of the community of mammals inhabiting an area (Palencia et al., 2022; Wearn et al., 2022) . The REM models the random encounters between animals and cameras and estimates density accounting for other factors that affect the encounter rate (i.e., animal movement and camera trap detection capability). The REM equation is as follows:

$$\frac{y}{t} * \frac{\pi}{v * r * (2 + \theta)} \quad (1)$$

where y is the number of encounters, t is the total survey effort, v is the day range, and r and θ refer to the effective radius and angle of the camera detection zone, respectively. An individual of the target species entering the detection zone was considered an encounter (y). As our sampling period overlapped with the calving season, we discarded year-born individuals from the analysis, and thus we estimated the density of adult (>1 year) individuals. To estimate t , we quantified the total number of days that each camera was operative during the sampling periods. We discarded those camera days in which a given camera was not operative due to empty batteries, full memory, and/or stolen. To estimate the detection zone, we only considered the animal's position in the first picture in which each one was recorded. Distances and angles for the first detection were modelled under distance sampling theory to finally estimate the effective detection zone size (Buckland, 2001; Rowcliffe et al., 2014).

The R packages *activity*, *distance* and *trappingmotion*, and some functions in *CTtracking* repository (<https://github.com/MarcusRowcliffe/CTtracking>) were used. The R code, working data, and vignette to run a REM analysis are available in the following link <https://github.com/PabloPalencia/CameraTrappingAnalysis/tree/main/REM>.

2.3.3. Spring Recruitment. We selected an interval longer than 10 min to consider two consecutive records of wild boar as independent events. After the first record of a group of piglets, we recorded in each event (i) the sex of the adult individuals on the basis of external signs and (ii) the litter size (number of piglets) when present.

2.3.4. Biosafety: Human Movement Restriction. Using the abovementioned 43 camera traps deployment, we evaluated the presence of humans and domestic dogs in the pictures recorded during the entire sampling period. Those deployments in which the camera was stolen were quantified as positive to human presence despite we did not have the photographic evidence. On every single camera, we also quantified the time gap between a human and the previous wild boar encounter.

3. Results

A total of 980,883 pictures were recorded during an effective sampling effort of 4037 camera-days. A total of 12 mammal species were recorded. Roe deer was the more frequent, with more than 1500 encounters, followed by the European hare (*Lepus europaeus*) with 228 encounters. We also detected other rare and endangered species such as wild cat (*Felis silvestris*), polecat (*Mustela putorius*), and weasel (*Mustela nivalis*). See a

summary of all the mammals recorded in Appendix S2 and the raw data to replicate the analysis in Appendix S3.

3.1. Movement Patterns. The activity level was 0.54 ± 0.06 (standard error, SE) for roe deer, 0.49 ± 0.07 for wild boar, and 0.60 ± 0.10 for wolf (Figure 2). This indicates that the roe deer spent 12.96 hours active per day, wild boar spent 11.76 hours, and wolf spent 14.4 hours. Regarding the day range, the roe deer showed the lowest daily displacement ($4.64 \text{ km} \cdot \text{day}^{-1} \pm 0.97$), $9.07 \text{ km} \cdot \text{day}^{-1} \pm 1.80$ for wild boar, and $37.74 \text{ km} \cdot \text{day}^{-1} \pm 6.91$ for wolves. While two movement behaviours were observed in wild boar (mean speed slow behaviour: $0.09 \text{ m} \cdot \text{s}^{-1} \pm 0.03$; fast behaviour: 0.87 ± 0.08), only one behaviour was observed in roe deer ($0.10 \text{ m} \cdot \text{s}^{-1} \pm 0.02$) and wolf ($0.73 \text{ m} \cdot \text{s}^{-1} \pm 0.10$) (Figure 2).

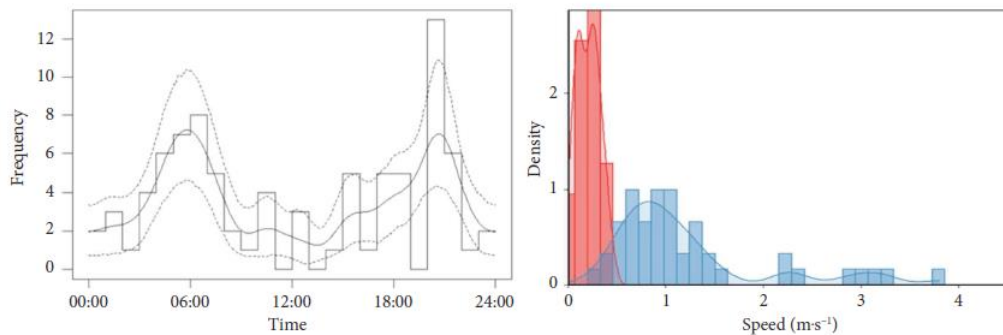


Figure 2: Movement parameters for wild boar. The left panel represents the activity pattern (continuous line being the mean, dashed line being 95% confidence interval). The right panel represents the two movement patterns observed in the population. The red colour represents slow movements (e.g., feeding), and the blue colour represents fast movements (e.g., displacement between habitat patches).

3.2. Mammals' Population Density. Estimated densities were $0.27 \text{ ind} \cdot \text{km}^{-2} \pm 0.11$ for wild boar, $6.35 \text{ ind} \cdot \text{km}^{-2} \pm 1.88$ for roe deer, and $0.07 \text{ ind} \cdot \text{km}^{-2} \pm 0.03$ for wolf.

3.3. Spring Recruitment. The first evidence of wild boar reproduction was recorded on May, 6th, when we recorded a female with three piglets in one camera. After that, we recorded 72 independent wild boar events of one or two adult individuals (average $1.26 \text{ ind} \cdot \text{group}^{-1}$). Specifically, we recorded 17 events of males, 41 events of females and piglets (24 of them only piglets), and 14 events in which we were not able to identify sex. From the 41 events of females and piglets, we recorded piglets in 32 of them, with an average litter size of $1.72 \text{ piglets} \cdot \text{group}^{-1}$ (min: 1; max: 4).

3.4. Human Restriction Laws. Human presence was detected in 19 camera traps, and seven of them were stolen. A maximum of 14 people was detected in a single camera. Domestic dogs, always off leash, were detected in eight cameras. Humans were detected doing different open-air leisure activities such as trekking, mushroom picking, family walks, or walking the dog (Figure 3). Wild boar presence was confirmed in 50% of the cameras in which humans were observed. Except for one wild boar-human interaction spaced by 125 days, all the others occurred in less than 12 days (mean ± 6.4), with a minimum time gap of 12 h.

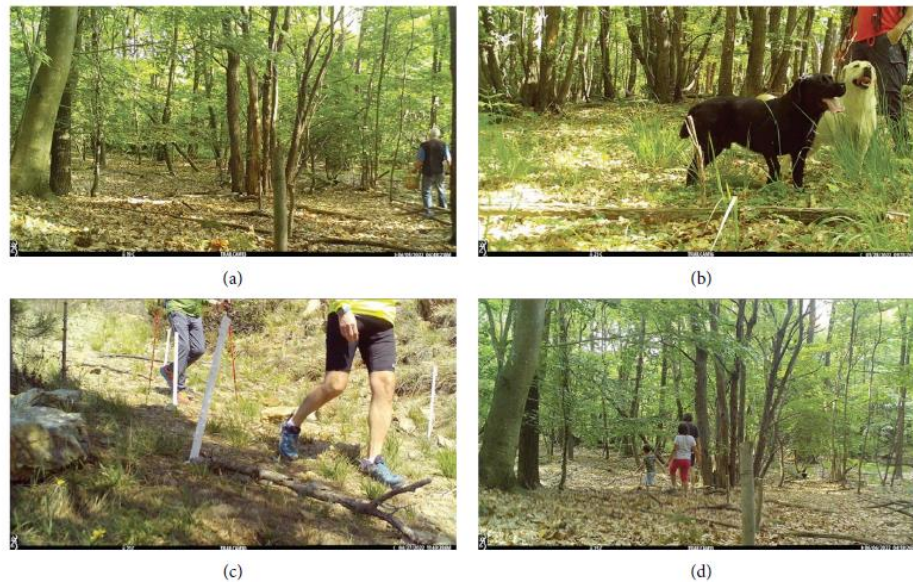


Figure 3. Example of pictures evidencing human presence and domestic dogs on leash in an area restricted to human movements due to the African swine fever outbreak. Different outdoor activities were recorded, such as mushroom picking (a), walking the dog (b), trekking (c), and family walks (d).

4. Discussion

In this study, we evidenced the utility of camera traps to monitor populations infected with ASFV by reporting both ecological and management-related parameters.

The ASFV (genotype II) was first detected in mainland Italy on 29 December, 2021, in a male adult wild boar. In the next few days, other wild boars were found dead in a buffer zone of 20 km (Iscaro et al., 2022). In 2022, other two outbreaks have been reported in central Italy (farther than 500 km from the first report) and the spill-over to domestic pig farms occurred. These long-distance jumps reinforced the hypothesis that the transmission occurred via the human factor. Emergency measures to prevent the spread of the diseases were undertaken by Italian authorities, especially focused on passive surveillance and biosecurity measures, fencing, and human activity regulation (Italian Ministry of Health, 2022). Focusing on human restrictions, hunting and other outdoor activities were prohibited in the infected area and neighbouring regions (Figure 1). Concerning this, our camera traps provided evidence of a restriction violation. Human pictures were present in 19 camera deployments, showing a great variety of outdoor activities such as picking up mushrooms, trekking, and/or family walks (Figure 3). The human presence was frequent in some cameras, with a maximum of 14 people recorded in one camera. Additionally, free-ranging dogs were also observed (Figure 3). Previous studies have shown that the survival times of the virus on the soil strongly depend on the type of soil (e.g., sand vs. humus-rich one), with average survival times ranging from less than three days to up to two weeks (Carlson et al., 2020). In contaminated water, the infectious virus was detectable for more than two weeks (Carlson et al., 2020). Here, the average time gap between a wild boar and the subsequent human encounter was 6.4 days, with some encounters spaced just 12 h. Thus, it has been possible the mechanical

spread of the virus by clothing or mud rest (Woźniakowski et al., 2021). We would like to emphasize that all the cameras except one were not pointing to human trails; thus, we expect that the human presence in the infected (and restricted) area should be more frequent in hiking trails and paths. Additionally, humans usually avoid being recorded by the cameras by passing behind them. Thus, the human presence in the study area could be even underestimated. Despite other actions such as 270 km of fencing has been implemented to control the spread of the virus, allocating funds to implement vigilance for restriction rules and increasing dissemination campaigns to stakeholders and the general public are instead needed to prevent the spread of the virus according to our results. It has been demonstrated that fencing is neither the most effective action for widely affected areas (Jo & Gortázar, 2021; Mysterud & Rolandsen, 2019; Sauter-Louis et al., 2021) nor to control the movements of wild boar (Laguna et al., 2022).

Regarding the estimation of ecological parameters, camera traps have been broadly used to monitor different ecological, behavioural, and sanitary aspects of wild boar. Specifically, it has been used to monitor population densities and abundance (Higashide et al., 2021; Palencia et al., 2021b; Pascual Rico et al., 2022); movement parameters such as activity level and daily distance travelled (Ohashi et al., 2013; Palencia et al., 2019, 2021a); species interactions (Cadenas-Fernández et al., 2019; Carrasco-Garcia et al., 2016; Triguero-Ocaña et al., 2020); reproduction success (Barasona et al., 2020); and diseases transmission risk points (Jiménez-Ruiz et al., 2022; Kukiela et al., 2013) among others. Even more recently, some studies have discussed the utility of camera trapping to be implemented in integrated wildlife monitoring programs targeted at wildlife health and host community monitoring (Barroso et al., 2023). Here, we reinforced the utility of camera traps in the ASF epidemic (Bollen et al., 2021; Cadenas-Fernández et al., 2019; Morelle et al., 2020). Despite the management of ASF is usually focused on the wild boar and pork industry (AHAW et al., 2020), the ecological impact of ASF emergence in a wild boar population could affect the entire ecosystem. Wild boar is considered an “ecosystem engineering” (Sandom et al., 2013), and a drastic decrease in wild boar abundance due to ASF high mortality is expected to cause significant changes. For instance, a recent study described that wolves’ diet changed to more predation on both roe and red deer after ASF emergence in Belarus (Klich, et al., 2021a). Similarly, the emergence of ASF in Poland derived in a higher number of livestock attacks by wolves (Klich, et al., 2021b). Thus, the utility of multispecies methodologies to monitor both wild boar population size but also other species involved in the dynamic of the ecosystem will improve the better understanding of the system as well as the management actions implemented (Allen et al., 2011). The random encounter model is a camera trap-based method that allows the estimation of population density without the need to individually identify animals (Rowcliffe et al., 2008) and spatial autocorrelation in the camera trap placements. This provides the flexibility of a given survey design to estimate the density of more than one species (Palencia, 2021; Palencia et al., 2021b, 2022; Rowcliffe et al., 2008; Wearn et al., 2022). Reliable data on the abundance of multiple species after an ASF outbreak will improve decision-making. To the best of our knowledge, this is the first study in which the wild boar, other ungulates (here roe deer),

and main predator (here wolf) population densities have been estimated. While the REM has been already applied and validated for wild boar and roe deer (ENETWILD-consortium et al., 2022a; Palencia et al., 2021b, 2022; Pfeffer et al., 2018), this is the first study in which the REM has been applied to estimate wolf density. The REM has not been yet tested against wolf reference densities; but, it has been previously applied to other carnivores and/or apex predators (*Felis silvestris*—(Anile et al., 2014); *Panthera leo*—(Cusack et al., 2015); *Puma concolor*—(Loonam et al., 2021)). The point density estimated here is equivalent to that reported by genetic sampling in the study area (Marucco et al., 2022). Regarding the wild boar density, a previous study based on the capture-recapture method estimated a density in the study area ranging from 3.45 to 6.90 ind·km⁻² in 2006 (Bertolino, 2008). According to the general population trend of wild boar in Europe (Massei et al., 2015), it is expected that the wild boar abundance has increased in the subsequent years till the ASF outbreak. Inside the study area, the first ASF-positive carcasses were reported in early January 2022 (Figure 1). Our sampling period covers from two to six months later; thus, the wild boar density reported here is expected to be one of the lower after ASF emergence and significantly lower than before the ASF emergence. Previous studies have reported a drop in wild boar density by 84% and 95% within one year following the ASF outbreak (Morelle et al., 2020). Subsequent monitoring programs could be recommended to monitor both the wild boar and other species populations.

Regarding the movement patterns, the activity level and the day range estimated for the target species are in the range of those reported in other areas (Barroso et al., 2023; ENETWILD-consortium et al., 2022a; Palencia et al., 2022; Palencia et al., 2021a, 2021b; Pfeffer et al., 2018). Wild boar movement has been suggested to be only responsible for local transmission (Podgórski & Śmietanka, 2018; Taylor et al., 2021) and other pathways (such as the human ones discussed above) be more dominant in medium- and long-distance spread of the disease. Some authors have also discussed the effect of animal movement, especially wolves, which usually display longer movements (Kusak et al., 2005). Broadly, a better understanding of wildlife movement patterns will help to improve the prediction capability of the spread of the virus (Nigsch et al., 2013). Daily distances travelled by wild boar are relatively higher than those estimated in other wild boar populations across Europe (ENETWILD-consortium et al., 2022a). This is consistent with the longer movement observed in marked animals (Bertolino, 2008). In the capture-recapture study carried out in 2006, four individuals have been recaptured far away, 15 km from the study area, with a maximum of one male found at 28 km and a female found at 25 km (Bertolino, 2008). These movement patterns (together with a likely late detection and human presence in the infected area) could explain the wide affected area, currently more than 3000 km² (Figure 1).

Finally, regarding spring recruitment, the values reported in our study (i.e., 1.72 average litter size and 78% of females giving birth to piglets) are in the range of those previously published (ENETWILD-consortium et al., 2022a). Through modelling, it has been demonstrated that ASF persistence is significantly and positively influenced by spring recruitment (Gervasi & Guberti, 2021). While a recent review concluded that

the average percentage of pregnant females is lower than 50% for all the age classes (ENETWILD-consortium et al., 2022a), the value observed in our population was much higher. We acknowledge that pseudoreplication issues (i.e., the same female and piglets group recorded more than once) could emerge when using camera traps and monitoring species without natural marks that allow individual identification. However, while these multiple records could inflate the sample size, bias is not expected in average litter size, not on a percentage of females with piglets. An increase in re-productive performance will also generate a higher population growth during the endemic phase, increasing the chances for a second epidemic wave (Gervasi & Guberti, 2021).

Despite that it has not been addressed in this study, camera traps are also a cost-efficient tool to monitor wild boar and domestic pig interactions (Carrasco-Garcia et al., 2016; Triguero-Ocaña et al., 2020) and wild boar occupancy (Bollen et al., 2021). Previous studies have already monitored wild boar-domestic pig interactions in the context of ASF and free-ranging pigs, concluding its utility and the need to include this interface in the epidemiological assessments of ASF (Cadenas-Fernández et al., 2019) occupancy estimates derived from camera trap data will also be useful to better understand wild boar- environment relationships and to select those areas preferentially occupied when intervention actions such as strengthening passive surveillance or culling are implemented (Wevers et al., 2021).

In conclusion, our study reinforced the utility of camera traps to monitor populations infected by ASFV. Despite the initial inversion (ca. 150–200 € per device), camera trapping is a cost-efficient methodology, especially from the long-term point of view. By now, only Czech Republic and Belgium have recovered the ASF-freedom status, being the endemic status the most frequent in Europe after the virus infected an area (Sauter-Louis et al., 2021). The noninvasiveness characteristics together with the multispecies potential are also relevant advantages in the ASF context. New analytical procedures in which individual recognition is not needed are also especially relevant for wild boar (Palencia et al., 2021a; Rowcliffe et al., 2008, 2016). Broadly, camera traps provide reliable data that could be subsequently considered in prevention, control, and eradication plans. We encourage wildlife managers, administrators, and decision-makers to consider a camera trap as a reference tool to estimate population-specific parameters, which are of prime importance for optimizing ASF management efforts.

3.4. The contribution of citizen science in the surveillance of wildlife and related arthropods.

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The contribution of *citizen science* in the surveillance of wildlife and related arthropods

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Abstract. Environmental and anthropogenic factors may significantly affect the diffusion of wild animals, enhancing the interface of human–wildlife interactions and driving the spread of pathogens and vector-borne diseases between animals and humans. However, in the last decade, the involvement of citizens in scientific research (the so-called *citizen science* approach, hence- forth abbreviated as CS) provided a network of large-scale and cost-effective surveillance programmes of wildlife populations and their related arthropod species. Therefore, this review aims to illustrate different methods and tools used in CS studies, by arguing the main advantages and considering the limitations of this approach. The CS approach has proven to be an effective method for establishing density and distribution of several wild animal species, in urban, peri-urban and rural environments, as well a source of information regarding vector–host associations between arthropods and wildlife. Extensive efforts are recommended to motivate citizens to be involved in scientific projects to improve both their and our knowledge of the ecology and diseases of wildlife. Following the *One Health* paradigm, collaborative and multidisciplinary models for the surveillance of wildlife and related arthropod species should be further developed by harnessing the potentiality of the CS approach.

What does *citizen science* mean? Theoretical considerations

The COVID-19 pandemic has enlightened the world on the risks of spill-over and transmission pathways of zoonotic agents through several interaction phenomena at the human–wildlife interface (One Health High-Level Expert Panel, 2023). This is particularly noteworthy when considering that 70% of zoonoses originate from wildlife (Jones et al., 2008; Morse et al., 2012) and the increasing density of wild species in urban and peri-urban settlements may act as a trigger for the circulation of zoonotic agents, including those transmitted by arthropods (Wegner et al., 2022). Furthermore, several sociodemographic factors, such as agricultural strategies, wildlife management, deforestation and global warming may significantly affect the diffusion of wildlife and arthropod–host associations (Dantas-Torres et al., 2013). These aspects indicate that the monitoring of wildlife populations and their arthropod species is crucial for preventing the potential spread of emerging or re-emerging vector-borne pathogens (VBPs)

(Santoro et al., 2019; Sgroi et al., 2021).

In this scenario, the *citizen science* approach (hereafter CS) facilitates the collection of large-scale data in a cost-effective manner whilst, raising awareness of zoonoses prevention among the general public (Hamer et al., 2018). Several interpretations of this approach are to date available in the literature, whereby there are 34 different definitions of CS reported (Haklay et al., 2021). For example, UNESCO defines CS as *the participation of a range of non-scientific stakeholders in the scientific process. At its most inclusive and most innovative, CS involves citizen volunteers as partners in the entire scientific process, including determining research themes, questions, methodologies and means of disseminating results* (Haklay et al., 2021). Since CS is currently developing so rapidly, the discussions regarding definitions and criteria are so varied, and it is difficult to narrow this down to a single definition (Vohland, 2021). However, ubiquitous in all definitions is the point that in any CS project, activities are voluntary undertaken by members of the general public who are represented by non- scientific stakeholders interested in a specific problem-solving. Although the practices themselves are much older, CS as a definition originated in the 1990s as a result of the work of scientists such as Alan Irwin and Rick Bonney who recognized the value of data collected by amateur naturalists and its implications in research (Brossard et al., 2005). In the last decade, the term CS became extremely popular due to a rise in the number of publications and research projects. As a result, CS is a routine practice supported by social media (acting as 'brokers') and public institutions, such as the European Citizen Science Association (ECSA) which promotes the growth of an interdisciplinary and international CS community (Frigerio et al., 2018). Therefore, beginning by discussing a symposium during the *Italian Congress of Parasitology 2022* (SoIPa Congress), this review aims to provide an overview on the contribution of CS as a valuable tool in the surveillance of wild animals and their related arthropod species, and highlight its advantages and disadvantages. During the CS approach, mutual exchanges occur between citizens and scientists whereby any information provided by citizens is transferred to scientists who return it in the form of results of public utility. For instance, when citizens collect data (e.g. on the presence of wild animals in an area or the occurrence of arthropods on wildlife), this information is then interpreted and integrated into sources of information for either academia, or public information (e.g. density evaluation of wildlife and risk of pathogen transmission or the presence of vectors in a given environment). Compared to traditional methods, multiple advantages may be considered in studies that involve CS. Firstly, the possibility for scientists to provide public health education and open access to scientific findings for participants. In addition, CS may be a way to spread medical knowledge to geographic areas where such information is poorly available, such as in rural settlements that are commonly characterized by limited public health services (De Vries et al., 2018). In these environmental contexts, CS may be a useful tool for supporting hard-to-reach populations (i.e. forestry workers, hunters, farmers), in collaboration with

local public institutions (Perry et al., 2022; Stufano et al., 2022). Secondly, scientists have the opportunity to obtain large amounts of samples and datasets, over wide territories in a cost-effective manner. In fact, the collection from wider geographic areas than possible by small teams of scientists is a common feature of CS studies (Dickinson et al., 2010; Hamer et al., 2018; Johnson et al., 2022; Sgroi & Veneziano, 2023). However, the involvement of a large number of volunteers does not necessarily increase economic costs by scientific institutions, as most programmes do not pay contributors, as they simply participate because of their enthusiasm and interest in science (Edwards et al., 2021; Hamer et al., 2018; Poh et al., 2022; Rafiq et al., 2019). At the same time, the direct collaboration between scientists and citizens promotes transparency and trust between the general public and scientific institutions (Hamer et al., 2018). The main advantages of the CS approach outlined above are summarized in Table 1.

Table 1. Advantages of *citizen science* approach for citizens and scientists/ scientific institutions in the surveillance of wildlife and related arthropods

For citizens	For scientists/scientific institutions
Health education and promotion	Large-scale datasets and amount of samples
Open access to scientific advance	Wide geographic sampling
Trust in public health institutions	Cost-effective research
Citizenship's interest in science	Optimization of research fundings

It is important to note that the challenges and limitations of CS studies may occur in the case that citizens are not properly trained or supported by experts. This can result in misinformation or insufficient volume of data or incorrect procedures in data collection with a consequent wane in public interest and loss of interest in participation over time (Laaksonen et al., 2017; Wozniak et al., 2015). Other limitations include the potential risk of infection to citizens during collection, transport or transport samples in the field and the need to guarantee anonymity and correct use of their personal information during a CS project. Finally, it is essential to check and verify the robustness and reliability of data collected by volunteers over the life of the programme, especially in long projects (Hamer et al., 2018).

The application of *citizen science* in wildlife census

Contrary to popular belief, census of wildlife goes back centuries through initiatives that today would be described as CS. For example, in late 16th century, the Spanish government created a survey (called 'relaciones topográficas'), which provided information on the presence of several animal species (Clavero & Revilla, 2014). To date, sightings of wildlife by citizens contribute for obtaining a plethora of information, such as population density (Scott et al., 2014), spatial overlap and interactions with humans and companion animals (Goswami et al., 2015) and, consequently, help in the prediction

of potential routes of pathogen transmission (Lawson et al., 2015).

Different tools and methods are set up for conducting census activities of wildlife in different countries of the world (Table 2). Most CS projects are based on opportunistic observations of live wild animals through GPS-telemetry (i.e. GPS-transmitters that are fitted to the animals with a collar/ear tag to track their movements) in various environmental contexts, including natural parks or faunal reserve (Ostermann-Miyashita et al., 2022; Rafiq et al., 2019). Other studies aim to create maps on the distribution of wildlife employing volunteers to fill specific road-killed schedules (ENETWILD-consortium et al., 2023a; Heigl & Dörler, 2022; Raymond et al., 2021). A substantial number of CS studies are based on camera trap (or camera capture) surveys, where participants collect images and/or videos of wild species that are further verified by experts (Caravaggi et al., 2018; Lasky et al., 2021; Swanson et al., 2016). Although difficult to apply on a large scale, the camera trap is commonly preferred as an independent, less disruptive and feasible tool for collecting reliable data on wildlife density (ENETWILD-consortium, 2021; ENETWILD-consortium et al., 2022a).

Table 2. *Citizen science* projects based on sightings of wildlife species classified according to taxonomic order

Wildlife species	Contribution of citizen scientists	Country	Reference
Artiodactyla			
Red deer (<i>Cervus elaphus</i>)	Sightings reported on website with date and coordinates	Great Britain	(Croft et al., 2019)
Reindeer (<i>Rangifer tarandus</i>)	Sightings reported on website with date and coordinates	Norway	(Cretois et al., 2021)
Roe deer (<i>Capreolus capreolus</i>)			
Wild boar (<i>Sus scrofa</i>)	Sightings reported on questionnaire survey with geographical information	Denmark	(Jordt & Alban, 2016)
Carnivora			
African lion (<i>Panthera leo</i>)	Assessment of lion tracks reported by cell phone with number of lions, age, sex and predation patterns towards domestic animals	Kenya	(Dolrenry et al., 2016)
Red fox (<i>Vulpes vulpes</i>)	Urban sightings reported on website with date, time and coordinates	Great Britain	(Scott et al., 2014)
Red fox	Urban sightings reported by phone call, e-mail and online platform, with number of animals, date, time, dens, traces and photographs	Austria	(Walter et al., 2018)
Wolf (<i>Canis lupus</i>)	Sightings based on collection of fecal samples by specific kits with date,	Finland	(Granroth-Wilding &

coordinates, number of trace marks around the sample and sample freshness value Laaksonen, 2017)

Diprotodontia			
Koala (<i>Phascolarctos cinereus</i>)	Sightings reported on website with date, coordinates and geographical information	Australia	(Dissanayake & Henning, 2019)
Monotremata			
Short-beaked echidna (<i>Tachyglossus aculeatus</i>)	Sightings reported on website with date, time, coordinates and photographs	Australia	(Perry et al., 2022)
Lagomorpha			
European hare (<i>Lepus europaeus</i>)	Sightings reported by camera traps with photographs	Ireland	(Caravaggi et al., 2018)
European rabbit (<i>Oryctolagus cuniculus</i>)			

The development of Internet and new digital technologies, including smartphone applications and geographical information systems, brought the popularity of CS wildlife sightings in a global dimension. In particular, social media is a key in the success of recruiting of participants to create a network where information is shared between communities of citizens and experts (Chenery et al., 2022). These networks are often represented by general social media platforms (e.g. Facebook, Twitter, Flickr) or specific wildlife monitoring channels (e.g. Zooniverse, iNaturalist, EchidnaCSI, iMammalia, MammalNet) (ENETWILD-Consortium et al., 2020c, 2022e; Frigerio et al., 2018). In particular, Zooniverse is a web platform containing different active projects (e.g. ‘Snapshot Serengeti’ and ‘Chimp & See’) in which volunteers analyse wildlife photographs and videos obtained through camera traps and upload them to an online system (Edwards et al., 2021).

While some platforms are generalized, as they allow volunteers to perform multi-species sightings (e.g. iNaturalist) (Callaghan & Young, 2022), others are focused on tracking and mapping selected species of wildlife, such as ‘Echidna Conservation Science Initiative’ (i.e. EchidnaCSI), a free mobile application to report sightings of European hedgehogs (Perry et al., 2022).

Although most platforms can be employed in any country in the world where an Internet connection is available, some are active exclusively in certain geographical areas. This is the case of the ‘Brazilian Wildlife Health Information System’ (i.e. SISS-Geo), a web platform for collaborative monitoring in Brazil, in order to generate preventive measures and computational models for predicting zoonoses of wildlife (Chame et al., 2019).

The reliability of CS studies for monitoring wildlife has been ascertained through several investigations. For example, the density of red foxes in urban and peri-urban areas of the UK observed by CS sightings overlapped with the data of a census previously outlined

with traditional surveys by scientists (Scott et al., 2014). Again, data on the distribution of several wildlife species obtained *via* Flickr users were in accordance with those provided by the National Biodiversity Network (NBN) Atlas in the UK (Edwards et al., 2021). In addition, CS projects represent a useful bottom-up approach in low-income and developing countries. In this regard, a community-based conservation study on African lions in Kenya through Maasai warriors (pastoralist men with no formal education) produced reliable data on the demographics and movements of this species, but also direct benefits to participants, such as literacy training, skill enhancement and sensitivity to wildlife (Perry et al., 2022). Additionally, CS sightings can be particularly useful in conditions where field activities by experts are not possible or allowed. For example, long-term restrictions during the COVID-19 lockdown promoted increased movements of wild animals near urban settlements (Manenti et al., 2020), together with the inability to run traditional surveillance plans of wildlife; however, during this period, CS sightings continued, confirming their value and utility (Stenhouse & Lewis, 2022). Lastly, CS projects may be a cost-effective strategy for wild populations surveillance, as demonstrated by a study where photographs by visitors proved to be a cheaper investigation framework compared to camera trap, call-in station and spoor survey for monitoring the circulation of animals in protected areas (i.e. natural parks and faunal reserves) (Rafiq et al., 2019).

Citizen science and arthropod surveillance in wildlife

The CS approach in arthropod surveillance frequently relies on the involvement of specific stakeholder groups (e.g. hunters), including in sample collection activities. For example, CS projects where citizens collect hard ticks (Ixodidae) from wildlife are useful to determine which animal species harbour a given tick species (Table 3) and, consequently, to predict which VBPs potentially circulate in a certain area. Data on vectors and VBPs in wildlife are made available *via* CS, such as arthropod–host associations (Heylen et al., 2017), reservoirs of zoonotic infections (Raizman et al., 2013), target organ of infection in the host (Zinck & Lloyd, 2022) and risk of infection for animals and humans in a geographic area (Nieto et al., 2018; Sgroi et al., 2022a, 2022b; Stufano et al., 2022).

Table 3. *Citizen science* surveys investigating the association between arthropods and wildlife according to taxonomic order

Wildlife species	Arthropod species	Contribution of citizen scientists	Country	Reference
Artiodactyla				
White tailed deer (<i>Odocoileus virginianus</i>)	<i>Ixodes scapularis</i> , <i>Amblyomma americanum</i>	Assistance in carcass inspection for tick removal at hunter check stations	USA	(Apperson et al., 1990)
White tailed deer	<i>Ixodes scapularis</i> ,	Ectoparasite removal and	USA	(Poh et al.,

	<i>Lipoptena cervi</i>	collection of animal data (county, township, date and time of harvest) at hunter check stations		2020)
White tailed deer	<i>Lipoptena cervi</i>	Ked removal by hunters with a specific ked-kit	USA	(Evans et al., 2021)
Fallow deer (<i>Dama dama</i>)				
Red deer (<i>Cervus elaphus</i>)	<i>Lipoptena fortisetosa</i>	Ked removal and collection of animal skin samples by trained hunters	Italy	(Andreani et al., 2021)
Roe deer (<i>Capreolus capreolus</i>)				
Red brocket deer (<i>Mazama americana</i>)	<i>Amblyomma dissimile</i>	Tick removal by hunters	Trinidad	(Sameroff et al., 2021)
	<i>Dermacentor marginatus,</i>			
Wild boar (<i>Sus scrofa</i>)	<i>Dermacentor reticulatus, Ixodes ricinus, Haemaphysalis concinna</i>	Tick removal by licensed hunters	Hungary	(Hornok et al., 2022)
Carnivora				
Coyote (<i>Canis latrans</i>)	<i>Dermacentor variabilis</i>	Tick removal by hunters and volunteers with collection kit and collection of animal data (age, sex, date, coordinates and tick attachment site)	USA	(Hertz et al., 2017)
Otter (<i>Lutra lutra</i>)	<i>Ixodes hexagonus</i>	Tick removal by volunteers, amateur entomologists, wildlife charities and collection of animal data (date, coordinates and tick attachment site)	USA	(Cull et al., 2018)
Eulipotyphla				
Hedgehog (<i>Erinaceus europaeus</i>)	<i>Ixodes hexagonus</i>	Tick removal by volunteers and wildlife charities and collection of animal data (date and coordinates)	Great Britain	(Hansford et al., 2023)

In addition to ticks, citizens can participate in the collection of dead animals that are then tested by scientists to assess their positivity for VBPs (Zinck & Lloyd, 2022). This method has been applied in a study in which rodent carcasses were collected by the public and delivered to scientists for testing different VBPs, proving that the jumping mouse and eastern deer mouse were the main reservoirs of *Borrelia burgdorferi sensu lato*

complex in Canada (Zinck & Lloyd, 2022). This study also investigated the different target organs of infection by *B. burgdorferi* s. l. compared to the emerging *Borrelia miyamotoi*. In particular, the study found that *B. burgdorferi* s. l. was able to spread in the hosts to a single tissue, whereas *B. miyamotoi* in multiple organs. In addition, this survey has suggested a potential vertical transmission (i.e. transplacental) of *B. miyamotoi* in mammals, being likely able to rapidly spread within wildlife populations (Zinck & Lloyd, 2022).

Furthermore, the association between keds (Hippoboscidae) and wildlife (Table 3) is relevant due to the suspected role of these insects in the transmission of VBPs. For example, *Lipoptena cervi* parasitizes both humans and ungulates (i.e. fallow deer, red deer, roe deer, white-tailed deer) and has been indicated as a potential candidate to vector bacterial and parasitic agents, although experimental studies are needed to clarify its role (Bezerra-Santos & Otranto, 2020).

Most CS projects are state-registered, thus scientists directly support citizens in field activities. This approach is used by a national tick surveillance plan in the UK by the Health Security Agency, who supports volunteers in tick sampling and assessing seasonal activity and host association of arthropods (Cull et al., 2018; Hansford et al., 2023).

In other CS studies, ready-to-use collection kits (i.e. combs, tweezers, vials with 70% ethanol) are given by scientists to groups of hunters who attend training courses and follow instructions to collect, store and deliver ectoparasites obtained from their harvests (Hertz et al., 2017), often using check stations, public hunting lodges or private slaughterhouses (Poh et al., 2022). An example of large-scale sampling of ectoparasites directly run by citizens is the Project Acari, where participants use a custom-designed 'tick kit' that includes a tick card, clear stickers to attach ticks on the card and a biohazard bag with plastic envelope for return shipping (Chauhan et al., 2020). Up to date, this study has involved 900 citizens and obtained 322 tick cards, with a total of 2417 ticks, from 32 states of the USA. The use of tick-cards could be used in the surveillance of ectoparasites of wild animals, by the involvement of hunters or other non-scientific stakeholders, such as wildlife conservationists and naturalists. Similarly, Evans et al. (2021) has demonstrated the efficacy of a 'ked-kit' delivered to hunters for the collection of keds (*L. cervi*) from the white-tailed deer in the USA. Details on CS studies characterized by a direct engagement of citizens in the collection of ectoparasites from wild animals and related information are reported in Table 3.

One of the crucial points of CS projects focused on arthropods is the scientific quality and reliability of data provided by citizens who have little experience in this field. Although citizens are traditionally not educated about arthropods and VBPs initially, a coherent and well-designed project can significantly improve their theoretical and practical knowledge. For example, it has been demonstrated that as soon as deer hunters become familiar with ectoparasites, they are able to collect samples from a greater variety of geographic areas than researchers (Evans et al., 2021), probably due to their deeper knowledge of local rural contexts compared to scientists. Again, in a CS study, the prevalence of *Trypanosoma cruzi* in kissing bugs (Hemiptera) from wildlife nests of Texas (i.e. 63%) was similar to that retrieved in the same geographical areas by traditional research studies (i.e. 50.7%) (Curtis-Robles et al., 2015). This suggests that the scientific reliability of a project does not depend on whether it is a CS or traditional research but rather on the solid planning of the study itself.

Despite its potential, CS has limitations. For example, species such as kissing bugs, biting flies and mosquitoes do not remain after feeding on wildlife carcasses which makes the collection of these arthropods difficult (Poh et al., 2022). This gap may be partially bridged by identifying the transmitted pathogens rather than the arthropod itself in a given animal species. This approach has been employed to assess the presence of Triatominae (e.g. *Triatoma* spp., *Panstrongylus* and *Rhodnius* spp.) on the white-tailed deer in Texas, to detect *T. cruzi* DNA in the animal's heart (Gunter et al., 2018). However, while detecting a VBP provides information on the prevalence of infection in wildlife populations, it does not represent a vector surveillance, neither evidence for host–vector interactions. Finally, the morphological identification of arthropods at species level requires skilled experience; therefore, in all CS projects, the final identification of ectoparasites is run by experts (Johnson et al., 2022).

Future considerations

As a result of the climate challenges, human-induced environmental changes and restriction of natural habitats, the composition and urbanization of wildlife communities may inadvertently enhance human–wildlife interactions and drive the rise of emerging infectious diseases in humans, companion and farm animals (Sgroi et al., 2022b).

In this scenario, CS could be more beneficial than ever, thus this review has outlined examples on how this approach represents an impactful model in the surveillance of wildlife and their related arthropods, with respect to its disadvantages. The main limitation of CS is motivating citizens to participate. However, once this issue has been addressed, and the technique of engaging a large number of citizens in science has been improved upon, then the outcome will also be greater.

Following the *One Health* paradigm, collaborative and multidisciplinary workflows in the surveillance of wildlife and related arthropods should be further developed by harnessing the power of citizens (Lawson et al., 2015). For this reason, the top challenge

for research institutions and intergovernmental agencies is to open public accession lines to impactful and long-term CS projects. A deeper and coordinated effort is needed by scientists, stakeholders, local veterinary services and rescue centres, to improve the knowledge and training of citizens on ecology and wildlife disease, but also for extending the surveillance to non- communicable pathogens and neglected hosts (ENETWILD-consortium et al., 2023a).

To conclude, the involvement of the media and politicians in motivating and raising awareness of the importance of wildlife and disease management would surely hugely benefit the development of strategic wildlife monitoring plans at an international level.

4. Implementing camera traps to investigate the impact of wild ungulate and mesocarnivores on ticks and tick-borne diseases

4.1. An integrated approach to an emerging problem: implementing a whole year of camera trap survey in evaluating the impact of wildlife on tick abundance.

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An integrated approach to an emerging problem: implementing a whole year of camera trap survey in evaluating the impact of wildlife on tick abundance.

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Abstract. Tick-borne zoonoses are an emerging health issue. The expansion of ticks is mainly driven by climatic changes, but also by new approaches to the management of the natural environment, increasing the abundance of vertebrate host species and thus the potential exposure to tick bites for both humans and companion animals. In this context, a holistic approach to studying ticks' ecology is required. In the present work, we shed light on the link between environmental tick abundance (global and specific of *Ixodes ricinus* nymphs, as the highest zoonotic threat) and the temporal occupancy of wildlife host species retrieved from camera traps (namely wild ruminants, mesocarnivores and wild boar). We modelled this relationship by integrating abiotic factors relevant to tick survival, such as the vegetation cover and saturation deficit, and estimated the accuracy of prediction. To collect these data, we deployed camera traps in a peri-urban Natural Park in Northwest Italy to monitor wildlife for 1 whole year, while collecting ticks in front of camera traps by dragging transects every 2 weeks. Overall, wildlife temporal occupancy showed an additive impact on tick abundance for species that are preferential hosts (deer and mesocarnivores), and a detractive impact for wild boar, which also presented a lower tick burden, particularly with regards to the tick species collected in the environment (mainly *I. ricinus* and *Haemaphysalis punctata*). Accuracy of prediction was higher for *I. ricinus* nymphs rather than the global model. Temporal fluctuations in the tick population were also highlighted. Wildlife temporal occupancy was not constant and varied between seasons according to feeding habits. In conclusion, we highlighted the utility of camera trap data to investigate tick ecology and acarological risk. This information is crucial in informing monitoring and prevention strategies to decrease the risk of tick bites in humans, and thus zoonotic risk of tick-borne diseases.

1. Introduction

Tick-borne zoonoses (TBZs) including Lyme disease, babesiosis and tick-borne encephalitis are currently considered an emerging problem in several European countries (Bajer et al., 2022; Hartemink & Takken, 2016; Jánová, 2019; Vorou et al., 2007). Ticks are indeed expanding their altitudinal and latitudinal range, mainly due to temperature increase and loss of seasonality (Jaenson et al., 2012; Medlock et al., 2013; Medlock & Leach, 2015; Nuttall, 2022), being climatic and environmental factors the main drivers of tick survival and activity pattern (Kahl & Gray, 2022; Scharlemann et al., 2008).

In addition, anthropogenic factors influencing wildlife populations are also impacting the trends of ticks' expansion (Medlock et al., 2013): reforestation and land abandonment have increased wildlife abundance after a population contraction, particularly for deer species, yielding to ecological conditions conducive for the re-emergence of ticks (Eisen et al., 2017; Estrada-Peña, 2003). Animal welfare politics moving towards extensive animal breeding, increase of wildlife management, habitat destruction and popularity of outdoor sporting are among the several reasons that are bringing wildlife closer to urbanised areas, people and their animals (Becker et al., 2015; Muehlenbein, 2013). In sympatric environments for wildlife, domestic animals and people, a holistic approach is fundamental to understanding the mechanisms impacting the presence and abundance of questing ticks; this can be accomplished only by integrating high-quality wildlife population data with biotic and abiotic environmental information.

The debate about how to reduce tick abundance is still open (Černý et al., 2020). On a small scale (house yards for instance), chemical and biological methods have been experimented, without a definitive success (Keesing et al., 2022; Ostfeld et al., 2023). A review of their pitfalls and potentialities is presented by Ostfeld et al. (2006). Those methods are impractical at a large scale, therefore in the wider natural environment other options have been explored. For instance, vertebrate host reduction was tested on deer populations, but ultimately it did not provide the expected results and still needs further confirmation (Kugeler et al., 2016; Van Buskirk & Ostfeld, 1995; Wilson et al., 1984). On a small scale, integrated pest management actions have been proven to be highly effective (Stafford III et al., 2017), and this seems to be a suitable direction for the natural environment as well. There is much interest in integrating data from wild hosts, the environment and human activities to identify risk areas for tick bites on people (e.g. Mols et al., 2022).

With an increasing trend in the last few years, camera traps have been claimed by the scientific community to be the most efficient tool to monitor wildlife in terms of cost-effectiveness and reliability of results (ENETWILD-consortium et al., 2020c). They also present the advantage of providing high-resolution data with limited wildlife disturbance, allowing us to gather information that would be impossible to obtain otherwise. Worldwide, few examples have been produced of use of camera trap data to study the relationship between population composition and tick abundance (Domínguez et al., 2019; Esser et al., 2019; Hofmeester et al., 2017b; Iijima et al., 2022; Noden et al., 2024). In some cases, they have been examples of an integrated approach including small mammals (Takumi et al., 2019; Taylor et al., 2023). Hofmeester and colleagues (2017b) implemented camera traps to relate wildlife passage rate to tick abundance, highlighting a positive correlation and introducing camera trap data to the study of the relationship between densities of questing ticks and the availability of different vertebrate species. In this work, we aim to analyse the impact of wildlife temporal occupancy (TO) (i.e. the amount of time a species is seen in a given site) on questing ticks abundance, considering also abiotic factors and fluctuations in time. The information gathered might inform policy and practice to prevent and control transmission of relevant TBZs.

2. Materials and Methods

Study Area and Objectives

This study was implemented in La Mandria Natural Park, Northwest Italy, where we performed a one-year-long data collection, aiming to include the seasonal variation in the analysis. La Mandria Natural Park is a fenced park of 6571 ha at a mean altitude of 386 m a.s.l. (± 269 m), mostly covered in deciduous forest and fields of grassland for mowing. The annual precipitation in 2020 was 898.2 mm, and it presented an average temperature of 13.25°C. It welcomes an average of 2000 visitors every day, and wildlife management is constant throughout the year. Few horse farms and cultivated areas are present inside the park. Wild ungulate density for those areas was studied in the framework of the European Wildlife Observatory (ENETWILD-consortium et al., 2023b). The highest densities were obtained for wild boar (*Sus scrofa*), 15.26 ± 2.41 individuals/km² (with confidence intervals), and red deer (*Cervus elaphus*) 11.03 ± 3.17 individuals/km², followed by fallow deer (*Dama dama*), 3.31 ± 1.16 individuals/km², and roe deer (*Capreolus capreolus*) 1.90 ± 0.97 individuals/km². Those densities were obtained by implementing the random encounter method (Rowcliffe et al., 2008), which allows to estimate the density of those species without the need of individual recognition, and contextually sampling for the present work.

Detailed objectives for the present study were to (i) determine the relationship between tick abundance and wildlife TO, considering abiotic factors and, especially, temporal fluctuations; (ii) detail the above relationship for *I. ricinus* nymphs only, as the developmental stage and species posing the highest zoonotic threat; (iii) identify variation in temporal occupancy for wild species monitored through camera traps; (iv) quantify tick burden for culled wildlife in the park.

Sampling design

Information on land cover was retrieved from CORINE Land Cover (CLC), available for the study area from Geoportale Piemonte (<https://www.geoportale.piemonte.it/cms/progetti/land-cover-piemonte>). CLC (Corine Land Cover Piemonte level) was reclassified into two macro types of vegetation: deciduous forests and human activity parcels (fields of grassland for mowing). We identified an adequate number of sampling points to cover the area homogeneously, randomly placed with respect to animal movement and proportionally representative of different macro types of vegetation. This led to a total of 14 sampling points in the study area (Figure 1). In each sampling point, a camera trap (either Browning FORCE EDGE - Model BTC-7E, or Browning DARK OPS APEX - Model BTC-6HD-APX) was deployed facing north, 50 cm above the ground, with the sensor angled parallel to the slope. Cameras were set to be operative all day, to record a burst of eight consecutive pictures (rapid fire setting) at each activation, with the minimum time laps (0.22 sec) between consecutive activations. Nocturnal pictures were illuminated with infrared flash (low glow). Neither baits nor attractants were used. The date and time of each capture were automatically stamped onto each picture.



Figure 1. Map of the study area with the CLC categories: yellow for human activity parcels (grassland for mowing), green for deciduous forest. Sampling points are indicated as red dots.

Contextually with the camera trap placement, two tick dragging transects were performed on site: a 10 m² square in front of the camera trap (being 10 m the furthest distance at which data could be obtained with almost perfect detectability of animals) and in a circle of 26 m radius and centre in the camera trap (the maximum field of view of the camera trap), to sample the whole area around the site. Ticks collected from dragging were stored in ethanol 70% and later identified by morphological keys (Estrada-Peña et al., 2018; Manilla & Iori, 1992, 1993). A graphic representation of the transects is presented in Figure 2. Together with camera traps, we placed a sensor (temperature and humidity data logger RS PRO, USB) to register hourly the temperature and humidity 15 cm above the ground.

Camera traps were checked to retrieve SD cards and control batteries every 2 weeks for 1 year (August 2020-August 2021) and, on the same occasion, the tick-dragging transects were repeated. We performed a total of 26 repetitions during the whole study.

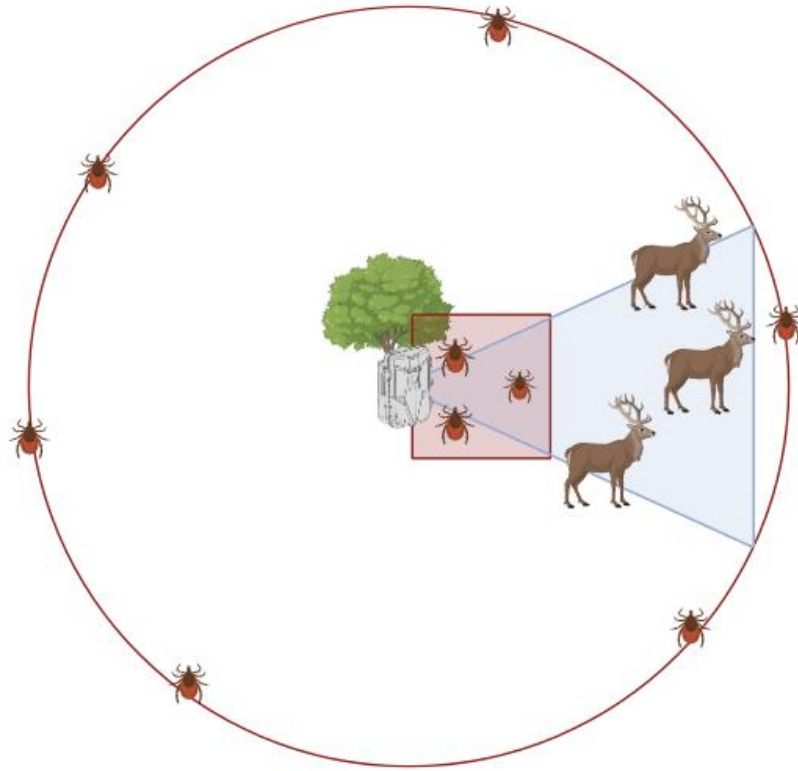


Figure 2. Graphical representation of the sampling design, including tick dragging transects, referred to the camera trap deployment and field of view. Animals that are in the field of view are recorded by the camera trap, while a 100 m² (10x10 m) square dragging transect is performed in front of it and a circle of 26 m radius dragging transect is performed around the camera trap.

Dataset Preparation

For statistical purposes, we considered an observation as the data collected at each sampling point and repetition. Therefore, each observation was characterized with: a) tick abundance, b) wildlife TO, c) mean normalized difference vegetation index (mean NDVI), d) maximum saturation deficit (maxSD, following Perret and colleagues (2004)) and e) month. Data was prepared as follows:

- i. Tick abundance. Tick counts from both transects were then summed by sampling point and repetition and the abundance was calculated referring to the area covered by both transects.
- ii. Wildlife TO. We extracted TO for wild species with good detectability on camera traps, namely wild boar, red deer, roe deer, fallow deer, red fox (*Vulpes vulpes*), badger (*Meles meles*), pine marten (*Martes martes*) and beech marten (*Martes foina*). Camera trap pictures were analysed to extract, for each individual: sampling point, date of passage and time spent in front of the camera (calculated as the number of seconds between the first picture and the last). As the aim of this analysis was to calculate the species TO, no individual recognition was required and, for simplicity, an individual exiting the field of view and entering it again was considered as a new individual, so that only the actual time spent in front of the camera trap was considered. In case that more than one individual appeared in the picture, the time of each animal was calculated separately and eventually summed. We summed the seconds

for each species per sampling point and repetition. For statistical purposes, we merged seconds for all deer species and mesocarnivores (red fox and mustelids), so that three TO data types were implemented in the analysis: wild boar, wild ruminants and mesocarnivores.

- iii. Mean NDVI. These data were retrieved with the R (R Core Team, 2023) *MODISsp* package (Busetto & Ranghetti, 2016) at a resolution of 250 m and 16-day intervals. Dates were matched with sampling dates and point coordinates, and the mean was calculated per sampling point and repetition.
- iv. MaxSD. With the sensor registered, for each sampling point, the hourly mean temperature and humidity, and calculated the daily mean SD (as in (Sands et al., 2021) per sampling point, from which we derived the maxSD per sampling point and repetition. Mean and minimum SD were considered but ultimately discarded, based on the high collinearity with max SD and being the max SD the most limiting factor to tick activity (Perret et al., 2004).

Statistical Analyses

We described the time fluctuations in ticks' abundance and tested abundance correlation among stages (Spearman's rank correlation). Also, we tested different abundance between the two CLC categories with a Kruskal-Wallis test.

We modelled the impact of six explanatory variables (max SD, mean NDVI, TO for wild boar, wild ruminants and mesocarnivores and month) on tick abundance (global model) and, as a second step, on *I. ricinus* nymphs abundance. We proceeded alike for both models. We excluded outliers and months that, for tick ecology reasons, returned no ticks, thus creating a bias towards zero values not determined by wildlife presence (November, December and January). We also checked data for normality and applied to each variable a normalization transformation if needed, with the *bestNormalize* package (Peterson, 2021) in R (R Core Team, 2023). Relationships among explanatory and response variables were modelled using general additive mixed models (GAMMs) (Wood, 2017). GAMMs extend generalized linear models (GLMs/GLMMs) by using smooth functions to define nonlinear relationships between the response and explanatory variables, and by combining predictor variables additively (Wood, 2017). This approach is particularly useful to better describe relationships in ecological systems. Non-linear relationships are expressed as "smooths", which are evaluated by visual inspection of a plot rather than evaluation of coefficients, as done in linear regression (Wood, 2017). We tested the performance of the variables as linear predictors and smooth terms and, however, expected a linear relationship between maxSD (Perret et al., 2004) or mean NDVI and tick abundance, and a non-linear relationship for month (Kahl & Gray, 2022) and wildlife TO. We also tested the effects of the interaction between different variables. We tested spatial random effects for the sampling point. Moreover, we explored different variance weights, including by sampling point, by month and by season and paired interactions between them. We explored different variable interactions for linear and non-linear predictors, as well as variance weight combinations. Model selection was based on three parameters: i) the statistically significant (ANOVA test) lowest Akaike information criterion (AIC), ii) a satisfactory R^2

(considering the complexity of the biological phenomena observed) and iii) good performance of residuals analysis and smooth graphs. Analysis was performed in R (R Core Team, 2023) with the function *gam* of the *mgcv* package (Wood, 2017). In addition to R^2 , to evaluate the predictivity of the models, we split our dataset in train (70% of the observations homogeneously through seasons) and test (30% of the observations). We defined our model on the train dataset and predicted values on the test dataset. We compared the test observed and predicted values with Spearman correlation, and the mean absolute error (MAE).

After analysing the impact of wildlife TO on tick abundance, we also described the differences in wildlife TO in relation to CLC classes and seasons using, due to non-normality, the Kruskal–Wallis non-parametric test of difference.

Tick Collection On Culled Wildlife

To evaluate the tick burden on wildlife and get a more complete picture of the study area, we sampled the carcasses culled by park rangers for six months (December to June). Only wild boar and red deer were available for the purpose, as both species are culled for numerical control throughout the whole year. We collected ticks and stored them in ethanol for morphological identification as for questing ticks. A descriptive analysis was performed to identify tick load and tick species composition on sampled wildlife and a Mann–Whitney test was performed to identify the difference in tick burden between the two animal species.

3. Results

Tick Transects and Camera Trap Pictures

We registered a total of 300 observations (the data collected at each sampling point and repetition, as the combination of ticks, TO calculation and environmental variables detection). Almost 80% of collected ticks belonged to larval stages. The most abundant genera were *Ixodes* (*I. ricinus*), which made 63% of collected ticks, and *Haemaphysalis* (*H. punctata* and *H. concinna*), which made 36% of collected ticks (Table 1).

Table 1. Total number of ticks collected with dragging, per developmental stage and species.

Total amount of collected ticks	
Larvae	2069
Nymphs	480
Adults	48
<i>Ixodes ricinus</i>	1631
<i>Ixodes hexagonus</i>	5
<i>Haemaphysalis punctata</i>	648
<i>Haemaphysalis concinna</i>	247
<i>Dermacentor reticulatus</i>	6

Ticks presented a different seasonal trend depending on the life stage, with a peak between the end of spring and the beginning of summer and a second peak for larvae in August (Figure 3, Supplementary Figure 1 for species detail). Larval abundance exhibited a significant positive correlation with nymphal abundance ($p < 0.05$), but not with adult abundance ($p > 0.05$). Additionally, nymphal and adult abundances were also significantly correlated ($p < 0.05$).

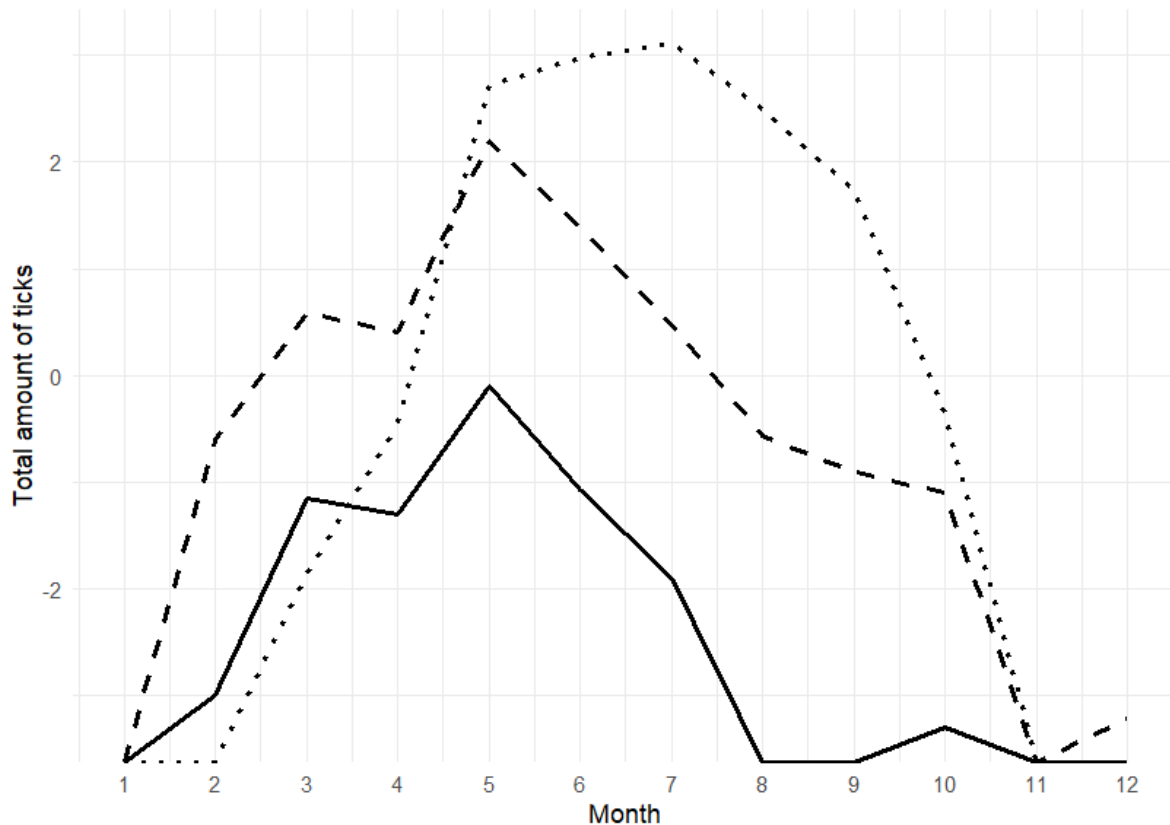


Figure 3. Temporal trend of ticks collected in La Mandria. The graph shows the trends per developmental stage, plotting the logarithmic mean number over the month of collection: solid line, adults; dashed line, nymphs; dotted line, larvae.

Ticks abundance in grassland for mowing fields was lower ($p < 0.05$) than in deciduous forest.

Regarding wildlife TO, we registered the highest for wild boar (706,688 s in the whole study period) and red deer (108,938 s), followed by fallow deer (58,017 s) and roe deer (5,437 s). All ungulates were recorded in all sampling points, with the exception of roe deer, which was only recorded in 11 out of 14 sampling points. Mesocarnivores were recorded in all sampling points with a total of 7,524 s.

Temporal Occupancy Model

Following the best model selection through R^2 , AIC and residual analysis (Supplementary Table 1 and Supplementary Figure 2), tick abundance in both models was best predicted by maxSD and meanNDVI as linear predictors, month as a non-linear

predictor and the interaction of TO for mesocarnivores, wild boar and wild ruminants with season as non-linear predictors. The models, based on a Tweedie distribution (Dunn & Smyth, 2005), implemented the sampling point as a random variable and weighted variance by the interaction of the sampling point and month. All linear predictors and smooth terms were statistically significant (Supplementary Table 2 for coefficients).

The variables effect was similar for global and *I. ricinus* nymphs model. The smooth function for the variable “month” reproduced, in both models, the peaks of the raw trend (Figure 4a and 4e). TO for mesocarnivores shows an additive effect for most seasons, and a detractive effect at low TO during spring (Figure 4b and 4f). Similar results were obtained for wild ruminants, but the trend was constant through all seasons, with a detractive effect at low TO and an additive effect at high TO (Figure 4d and 4h). As for wild boar, TO increase shifts its effect to being detractive to ticks abundance, with a marked detractive effect at higher TO and an additive effect at lower TO during spring (Figure 4c and 4d).

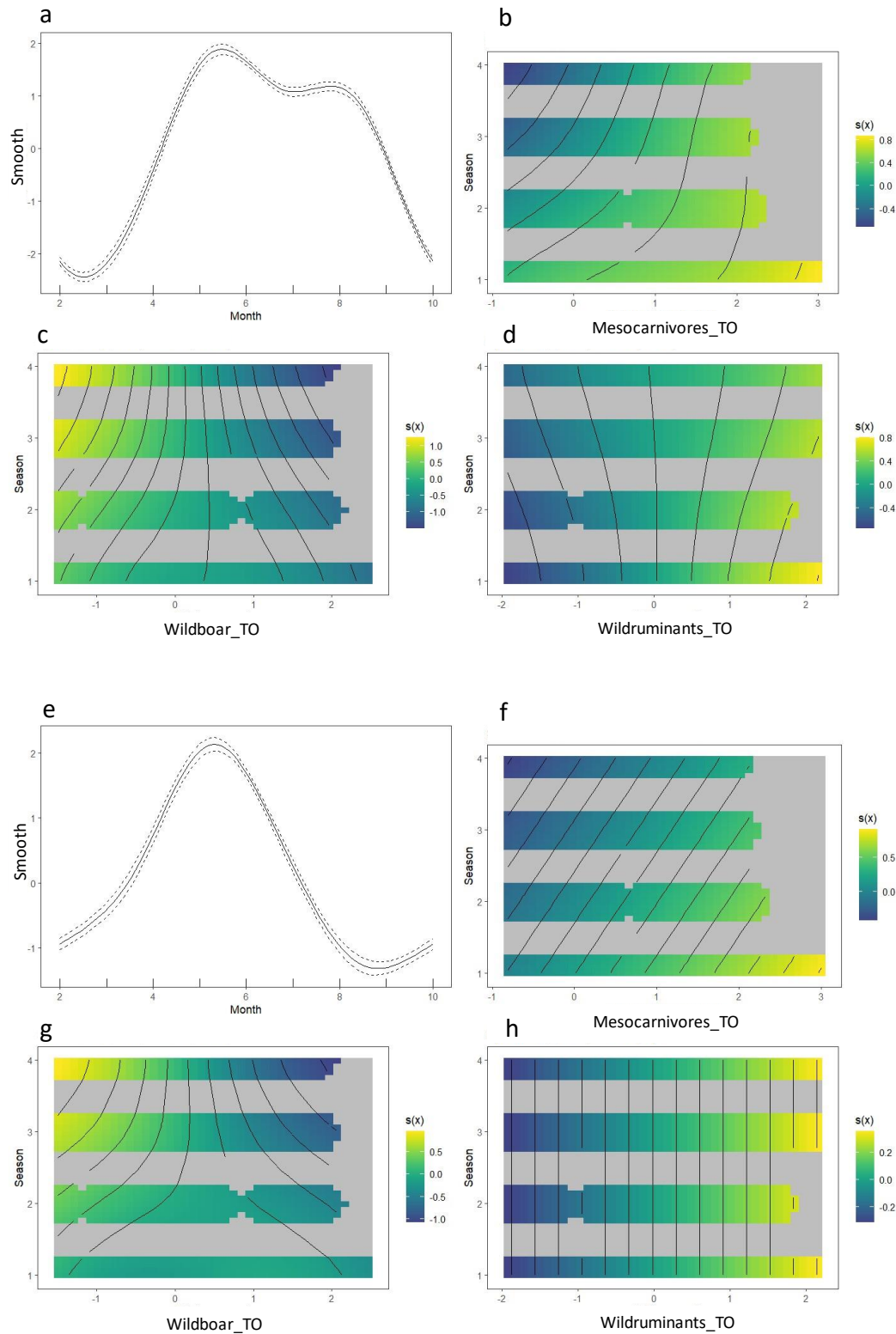


Figure 4. 2D smooth effect plots. Plots a-d: global model. Plots e-h: *I. ricinus* nymphs model. (a, e): month effect on tick abundance. Smooth effects on tick abundance are presented, by season, for mesocarnivores (b, f), wild boar (c, g) and wild ruminants (d, h) TO. Season codes are: 1 (summer), 2 (autumn), 3 (winter) and 4 (spring). TO ranges from negative to positive values due to data transformations. TO for mesocarnivores and wild ruminants shows an additive effect (light green-yellow) for most seasons, and a detractive effect (dark green – blue) at low TO. The increase in wild boar TO shifts its effect to being detractive to ticks abundance.

In the global model, the deviance explained was of 39%, Spearman correlation between predicted and observed data of 0.36 ($p = 0.003$) and MAE of 15. *I. ricinus* nymphs model returned 39.1% deviance explained, Spearman correlation between the predicted and observed data was of 0.44 ($p = 0.0003$) and a MAE of 1.93.

Variations of TO

The only wild ruminant displaying different TO was red deer, which seemed to spend more time in deciduous forests during summer and winter. Wild boar spent more time in deciduous forests during summer and in parcels with human activity (fields of grassland for mowing) during spring, which corresponded to the habitat where mesocarnivores spent more time in winter.

Tick Collection on Culled Wildlife

We sampled 145 animal carcasses culled by park rangers, 24 red deer and 121 wild boar, for half a year (December to June). Of these, 22 red deer ($91.67\% \pm 11.06\%$, 95% confidential interval) and 42 wild boar ($34.71\% \pm 8.48\%$) were infested by ticks. *D. marginatus* was the main species found on wild boar ($38.84\% \pm 8.14\%$ of individuals had at least one *D. marginatus* tick), while *I. ricinus* was for red deer ($75\% \pm 17.03\%$ of individuals with at least one *Ixodes* tick), as shown in Figure 5.

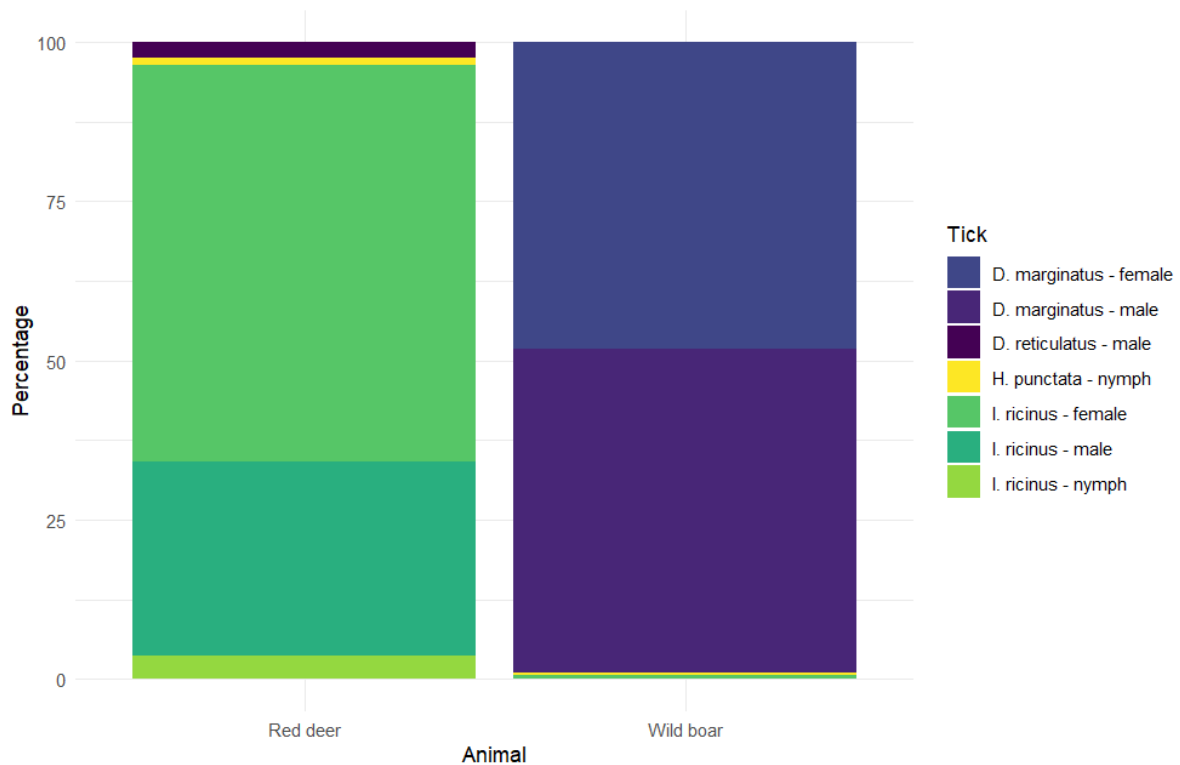


Figure 5. Percentage of tick individuals per species and life stage over the total number of tick collected.

At least one individual for each tick genus was found on both ungulates (Table 2). Most ticks were adult (either male or female), but a nymph of *H. punctata* was found on both ungulate species and three nymphs of *I. ricinus* were found on red deer. Through a non-

parametric statistical test (Mann–Whitney test), we identified a significant difference in parasitic load between the two species for *Ixodes* spp.

Table 2. Tick species distribution as average abundance of ticks per tick species found on red deer and wild boar, with 95% confidential interval.

	Red deer	Wild boar
<i>D. reticulatus</i> – adult male	0.17 (± 0.33)	0
<i>D. marginatus</i> – adult male	0	0.83 (± 0.41)
<i>D. marginatus</i> – adult female	0	0.78 (± 0.46)
<i>I. ricinus</i> – adult male	2.08 (± 1.24)	0
<i>I. ricinus</i> – adult female	4.25 (± 2.88)	0.01 (± 0.02)
<i>I. ricinus</i> – nymph	0.25 (± 0.35)	0
<i>H. punctata</i> - nymph	0.08 (± 0.16)	0.01 (± 0.02)
No Tick found	0.17 (± 0.22)	0.72 (± 0.08)

4. Discussion

Camera trap data allowed us to model the impact of wildlife TO on questing ticks abundance, factoring in also other biotic and abiotic factors. We highlighted that, for the preferential hosts of the collected tick species (mesocarnivores and wild ruminants, Salman & Tarrés-Call, 2013), the increase in TO changed its effect with a positive trend, with animals removing ticks at low TO and having an additive effect when increasing TO. On the contrary, wild boar, which was less frequently parasitized by *I. ricinus* and *Haemaphysalis* spp. (Salman & Tarrés-Call, 2013), showed an opposite trend, with a detractive or a null effect in most cases. These results were in accordance with the ones obtained in previous studies with camera trap data, showing the positive impact of deer species on tick abundance (Hofmeester et al., 2017a, 2017b; Takumi et al., 2019). In this case we confirmed the necessity to merge all deer species (as in Takumi et al., 2019), but we kept wild boar separate, obtaining quite opposite effect on questing tick abundance, which was, as already stated, justifiable by the general low parasitic burden of major species for this study. In relation to mesocarnivores (fox in particular), previous studies have detected a detractive impact on *I. ricinus*, while our results showed an opposite trend. Given that *I. ricinus* is frequently found on foxes too, epidemiological and habitat characteristics may have influenced the contradictory result.

The population structure of ticks, as well as its temporal fluctuations, with peaks corresponding to months with temperature and humidity in the range of tick suitability, and scarce or even null presence in colder months, were consistent with previous studies (Gray, 2008; Kahl & Gray, 2022; Perret et al., 2004; Schulz et al., 2014). Sampling points in fields of grassland for mowing did return a low number of ticks, most likely because of the absence of shelter with low grass, which exposes ticks to more extreme temperatures and lower humidity; as well as scarce sensitivity of dragging transects

(compared with other methods) with high grass, where the most abundant stage (larvae) quest closer to the ground (Tack et al., 2011).

In terms of tick species composition, *I. ricinus* was, as expected, the most abundant tick collected in the environment. On the other side, through dragging we did not register *D. marginatus* in an area with high density of wild boar (which is among its preferential host species in continental and temperate climates (Salman & Tarrés-Call, 2013)). Carcass sampling did return instead of several wild boar carrying *D. marginatus* (Figure 5). These findings might corroborate the speculation over the nidicolous status of larval and nymphal stadia for this genus (Sands et al., 2021), a behaviour that would lower the sensitivity of dragging transects and explain the low environmental presence despite the high parasitic load on wild boar. Moreover, culling activity may constrain a good sampling representability: in the park, 10 times more wild boar are culled than red deer, which are eventually little represented, and no roe deer, an important host for *I. ricinus* (Cull et al., 2018) is culled at all. *I. ricinus* is indeed a species that is found on wild boar with low frequency, as shown by the sampling of culled animals in the park and by literature (Accorsi et al., 2022; Castillo-Contreras et al., 2022; Fabri et al., 2021).

Different tick stages have feeding preferences for different hosts (Kahl & Gray, 2022; Mihalca & Sándor, 2013), and a broad range of small-animal species (from small mammals to birds) has been showed as the main host of immature stages for both *I. ricinus* and *H. punctata* (Kahl & Gray, 2022; Mysterud et al., 2021). However, all stages have been found feeding on deer, although at different body locations (Carpi et al., 2008; Mysterud et al., 2014). Therefore, we face a complex interaction between environmental factors and animal species that determines the abundance of ticks at different stages, as it has already been explored elsewhere (e.g. Peralbo-Moreno et al., 2022). This complexity must be considered to explain our choice of preserving a global model: we addressed the impact of wild ungulates and mesocarnivores TO on the global population of questing ticks. For a finer scale work, information on ticks' life cycle such as moulting and egg hatching time in different environmental conditions would provide a useful reference. Additionally, the inclusion of small mammals would be another significant step forward to encompass hosts for immature tick stages and reservoirs for significant TBZs, such as Lyme disease (Rizzoli et al., 2011).

We tested the model for *I. ricinus* nymph, as the species vector for most zoonotic tick-borne diseases (Salman & Tarrés-Call, 2013) and the developmental stage that most frequently parasitises humans (Battisti et al., 2019). Nymphs are considered the most important stage in transmitting Lyme disease and TBE to humans (Wilhelmsson et al., 2013). This is because in nature nymphs are much more numerous than adult ticks, and because nymphs, compared to adult female ticks, are more easily overlooked due to their smaller size and less conspicuous colouration. Despite similar outputs for both models, the predictivity of the finer one was higher. Thus, due to different behaviours and ecology of tick species and developmental stages, breaking down the response variable allows us to obtain outputs with higher accuracy. The abundance of following stages (larvae with nymphs and nymphs with adults) was closely correlated, potentially

explaining the similar outputs generated by the two models. In contrast, additional factors such as survival rates, may have influenced the absence of correlation between adults and larvae.

Considering all habitat related factors that may determine the presence of ticks in the environment, model predictivity was quite satisfactory and represented an indicator of the usefulness of camera trap data to define acarological risk. A step forward to a practical application of these results is the analysis of wildlife TO variability in the study areas according to different habitat types. Our findings were in accordance with previous studies about habitat selection, especially regarding wild boar preferences in different seasons (i.e. Acevedo et al., 2006; Thurfjell et al., 2009), probably following feeding and sheltering preferences. Understanding which sites are preferred by wildlife represents useful information to assess the risk of tick bites for people accessing the natural environment. However, it is important to consider that the modest extension of the study area and habitat and management conditions of natural areas impose limits to the generalizability of the study's findings. The identification of risk areas for ticks' presence and consequently TBZs transmission, entering itself into the broader approach of integrated management, is a promising but yet not much-explored field. However, a more detailed ecological analysis, including landscape analysis such as habitat fragmentation and connectivity, is needed to predict TO differences in different sites and therefore tick bite risk. As camera traps are getting more popular, for their efficiency in retrieving highly valuable data, deployment sites are increasing. In these areas, information retrieved from camera traps might be used to investigate the relationship between wildlife TO and tick abundance, identifying risk zones for tick bites and providing evidence-based information for effective decision-making in the context of public health.

5. Conclusions

In this study, we emphasise the impact and predictivity of time spent by wildlife in a specific site, extracted from camera trap data, on the abundance of ticks in the environment. This effect was mainly related to ticks' host preferences and seasonality. The information retrieved from camera traps was crucial in investigating ticks' ecology, demonstrating another application for this already useful tool. To properly achieve a holistic approach to assess the risk of tick bites in humans in natural areas, which is proving to be the best approaches to limit the spread of TBZs, more detailed environmental data is required, as well as small mammal populations data, traditionally considered important tick hosts and TBDs main reservoirs.

4.2. Relating wildlife camera trap data to tick abundance: testing the relationship in different habitats.

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Relating wildlife camera trap data to tick abundance: testing the relationship in different habitats

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Simple Summary: This study addresses the growing risk of tick bites, which is becoming more prevalent due to changes in landscape, leading to an increase in wildlife that supports tick populations, raising the risk of disease transmission to humans and animals. To better understand this, we explored the ecology of ticks by examining the relationship between wildlife presence and tick abundance in two regions: an alpine hunting district and a natural park in the Apennines. We used camera traps to monitor wildlife and conducted tick sampling nearby. Additionally, we considered factors like altitude, vegetation, and climate. Our findings highlight the significant role of altitude and wildlife presence—both influenced by habitat and land management—in the global abundance of ticks in the environment. In particular, the species most impacting tick presence was roe deer. These insights could be valuable for managing natural environments, helping to reduce the risk of tick-borne diseases, and they show the utility of camera trap data, which are gathered with great detail and little disturbance of wildlife.

Abstract: The increase in acarological risk of tick bites is significantly driven by profound changes in landscape, which alter the density and distribution of wildlife that support tick populations. As a result of habitat shifts and land abandonment, which create environments conducive to tick proliferation, the risk of disease transmission to humans and animals is increasing. In this context, it is important to explore tick ecology applying a comprehensive methodology. In this study, we examined the relationship between wildlife temporal occupancy and tick abundance in two distinct regions: an alpine hunting district and a natural park in the Apennines. For each sampling point, we calculated wildlife temporal occupancy from camera trap pictures and estimated ticks' abundance from dragging transects in the area immediately surrounding camera traps. In modelling the relationship between those two variables, we included abiotic factors such as saturation deficit, normalized difference vegetation index and altitude. Results show the importance of altitude and wildlife temporal occupancy (itself related to different habitat and land management characteristics) on the ecology of questing ticks. If employed in management decisions for natural environments, such information is useful to modulate the acarological risk and thus the risk of tick-borne pathogens' transmission.

Keywords: camera traps; tick abundance; tick-host association

1. Introduction

The recent altitudinal and latitudinal expansion of tick populations has become a significant concern due to the zoonotic pathogens they carry, which are classified as emerging infectious diseases (Eisen et al., 2017; Vannier & Krause, 2015). By influencing different aspects of tick biology (survival, development, reproduction and dispersal) abiotic factors, habitat (in a broad sense, including human interventions), and the presence of wild vertebrate hosts are responsible for this range expansion of ticks (Medlock et al., 2013).

The altitudinal factor allows us to study ticks' abundance at the extremes of their distribution and therefore draw conclusions on how climate change will impact those vectors. For instance, higher altitudes appear to constrain tick eggs hatching and moulting to further stages (Materna et al., 2008), even independently from the host presence (Gilbert, 2010).

In addition, habitat or landscape modification, through its impact on tick hosts' presence and abundance, may determine ticks' presence and expansion, as well as impact its abundance (Medlock et al., 2013). Scientific work and anecdotal considerations (collected by Medlock and colleagues (2013)) show how the tick population has increased across Europe, probably linked to the impact of human activities on the environment and habitat modification. In addition to this, wildlife management can be in some cases a strategy to reduce the acarological risk for people. While some studies have shown that deer removal (either by culling or fencing) can reduce the number of immature stages of ticks in the environment (Lise Gern et al., 2008; Gilbert, 2010; Materna et al., 2008), this relationship may be weak in some contexts (Rand et al., 2003) or with temporal limitations (Ginsberg et al., 2020).

With an increasing trend in the last few years, camera traps have been claimed by the scientific community to be the most efficient tool to monitor wildlife in terms of cost-effectiveness and reliability of results (ENETWILD-consortium et al., 2020). They have been integrated into studies addressing tick ecology worldwide, thanks to the high quality of wildlife data that they can gather (Domínguez et al., 2019; Esser et al., 2019; Hofmeester et al., 2017b; Iijima et al., 2022; Noden et al., 2024), sometimes also integrating small mammal trapping (Takumi et al., 2019; Taylor et al., 2023). Hofmeester and colleagues (2017b) implemented camera traps to relate wildlife passage rate to tick abundance, highlighting a positive correlation and introducing camera trap data to the study of the relationship between the densities of questing ticks and the availability of different vertebrate species.

In this scenario, it is important to apply holistic approaches to study the ecology of ticks and, consequentially, tick-borne diseases. In the present work we aim to examine the relationship between ticks' abundance and host presence, by implementing the high-

quality data obtained from camera traps to estimate wild ungulates and mesocarnivores' temporal occupancy (TO). Findings may be of use as tools to decrease the risk of tick biting in decision-making processes for habitat and wildlife management.

2. Materials and Methods

2.1 Study areas

No ethical approval was necessary for this study, as it did not involve any direct manipulation of or intervention with wild animals.

Two study areas in the same geographical region (north-west Italy) were selected: Val Maira Hunting District Cuneo 3 (CACN3 – Italy) and Capanne di Marcarolo Natural Park (Aree Protette Appennino Piemontese, Italy). Val Maira is an alpine Hunting District comprising 56700 Ha, featuring an open valley with a mean altitude of 1650 m a.s.l. (considered in our study area from 500 m a.s.l. to 2500 m a.s.l.). The annual precipitation for 2020 was 788.2 mm and the mean temperature was 10.19°C. The valley registers a population of 11.500 inhabitants (20.28 inh/km²) and several agropastoral activities. Capanne di Marcarolo is a natural park comprising 8216 Ha in the Appennines, with a mean altitude of 754 m a.s.l. (from 300 m a.s.l. to 1000m a.s.l.). The annual precipitation for 2020 was 1612.4 mm and the mean temperature was 10.92°C. Around 30 people reside in the park the whole year long and several agropastoral activities are present. Wild ungulate density for those areas was studied in the framework of the European Wildlife Observatory (ENETWILD-consortium et al., 2023b) and estimated contextually to the sampling for the present work. In Val Maira, there is a higher density of wild boar (10.09 ± 3.37 in/km²) and roe deer (15.9 ± 4.38 in/km²), with a lower density of red deer (0.79 ± 0.77 in/km²). Density for roe deer in Capanne di Marcarolo is 6.35 ± 1.88 in/km², and for wild boar, density is of 0.27 ± 0.11 in/km², while no red deer was recorded in the park (Palencia et al., 2023b). Other species present in both areas are red fox (*Vulpes vulpes*), badger (*Meles meles*), European pine marten (*Martes martes*) and beech marten (*Martes foina*).

2.2 Sampling design

To homogenously cover the study area and its macrohabitats with an adequate number of camera traps (we used Browning FORCE EDGE - Model BTC-7E, or Browning DARK OPS APEX - Model BTC-6HD-APX), we selected a total of 38 sampling points in Val Maira and 41 in Capanne di Marcarolo (Figure 1). Sampling points were selected in close (forest) and open (meadows) habitats, proportionally to their extension in each park. Cameras were deployed facing north, 50 cm above the ground, with the sensor angled parallel to the slope. Cameras were set to be operative all day, to record a burst of eight consecutive pictures (rapid fire setting) at each activation, with the minimum time laps (0.22 sec) between consecutive activations. Nocturnal pictures were illuminated with infrared flash (low glow). Neither baits nor attractants were used. The date and time of each capture were automatically stamped onto each picture.

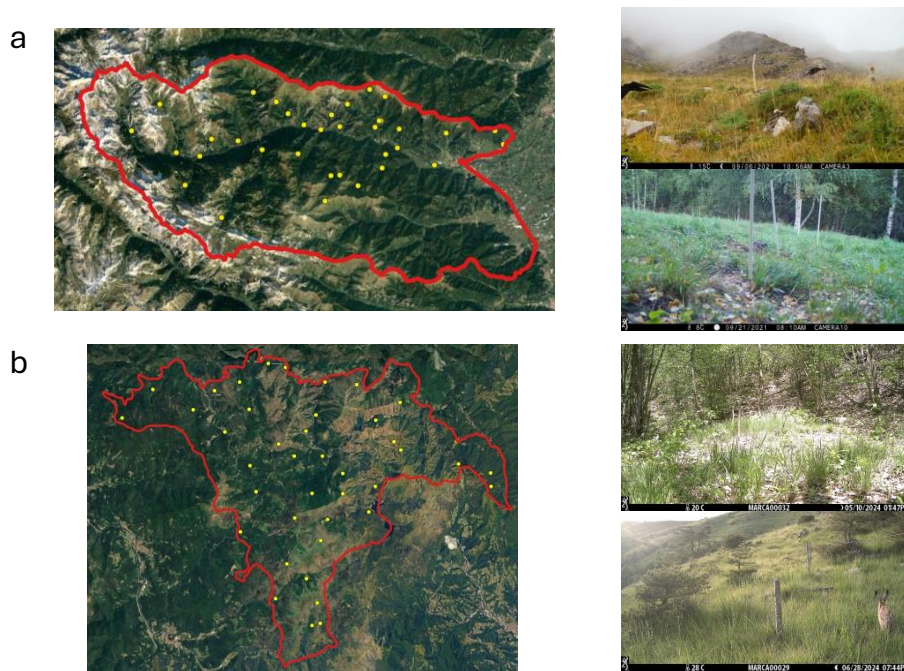


Figure 1. borders of Val Maira (a) and Capanne di Marcarolo (b) study areas, with sampling points distribution. Pictures on the right show the type of habitat sampled.

Questing ticks were collected with two tick dragging transects, in a 10m² square and a 26m radius circle, respectively in front and around the CT (Figure 2), and a data logger (Temperature and Humidity Data Logger RS PRO, USB) was set to register hourly the temperature and humidity 15 cm above the ground. We performed a first tick dragging transect (T_0) at the deployment of the CT, to clean the area from questing ticks, and a second transect (T_1) after one month, at the removal of the CT. This duration was chosen to strike a balance between collecting enough wildlife data for all species (including wild boar, which were less abundant in Capanne di Marcarolo) and obtaining tick abundance data with sufficient resolution to be correlated with wildlife TO. Due to the need to avoid hunting and other activities in the study areas, in Val Maira, this study was performed from the end of August 2021 to the beginning of October 2021 and camera traps were rotated to cover all sampling points, while in Capanne di Marcarolo it was performed in July 2022.

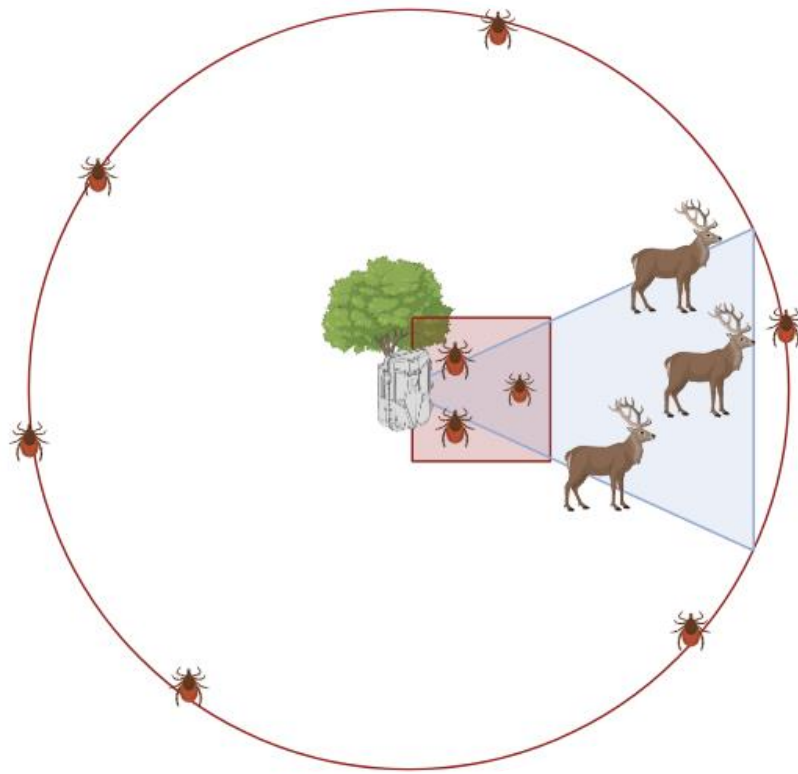


Figure 2. Graphical representation of the sampling design, including tick dragging transects referred to the camera trap deployment and field of view. Animals that are in the field of view are recorded via the camera trap, while a 100m² (10x10 m) square dragging transect is performed in front of it and a dragging transect in a circle with a 26 m radius is performed around the camera trap.

2.3 Data preparation

For statistical purposes, we considered an observation as the sum of data collected at each sampling point at T1. Therefore, each observation was characterized by the following: a) tick abundance from both transects at T1, b) wildlife temporal occupancy (TO), c) mean normalized difference vegetation index (NDVI), d) maximum saturation deficit (maxSD), and e) altitude. Data were prepared as follows:

- a) Tick abundance. Ticks collected from dragging were stored in ethanol 70% and later identified with morphological keys (Estrada-Peña et al., 2018; Manilla & Iori, 1992, 1993). Ticks were identified with a Nikon SMZ1500 stereomicroscope (Nikon Corporation, Tokyo, Japan) and Zeiss Axiolab 5 microscope (Carl Zeiss AG, Oberkochen, Germany). Only ticks collected at T1 were considered in the analysis, and abundance was calculated with the sampling point referring to the total area sampled via the two dragging transects.
- b) Wildlife TO. We focused on species that had good detectability in camera traps, thus extracting TO for wild boar, red deer, roe deer, red fox, badger, pine marten and beech marten. Camera trap pictures were analyzed to extract, for each individual, the following information: sampling point, date of passage, and time

spent in front of the camera (calculated as the number of seconds between the first picture and the last). As the aim of this analysis was to calculate the species TO, no individual recognition was required and, for simplicity, an individual exiting the field of view and entering it again was considered as a new individual, so that only the actual time spent in front of the camera trap was considered. In the case that more than one individual appeared in the picture, the time of each animal was calculated separately and eventually summed. As species were unevenly represented in the two study areas, we merged seconds per sampling point for mesocarnivores and we decided to discard the red deer for its reduced presence and being localized in one study area only, thus implementing in the analysis the TO for roe deer, wild boar and mesocarnivores.

- c) Mean NDVI. These data were retrieved with the R (R Core Team, 2023) package MODISTsp (Busetto & Ranghetti, 2016) at a resolution of 250 m and 16-day intervals. Dates were matched with sampling dates and point coordinates, and the mean was calculated.
- d) MaxSD. We registered, for each sampling point, the daily mean temperature and humidity, and calculated the daily mean SD (as in (Sands et al., 2021) per sampling point, from which we derived the maxSD per sampling point in the whole period. Mean and minimum SD were considered but ultimately discarded, based on the high collinearity with max SD and considering that the max SD was the most limiting factor to tick activity (Perret et al., 2004).
- e) Altitude. Altitude was registered with a GPS device (GPSmap 60CSx, Garmin Ltd, Olathe, Kansas, USA) for each sampling point.

We also retrieved CORINE (coordination of information on the environment) land cover (CLC) data from Geoportale Piemonte (<https://www.geoportale.piemonte.it/cms/progetti/land-cover-piemonte>, accessed on 21st September 2023). To limit variability, we grouped the CLC data (from the LCP level) into three classes: evergreen forests, deciduous forests and human activities patches (pastures).

2.4 Statistical analysis

We modelled the impact of six explanatory variables (max SD, mean NDVI, TO for wild boar, roe deer and mesocarnivores, and altitude) on tick abundance, adding the site as a further explanatory variable. We cleaned the database off outliers and points where none of the species of our interest was recorded. We also checked data for normality and applied to each variable a normalization transformation when needed, with the bestNormalize package (Peterson, 2021) in R studio. Relationships among explanatory and response variables were modelled using GAMMs (General Additive Mixed Models (Wood, 2017)). GAMMs extend generalized linear models (GLMs/GLMMs) by using smooth functions to define nonlinear relationships between the response and explanatory variables, and by combining predictor variables additively, which makes

them particularly suitable for ecological study (Elias et al., 2021; Wood, 2017). We tested the performance of the variables as linear predictors and smooth terms, while, however, expecting a linear relationship with maxSD, altitude or mean NDVI, and a non-linear relationship with wildlife TO. We also tested the effects of the interaction between different variables. We tested spatial random effects for the sampling point. Moreover, we explored different variance weights, including altitude, site and interactions between them. To consider sampling area differences, the site was implemented in interaction with other explanatory variables. Model selection was based on three parameters: i) the statistically significant (ANOVA test) lowest AIC (Akaike information criterion), ii) a satisfactory R² (considering the complexity of the biological phenomena observed) and iii) good performance of residuals analysis and smooth graphs. Analysis was performed in R studio with the function gam of the mgcv package (Wood, 2017).

After analyzing the impact of wildlife TO on tick abundance, we also tested differences in wildlife TO in relation to CLC classes and altitude with a Kruskal-Wallis test (McKight & Najab, 2010) due to the non-normality of data.

3. Results

3.1 Tick transects and camera trap pictures

We registered a total of 32 observations in Val Maira and 37 in Capanne di Marcarolo. The majority of ticks were in larval stages. The most abundant genera were *Ixodes* (*I. ricinus*) and *Haemaphysalis* (*H. punctata* and *H. concinna*). *Ixodes* spp. comprised 96% of ticks collected in Val Maira and 46% in Capanne di Marcarolo, while *Haemaphysalis* spp. made 4% of collected ticks in Val Maira and 54% in Capanne di Marcarolo (Table 1). The difference in tick abundance among the two areas was not statistically significant (Kruskal-Wallis, $p < 0.05$). However, Val Maira registered a lower average abundance (1.45 ticks/m²) than Capanne di Marcarolo (3.37 ticks/m²).

Table 1. Total number of ticks collected with dragging, per developmental stage and species.

	Val Maira	Capanne di Marcarolo
Larvae	143	735
Nymphs	49	21
Adults	6	3
<i>Ixodes ricinus</i>	190	350
<i>Haemaphysalis punctata</i>	7	406
<i>Haemaphysalis concinna</i>	0	3
<i>Dermacentor reticulatus</i>	1	0

Tick abundance varied differently in the two areas according to altitude. In Val Maira, we registered a peak between 1000m a.s.l. and 1700m a.s.l. (Figure 3 A), while in Capanne di Marcarolo the abundance increased more homogeneously with altitude (Figure 3 B).

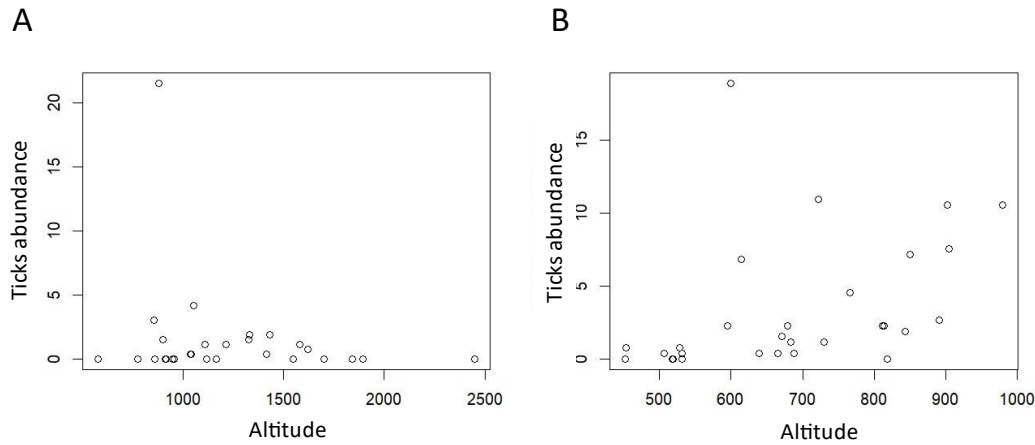


Figure 3. Dotplot of tick abundance in relation to altitude. A) Val Maira and B) Capanne di Marcarolo

The highest temporal occupancy was registered for roe deer, followed by wild boar, and we recorded a low presence of red deer (or absence, as in the case of Capanne di Marcarolo), although with great differences among the two study areas (Table 2). Mesocarnivores were registered in most sampling points.

Table 2: Total number of seconds registered via camera traps by species and sampling area. In parenthesis is, the number of sampling points where at least one individual of the species was recorded. As camera traps pictures often do not enable to distinguishing between stone and pine marten, mesocarnivores seconds were aggregated.

	Val Maira	Capanne di Marcarolo
Mesocarnivores	2644 (26/38)	511 (20/41)
Red deer	818 (8/38)	Not present
Roe deer	7607 (20/38)	6361 (26/41)
Wild boar	3021 (16/38)	2432 (18/41)

3.2 Temporal occupancy model

Following best model selection through R^2 , AIC and residual analysis (Table S1, S2, Figure S1), tick abundance was best predicted with mesocarnivores TO, maxSD, meanNDVI and altitude as linear predictors, however only the latter showed a statistically significant (negative) impact on tick abundance. Wildlife TO for roe deer and wild boar was implemented in the model as a nonlinear predictor, interacting with altitude separately by the site. Altitude showed a significant detractive impact, while NDVI had an additive impact, although not statistically significant. For smooth terms the only significance was recorded for roe deer TO. In Val Maira, we recorded an additive effect at lower altitudes and lower roe deer TO, and it progressively decreased until a detractive effect (Figure 4a). In Capanne di Marcarolo, the roe deer effect is less marked (Figure 4b). No data were recorded in Capanne di Marcarolo above 980m a.s.l. and the effect is detractive at low altitudes.

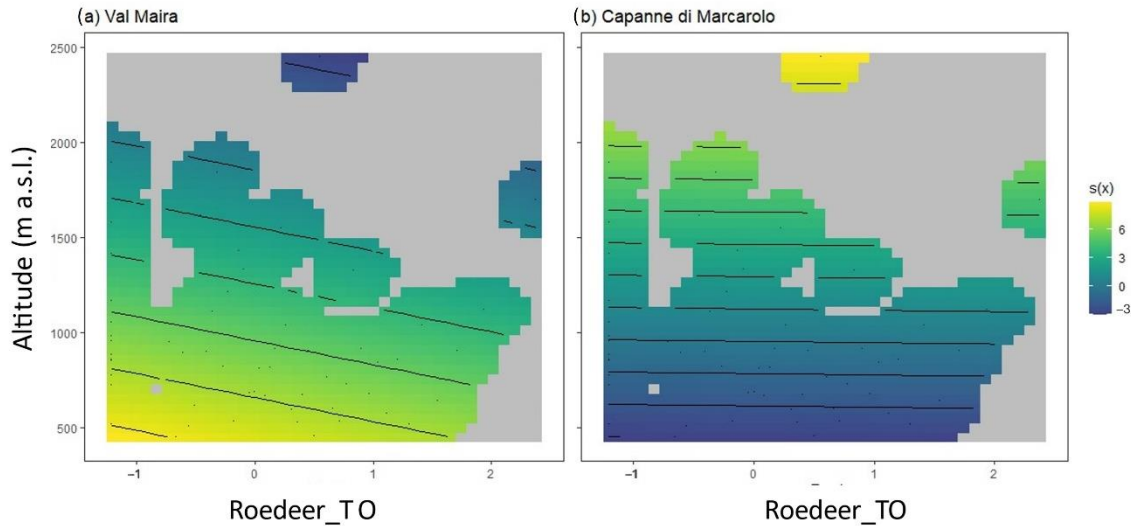


Figure 4. 2D smooth effect plots. Smooth effects on tick abundance are presented for roe deer TO and altitude at Val Maira (a) and Capanne di Marcarolo (b). Temporal occupancy ranges from negative to positive values due to transformations to normalize data. In b, data above 980m a.s.l. are an interpolation of the model, as no information was recorded in the park.

3.3 Variations of TO

The only significant difference among wildlife TOs in the CLC classes was for wild boar, which seems to be spending more time in deciduous forests in Val Maira. Regarding the fluctuation of TO according to altitude, the range from 1000 m a.s.l. to 1500 m a.s.l. records the highest presence for all species (Figure 5). However, roe deer seems to be particularly active both at lower and, though with less intensity, higher altitudes.

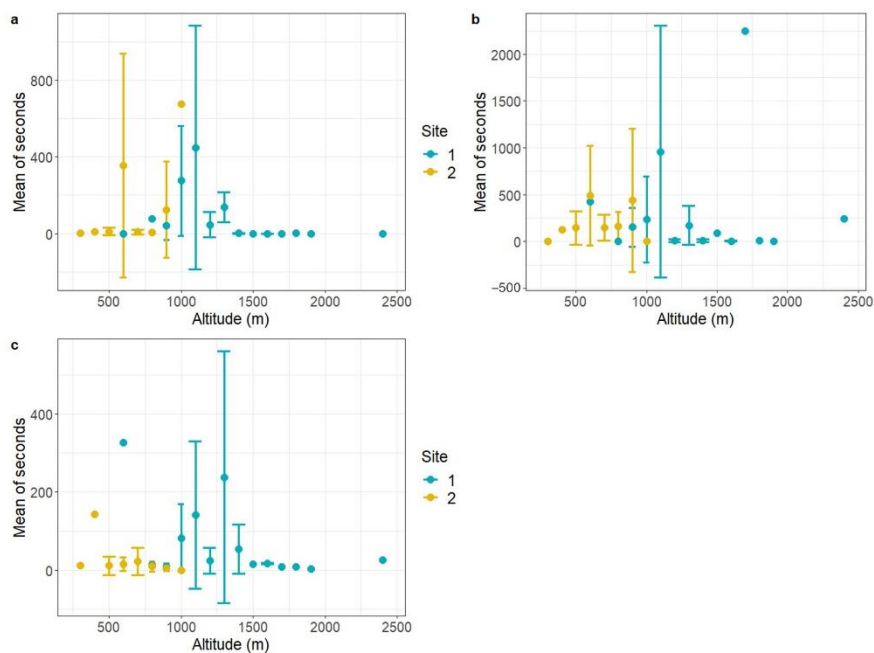


Figure 5. Mean TO according to altitude. Mean TO for 100 m altitude gradient was calculated with relative error bars for wild boar (a), roe deer (b) and mesocarnivores (c), separated for Val Maira (Site 1) and Capanne di Marcarolo (Site 2).

4. Discussion

In this study, we modelled the impact of wildlife TO on tick abundance in two different study areas: an alpine hunting district, where the altitude gradient is particularly broad, impacting the habitat and wildlife population, and an Apennine natural park, with lower biodiversity for ungulate species, a drier climate. We note that the impact of wild species on tick abundance depends on whether they are a preferential host or not, but we highlight a much more important role for abiotic factors, such as altitude.

The two most abundant genera are *Ixodes* and *Haemaphysalis*. In Val Maira there is a neat predominance of *Ixodes ricinus*, a result which is anyways consistent with the literature findings (Salman & Tarrés-Call, 2013). However, the trend inverts in Capanne di Marcarolo, where *H. punctata* becomes the main species, almost matching the number of *I. ricinus*. This supports *Haemaphysalis* spp. being reported mainly in the Mediterranean area, as well as its preference for humid and well-vegetated areas (Salman & Tarrés-Call, 2013): in Capanne di Marcarolo, most ticks were found in deciduous forests with shrubs or dead leaves to keep humidity and little findings were reported in other open habitats, which, in a Mediterranean habitat, are too dry for tick activity.

GAMMs are particularly useful to model the non-linear relationship that often occurs in non-biological environments. This approach, as implemented for example by Elias and colleagues (Elias et al., 2021), offers a significant benefit over Generalized Linear Mixed Models (GLMMs), which are limited to modelling linear relationships, or simpler statistical correlations that do not account for all variables simultaneously—an essential consideration when investigating complex phenomena. In our study, we captured a snapshot of the relationship between wildlife presence and tick abundance by constructing a global model that included all tick stages without differentiation. However, for more detailed investigations, future studies should consider incorporating hatching and moulting times at different altitudes and environmental conditions, to more precisely correlate wildlife presence with different tick developmental stages. Furthermore, expanding the study design to include additional host species, such as small mammals crucial to tick maintenance in the environment, could enhance the breadth of the research by targeting a wider host spectrum. Regarding wild boar, we did not record any statistically significant effect in the model, which may be in accordance with the scarce role of this species in supporting *I. ricinus* population, not being a preferential host (Accorsi et al., 2022; Castillo-Contreras et al., 2022; Fabri et al., 2021). Wild boar presence in Capanne di Marcarolo is rather limited (Palencia et al., 2023b) and the few individuals left in the park were not sufficient to play a role in impacting tick abundance anyways. Similarly, mesocarnivores also do not show any statistically

significant impact, probably due to their scarce TO in the sampled areas, compared to roe deer.

According to the model, ticks' abundance decreased with increasing altitude, where the environment becomes less favourable for them (Danielová et al., 2006). When looking at the data, we can spot instead a more bell-shaped trend, with an increase up to 1000m a.s.l. and a decrease after 1700m a.s.l., registering the highest abundance where temperature and humidity combine with moderate altitude. Indeed several studies on *I. ricinus* in mountain areas of different regions have shown how altitude has a detractive impact on ticks presence (De Pelsmaeker et al., 2021; Gilbert, 2010; Materna et al., 2008; Shchuchinova et al., 2015; Sun et al., 2017; Zubriková et al., 2024), also influencing developmental/eggs hatching time and success (Materna et al., 2008). Of course, such behaviour is particularly interesting under the scope of climate change threats, as increasing temperatures are creating favourable habitats for ticks at higher altitudes, shifting uphill their limit of distribution (De Pelsmaeker et al., 2021; Garcia-Vozmediano et al., 2020; Medlock et al., 2013). The impact on ticks' presence and activity of parameters such as exposition of the slope (Jouda et al., 2004) and solar radiation (Kiewra et al., 2014) has previously been proved, which we also tested in the model, but were ultimately not added due to poor performance of the outputs and statistical similarity with altitude effects.

Habitat characteristics, especially vegetation presence on the ground and tree/shrub cover (higher NDVI), have shown, together with altitude, a positive impact on tick abundance. The lack of statistical significance can be related to the scale of NDVI (250m) being broader than the actual sampled area (10m² and 26m radius circle) and therefore in some cases not representing the actual vegetation cover for the specific sampled area. Indeed such a relationship (NDVI positively correlating with tick abundance) was already highlighted by other works in Europe (Alonso-Carné et al., 2016; Kjaer et al., 2019; Rosà et al., 2018; Vucelja et al., 2023), as vegetation can maintain a better balance of temperature and humidity for the survival and activity of ticks. Also, larger mammals tend to spend more time resting or looking for food in covered areas (wild boar for instance were recorded significantly more in deciduous forests), which could relate to the higher abundance of ticks.

Altitude seems to have the highest impact on tick abundance, with a moderate effect of the interaction with roe deer. In Capanne di Marcarolo, the effect at higher altitudes is indeed just an interpolation of the model. Below 1000m a.s.l. a detractive impact is still reported, despite not being related to the increase in temporal occupancy: the effect of roe deer in Capanne di Marcarolo is overall quite negligible. In Val Maira roe deer temporal occupancy increases ticks abundance at low altitudes, with a progressively less-strong additive impact with the increase in temporal occupancy. At higher altitudes, this effect converts into being progressively more detractive. Apparently, with the principal limiting element for ticks' activity being abiotic factors, the summatory

presence of the host may remove ticks from the environment: the more time deer species spend in front of a camera trap, the more ticks tend to jump on the host, especially at higher altitudes, while at a lower altitude, even brief roe deer presence still adds environmental ticks. Such a result does not surprise if we consider that those species are preferential hosts for *I. ricinus* and *H. punctata*. Since deer species are a preferential host for the main tick species collected in this study and the sampling periods in both study areas exhibit a decreasing phase in ticks activities, it is possible that the lower host density, the broader range of different habitats and altitudes, and the scattered wildlife presence in a wider sampling area all contribute to this different result.

A significant difference in terms of tick abundance and model outputs can be spotted between the two study areas. Despite their similarities, differences in wildlife densities and habitat types may still have significant impacts on the number of questing ticks in the environment and the tick-host relationship.

5. Conclusions

In the present work we highlight how environmental characteristics (habitat macro type and its consequences on climate and vegetation, altitude, land use and management), that ultimately affect wildlife density and distribution (constraining its movements or focusing its presence in particular areas), may affect the relationship between wildlife TO and the abundance of questing ticks. Since habitat and wildlife management is the most useful tool to impact the risk of tick bite for people in the natural environment, our results highlight the importance of contextualizing the ecology of the ticks in relationship with their vertebrate hosts to the specificities of the habitats for decision-making on habitat intervention.

4.3. Assessing zoonotic risk in a fenced natural park in northwestern Italy: integrating camera traps for a vector-host approach to tick-borne pathogens.

Vada, R., Zanet, S., Occhibove, F., Trisciuglio, A., Varzandi, A.R. & Ferroglio, E. (2025). Assessing zoonotic risk in a fenced natural park in northwestern Italy: integrating camera traps for a vector-host approach to tick-borne pathogens. *Frontiers in Veterinary Sciences*, *in press* (accepted on 29/01/2025)

Assessing Zoonotic Risk in a Fenced Natural Park in Northwestern Italy: Integrating Camera Traps for a Vector-Host Approach to Tick-Borne Pathogens

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Keywords: Ixodidae, wildlife, humans, recreational areas, tick-borne zoonoses

Abstract. Tick-borne diseases are among the most widespread emerging zoonotic diseases, and their circulation in the environment is influenced by a broad range of abiotic and biotic factors, including the abundance of vectors and vertebrate hosts. In this study, we estimated the prevalence of tick-borne pathogens and the impact of wildlife head count on their circulation in a lowland natural area in North-Western Italy. We used ticks and camera trap pictures collected from 14 sampling points every two weeks for one year, and detected pathogens through molecular analyses: *Babesia capreoli*, *B. microti*-like, *Borrelia burgdorferi* sensu lato, *Rickettsia* of the spotted fever group (SFG), *Theileria capreoli* and *Anaplasma phagocytophilum*. We modelled the presence of *B. capreoli*, *B. microti*-like, *B. burgdorferi* s.l. and SFG *Rickettsia* on head counts of wild ungulates and mesocarnivores, testing a global model including all collected ticks and an *I. ricinus* nymphs-only model, being this (species and developmental stage) the greatest source of zoonotic infection risk. The highest prevalences were obtained for *B. microti*-like (13%) and SFG *Rickettsia* (11%) and, for most pathogens, no differences were detected among tick species and developmental stages. Mesocarnivores showed an additive effect on *B. microti*-like and *B. burgdorferi* s.l., while wild ungulates, non-competent for transmission of our target pathogens, showed a dilutive effect. These findings confirm the circulation of relevant tick-borne pathogens in the study area and show the use of camera trap data in predicting tick-borne pathogens' risk by targeting host species, which may have an indirect impact and are more easily addressed by monitoring and control strategies.

1. Introduction

In recent years, tick-borne zoonoses have emerged as significant threats to human health, exhibiting increasing prevalences alongside the geographical expansion of their vectors (Eremeeva & Dasch, 2015; ECDC, 2020; Stroffolini et al., 2021). Wildlife species can boost the circulation of these pathogens, serving as both reservoirs and hosts for the vectors. However, scant information exists regarding potential differences in pathogen reservoir competence of various species, which is commonly investigated through xenodiagnosis. For instance, *Babesia divergens* is detected in red deer (*Cervus elaphus*) with relevant frequency, similar to *B. capreoli* in roe deer (*Capreolus capreolus*) (Hrazdilová et al., 2020; Zanet et al., 2020; Zintl et al., 2011). In contrast, red fox (*Vulpes vulpes*) is believed to act as a reservoir for some *Babesia microti*-like, such as the previously classified as *B. vulpes* (Cardoso et al., 2013; Goethert, 2021), and, moderately, for *Borrelia burgdorferi* sensu lato

(s.l.) (Heidrich et al., 1999). The wild boar (*Sus scrofa*), deer species and mesocarnivores are all believed to maintain *Anaplasma phagocytophilum* (Cafiso et al., 2021a; Ebani et al., 2011; Nahayo et al., 2014). Wild boars have tested positive for certain pathogens such as *B. vulpes* or *B. capreoli*, although they are not considered reservoir hosts for those (Castillo-Contreras et al., 2022; Kurtenbach et al., 1998b; Sgroi et al., 2023). Spotted Fever Group *Rickettsia* and *B. burgdorferi* s.l. register as reservoir species small mammals and birds (Salman & Tarrés-Call, 2013). Deer species are not reservoirs for or commonly infected by *B. microti*-like (Piesman et al., 1979), *B. burgdorferi* s.l. (De Keukeleire et al., 2017; Jaenson & Tälleklint, 1992) or SFG *Rickettsia* (Skarphedinsson et al., 2005b). Similarly, tick species might be specialist vectors for selected pathogens, e.g. *Ixodes ricinus* is considered competent for multiple pathogens, while *Haemaphysalis punctata* is the main vector of some SFG *Rickettsia* (Bajer & Dwużnik-Szarek, 2021; Salman & Tarrés-Call, 2013).

In the study of wildlife populations, camera traps (CTs) have been recognised to provide good quality data to characterise wildlife communities (ENETWILD-consortium et al., 2020a). This can be of utmost use in tick-host interaction studies (Ferroglia et al., 2024b; Vada et al., 2024b). Nevertheless, in the European context, very few studies have linked CTs-derived wildlife data to the presence of tick-borne pathogens. Among these, Takumi and colleagues (Takumi et al., 2019, 2021) have employed camera trap data to study the correlation between vertebrate host availability and density of tick-borne pathogens, including *Borrelia* spp. and *A. phagocytophilum*, showing a positive correlation with bank voles and wild ungulates, respectively.

The objective of this study is to assess how effectively camera trap data, specifically head counts of wildlife, can predict the presence of tick-borne pathogens in environmental ticks. This analysis is centred on species readily monitored by such tools, which are also relevant by indirectly influencing tick-borne disease dynamics.

2. Materials and methods

Sampling area and study design

The park (45° 8' 45 ", 7° 36' 2", Figure 1), spanning 6,571 hectares at an average elevation of 386 meters above sea level, with an elevation gradient of 269 meters, is characterised by a temperate lowland climate and is enclosed by fencing (ENETWILD-consortium et al., 2023b). It predominantly features deciduous forests and grasslands managed as hay meadows. The park attracts approximately 2,000 visitors daily and maintains consistent wildlife management throughout the year. While a few horse farms and cultivated plots are situated within the park, access by other domestic animals is restricted (including pets). Data on the density of wild ungulates within the park has been recently established via camera trapping by the European Wildlife Observatory (ENETWILD-consortium et al., 2023b).

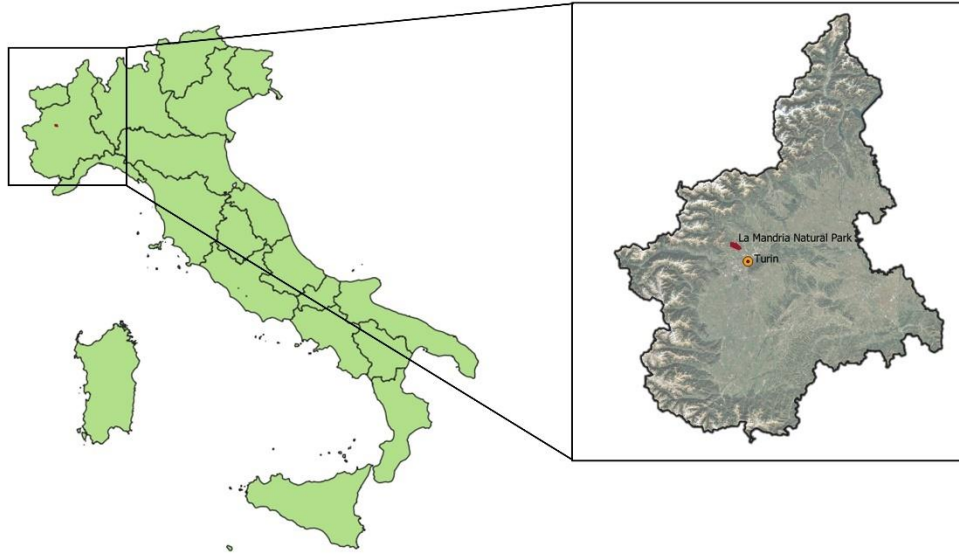


Figure 1. Location of La Mandria Natural Park in the Piedmont Region (Italy), with reference to the regional capital, Turin

The camera trap images analysed in this study originated from a separate field study conducted by Ferroglio and colleagues (Ferroglio et al., 2024b). This study utilized 14 sampling points, evenly distributed between open (hay meadows) and closed (deciduous forests) habitats. Camera traps at each sampling point operated continuously for a year, from August 2020 to August 2021; for detailed information on the deployment of these traps, refer to Ferroglio et al. (Ferroglio et al., 2024b). Alongside the camera trapping, ticks were systematically collected every two weeks throughout the entire study period using dragging transects. As described by Ferroglio and colleagues (Ferroglio et al., 2024b), we implemented a 1 m² cloth, dragged to cover a 10 m² surface in front of the camera trap and a 26 m circle around it. Cloth was repeatedly checked to collect ticks, which were stored in 70% EtOH for further identification (time of storage: 1 to 2 months).

Tick-borne pathogens detection and prevalence estimation

Alongside the performance of field work, ticks were identified with dichotomous keys (Estrada-Peña et al., 2018; Manilla & Iori, 1992, 1993), washed to remove any EtOH residual, which would inhibit the PCR reactions, and stored at -20°C for further analysis (performed after the end of field work). To optimize the effort, the genomic DNA was extracted from ticks grouped into uniform pools based on specific criteria: dragging transect, sampling point, repetition, species, developmental stage, and sex for adult ticks. The extraction was performed on the entire body of the ticks in the pool, using the blackPREP Tick DNA/RNA kit (Biosense, Italy) according to the manufacturer's instructions. Subsequent PCR tests were conducted on these samples to detect tick-borne pathogens, namely *B. divergens/capreoli*, *B. microti*-like, *Theileria capreoli*, *A. phagocytophilum*, *B. burgdorferi* s.l., SFG *Rickettsia*. Table 1 illustrates the references and targeted genes of the primers implemented to detect pathogens' DNA, while specific

protocols are detailed in Supplementary Table 1. Our PCR protocol did not differentiate *B. capreoli* from *B. divergens*, as it did not target the specific region containing Single-Nucleotide Polymorphisms (Hilpertshauser et al., 2006). However, considering the specific features of the study area (absence of domestic ruminants and the surrounding fence) and the documented extensive circulation of *B. capreoli* (Zanet et al., 2014), we considered positives to represent *B. capreoli* alone and like so were included in the statistical analysis. Originally designed to detect *B. microti*, primers from Persing and colleagues (Persing et al., 1992) encompass the whole *B. microti*-like group, such as *vulpes*-like and *Munich*-like clades (Goethert, 2021), which are of interest both on a zoonotic point of view and for the wildlife species targeted in the present study, specifically the red fox. Primers for *T. capreoli* were designed in the current work to be species specific. All primers for Piroplasmids species were intended to avoid interference of co-infections with other protozoan microorganisms. Similarly, when targeting Anaplasmatidae, we selected specific primers for *A. phagocytophilum*, the species with the highest public health relevance, to avoid interference of symbiotic bacteria such as *Candidatus Midichloria* spp. On the other hand, primers targeting *B. burgdorferi* s.l. and SFG *Rickettsia* encompass the whole group of microorganisms (e.g. *B. afzelii* and *B. lusitaniae* in the first case, and *R. monacensis* and *R. conorii* in the second), which are all relevant for public health as zoonotic pathogen, and are not commonly hosted by the wild species targeted in the study (Salman & Tarrés-Call, 2013). All PCR tests included a confirmed positive control for the target pathogen and a no-template negative control. All standard measures were taken to minimize the risk of contamination. Amplicons were analysed by agarose gel electrophoresis (2%) and visualized by staining with Gel Red Nucleic Acid Gel Stain (VWR International Milano, Italy).

Table 1. Primers implemented in the study, with gene targeted, primers name and publication reference.

Pathogen	Primers' names (5' – 3')	Reference
<i>B. divergens</i>	Gene: 18s (forward and reverse from the paper)	(Hilpertshauser et al., 2006)
<i>B. microti/microti</i> -like	Gene: 18s (Bab1 and Bab4)	(Persing et al., 1992)
<i>A. phagocytophilum</i>	Gene: <i>groEL</i> (EphplgroEL(569)F and EphplgroEL(1193)R)	(Alberti et al., 2005)
<i>B. burgdorferi</i> s.l.	Gene: spacer region between 5S and 23S <i>rRNA</i> genes (23SN1 and 23SC1)	(Rijpkema et al., 1995)
SFG <i>Rickettsia</i>	Gene: surface protein <i>rOmpA</i> (190-70 and 90-701)	(Roux et al., 1996)
<i>T. capreoli</i>	Gene: 18s (TcapreoliF and TcapreoliR)	This paper. Reference sequence: AY726011.

For each pool and pathogen, we recorded the binary outcome of the PCR (positive-negative) and we estimated the pathogen prevalence within the pool using the package PoolTestR (McLure et al., 2021) for RStudio (34). This package returns Bayesian estimates of the prevalence with its 95% Credibility Intervals (Cr.I.), knowing the number of ticks belonging to each pool and the test outcome (McLure et al., 2021). Differences in prevalence among tick species and among developmental stages were explored through Chi-squared or Fisher's test, depending on the distribution matrix (McHugh, 2013).

Tick-borne pathogens models

For each repetition and sampling point, we computed the total head count of individuals passing by the camera trap. Each instance of an animal exiting and re-entering the camera's field of view was treated as a new individual, as individual recognition was not possible. All age classes were included in the analysis. We extracted data for red deer, roe deer, fallow deer (*Dama dama*), wild boar, red fox, European badger (*Meles meles*), pine marten (*Martes martes*) and beech marten (*Martes foina*), as CT deployment was not sensitive for distinguishing animal species of smaller size, such as rodents and birds, or for detecting their presence in the whole field of view. We grouped data for mesocarnivores and wild ruminants.

Our database considered, as the response variable, the binary outcome of the PCR test (positive-negative). Explanatory variables involved:

- sampling season and point
- wildlife head counts (as number of passages) for wild boar, mesocarnivores and wild ruminants
- the number of ticks in the pool, as the higher number of ticks in the pool, the higher the probability of a positive result.

Vegetation and environmental parameters were not included in the analysis for several reasons. Firstly, the sampling points were uniformly distributed across vegetation types, ensuring homogeneity in coverage. Secondly, the habitat within the park exhibited overall uniformity due to its limited spatial extent and minimal elevation gradient. Lastly, the influence of vegetation on the presence of tick-borne pathogens is likely indirect, as vegetation primarily affects reservoir host distribution or vector abundance rather than directly influencing pathogen presence

We implemented a General Linear Mixed Model with a binomial family with lme4 package in R studio (Bates et al., 2015). We considered the sampling point as the random variable and the variance was weighted on the season in which ticks were collected, to account for seasonality in tick and host population.

To test how well the model performed, and, consequently, how useful camera trap data could be to predict the presence of tick-borne pathogens, we evaluated three parameters: (i) conditional and marginal R², to test how much the model was satisfactory in explaining the variance; (ii) accuracy and (iii) AUC (Area Under Curve, for which thresholds interpretation was presented by works such as Çorbacioğlu and colleagues

(Çorbacioğlu & Aksel, 2023)), tested by splitting the database into train (70% of data) and test (30% of data) datasets.

We modelled (i) all tick pools, regardless of species and developmental stages (global model) and (ii) *I. ricinus* nymphs pools, as particularly relevant in terms of zoonotic risk (*I. ricinus* nymphs model) (Battisti et al., 2019; Wilhelmsson et al., 2013). We ultimately only created models for the four most prevalent pathogens (*B. capreoli*, *B. microti*-like, *B. burgdorferi* s.l. and SFG *Rickettsia*).

3. Results

Tick-borne pathogens detection and prevalence estimation

We analysed a total of 2537 ticks divided into 413 pools, including 282 pools of *I. ricinus*, of which 112 (424 individuals) were identified in the nymphal stage (23). The two most prevalent pathogens were *B. microti*-like (Bayesian prevalence 13.12% with Cr.I. 11.05-15.28%) and SFG *Rickettsia* (10.79%, Cr.I. 9.14-12.64%), followed by *B. capreoli* (5.47%, Cr.I. 4.2-6.78%) and *B. burgdorferi* s.l. (2.57%, Cr.I. 1.83-3.45%). Finally, prevalence below 1% of *T. capreoli* and *A. phagocytophilum* were recorded (respectively 0.23%, Cr.I. 0.02-0.71%, and 0.34%, Cr.I. 0.14-0.62%).

Ixodes ricinus and *H. punctata* recorded at least one positive pool for each pathogen (Table 2). *B. capreoli*, *B. microti*-like, and SFG *Rickettsia* were found in samples from every tick species tested, including *D. reticulatus*, *H. concinna*, *I. hexagonus*, and the *R. sanguineus* complex (*R. sanguineus* sensu stricto, *R. pusillus*, *R. turanicus* according to the keys implemented in this study (Manilla & Iori, 1992, 1993)). Additionally, *T. capreoli* was detected in *H. concinna*, *H. punctata*, and *I. hexagonus*, while *B. burgdorferi* s.l. was identified in *H. concinna* and *H. punctata*. According to the Chi-squared and Fisher's test, the prevalence rates of only *B. burgdorferi* s.l. were significantly different ($p < 0.05$) among developmental stages and the ones of *B. microti*-like alone did significantly vary ($p < 0.05$) among the tick species.

Table 2. Bayesian estimated prevalence of pathogens per each tick species and developmental stages, with 95% Cr.I in parenthesis. In squared brackets, the number of pools per group (left column) and of positive pools (other columns).

Tick species	<i>B. capreoli</i>	<i>B. microti-like</i>	<i>B. burgdorferi</i> <i>s.l.</i>	SFG <i>Rickettsia</i>	<i>A.</i> <i>phagocytophilum</i>	<i>T. capreoli</i>
<i>I. Ricinus</i> [393]						
Larva [154]	3.5% (2.3 - 5%) [29]	6.5% (4.9 - 8.2%) [54]	0.4% (0.1 - 0.8%) [4]	7.5% (5.8 - 9.6%) [60]	0.2% (0 - 0.6%) [2]	0.5% (0.2 - 1%) [5]
Nymph [184]	7.5% (5 - 10.7%) [26]	30.9% (24.3 - 37.8%) [68]	8.2% (5.5 - 11.5%) [25]	31.2% (24 - 39.1%) [60]	0.7% (0.1 - 1.7%) [2]	0.8% (0.2 - 2%) [3]
Adult [55]	23.1% (12.1 - 36.7%) [9]	53.5% (37.4 - 69.5%) [20]	22.9% (11 - 37.1%) [9]	38.4% (24.1 - 52.7%) [15]	6% (1.1 - 14%) [2]	[0]
<i>I. hexagonus</i> [4]						
Nymph [4]	24.4% (2.5 - 60.1%) [1]	42.1% (8.7 - 77.2%) [2]	[0]	24.9% (3.1 - 62.8%) [1]	[0]	[0]
<i>H. concinna</i> [27]						
Larva [9]	1.1% (0.2 - 3%) [2]	1.1% (0.2 - 2.9%) [2]	0.7% (0 - 2.1%) [1]	2.1% (0.7 - 4.4%) [4]	[0]	[0]
Nymph [15]	12.8% (4.3 - 25.8%) [4]	4.2% (0.3 - 13%) [1]	4.3% (0.4 - 13.1%) [1]	19.3% (8.2 - 33.8%) [6]	[0]	10.2% (2.4 - 22.2%) [3]

While the percentage of positive pools of SFG *Rickettsia* and *A. phagocytophilum* were persistent through the study period, we observed a peak during the summer months for *B. burgdorferi* s.l. and the two *Babesia* species (Figure 2). However, some differences may be spotted while decomposing the trend according to developmental stages. For *B. burgdorferi*, positive larvae peaked in June, while among the other stages, positive pools were more uniformly distributed in warmer months (May-September). For *B. microti*-like, it peaked in all stages around June-July. Finally, for *B. divergens* the peak in nymphs and adults occurred earlier in the year (from May to July) than in larvae (July). *T. capreoli*, which was almost only recorded in larvae, showed higher positivity later in the year.

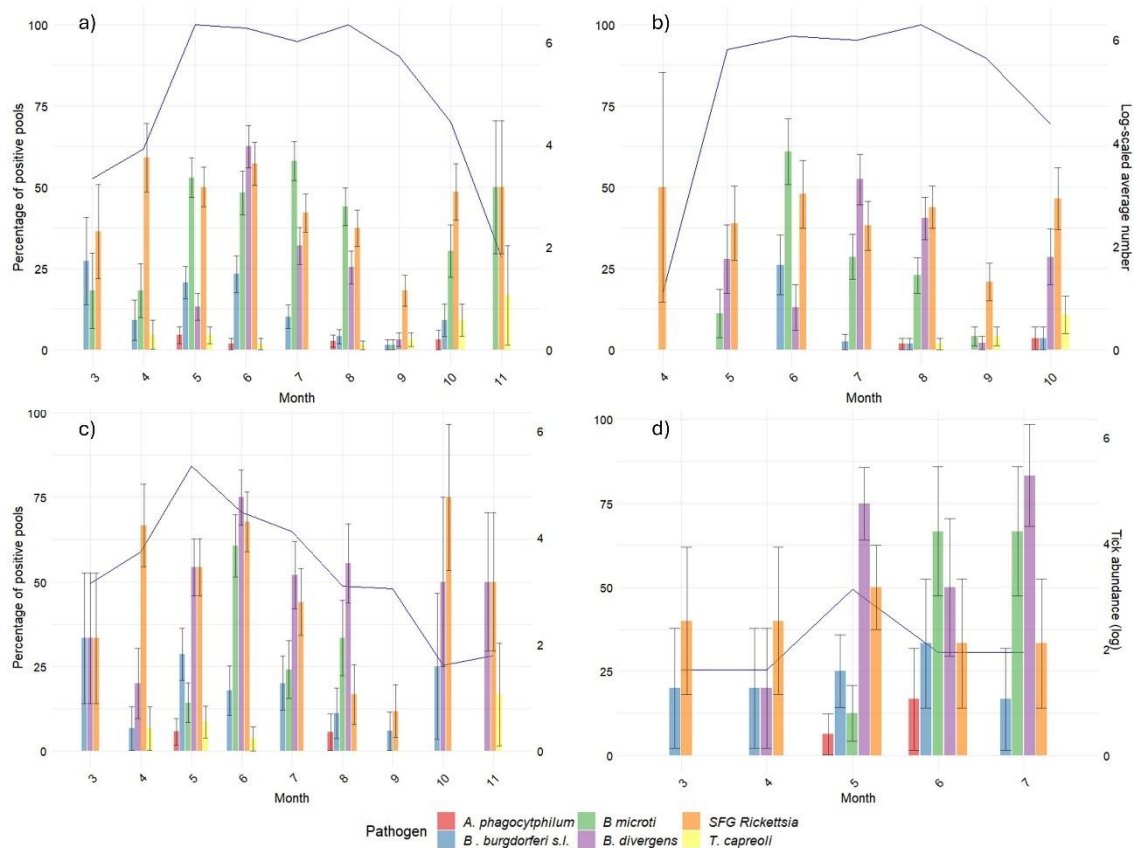


Figure 2. Pathogens positivity across the months per developmental stage. For each of the targeted pathogens, the percentage of positive pools over the total number of collected pools in each month. Winter months (December, January and February) were excluded due to lack of tick activity. The blue line (secondary y-axis) represents ticks' abundance (log-scaled average of number of ticks collected in each sampling point). Graph a) represents the total number of ticks, graph b) represents the number of larvae, graph c) the number of nymphs and graph d) the number of adults.

Tick-borne pathogens models

Based on the best performance of the models, the number of ticks in the pool, wild boar and mesocarnivores head counts were included in all models (Table 3), while deer species head counts were included in *B. capreoli*, SFG *Rickettsia* and the global model of *B. burgdorferi* s.l.

Table 3. Variables included in each model. Considered explanatory variables are wild boar, deer species and mesocarnivores head counts and the number of ticks in the pool. Both the global model and the model for *I. ricinus* nymphs are described.

Model	Pathogen	Wild boar	Deer species	Mesocarnivores	Tick pool
Global model	<i>B. capreoli</i>	✓	✓	✓	✓
	<i>B. microti</i> -like	✓		✓	✓
	<i>B. burgdorferi</i> s.l.	✓	✓	✓	✓
	SFG <i>Rickettsia</i>	✓	✓	✓	✓
<i>I. ricinus</i> nymphs model	<i>B. capreoli</i>	✓	✓	✓	✓
	<i>B. microti</i> -like	✓		✓	✓
	<i>B. burgdorferi</i> s.l.	✓		✓	✓
	SFG <i>Rickettsia</i>	✓	✓	✓	✓

Overall, the coefficients indicated a similar effect (either dilutive or additive) of the same host species across both the global model and the *I. ricinus* nymphs model, despite statistical significance (Figure 3). Wild ruminants and wild boar exhibited a dilutive effect (negative coefficient) on the prevalence of all assessed pathogens. In contrast, the influence of mesocarnivores varied between the global model and the *I. ricinus* nymphs model; overall, they demonstrated a statistically significant dilutive effect for *B. divergens*, while showing a statistically significant additive effect for *B. burgdorferi* s.l. and *B. microti*-like. Furthermore, the total number of ticks in the pool had a positive association with the presence of pathogens within the pool, except the global model of *B. burgdorferi* s.l., where a detractive impact was detected.

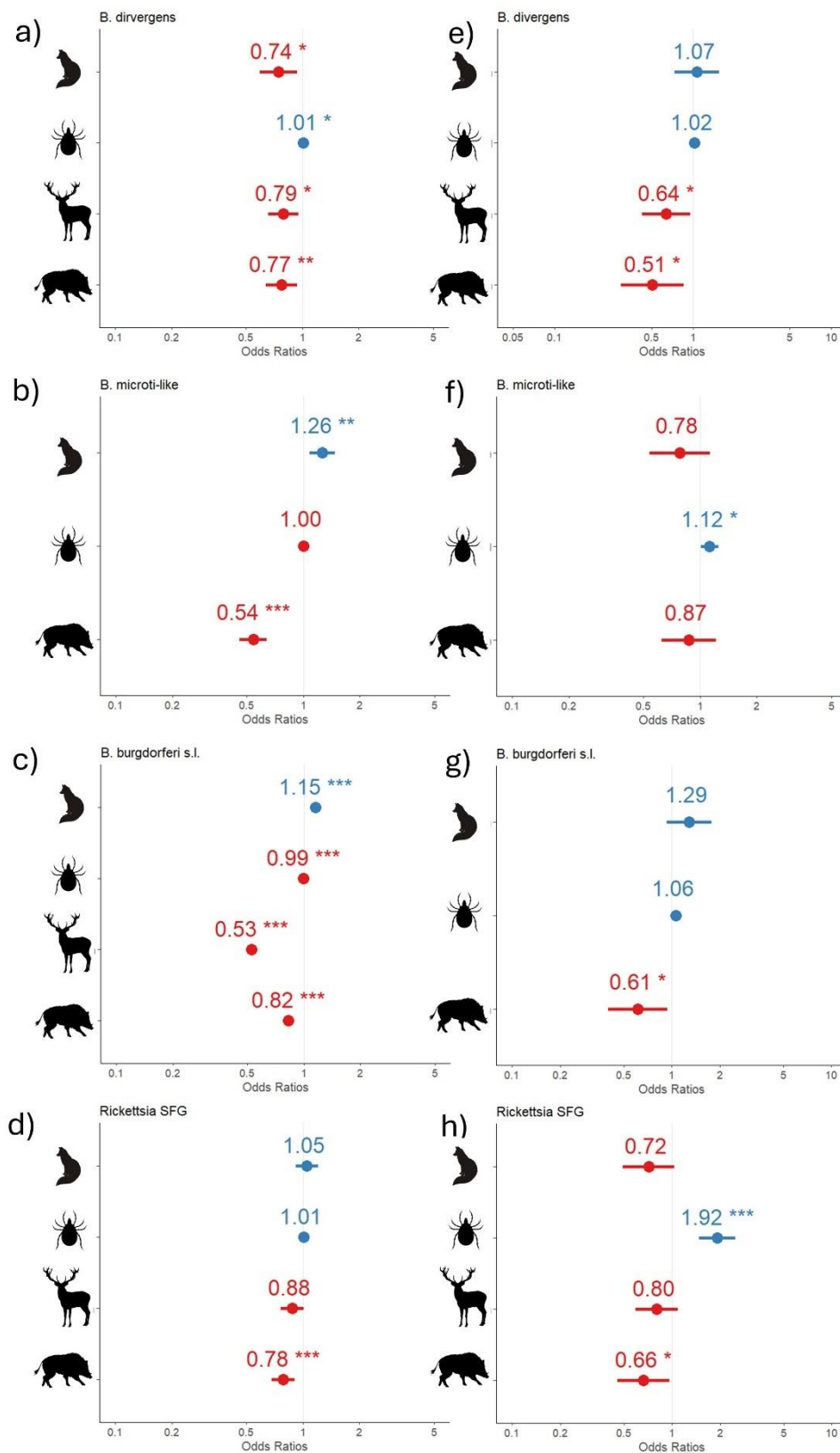


Figure 3. Models result coefficients. Coefficients (blue, positive; red, negative) of the models for each pathogen, in the global model (a-d) and *I. ricinus* nymphs' model (e-h). Asterisks identify statistical significance. Silhouettes on the x-axis represent: mesocarnivores head count (fox), total number of ticks in the pool (tick), wild ruminants head count (red deer) and wild boar head count (wild boar).

Model performance, evaluated using R^2 , accuracy, and AUC, was highest for the models focused on *I. ricinus* nymphs, apart from those predicting *B. burgdorferi* s.l. Notably, SFG *Rickettsia* exhibited the lowest accuracy and AUC, but the highest R^2 among the models analysed. The model for *B. capreoli* demonstrated the highest accuracy, while the *B. burgdorferi* model achieved the highest AUC, exceeding 0.7, marking it as the only model to surpass this threshold of good predictivity (Table 4).

Table 4. R^2 values, accuracy and AUC for each model.

	Global model	<i>I. ricinus</i> nymphs model
<i>B. divergens</i>		
Marginal R^2 / Conditional R^2	0.070 / 0.204	0.188 / 0.227
Accuracy	0.74	0.9
AUC	0.65	0.65
<i>B. microti</i> -like		
Marginal R^2 / Conditional R^2	0.098 / 0.171	0.137 / 0.485
Accuracy	0.6	0.69
AUC	0.62	0.67
<i>B. burgdorferi</i> s.l.		
Marginal R^2 / Conditional R^2	0.098 / 0.388	0.118 / 0.429
Accuracy	0.91	0.86
AUC	0.66	0.74
<i>Rickettsia</i> SFG		
Marginal R^2 / Conditional R^2	0.031 / 0.049	0.862 / 0.876
Accuracy	0.56	0.57
AUC	0.59	0.63

4. Discussion

Our models showed that wildlife presence affects tick-borne pathogens' prevalence, and, consequently, camera trap data can be useful to predict their risk in the environment.

The prevalence for *Rickettsia*, *A. phagocytophilum*, *Babesia* spp. and *B. burgdorferi* s.l. were consistent with studies on *I. ricinus* ticks collected from dogs (Zanet et al., 2020), humans (Audino et al., 2020) and wildlife (Battisti et al., 2020; Zanet et al., 2014) in North-Western Italy, and with previous studies in the same study area (Bellato et al., 2021). The prevalences detected for *T. capreoli* were consistent with the ones reported in wild deer species in Spain (Díaz-Cao et al., 2022; Hornok et al., 2017). Temporal fluctuations in pathogen presence did follow the seasonal peaks of the developmental stages in which they were more often detected (Ferroglia et al., 2024b), which is also in accordance with the pool size variable in the model showing an additive effect on pathogens' presence. Pathogens capable of transovarial transmission, such as *B. divergens* and SFG *Rickettsia* (Bonnet et al., 2007; Hauck et al., 2020), exhibit a more evenly distributed positivity rate throughout the months of tick activity. In contrast, *B. microti*-like, which lacks transovarial transmission (Gray et al., 2002), shows a peak in positivity rates

concentrated around June. Despite its transovarial transmission, *B. burgdorferi* s.l., in accordance with results obtained by Szekeres and colleagues in Germany (Szekeres et al., 2017), was predominantly found in nymphal pools and its temporal fluctuations varied accordingly with this developmental stage, with a peak around June. This concurrence was also highlighted by Hartemink and colleagues in the Netherlands (Hartemink et al., 2021).

A limitation of the Minimum Infection Rate (MIR) estimation is that it is derived from the number of ticks within a pooled sample ($MIR = 1/n^\circ$ ticks). Consequently, as the number of ticks tested together increases, the denominator rises, leading to a lower calculated prevalence. Although the Bayesian approach partially mitigates this issue, it does not eliminate the bias introduced by the pool size: for instance, a study on tick-borne zoonoses in the same study area testing individual ticks detected higher prevalences for SFG *Rickettsia* and *B. burgdorferi* s.l. (Bellato et al., 2021). This is particularly relevant for larval pools, which may encompass a substantial number of ticks, potentially resulting in an underestimation of the true prevalence. This approach is however more precise than other traditionally implemented methods and still allows comparison among tick species or different locations, especially in situations where single tick testing would be poorly effort-effective (Fracasso et al., 2023). Besides these considerations, it is noteworthy that an increased number of ticks positively affects the probability of a positive outcome of a biomolecular test, remarking how tick hotspots may represent a major risk for tick-borne disease transmission. The sole exception was the effect observed for *B. burgdorferi* s.l. in the global model, where this effect was highlighted as detractive. The scarcity of positive detections during periods of higher larval abundance may have influenced this outcome.

In some cases, pathogens were found in tick species that are not recognised as competent vectors: for instance, *Babesia* spp. were found in all species, despite only *I. ricinus* has been demonstrated to be capable of transmission back to the vertebrate host (Bajer & Dwuznik-Szarek, 2021; Salman & Tarrés-Call, 2013). Even though little is known about the actual competence of less common tick species, it is important to clarify that the presence of pathogen DNA in the vector is not proof of the transmission capability, and it just mirrors a pathogen's circulation in the study area. This can explain the absence of a statistically significant prevalence difference among tick species for most pathogens. According to the literature, *B. burgdorferi* was detected in *H. punctata* (Tälleklint, 1996), *B. microti* in *D. reticulatus* (Wójcik-Fatla et al., 2012) and *B. bigemina* in *H. punctata* (Yin et al., 1996), thus confirming our findings that pathogen infection and transmission do not necessarily coincide.

Wild ungulate species targeted by our camera trap data collection are likely not reservoirs for the four modelled pathogens (Castillo-Contreras et al., 2022; De Keukeleire et al., 2017; Piesman et al., 1979; Sgroi et al., 2023; Skarphedinsson, 2005a), but they act as maintenance hosts for tick population (Ferroglia et al., 2024b; Hofmeester et al., 2017a, 2017b). In light of informing management actions aimed at reducing the number of ticks, we choose them as relevant variables to model. Despite *B. capreoli* having been reported

in red deer (Zanet et al., 2014), there are several cases where only roe deer is found positive for this pathogen (Kauffmann et al., 2017; Silaghi et al., 2020; Tampieri et al., 2008). Additionally, *B. capreoli* is not reported in red deer in areas where roe deer is absent (Zintl et al., 2011), suggesting a limited role of this species as a maintenance host. This most likely explains our finding of a dilutive effect of deer species altogether, as roe deer density in the park was 1.90 ± 0.97 ind/km², much lower than other species (ENETWILD-consortium et al., 2023b), and the ratio between roe and red deer head counts in our study of 171:1212, and with fallow deer of 171:854. In Europe, *B. microti*-like species include *B. vulpes* and *B. microti* Munich strain (Goethert, 2021). Red fox, the mesocarnivore species mostly recorded in our study, may harbour *B. vulpes*, which is a possible reason for the additive effect of its presence on *B. microti*-like in environmental ticks. Additionally, red fox presence may be linked to its micromammal preys, which can be an underlying factor for the positive coefficient on *B. burgdorferi* s.l. and *B. microti*-like presence. Indeed, both pathogens have micromammals, such as ground-dwelling rodents and shrews, as major reservoirs (Goethert, 2021; Salman & Tarrés-Call, 2013). Including small mammals in such types of studies will be a fundamental step forward in understanding the ecology of these tick-borne pathogens, as performed by Takumi and colleagues (2019).

In this study, and for the reasons outlined, we did not incorporate environmental variables into the analysis. While temperature and humidity significantly influence tick activity and abundance (Zajac et al., 2021), and consequently the prevalence of tick-borne pathogens by increasing tick population density and contact rates between hosts, habitat characteristics primarily affect host presence, density, and temporal occupancy (Cargnelutti et al., 2002; Ferroglio et al., 2024b; Lovari et al., 2017). These, in turn, indirectly influence tick abundance and pathogen presence when hosts serve as competent reservoirs (Diuk-Wasser et al., 2021; Ehrmann et al., 2018; Ferroglio et al., 2024b; Vada et al., 2024b). Although parameters such as vegetation types or habitat fragmentation indices could be included in the analysis as proxies for host presence, they would lack the precision of data obtained directly from camera traps. Our results highlighted how camera trap data represent a valid tool to predict the presence of tick-borne pathogens and, consequently, draw insights about the zoonotic risk and further control strategies. The model performance in predicting pathogens' presence is improved by decomposing the response variable, meaning that pathogen-host association may vary depending on the developmental stage and species of the vector. More accurate predictions and new insights on the pathogen-host interaction would benefit from ad-hoc models targeting the single species and developmental stages. This approach was not possible in the current study due to limited numbers of other tick species.

5. Conclusion

Our study verified the presence of tick-borne pathogens in a fenced natural park, a site frequented by many visitors engaging in various outdoor activities. While rodents are known to be primary reservoirs and maintenance hosts for several of these pathogens,

our research concentrated on species that indirectly influence pathogen transmission. These species (wild ungulates in particular and red fox on a lesser degree) are more readily observed and managed, particularly through camera trapping and hunting. Our findings demonstrate a clear connection between pathogen prevalence and these species, underscoring the value of camera trap data in providing detailed insights into wildlife populations for studies in disease ecology.

5. Expanding the interface: the human dimension

5.1. A One-Health approach to surveillance of tick-borne pathogens across different host groups

Vada R., Zanet S., Trisciuglio A., Varzandi A.R., Calcagno A., Ferroglio E. A One-Health approach to surveillance of tick-borne pathogens across different host groups. *Submitted to: BMC Veterinary Research*

A One-Health approach to surveillance of tick-borne pathogens across different host groups

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Abstract. Tick-borne pathogens are increasingly impacting wildlife and human populations' health due to expanded vector ranges and the growing popularity of outdoor activities. This study aimed to survey the prevalence of tick-borne zoonotic (TBZ) microorganisms in a rural area, targeting different host groups involved in their circulation, namely wildlife, domestic animals, humans and questing ticks, addressing the risk associated to hunting and outdoor leisure activities in an alpine environment. The study was performed in an alpine district in Northwestern Italy. We collected 124 pools of questing ticks using dragging transects across 38 locations distributed in the district. In addition, we obtained 133 spleen samples from game ungulate species (wild boar, red deer, and chamois), 67 blood samples from volunteer rural inhabitants, and samples from 38 privately-owned hunting dogs. All samples were tested by PCR for *Babesia divergens*/*B. capreoli*, *B. microti*-like, *B. venatorum*, *Borrelia burgdorferi* sensu latu, *Rickettsiae* Spotted Fever Group (SFG) and *Anaplasma phagocytophilum*. All investigated pathogens, except *B. burgdorferi* s.l. (found only in questing ticks), were detected in rural inhabitants. *B. divergens* and *B. venatorum* were highly prevalent in wild ruminants, while *B. microti*-like organisms appeared sporadically in wild boar and dogs. SFG *Rickettsiae* were most common in dogs, with occasional cases in wild boar. *A. phagocytophilum* showed low prevalence in ticks and wildlife, but higher detection rates in humans and dogs. Our results underscore the interconnected nature of tick-host dynamics in the spread of TBZ and highlight the need for an expanded surveillance approach, potentially including other significant reservoirs.

1. Background

With tick vectors increasing in abundance and spreading geographically (Madison-Antenucci et al., 2020; Medlock et al., 2013), tick-borne zoonoses (TBZ) have emerged as some of the most impactful vector-borne diseases across Europe (Hartemink & Takken, 2016). For instance, Lyme disease, presenting with a relatively high and widespread prevalence already, has increased its incidence in the past 10 years (Rochlin & Toledo, 2020; Vandekerckhove et al., 2021). Human babesiosis, another important emerging disease of global significance, is also recorded in increasing countries and prevalence Europewide (Bajer et al., 2022).

In addition to the geographical expansion of tick vectors and the increase in wild host populations, the popularity of outdoor activities, further amplified by restrictions during the COVID-19 pandemic (Fernandez et al., 2021), is increasing the risk of tick bite for humans along facilities in the natural environment (e.g. Janzén et al., 2023; Vada et al., 2024a; Van Gestel et al., 2021) and beyond, and ultimately the transmission of TBZ from wildlife. Different wild species may act as reservoirs for TBZ, depending on the specific pathogen. While small mammals such as rodents, insectivores, or even birds maintain the causative agents of Lyme disease or Mediterranean Spotted Fever in the environment (Salman & Tarrés-Call, 2013), red deer (*Cervus elaphus*) is considered a potential reservoir for *Babesia divergens* (Gray et al., 2021), reaching prevalences up to 27% in Czech Republic (Hrazdilová et al., 2020) and, together with wild boar (*Sus scrofa*), for *Anaplasma phagocytophilum* (de la Fuente & Gortazar, 2012; Grassi et al., 2021). The same pathogen has also been detected at high prevalence (77.8%) in alpine chamois (*Rupicapra rupicapra*) (Grassi et al., 2021). Moreover, the presence of deer species and red fox (specifically, temporal occupancy of a site) has been shown to increase questing ticks' abundance (Ferroglia et al., 2024b; Vada et al., 2024b), which, in return, impacts the risk of tick bite and TBZ transmission.

Due to their multi-host and multi-pathogen nature, tick-borne zoonoses require a comprehensive, integrated surveillance system to effectively monitor and inform decision-making for prevention strategies. Integrated surveillance data targeting TBZ encompass human and animal health, pathogen presence, vector surveillance, and environmental factors like climate and wildlife abundance (Semenza & Zeller, 2014). Such data can also be integrated with modelling strategies, which can help predict outbreaks (Clow et al., 2019; Hai et al., 2014). Additionally, standardized, cross-border data collection enables data integration and minimizes bias.

In this study, we estimated the prevalence of the most common tick-borne zoonoses in Northwestern Italy, including several of the most widespread pathogens in Europe: *B. divergens*, *B. microti* and *microti*-like, *B. venatorum*, *A. phagocytophilum*, *Borrelia burgdorferi sensu latu* and *Rickettsiae* causing the Mediterranean Spotted Fever (SFG *Rickettsiae*). Within an alpine setting, our analysis targeted key host groups related to the transmission through outdoor and, particularly, hunting-related activities, namely environmental ticks, rural inhabitants, hunting dogs and game ungulates. By highlighting similarities or differences in the presence of TBZ microorganisms in the different hosts, this study aimed to provide useful information to refine a comprehensive surveillance of these pathogens.

2. Materials and methods

Study area

This study was performed in an alpine hunting district in Northwestern Italy (C.A. CN3, Piedmont). It covers an alpine valley of 567 km², with altitude ranging from 500m above the sea level up to more than 3000m above the sea level (a.s.l.) and an alpine and continental climate. The annual precipitation in 2020 was 788.2 mm, and the mean

temperature was 10.19 °C. The valley registers a population of 11.500 inhabitants (20.28 inh/km²) and several agropastoral activities. The hunting plan targeting ungulate species involves wild boar, roe deer (*Capreolus capreolus*), red deer and chamois.

Sampling

The project received approval from the Ethics Committee of the University of Turin (Prot. n. 0346675, 26/06/2023 and Prot.0001785, 01/06/2023), and all participants and dog owners signed a written informed consent. All data were anonymized with unique alphanumeric code applied on samples, and Good Clinical Practice recommendations were followed. The cross-sectional study targeted the different host groups:

- Questing (environmental) ticks. Ticks were collected during August-October 2021 through 38 sampling points covering the whole valley, up to 2500m a.s.l. At each sampling point, ticks were collected by dragging transects (a 10m side square and a 26m radius circle around the GPS point) and stored in 100% ethanol. They were identified with dichotomous keys (Estrada-Peña et al., 2018; Manilla & Iori, 1992, 1993) and pooled by sampling point, species, and developmental stage.
- Game ungulates. With the collaboration of local hunters and with the support of the hunting district management, an opportunistic collection of spleen from regularly hunted species was performed, namely wild boar, red deer, and chamois. All animals were hunted during the hunting season 2021/2022; samples were individually identified and stored at -20°C until further analysis. The sample size for each species was proportional to the number of culled specimens available for sampling.
- Rural inhabitants. Together with game management authorities, hunters were voluntarily enrolled in the study. A sampling day was organized locally in the summer of 2023. Besides hunters, to increase the sample size, their extended families and other people related to the hunting district were also included in the cohort. All participants engaged in outdoor recreational activities at least once per week, either hunting, fishing, mushroom picking, or camping/hiking/cycling. Whole blood was collected and stored at -20°C until further analysis.
- Dogs: participants could also voluntarily enrol their dog(s) in the study. An aliquot part of whole blood collected by routine clinical practices by private veterinarians was dedicated to this study, and tubes were stored at -20°C until further analysis. We additionally investigated how often the animal was involved in outdoor activities (from less than once to 7 times per week) and assessed the use of antiparasitic treatment registered for Ixodidae ticks (between “never,” “sometimes,” and “always”).

Biomolecular detection of microorganisms

Genomic DNA of vertebrate hosts was extracted from 200 ul of blood (humans and dogs) and from 10 mg of spleen (wildlife) with the PureLink Genomic DNA Mini Kit (Invitrogen, CA, USA), according to the manufacturer's instructions. The genomic DNA

of ticks was obtained by extraction from the whole pooled ticks with the blackPREP Tick DNA/RNA kit (Biosense, Italy). The concentration and quality of extracted DNA were evaluated with Nanodrop One (ThermoFisher, MA, USA). Extracted DNA was then stored at -20°C until further analysis.

PCR protocols were implemented to detect microorganisms' genomic DNA through all groups, despite some pathogen-hosts combination not having been yet reported in literature. A non-template negative control and a positive control (either DNA extracted from cultivated pathogen or positive samples previously confirmed by amplicon sequencing) were included in all PCR reactions, and all standard precautions were taken to minimize the risk of contamination. Amplicons were analysed by agarose gel electrophoresis (2%) and visualized by staining with Gel Red Nucleic Acid Gel Stain (VWR International Milano, Italy). Primers implemented are reported in Table 1, while the complete protocols are presented in Supplementary Table 1.

Table 1. Primers implemented in the current work, with reference to the targeted gene and the work where the primer was firstly described.

Pathogen	Gene	Reference:
<i>B. divergens</i>	18s	(Hilpertshauser et al., 2006)
<i>B. microti</i>	18s	(Persing et al., 1992)
<i>B. venatorum</i>	18s	(Michelet et al., 2014)
Anaplasmataceae	16s rRNA	(Battisti et al., 2020; Goodman et al., 1996)
<i>A. phagocytophilum</i> *	groEL	(Alberti et al., 2005)
<i>B. burgdorferi</i> s.l.	spacer region between 5S and 23S rRNA genes	(Rijpkema et al., 1995)
SFG Rickettsia	surface protein rOmpA	(Roux et al., 1996)

* To avoid false positive results due to the detection of symbiotic bacteria like *Candidatus* *Midichloria*, in ticks we implemented specific primers for *A. phagocytophilum*, while *Anaplasmataceae* primers were implemented in vertebrate hosts.

Anaplasmataceae and SFG *Rickettsiae* species were further identified by sequencing (Macrogen Europe, The Netherlands) and further comparison of obtained sequences with those deposited in GenBank®. The targeted section of the 18s gene for *B. divergens* did not include the single-nucleotide-polymorphisms (SNPs) with *B. capreoli* and, therefore, could not allow to differentiate species through sequencing. All protocols implemented an end-point PCR, with the only exception of *B. venatorum*, that implemented a real-time PCR.

Statistical analysis

Prevalence with 95% confidence intervals (95% CI) in vertebrate hosts (rural inhabitants, dogs and game ungulates) was calculated through the Wilson Score method. For tick pools, prevalence was calculated as Minimum Infection Rate (MIR). Due to their zoonotic relevance, we also tested prevalence in nymphs and adults separately. To point

out any statistical difference among groups, we implemented a paired comparison through a Chi-square test for vertebrate host groups (R Core Team, 2023). Fisher's exact test and correlation were implemented (R Core Team, 2023) to test the association between survey responses and positivity to PCR in dogs.

3. Results

Sampling population description

We sampled 532 ticks divided into 124 pools (Table 2).

Table 2. Number of ticks collected by species and developmental stage and corresponding number of pools.

	Count	Number of pools
<i>Ixodes ricinus</i>		
Larvae	339	51
Nymphs	126	42
Adults	19	15
<i>Haemaphysalis punctata</i>		
Larvae	46	14
<i>Dermacentor marginatus</i>		
Nymphs	1	1
Adults	1	1

We sampled a total of 67 rural inhabitants, of which 54% practiced hunting activities. The sex ratio of rural inhabitants was skewed towards males (49-18), and the age average was about 50 years.

We sampled a total of 38 dogs, with an even sex ratio between males and females and an average age of 5 years. 66.7% were engaged in outdoor activities at least once a week (33.3% less than once per week, 20.8% once per week, 8.3% twice, 29.3% three times and 8.3% daily), and seasonality in outdoor activity being linked to hunting activity (i.e. only from September to December). Antiparasitic treatment registered for Ixodidae ticks was regularly administrated in 84.4% of sampled dogs, while it was administrated at sub-optimal frequencies in 15.6% of cases.

We sampled a total of 133 game ungulates, including 117 wild boar, 13 red deer and 3 chamois.

Biomolecular detection of microorganisms

Red deer showed a statistically significant higher prevalence (Figure 1) of *B. divergens* than rural inhabitants (Chi-square test's $p < 0.001$), wild boar (Chi-square test's $p < 0.001$) and questing ticks (Chi-square test's $p < 0.001$), while no statistically significant difference was observed among other groups.

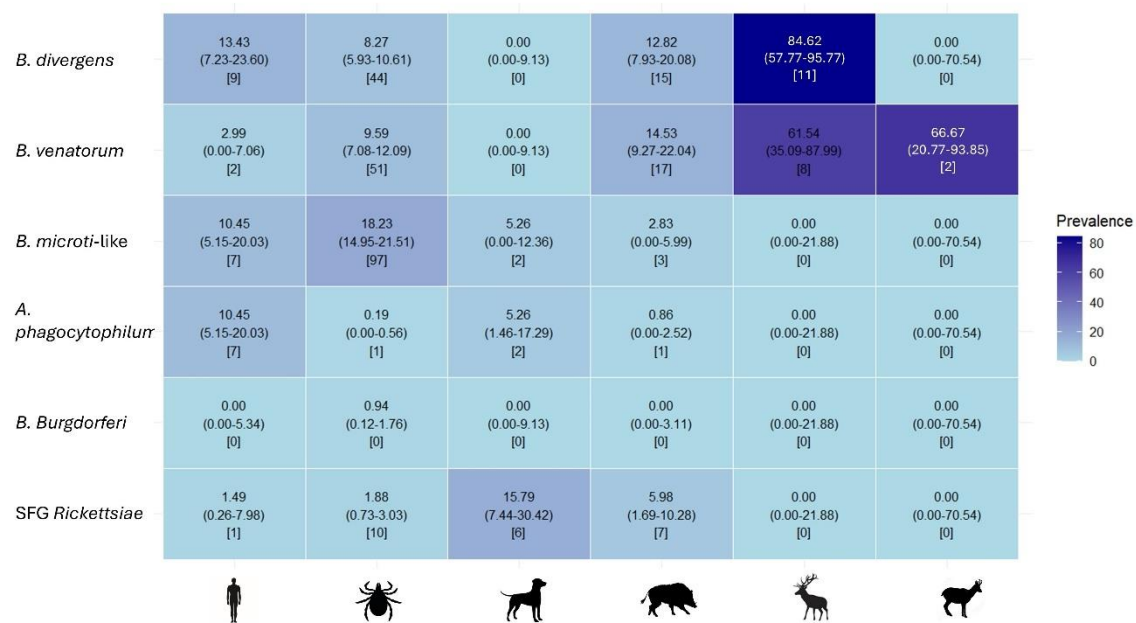


Figure 1. Heatmap of the prevalences of the detected microorganisms across groups. In round brackets, 95% C.I.; in square brackets, the number of positive specimens or ticks. In order: rural inhabitants, questing ticks, dogs, wild boar, deer and chamois.

Wild ungulates showed a significant higher prevalence of *B. venatorum* than rural inhabitants (Chi-square test's $p = 0.01$ for wild boar, Chi-square test's $p < 0.001$ for red deer and Chi-square test's $p = 0.007$ for chamois) (Figure 1). Additionally, red deer showed a significant higher prevalence than wild boar (Chi-square test's $p < 0.001$) and questing ticks showed a significant lower prevalence than red deer (Chi-square test's $p < 0.001$) and chamois (Chi-square test's $p = 0.027$).

Ticks registered the highest prevalence of *B. microti*-like, which was statistically higher than dogs (Chi-square test's $p = 0.045$) and wild boar (Chi-square test's $p < 0.001$) (Figure 1). Additionally, prevalence in rural inhabitants was also higher than in wild boar (Chi-square test's $p = 0.47$).

Anaplasmatidae tests were confirmed to be *A. phagocytophilum*. The sequenced amplicons (accession numbers: PQ657427 and PQ657428) showed a query coverage ranging from 93% to 100% and sequence identity between 95.49% and 100% when compared to GenBank sequence KP245908.1, which was previously identified in ticks from Poland (Stańczak et al., 2015). A higher prevalence was observed in rural inhabitants and domestic dogs (Figure 1). In particular, rural inhabitants showed a significantly higher prevalence (Chi-square test's $p = 0.004$) than wild boar, for which only a single specimen tested positive, and environmental ticks (Chi-square test's $p < 0.001$), while dogs showed a statistically higher prevalence than environmental ticks (Chi-square test's $p = 0.13$). No positive cases were detected in red deer or chamois.

Detection of *B. burgdorferi* did not return results in the vertebrate hosts (Figure 1). The highest prevalence of SFG *Rickettsiae* was detected in dogs, with a statistical significance

with respect to rural inhabitants (Chi-square test's $p = 0.009$) and environmental ticks (Chi-square test's $p < 0.001$) (Figure 1). Regarding wildlife, only wild boar was found positive to the pathogen and its prevalence was also statistically higher than in ticks (Chi-square test's $p = 0.02$). Amplicons sequences were identified as *R. monacensis* (GenBank: LN794217.1; query cover of 100% and a percentage of identity from 97% to 100%; accession numbers of the sequences obtained from the current study: from PQ684452 to PQ684460).

Within groups, fewer statistical significance was detected among pathogen prevalences (Figure 1). In rural inhabitants, *B. divergens* prevalence was significantly higher than SFG *Rickettsiae* (Chi-square test's $p = 0.02$). *B. microti* in wild boar was statistically lower than *B. divergens* (Chi-square test's $p = 0.012$) and *B. venatorum* (Chi-square test's $p = 0.002$) and *A. phagocytophilum* was significantly lower than *B. divergens* (Chi-square test's $p = 0.002$), *B. venatorum* (Chi-square test's $p < 0.001$) and SFG *Rickettsiae* (Chi-square test's $p = 0.04$).

Regarding ticks' developmental stages, prevalences in adults were higher than in nymphs and prevalences in both stages were higher than in the total tick population (Figure 2). No nymphs or adults were positive for *A. phagocytophilum*.

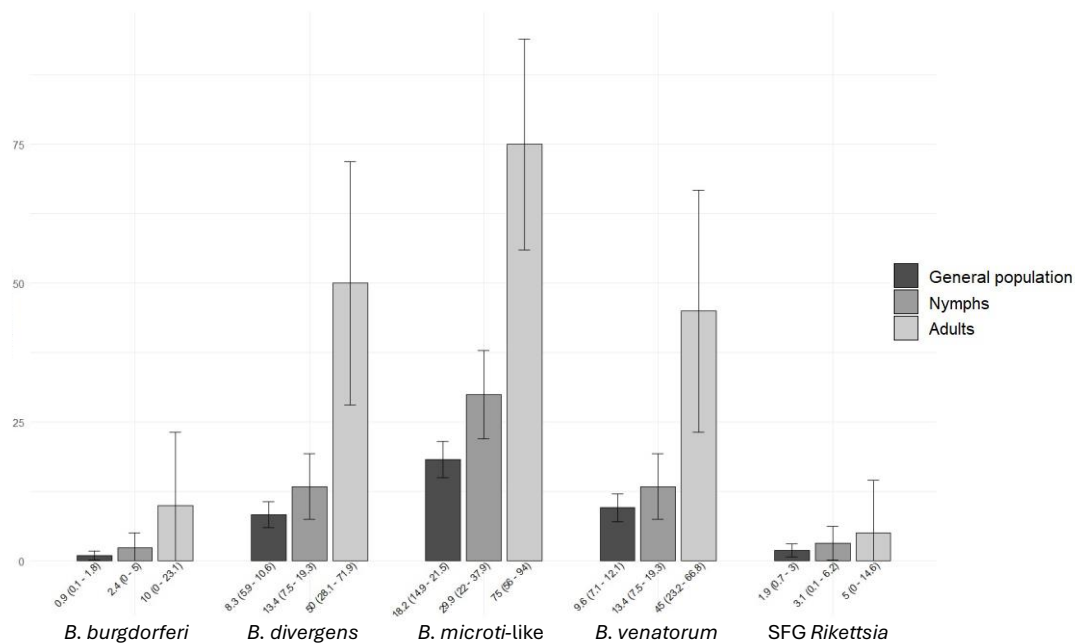


Figure 2. MIR expressed as percentage of the targeted pathogens in the general ticks population, nymphs and adults. Below each bar, the MIR value with 95% C.I. We do not report *A. phagocytophilum* as it was only recorded in larvae.

Analysis of the dog owners' questionnaires revealed no significant association ($p < 0.05$) between PCR positivity and antiparasitic use or the weekly frequency of outdoor activities.

4. Discussion

Our findings underscore the circulation of key tick-borne zoonotic pathogens across distinct host groups involved in their transmission. Except for *B. burgdorferi* s.l., which was only detected in questing ticks, rural inhabitants tested positive for all other investigated pathogens. Notably, *B. divergens* and *B. venatorum* exhibited substantial prevalence in wild ruminants, while *B. microti*-like organisms were sporadically detected in wild boar and domestic dogs. Regarding SFG *Rickettsia*, the highest prevalence was observed in dogs, although occasional detections also occurred in wild boar. *A. phagocytophilum* was found in questing ticks and wildlife at a low prevalence but showed markedly higher detection rates in humans and domestic dogs.

Human babesiosis has been recognized in Europe as an emerging zoonosis (Hildebrandt et al., 2021). A serosurvey conducted in Northern and Central Italy to investigate the presence in humans of antibodies against *Babesia* species has detected a high reactivity to *B. microti* and *B. divergens* antigens in foresters and hunters, reaching respectively 32% and 12% (Gabrielli et al., 2014). Although all participants in our study were healthy individuals without symptoms, we identified a notable prevalence of *B. divergens*, the most common zoonotic *Babesia* species across Europe, and, in addition, we detected lower yet relevant prevalences of *B. microti*/*microti*-like and *B. venatorum*, confirming that also these zoonotic species are circulating within the human population at notable levels. Zoonotic *Babesia* species are indeed often present in ticks removed from humans, with MIR reaching up to 31% (Battisti et al., 2019), a finding which is also remarked by our study results, making questing ticks good targets for surveillance of such pathogens.

For *B. divergens* and *B. venatorum*, red deer and chamois are remarked by our study results as frequent hosts. Indeed, *B. divergens* has been found with relevant prevalences, from 5 to 27%, in red deer and cervids (Cézanne et al., 2017; Gray et al., 2021; Hrazdilová et al., 2020), and *B. venatorum* has been documented in roe deer (Cafiso et al., 2021a; Hrazdilová et al., 2020; Remesar et al., 2019; Zanet et al., 2014) and chamois (Michelet et al., 2014). Given that these species are subject to regulated hunting plans, the relative ease of obtaining spleen samples from them makes them suitable candidates for inclusion in wildlife-based surveillance programs for zoonotic babesia species. However, surveillance in cervid species should target genomic regions of *Babesia* that contain SNPs distinguishing *B. capreoli* from the zoonotic *B. divergens*, as the former, though non-zoonotic, can be detected at high prevalence (up to 46%) in deer species (Remesar et al., 2019; Zanet et al., 2014). The role of wild boar for the transmission of zoonotic babesia species, and its relevance to surveillance actions, is still uncertain: while their detection is still uncommon, *B. vulpes* (now classified as *B. microti*-like) has been reported in Italy (Sgroi et al., 2023), as well as *B. divergens* in the Czech Republic (Hrazdilová et al., 2021), findings which corroborate the ones of our study but still lead to an inconclusive opinion about their role for the circulation of these pathogens. A relevant exposure of hunting dogs to *B. microti* was remarked in Spain, 15% to 60% (Checa et al., 2019; Miró et al., 2015), France, 0.7% (René-Martellet et al., 2015) and Serbia, 10% (Gabrielli et al., 2015). Particularly, the presence of clinical cases in dogs (Miró et al., 2015) remarks the value of a surveillance for *B. microti*-like aimed not only at defining the ultimate exposure risk for

humans through holistic approaches, but also the risk of developing a disease for pet dogs.

The high prevalence of SFG *Rickettsiae* in dogs detected in this study is consistent with results obtained elsewhere: a study on ticks removed from 221 dogs in different Italian locations has detected SFG *Rickettsiae* as the pathogen present with the highest (51.7%) prevalence (Ferroglia et al., 2024a). In the present work, this finding may suggest a “domestic” cycle of SFG *Rickettsiae* besides the natural environment, supported by the fact that the primary vector of SFG *Rickettsiae* is ticks of genera *Rhipicephalus* (Salman & Tarrés-Call, 2013), which is more commonly found in domestic environments. Reports of SFG *Rickettsia* species in wild boar are documented in the literature (e.g. *R. monacensis* by Matei et al., 2023), along with detections of relevant prevalences in tick species collected from wild boar (Castillo-Contreras et al., 2022), findings which align with our results.

Although the detection of circulating *B. burgdorferi* s.l. DNA with an end-point PCR in blood is sometimes inconclusive due to transient or too low spirochaetaemia (Aguero-Rosenfeld et al., 2005; Eshoo et al., 2012; Zygnier et al., 2009), the identification of the spirochete in ticks highlights the ongoing exposure risk for humans and supports the use of ticks as optimal targets for surveillance efforts. The absence of positive spleen samples from wild ungulates may either corroborate their non-significant role as hosts or again suggest a non-appropriate tissue matrix. However, opportunistic sampling of other tissues such as cerebrospinal fluid or lymph nodes would require an increased effort of specialists to collect samples and would not be feasible through a citizen science approach involving participation of hunters and hunting associations.

Eight clusters encompassing five ecotypes of *A. phagocytophilum* have been identified so far, according to the literature. Among these, Cluster I is recognized as zoonotic and has been detected in wild ungulates, including deer species and wild boar, raising suspicions of a zoonotic role for these species, along with domestic animals such as dogs and livestock (Jaarsma et al., 2019; Matei et al., 2019). Despite the potentiality of harbouring zoonotic ecotypes of the pathogen, of all tested culled wildlife only one individual was detected as positive for *A. phagocytophilum*. Considering that there is a certain concordance between environmental ticks and wildlife and a notable difference in humans and dogs, this could again indicate some different sources of infections for people and their pets. Additionally, it should be noted that *A. phagocytophilum* often exhibits fluctuating detectability through biomolecular techniques (Dondi et al., 2014; Rejmanek et al., 2012), which may have influenced the low prevalence detected by our PCR techniques.

Human cases of babesiosis, anaplasmosis and rickettsiosis are anecdotal in the European Region and this seems in contrast with the prevalence of circulating DNA we observed in animals, vectors, and healthy volunteers. While asymptomatic infections have been observed, it is possible that these TBZs are largely underdiagnosed (for lack of knowledge and limited capacity for laboratory diagnosis) and underreported. Since the

initiation of systematic nationwide data collection in January 2011, the United States has reported an increase in human babesiosis cases (CDC, <https://www.cdc.gov/babesiosis/maps-graphs/index.html>, accessed on 03/02/2025). Variations in reported cases may not directly reflect true changes in disease incidence but could be influenced by clinician awareness and state-specific public health resources and reporting requirements (CDC, 2022). This underscores the necessity of ongoing surveillance to generate robust epidemiological data and enhance awareness. With regard to wild hosts, this study focused on game ungulates, given their suitability for systematic sampling for the accessibility of their samples. However, the absence of *B. burgdorferi* s.l. detection and the limited positivity for SFG *Rickettsia* and *B. microti*-like organisms highlight the importance of including non-game species, such as small mammals, in surveillance programs, as they are recognized reservoirs or key hosts for several tick-borne pathogens (Salman & Tarrés-Call, 2013).

Wildlife plays a key role in the maintenance of certain TBZ, as highlighted by our findings on *Babesia* species, for instance. Wild ungulates, in particular, can contribute not only to the circulation of these pathogens (Vada et al., 2025) but also to the maintenance of tick populations (Ferroglia et al., 2024b). Their increasing densities, driven by factors such as land abandonment and rewilding, may elevate the risk of human exposure to TBZ. These dynamics should be carefully considered in the development of wildlife management.

5. Conclusions

The rising popularity of outdoor activities may facilitate the circulation of TBZ pathogens from wildlife to humans, with an expanded and more frequent use of shared spaces and an increase in the vector distribution and abundance, demanding appropriate surveillance. Although our results show that targeting questing ticks is often the most suitable method for TBZ surveillance, wildlife hosts can serve as a valuable complement for certain pathogens. For example, wild game species can be particularly informative for monitoring *Babesia* spp., while for others, such as SFG *Rickettsia* and *B. burgdorferi* s.l., additional sampling of diverse host species is recommended. While the detection of TBZ in less common host species can contribute to the existing literature (such as *B. microti*-like in wild boar or dogs, or SFG *Rickettsia* in wild boar), the role of these hosts is still unclear.

5.2. “The clock is ticking”: High Prevalence of Tick-Borne Zoonoses in Healthy Adults with Varying Tick-Bite Exposure in Northwestern Italy

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“The clock is ticking”: High Prevalence of Tick-Borne Zoonoses in Healthy Adults with Varying Tick-Bite Exposure in Northwestern Italy

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Abstract. Tick-borne zoonoses are among the main vector-borne and emerging diseases in Europe. In this work, we report the biomolecular prevalence (PCR) and seroprevalence of *Babesia divergens*, *B. microti*, *B. venatorum*, *Borrelia burgdorferi*, Spotted Fever Group (SFG) *Rickettsiae*, Anaplasmataceae and Tick-borne encephalitis virus in a cohort of healthy adults from North-Western Italy. Participants were differently exposed to tick bites through their engagement with natural environments. We included 144 participants, encompassing people from professional fields such as forestry management professionals (n=26) and research biologists (n=43) and people doing recreational activities in the natural environment, such as hunters (n=36) and group doing outdoor activity only occasionally (n=39). 65% of participants reported at least a tick bite per year. Time spent outdoor was remarked as an exposure factor and checking the body for ticks upon returning home as a protective measure. Regardless of the agent, biomolecular prevalence was 30%, while seroprevalence of 44%. In both, we detected the highest prevalence for *B. divergens* (11.5% in PCR, 22.2% in serology) and *B. microti* (10.1% in PCR, 13.9% in serology), followed by SFG *Rickettsiae* (9.4% in PCR, 13.2% in serology), *A. phagocytophilum* (7.2% in PCR, 12.5% in serology), *B. burgdorferi* s.l. (4.3% in serology), *B. venatorum* (5% by PCR), and no TBEv autochthonous infection. Significant intergroup differences were evident, particularly highlighting at biomolecular analysis a higher prevalence in professionally exposed participants. This data is relevant to raise awareness in the general public, to inform medical practitioners and for epidemiological and public health surveillance actions and disease control.

Keywords: acarological risk, risk factors, babesiosis, Lyme disease, Mediterranean spotted fever, human granulocytic anaplasmosis

1. Introduction

With the increasing abundance and expanding geographic range of their vectors, tick-borne zoonoses (TBZ) have become some of the most significant vector-borne diseases in Europe (Hartemink & Takken, 2016). TBZ encompasses a wide range of causative agents, from viruses to protozoa. Historically, research has primarily focused on Lyme borreliosis and tick-borne encephalitis (TBE) due to their high prevalence and public health impact (Rochlin & Toledo, 2020). Although the overall public health burden of TBZ remains largely unquantified, Lyme disease burden was estimated in tens of

millions of euros in several European countries (Mac et al., 2019). An analysis of hospital discharge records from 2000 to 2012 in Northwestern Italy identified a combined healthcare cost associated with tick-borne diseases of approximately €650,000 (Ferroglio et al., 2016). Simultaneously, the emergence and re-emergence of new tick-borne pathogens in several countries have been increasingly recognized (Madison-Antenucci et al., 2020; Rochlin & Toledo, 2020). Human babesiosis, for instance, has been now reported in almost all regions of Europe. In addition to the well-known pathogen *Babesia divergens*, which causes human babesiosis in Europe, increasing numbers of cases have been linked to other species, such as *B. venatorum* and *B. microti* (Bajer et al., 2022).

The increasing popularity of outdoor activities (Fernandez et al., 2021) coupled with the spread of ticks to higher elevations (Medlock et al., 2013) and their extended season of activity (Maurelli et al., 2018), is increasing the risk of tick bites for people who spend time in natural settings. This heightened risk affects not only those engaged in occupational activities but also individuals participating in leisure activities including hunting (Panczuk et al., 2019), and outdoor sports, from hiking (Blasko-Markic & Socan, 2012; Jore et al., 2020) to dog walking (Nygren et al., 2022). In general, the level of exposure may vary based on several behavioural factors, such as type and frequency of outdoor activity, choice of clothing, use of protective measures and type of habitat crossed (Velay et al., 2024).

For the six most common TBZ causative agents in Northern Italy and Europe, namely, *B. divergens*, *B. microti* (including *B. microti*-like), *B. venatorum*, *B. burgdorferi* s.l., SFG *Rickettsiae* and *Anaplasmataceae*, and for TBEv, we aimed to define the infection prevalence and/or seroprevalence in a population of healthy individuals with either occupational or recreative exposure to tick bite and different exposure intensity. To the best of our knowledge, few studies have addressed, combining biomolecular and serological approaches, the screening of healthy populations.

2. Materials and methods

Sample collection

This study employed a cross-sectional randomized opportunistic sampling approach, targeting adult individuals residing in the Piedmont region who frequently engage in natural environments. Participants were recruited voluntarily during summer-autumn 2023 through associations linked to the University of Turin. Recruitment was designed to ensure representation across four distinct groups with varying levels of exposure to tick bites in natural settings:

- Professionals working in natural environments, such as biologists and biology postgraduate students, as well as forestry management professionals (FMP), both recruited through their respective professional orders. Groups were kept separate due to different type of activity and different involvement throughout the year.
- People from non-environmental work sectors engaged in recreational outdoor activities off-road, such as hunters and people occasionally practising outdoor activities (OOA). Hunters were kept as a separate group due to their higher frequency of activity and their tendency to operate off-trail.

Power analysis, set to identify a medium effect between groups ($h=0.5$), with a significance level of 0.05 and a power of 0.8, identified an optimal group size of 40 individuals. The project received approval from the Ethics Committee of the University of Turin (Prot. n. 0346675, 26/06/2023), all patients signed a written informed consent, data were anonymized, and Good Clinical Practice recommendations were followed.

During the sampling procedures, blood was collected from each participant in two tubes: one EDTA tube (6 ml) for biomolecular detection of pathogens, and one tube without anticoagulant (6 ml) for serological analysis. At the same time, participants were requested to fill out a questionnaire designed to collect demographic information and details about habits that could lead to exposure to tick bites, as well as their related medical history (Supplementary Table 1).

Biomolecular analysis

EDTA tubes were stored at -20°C until further analysis. DNA extraction was performed from 200 μl of blood, with the PureLink Genomic DNA Mini Kit (Invitrogen, CA, USA), according to the manufacturer's instructions. The quality and quantity of extracted DNA were evaluated with Nanodrop One (ThermoFisher, MA, USA).

Pathogen DNA detection was conducted using End-Point or Real-Time PCR. All standards precautions were taken to minimize the risk of cross-contamination or laboratory environment contamination, and non-template (negative) controls as well as positive control samples were included in all analytical procedures. Positive controls were either DNA extracted from cultivated pathogen or positive samples previously confirmed by amplicon sequencing. Amplicons were analysed by agarose gel electrophoresis (2%) and visualized by staining with Gel Red Nucleic Acid Gel Stain (VWR International Milano, Italy).

For *Babesia* spp. detection, a semi-nested PCR targeting the V4 hypervariable region of the 18S rRNA using primers RLB-F2, RLB-R2 and RLB-FINT was performed (Schnittger et al., 2004). Samples testing positive for this genus-specific protocol were further analysed to differentiate between the three targeted *Babesia* species. All primers targeted the 18s region. For *B. divergens*, we used primers described by Hilpertshauser and colleagues (Hilpertshauser et al., 2006). For *B. microti*, we employed primers Bab1 and Bab4 (Persing et al., 1992). For *B. venatorum*, we conducted real-time PCR using primers Bab_EU_RNA18S_F and Bab_EU_RNA18S_R and the probe Bab_EU_RNA18S_P (Michelet et al., 2014). Anaplasmatidae were detected by end-point PCR by using primers PER1 and PER2 targeting the 16S rRNA (Battisti et al., 2020). For SFG *Rickettsia*, we targeted a fragment of the surface protein rOmpA with primers 190-70 and 90-701 as described by Roux and colleagues (Roux et al., 1996). *B. burgdorferi* s.l. was identified using a touch-down nested PCR with primer pair 23SN1, 23SC1, 23SN2 and 5SCB, targeting spacer region between 5S and 23S rRNA genes (Rijpkema et al., 1995). To identify pathogen species and confirm results, positive amplicons for Anaplasmatidae, *B. burgdorferi* s.l. and SFG *Rickettsia* were sequenced (MacroGen Europe, Amsterdam, The Netherlands) and compared with those available in GenBank®. Specific protocol details and adaptations from the literature are presented in Supplementary Table 2.

Given that north-western Italy is currently not considered endemic for the virus, we did not target TBEv for direct identification.

Serological analysis

Sera was extracted from the tube without anticoagulant and stored in individual vials at -20°C until analysis. Human IgG antibody detection was performed using commercially available kits that were already implemented in the literature (Supplementary Table 3). We used IFAT kits for detecting IgG against *B. divergens*, *B. microti* and *A. phagocytophilum*, and ELISA kits for TBEv, SFG *Rickettsiae* and *B. burgdorferi* s.l. Due to the absence of a commercially available assay targeting all SFG *Rickettsiae*, we used an ELISA assay specific for *R. conorii*, as the manufacturer reported a high degree of cross-reactivity with other other SFG species. Results were thus referred to as SFG *Rickettsiae*. For similar reasons, we selected an assay targeting the Anaplasmataceae species most relevant for zoonotic risk, namely *A. phagocytophilum*. However, cross-reactivity with *A. platys* was reported by the producer. According to the manufacturer, the TBE ELISA assay may exhibit cross-reactivity with other flaviviruses (which were excluded through viral seroneutralization (Ergunay et al., 2016)), while the *B. burgdorferi* assay may cross-react with *Leptospira* spp., *Treponema pallidum*, and rheumatoid factor. Although no cross-reactivity has been reported for the *B. microti* assay, the *B. divergens* IFAT may cross-react with *Plasmodium* spp. and other *Babesia* species. To the best of our knowledge, for *B. venatorum* there was no commercial kit available at the time of the study (also reported by Wilhelmsson and colleagues (2020)). All samples were tested following the instructions provided by the respective manufacturers. Due to variability in our kits, we incorporated only the qualitative (positive-negative) outcomes into our analysis. For IFAT, titres of $\geq 1:64$ were classified as positive. TBE ELISA kit did not allow to differentiate vaccinated individuals.

Statistical analysis

We implemented a random forest model to evaluate the importance of the survey variables influencing the yearly frequency of tick bites detected by participants. Using the ranger package in R studio (R Core Team, 2023; Wright & Ziegler, 2015), we constructed a forest of 1,000 trees, focusing on understanding the relative contributions of each predictor to the model outcomes rather than on predictive accuracy. A 10-fold cross-validation was applied to ensure the robustness of our results while tuning the entry parameter to explore different feature combinations at each split. Variable importance metrics were computed to identify which factors had the most significant impact on the reported tick-biting frequency. To define the direction of the relationship between the factors and the response variable, we implemented a univariate analysis with General Linear Mixed Models (GLMM) of the Poisson family, with the individual identification as a random variable, through package lme4 in R Studio (Bates et al., 2015). The frequency of tick-bite per year was categorized into 5 levels: 0 if never, 1 if once or less, 2 if from 1 to 5, 3 if 5 to 10 and 4 if more than 10

As a further step, we investigated the effect of the yearly frequency of tick-bite and the group on the general positivity to any pathogen through biomolecular and serological analysis. In this case, we implemented a GLMM of binomial family, with the same

package as before. The difference in prevalence and seroprevalence among groups for the targeted pathogens was detected through a pairwise Chi-squared test in R studio, with statistical significance considered at $p < 0.05$.

3. Results

Sampled population

A total of 144 participants were sampled, including 36 hunters, 26 FMP, 43 biologists, and 39 OOA, making it 48% occupationally exposed and 52% exposed through recreational activities. The male-to-female ratio was 95:49. The most represented age group was between 20 and 30 years (40%). Residential exposure was reported by 54% of participants (Table 1). In addition, 91% of participants engaged in at least one outdoor activity associated with increased tick exposure and 73% reported spending outdoors less than 10 hours/day, with the most common frequency of outdoor activity being once per week or less (49%). Hunting and fishing were predominantly practised by older males.

Table 1. Distribution of participants' housing and recreational exposure through the four professional cohorts.

	Livestock farm	Proximity to the woods	Garden with wildlife access	Hunting	Fishing	Camping/hiking/cycling	Mushroom picking
OOA	1	4	7	0	0	28	8
F	0	2	4	0	0	18	5
20-30	0	1	4	0	0	13	2
31-40	0	0	0	0	0	4	2
41-50	0	0	0	0	0	1	0
51-60	0	1	0	0	0	0	1
M	1	2	3	0	0	10	3
20-30	0	1	1	0	0	5	2
31-40	0	0	1	0	0	2	0
41-50	0	0	0	0	0	2	0
51-60	1	1	1	0	0	1	1
Biologists	2	16	21	3	8	37	22
F	1	6	7	0	1	13	7
20-30	0	4	6	0	1	10	5
31-40	0	1	1	0	0	2	1
51-60	0	0	0	0	0	1	0
>60	1	1	0	0	0	0	1
M	1	10	14	3	7	24	15
20-30	1	7	9	2	6	17	10
31-40	0	1	4	0	0	4	2
41-50	0	1	1	0	0	2	2
51-60	0	1	0	1	1	1	1
>60	0	0	0	0	0	0	0

FMP	4	9	11	2	2	22	17
F	2	2	1	0	0	5	3
31-40	0	1	1	0	0	2	2
41-50	1	0	0	0	0	2	1
>60	1	1	0	0	0	1	0
M	2	7	10	2	2	17	14
20-30	0	0	0	0	0	1	1
31-40	1	1	3	1	1	3	4
41-50	1	4	4	1	1	6	7
51-60	0	2	2	0	0	3	2
>60	0	0	1	0	0	4	0
Hunters	5	17	21	36	15	20	27
F	1	2	2	2	0	2	2
<20	1	1	1	1	0	1	1
41-50	0	1	1	1	0	1	1
M	4	15	19	34	15	18	25
<20	0	1	1	1	1	1	1
20-30	0	2	1	2	2	2	2
31-40	1	3	1	4	0	2	3
41-50	1	2	3	6	2	4	3
51-60	0	2	5	7	2	4	6
>60	1	4	6	11	6	4	8

65% of participants reported experiencing at least one tick bite in the last twelve months (Table 2). Biologists are the category registering more tick bites (77%), while the OOA registered the lower (23%) and no more than 5 bites per year.

Table 2. Number of bites per group.

N. of bites	Biologist	FMP	Hunters	OOA
No tick bite	8	3	8	30
1 or less	13	7	9	5
from 1 to 5	10	13	12	4
from 5 to 10	5	0	1	0
More tan 10	5	3	3	0
Total bites	33	23	25	9
Total	43	26	36	39

Most participants (82%) reported being bitten by ticks in the woods, and 36% in meadowed areas. Lower frequencies were reported for cultivated fields (n=5) and urban or peri-urban parks (n=5). Among the different methods used to prevent tick bites and the transmission of tick-borne pathogens, the practice of conducting thorough tick checks upon returning home was the most commonly adopted strategy (Figure 1). Participants who experienced longer exposure to tick bites (measured by the hours per day and days per week spent engaging in outdoor activities) were more likely to consistently wear long clothing (Spearman correlation $\rho = 0.18$, $p = 0.03$) and check for ticks once they returned home (Spearman correlation $\rho = 0.34$, $p < 0.001$).

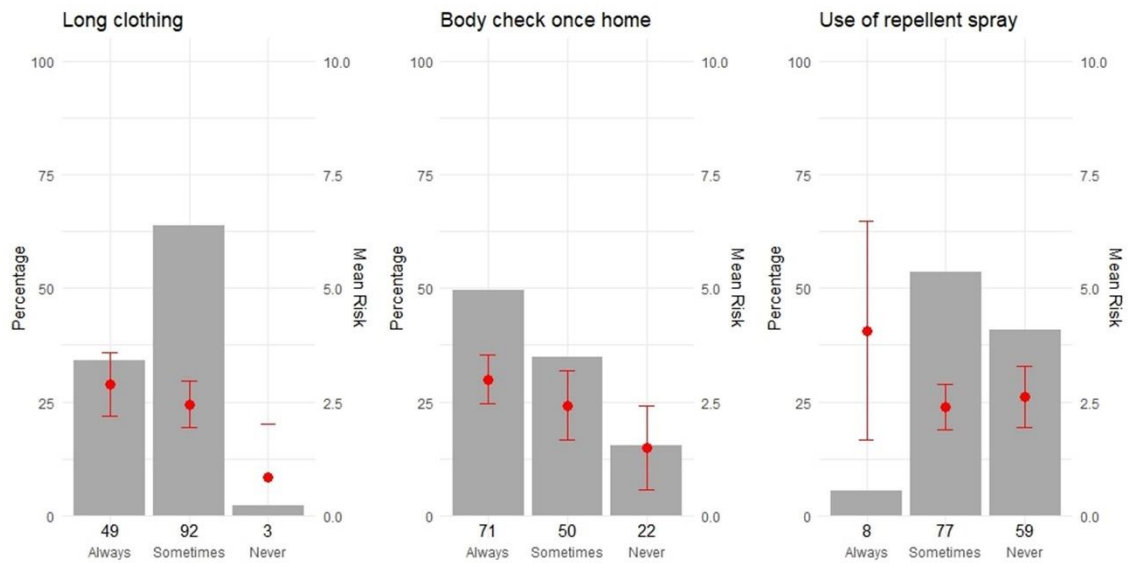


Figure 1. Frequency distribution (in percentage, illustrated by grey bars and referring to left y axis) of the use for the three protective behaviours investigated in the survey. For comparison, we introduced the mean risk for each category of frequency (red whiskered dots, referring to right axis).

After a tick bite, only 26 participants reported symptoms: 4 people reported severe symptoms (joint pain, fever, loss of appetite, headache) and 22 reported mild symptoms (redness, swelling, itching, erythema at the biting site). Five participants, all reporting symptoms, declared having received antibiotic treatment. 3/4 participants reporting severe symptomatology did receive antibiotic treatment as prescribed by their physicians. No statistically significant difference in terms of positivity to serology or PCR was detected among those who experienced symptoms and those who did not.

Univariate analysis showed that all variables had a positive impact on the annual frequency of tick bites, with higher importance for the habit of checking the body for ticks once home and the frequency of the activity per week, both with a statistically significant probability (Figure 2). Great importance is also shown by other preventive measures such as the use of repellent sprays and protective clothing, which however did not show a statistically significant coefficient. Occupational exposure also showed good importance and a significant positive coefficient.

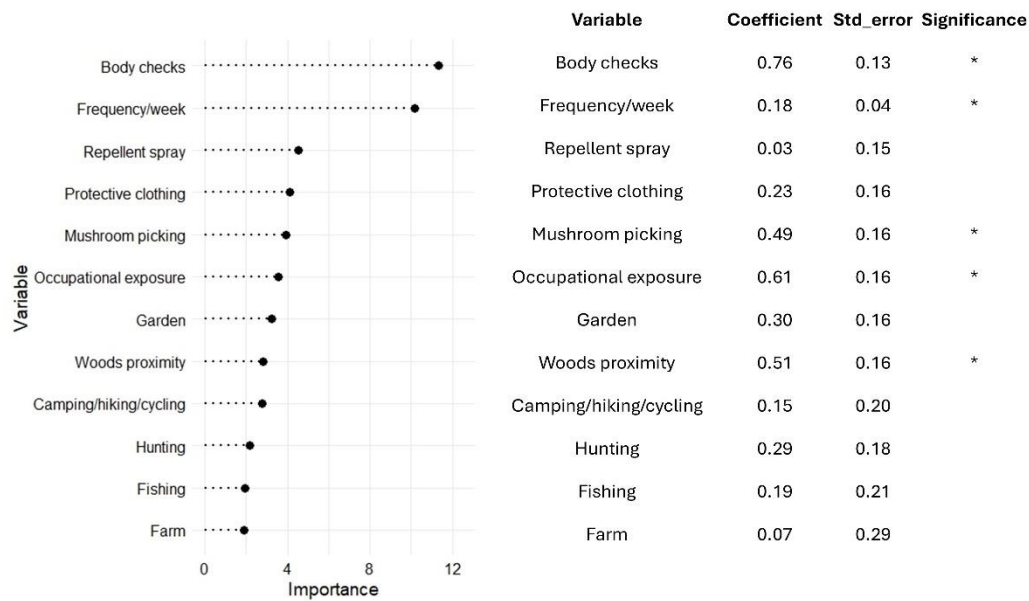


Figure 2. Importance value from the random forest for each variable (a) and coefficients of the univariate analysis (b), with standard error and significance.

Biomolecular analysis

Direct PCR detection of tick-borne pathogens revealed the highest prevalence rates for Piropasmids, listed in order of occurrence as *B. divergens* (11.5%, with 95% confidential intervals 7.2-17.9%), *B. microti* (10.1%, 95% C.I. 6.1-16.2%) and *B. venatorum* (5%, 95% C.I. 2.5-10%). This was followed by SFG *Rickettsiae* (9.4%, 95% C.I. 5.5-15.3%) and *A. phagocytophilum* (7.2%, 95% C.I. 4.0-12.7%). Sequencing did not confirm positive amplicons of *B. burgdorferi* s.l., thus eventually no positive individual was identified. Amplicons of Anaplasmatidae were identified as *A. phagocytophilum* (GenBank: KP245908.1; query cover ranging from 93 to 100% and a percentage of identity from 95.49 to 100%; accession number of the sequences obtained from the current study: from PQ657427 to PQ657433), amplicons of SFG *Rickettsia* as *R. monacensis* (GenBank: LN794217.1; query cover of 100% and a percentage of identity from 97% to 100%; accession numbers of the sequences obtained from the current study: from PQ684452 to PQ684460).

As shown in Figure 3, statistical difference was observed indicating that biologists were more frequently infected with *B. divergens* compared to other groups (biologists-hunters, Chi = 4.4, p = 0.04; biologists-FMP, Chi = 3.9, p = 0.047; biologists-OOA Chi = 3.9, p = 0.047), and FMP showed a higher prevalence of SFG *Rickettsiae* than other groups (FMP-Hunters, Chi = 13.8, p = 0.0003; FMP-biologists, Chi = 16.4, p < 0.001; FMP-OOA, Chi = 6, p = 0.02). Additionally, we detected a difference in *B. microti* prevalence between biologists and FMP (the pathogen was not detected in the latter, Chi = 4.4, p = 0.03). When considering together all individuals positive by PCR to any of the targeted pathogens, biologists and FMP had a significantly higher prevalence than hunters and OOA (FMP-hunters, Chi = 7.9, p = 0.005; biologists-hunters, Chi = 10.2, p = 0.001; FMP-OOA, Chi = 6.2, p = 0.013; biologists-OOA, Chi = 8.3, p = 0.04).

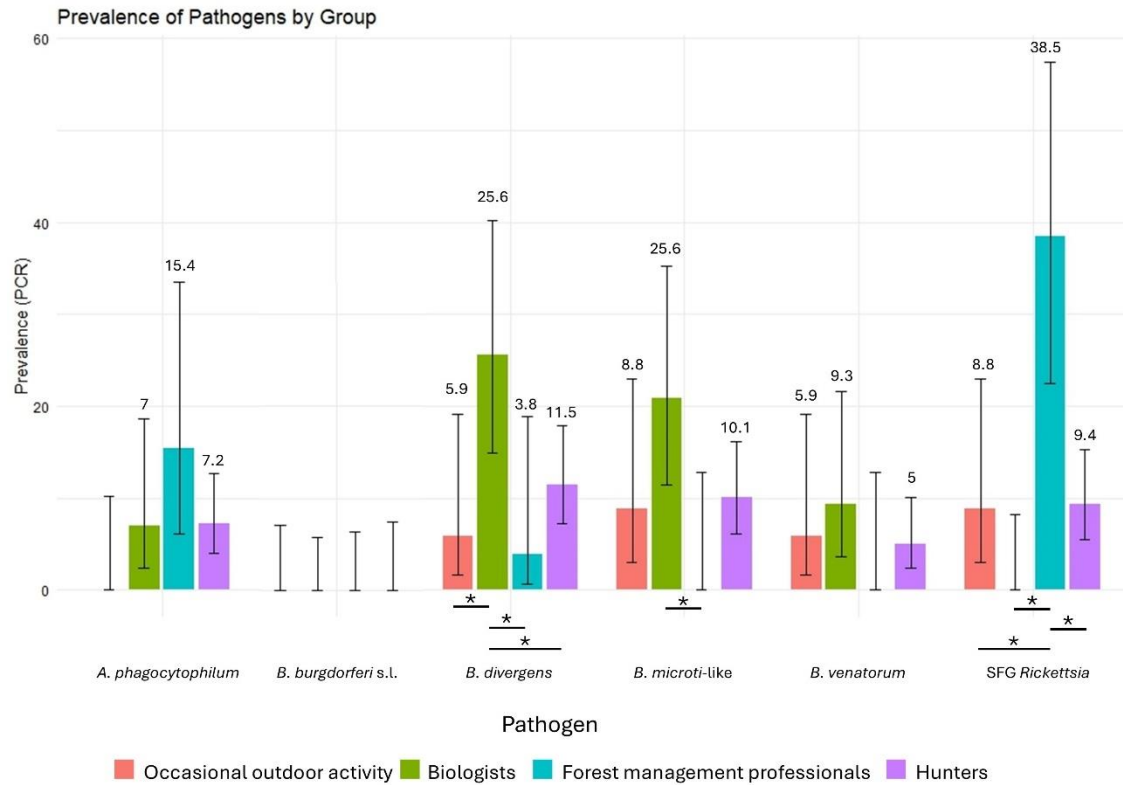


Figure 3. Prevalence of the targeted pathogens across groups (abbreviated, as Biologists, Forestry management professionals, Lower exposure group, Hunters), with confidence intervals. When a pathogen was not detected in the group, the upper limit of the confidence interval is plotted. Below the bars, black lines with asterisks indicate a significant ($p < 0.05$) Chi-squared result between the two groups at the lines' edges.

Coinfections were detected in 29.2% of positive cases ($n=14$), defined as the concurrent detection of circulating DNA of two or more pathogens. *Rickettsia* was detected together with *A. phagocytophylum* ($n=2$), while *B. divergens* was detected either with *B. microti* ($n=5$) and *B. venatorum* ($n=2$). In three individuals all three targeted *Babesia* species were detected simultaneously. Coinfection between *B. microti* and *B. venatorum* was detected in 2 individuals.

Univariate and multivariate regression coefficients for PCR results (considering all positivities) returned a significant positive association with the annual frequency of tick bite (coeff. 0.32, std. error 0.15, p -value = 0.034). Regarding the groups, the analysis detected a statistically significant positive correlation between PCR positivity and biologists (coeff. 1.4, st. err 0.5, $p = 0.005$) and FMP (coeff. 1.36, st. error 0.56, $p = 0.015$), and a non-significant negative correlation with hunters (coeff. -0.26, st. error 0.6, $p = 0.669$), using as reference the OOA.

Serological analysis

As for PCR, the highest seroprevalence detected (Figure 4) was that against Piroplasmids, with 22.2% (95% C.I. 16.2-29.7%) samples positives to IgG antibodies against *B. divergens* and 13.9% (95% C.I. 9.2-20.5%) against *B. microti*. Decreasing seroprevalences were detected for SFG *Rickettsiae* (13.2%, 95% C.I. 7.7-21.6%), *A.*

phagocytophilum (12.5%, 95% C.I. 8.1-18.9%), *B. burgdorferi* s.l. (4.3%, 95% C.I. 2-9.1%) and TBEv (2.8%, 95% C.I. 1.1-7, all positive samples vaccinated against TBEv, therefore not considered as true positives). Approximately one third (9/30) of the seropositive to *B. divergens* were also positive to *B. microti*. We detected a statistically significant difference between hunters and biologists for *B. divergens* (Chi = 6.6, $p = 0.01$) and *B. microti* (Chi = 10, $p = 0.002$), having hunters a higher exposure rate than biologists. For *B. divergens*, hunters have also a significantly higher seroprevalence than OOA (Chi = 5.4, $p = 0.02$). Considering all pathogens, we detected a significantly higher prevalence for hunters compared to biologists (Chi = 5.4, $p = 0.02$) and OOA (Chi = 8.2, $p = 0.004$).

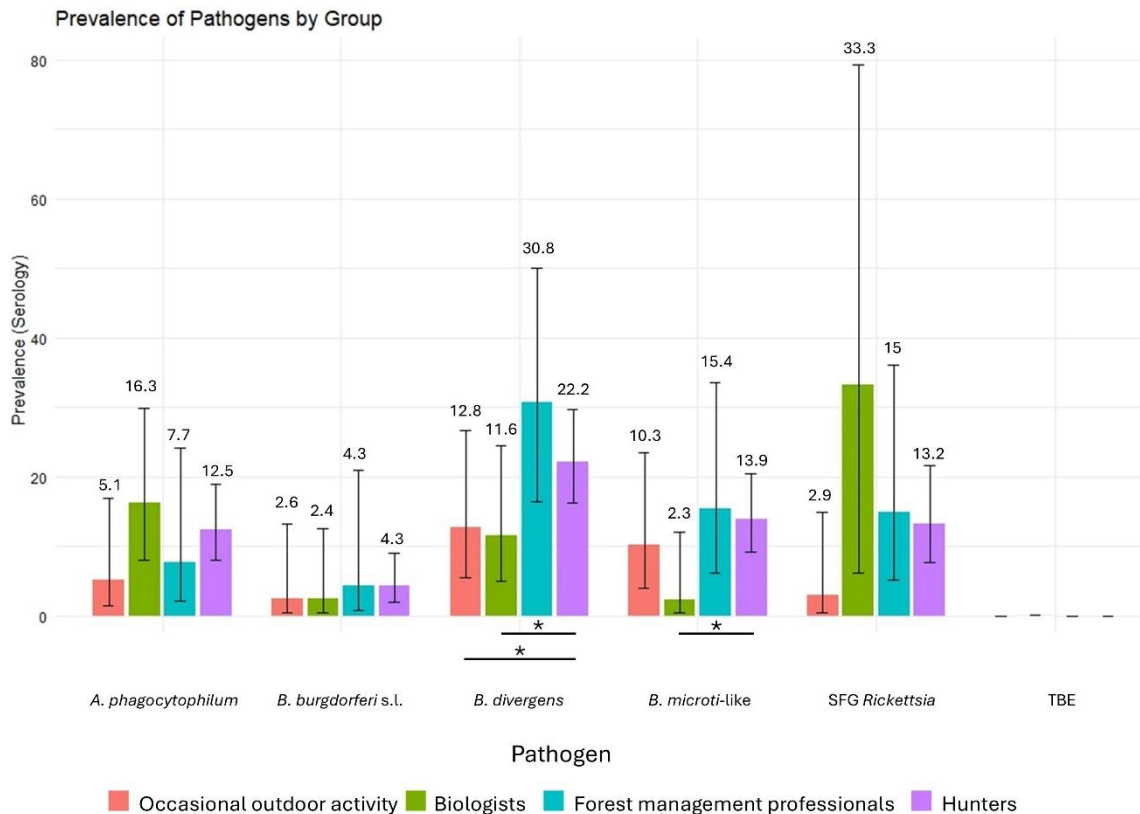


Figure 4. Seroprevalence of the targeted pathogens across groups (abbreviated, as Biologists, Forestry management professionals, Lower exposure group, Hunters), with confidence intervals. Below the bars, black lines with asterisks indicate a significant ($p < 0.05$) Chi-squared result between the two groups at the lines' edges.

Univariate analysis showed a non-significant positive correlation of positivity to any of the serological tests performed with the yearly frequency of tick bite (coeff. 0.17, st. error 0.15, $p = 0.24$). Additionally, based on reference levels of the OOA, we detected a positive but not significant correlation for biologists (coeff. 0.31, st. error 0.48, $p = 0.52$), and a positive significant correlation for FMP (coeff. 1.09, st. error 0.53, $p = 0.04$) and hunters (coeff. 1.51, st. error 0.5, $p = 0.003$).

Discussion

Our study provides an epidemiological assessment of TBZ exposure risk in a healthy population of adults across different types of outdoor activity (either professional or recreational), highlighting the potential risk for tick bites and the transmission of TBZ.

Nearly 90% of all study participants reported engaging in recreational outdoor activities, with varying degrees of exposure to tick bites. Indeed, especially from the COVID-19 pandemic on, outdoor activities have gained popularity, resulting in major exposure to TBZ (Fernandez et al., 2021; Nygren et al., 2022). In our study, weekly frequency of activity in the natural environment is the factor with the highest importance in positively affecting the risk of tick bite. This concept was already presented by a study in Germany (Nygren et al., 2022). Our analysis indicates that hunting does not have a relevant impact on tick bites, nor do hunters exhibit statistically higher prevalences of pathogens (if not, the contrary). This could be attributed to the period of the year in which hunting is practised in the study area (October to February), which coincides with reduced tick activity. However, when considering long-term exposure, as reflected by serological prevalence, hunters demonstrate a higher risk compared to other groups. Tick exposure risk was assessed in hunters in southern Italy, showing a mean of 1.6 ticks per person in the study period (Sgroi et al., 2022b). High *B. burgdorferi* exposure was also shown in Polish hunters, with a prevalence of IgG of 63.3% (Panczuk et al., 2019).

As reported by participants, ticks are mostly encountered in a natural environment (woods or meadows), rather than more anthropized settings such as cultivated fields and urban/peri-urban parks, similar to what was highlighted by Jore and colleagues in Scandinavia (Jore et al., 2020) or Mathews-Martin and colleagues in France (Mathews-Martin et al., 2020). However, with changes at the human-wildlife interface driving wild animals closer or even into urban settlements, ticks are entering urbanized areas and parks with increasing abundance (Medlock et al., 2013).

Although the use of repellent sprays remains relatively uncommon, behaviours such as conducting body checks upon returning home to promptly remove ticks, and wearing long protective clothing, are more widely adopted; this result is similar to that obtained by Blasko-Markic and Socan within a group of TBE patients (2012). These preventive practices are particularly prevalent among individuals who spend more time outdoors, suggesting an increased awareness and sensitivity to the risks associated with tick exposure. By more consistently checking bodies for ticks once home, more exposed people tend also to report a higher tick-finding rate throughout the year. In a study in Poland, researchers highlighted that socioeconomic factors influence the practising of tick-borne diseases prophylactic behaviours (Bartosik et al., 2008).

Serological and PCR-based prevalence data have a similar pattern, with the highest rates observed for *B. divergens* and *B. microti* (although one-third of the *B. divergens* seropositivities may be caused by cross-reaction with *B. microti*), followed by SFG *Rickettsiae* and *A. phagocytophilum*, and finally *B. burgdorferi* s.l. and *B. venatorum*. This trend is consistent with studies in other groups of hosts such as dogs (Zanet et al., 2020) or wild species (Melis et al., 2024; Zanet et al., 2014); yet differences related to species-specific vectors (i.e. statistically higher occurrence of *A. phagocytophilum* in ticks taken from ungulates) need to be considered in such comparison. Similar trends have been

detected also in environmental ticks (Cafiso et al., 2021b; Pistone et al., 2017). Moreover, we highlight how occupational exposure is a significant factor determining the risk of tick bite: the two groups working in the natural environment (biologists and FMP) have significantly higher pathogen prevalence at biomolecular analysis.

Clinical cases of human babesiosis and anaplasmosis are rarely reported in Italy (Beltrame et al., 2006; Herwaldt et al., 2003). A comprehensive review of cases of human babesiosis in Europe documented infections caused by each of the three *Babesia* species targeted in our study, including asymptomatic cases of *B. microti* and *B. venatorum* (Hildebrandt et al., 2021). A serosurvey conducted in Northern and Central Italy to investigate the presence in humans of antibodies has detected a high reactivity to *B. microti* and *B. divergens* antigens in foresters and hunters, reaching respectively 32% and 12% (Gabrielli et al., 2014). The underlying causes of the pathogen-specific discrepancies should be further investigated by examining the specific locations where their research activities are conducted.

According to the Italian Ministry of Health, in 2015 it was recorded an incidence of Rickettsiosis in 0.34/100,000 inhabitants (<https://www.epicentro.iss.it/zecche/FebbreBottonosa2001-2015>, accessed on 05/10/2024). A literature review of studies published up to 2021 detected 23 cases of rickettsiosis and 6 cases of anaplasmosis in humans, encompassing a broad variety of causative species (Guccione et al., 2021). The prevalence of *Rickettsia* spp. in ticks attached to humans was detected as 15% in another study from 2017 to 2019, and the one of *Anaplasma* spp. at 1.2% (Audino et al., 2021). Less recent studies detected *A. phagocytophilum* in Italy with prevalence values of 6.3% (Matei et al., 2019) and 8.8% (Cinco et al., 2006) in an at-risk group. Incidence of SFG *Rickettsiae* has been declining in almost all Mediterranean countries, with hypothesized reasons encompassing improved health conditions and domestic (livestock and pets) animal handling (Gomez-Barroso et al., 2019). Vector specificity and outdoor activity together with increasing temperatures (Gomez-Barroso et al., 2019), may be the cause of this relatively high prevalence in areas with continental climate. Being all TBE-positive individuals vaccinated against the virus, our data remark that TBE possibly has not yet expanded to the northwest of Italy, as the absence of reported human cases suggests (Calcagno, personal communication). A single serological study carried out in 2007 in the same region reported a seroprevalence for TBEV of 5.7% (Pugliese et al., 2007), even if cross-reactivity for other flaviviruses nor vaccination were evaluated.

Seroprevalence of *Borrelia* spp. was detected in around 7% of exposed workers in Italy (Santino et al., 2004; Tomao et al., 2005) and Spain (Lledo et al., 2019). Higher seroprevalences (up to 23%) were detected in other studies in Italy (Cinco et al., 2004; Stroffolini et al., 2021; Tabibi et al., 2013). The incidence seems also to be increasing: a positive trend in number of cases across the years of observation was recorded in a study across Italian provinces (Trevisan et al., 2023) and a mean annual incidence of 0.992/100,000 inhabitants was recorded in Verona Province, Northern Italy (Beltrame et al., 2021). Chances of detecting *B. burgdorferi* s.l. DNA in peripheral blood are quite scarce and different targets should be evaluated for screening procedures.

It is important to note that all participants were asymptomatic at the time of sampling. While some symptoms were reported as having appeared after the tick bite, the majority did not progress into a severe form of disease. Antibiotic treatment was infrequently administered, but with more consistency among those presenting severe symptomatology. This is not surprising as, for instance, pathogens such as *Babesia* spp. may in many cases cause no symptoms of disease (CDC, <https://www.cdc.gov/babesiosis/about/index.html>, accessed on 04/10/2024).

4. Conclusions

With the increasing abundance of ticks, the lengthening of their active season, and subsequent geographical expansion to higher altitudes and greater latitudes, exposure to tick bites and tick-borne zoonoses (TBZ) is rising, impacting not only those engaged in professional outdoor activities—who face the highest risk—but also individuals involved in outdoors leisure activities. As in many cases, microorganisms may not cause symptoms, but a comprehensive surveillance of their prevalence and circulation is essential for accurately assessing the risks posed by natural environments. Additionally, this information is fundamental in guiding the therapeutical approach towards these diseases. For instance, guidelines from the Infectious Diseases Society of America (IDSA) on human babesiosis suggest considering the possibility of coinfection with *B. burgdorferi* or *A. phagocytophilum*, or both, in patients with especially severe or persistent symptoms, despite administration of appropriate anti-babesial therapy (Wormser et al., 2006). Moreover, asymptomatic patients who test positive for babesiosis via smears and/or PCR should have these tests repeated and, if parasitemia persists for 13 months, a treatment regimen should be considered (Wormser et al., 2006). Within an ageing population and with higher prevalence of immune-suppressive conditions, babesiosis and anaplasmosis can present more often with severe symptoms. Continued efforts in preventive strategies, along with public awareness campaigns, are critical, especially as outdoor activities become increasingly popular. Our data may prompt several interventions:

1. Educational activities on the general population on the risks of TBZ and how to avoid them;
2. Educational activities on healthcare workers on the diseases associated with TBZ;
3. Collaborative surveillance and research studies involving veterinarians, physicians and public health professionals;
4. PCR screening of blood donors engaged in outdoor activities for leisure or work;
5. Molecular surveillance in patients with fever of unknown origin through next-generation sequencing for identifying the clinical burden of TBZ and the circulation of unexpected pathogens.

While some of these activities are already ongoing, this may be a call to take action in this field.

6. Discussion

6.1. Key messages

This thesis presents an overview of the possible application of integrated wildlife health surveillance principles applied to ticks and tick-borne pathogens. Camera traps were used to estimate the denominator data for wild ungulates and mesocarnivores and to model the presence of questing ticks and the prevalence of tick-borne pathogens. In a second step, it addressed the circulation of tick-borne zoonotic pathogens in the human dimension of the wildlife-livestock-human interface.

A few examples of the utility of camera traps have been produced worldwide and in Europe (Domínguez et al., 2019; Esser et al., 2019; Hofmeester et al., 2017b; Iijima et al., 2022; Noden et al., 2024). Apart from shedding light on the tick-host relationship, and sometimes also on tick-borne zoonoses-host dynamics, these studies, as well as our results, demonstrated the potentiality of data obtained with camera traps to model tick-host interactions and the impact of wildlife on ticks and, consequently, their effect on tick bite risk and tick-borne pathogens' transmission to people. It is important to remind, however, that other animals such as small mammals are important hosts which maintain ticks in the environment by feeding immature stages, and reservoirs of tick-borne pathogens, such as *B. burgdorferi* s.l. or SFG *Rickettsiae* (Brunner et al., 2008; Durden, 2006; Galfsky et al., 2019; Gern et al., 1998; Salman & Tarrés-Call, 2013). However, the relevance of wild ungulates and carnivores in maintaining adult stages of ticks and, in some cases, acting as pathogen reservoirs, together with the relative practicality of their monitoring through non-invasive and cost-effective techniques, makes this approach of particular use as a basis also for risk control strategies.

The good performance of camera trap data in our models highlights the importance of such detailed information. For sound predictions and localized risk assessment, high-resolution, punctual data is of course the best option. However, predictions and assessments at a broader scale require more extensive data. Gathering high-quality information may not be easy, especially in some more neglected areas or where resources are lacking. Statistical strategies can be implemented in these cases. For example, geostatistical modelling is particularly adequate in low-resources areas where the available data are limited to observations from a finite number of sampled locations (Diggle & Giorgi, 2019). About wildlife population data collection, an example was provided by the density maps produced by the ENETWILD Consortium, modelled on hunting yield statistics, which can be easily collected across broad areas, and validated through a network of study areas in which more precise camera trap techniques as the REM were implemented to obtain density values (ENETWILD-consortium et al., 2024a).

Tick-borne zoonoses are emerging pathogens and, as such, increase their prevalence and cause infections in animals and humans in areas from where they were previously believed absent (Hartemink & Takken, 2016). Sound epidemiological surveillance in the healthy population is still lacking, as most studies in humans do involve clinical cases or

ticks removed from people and not the subjects directly. However, in our studies we highlighted a relevant prevalence and seroprevalence of most of the target pathogens, which constitute a particular risk for immunosuppressed subjects. Since those pathogens circulate among wild species and environmental ticks, a clear epidemiological overview and, especially, a sound risk evaluation would involve several host species and surveillance compartments. Indeed, the usefulness of an integrated surveillance system for tick-borne diseases has been broadly remarked (Clow et al., 2019; Garcia-Vozmediano et al., 2022; Hai et al., 2014; Johnson et al., 2022), which would be beneficial for the establishment of evidence-based strategies (for either further surveillance and control), diagnosis, international coordination and public engagement.

Wildlife management and consequent wildlife densities can affect not only ticks' presence and abundance, but also host-pathogen interactions. Furthermore, developmental stages are sensitive to the densities of different hosts: for instance, questing larvae to rodents or questing nymphs to deer species (Pugliese & Rosà, 2008). Likewise, increasing deer densities seem to induce ticks to feed less on rodents and more on deer (Pugliese & Rosà, 2008). Area size may also play a role, as large fenced patches seem to result in lower densities of ticks in all stages, while small fenced patches can result in relevant increases in tick densities, depending on the tick species (Pugliese & Rosà, 2008). On the other hand, habitat factors such as altitude may be the limiting element to ticks presence and abundance despite the host presence, influencing, for instance hatching and moulting success (Medlock et al., 2013).

The circulation of tick-borne pathogens in relevant prevalence among humans poses the question of risk perception and consequent adoption of preventive measures. Risk perception of tick bites varies depending on the studies. For instance, in Scandinavia, the risk was perceived as even higher than the one reported in the literature (Slunge et al., 2019), while in another study on Staten Island, New York, participants believed having a low risk of a tick bite (Hassett et al., 2022). However, in the latter it was also highlighted that people do not cross high-risk areas for ticks (closed habitats, dense vegetation) and if they do, just for a short amount of time (Hassett et al., 2022). The seriousness of the issue was perceived less after tick bites of disease diagnosis (Lyme disease or TBE mostly), probably because of communication strategies adopted by media, which show only serious cases of the diseases (Hassett et al., 2022; Slunge et al., 2019; Slunge & Boman, 2018). Regarding preventive measures, higher exposure to tick bites seems to be associated with protective measures, especially the one of checking the body once home, while other strategies are less intensively implemented (Slunge et al., 2019; Slunge & Boman, 2018). In general, people seem to be able to identify ticks (as differentiating them from other acari) and there is good knowledge about some protective measures, which are more likely to be implemented if believed effective (Slunge & Boman, 2018). However, knowledge gaps and low-risk perception are still relevant issues and there is a need for more intervention in this direction (Hassett et al., 2022; Slunge et al., 2019). Particularly, differences in knowledge may be affected by gender, age and education/income level, information which could be useful for designing targeted

strategies (Slunge et al., 2019; Slunge & Boman, 2018). In general, since environmental intervention may be limited in wide areas such as natural parks, encouraging visitors to adopt preventive strategies and correct behaviour to limit the risk of a tick bite is an important pathway to go through, and it should also pass through a more capillary education and dissemination (Hassett et al., 2022)

Effective strategies to control tick-borne pathogens circulation require an integrated approach, especially when targeting broader areas than house yards, such as parks or natural environments. As already mentioned, addressing the general public with correct education about risks and available preventive measures is of fundamental importance (Černý et al., 2020; Hassett et al., 2022). These involve the use of proper clothing, repellent, prompt check and removal and, when available, vaccination. Involving healthy volunteers in screening procedures and studies is a strategy to facilitate knowledge dissemination and education. Complementary approaches would involve tick-host and habitat management. Reducing the densities of main tick hosts may be impactful in this direction, especially when targeting species which are already under a management plan such as deer (Černý et al., 2020; Kugeler et al., 2016). The relationship between tick abundance and wildlife presence highlighted in the present work could be useful to inform decisions in this direction. Acaricide application could, through various tools, address both larger-sized mammals such as deer and small mammals such as rodents (Černý et al., 2020), but it is also of fundamental importance to involve domestic animals. In addition, carefully planned landscape management could help create areas at lower risk, for instance by creating dry open areas, maintaining short-length grass and removing leaf litter, or separating areas at higher risk from the ones where people spend more time, such as with the implementation of barriers (Černý et al., 2020). In areas where human management is less intense, alternative strategies should be pursued, such as implementing biological agents, avoiding forest fragmentation and correctly informing visitors of their risk (Černý et al., 2020).

6.2. Further perspectives

Building upon the findings of the present work, several pathways have been identified to continue studying the circulation of vector-borne pathogens at the human-wildlife-domestic animal interface.

Small mammals, such as rodents or insectivores, are important hosts for immature stages of ticks and therefore for maintaining the environmental population of this vector. Moreover, they are reservoirs for some of the main tick-borne zoonoses. As already mentioned, adapting camera traps to monitoring small mammals requires a technical effort which would likely preclude monitoring larger body size mammals with the same tool (Glen et al., 2013; Ortmann & Johnson, 2021). Studies implementing camera traps to model the effect of wildlife presence on questing ticks' abundance usually sample small mammals with alternative strategies, such as live trapping (Takumi et al., 2019; Taylor et al., 2023). Including small mammals in the targeted host range would mean assessing their abundance and impact on ticks' presence, as well as sampling for ectoparasites and

also detecting the presence of tick-borne zoonoses in animal tissues. Such comprehensive sampling may also encompass reptile species such as lizards and wild birds, that host other immature tick stages. In addition to camera trap surveys and more traditional approaches such as live or footprint trapping, environmental DNA sampling can be evaluated (ENETWILD-consortium et al., 2023c).

To qualify as a vector, the tick must feed on infectious vertebrates and be able to successfully transmit those pathogens after moulting, when feeding again (Estrada-Peña et al., 2013). Such considerations have been presented in the current study, as almost all pathogens have been detected in all tested tick species. Similar considerations may be applied to vertebrate species, which can be distinguished between carrier hosts (not necessarily infective to ticks) or reservoir hosts (Estrada-Peña et al., 2013). Such is, for instance, the example of wild boar, which has been found positive for some tick-borne pathogens (e.g. *B. vulpes* or *B. capreoli*), but as an anecdotal event and is not considered capable for their transmission (Sgroi et al., 2023). Likewise, the fact that red deer is found positive for *B. capreoli* only in areas where roe deer is present, even at high prevalence, remarks the fact of this species potentially not being a reservoir for the pathogen (Zintl et al., 2011). Further studies are necessary to determine the competence as a host of vertebrate species and as vectors of tick species. These would require in vitro but also in vivo testing.

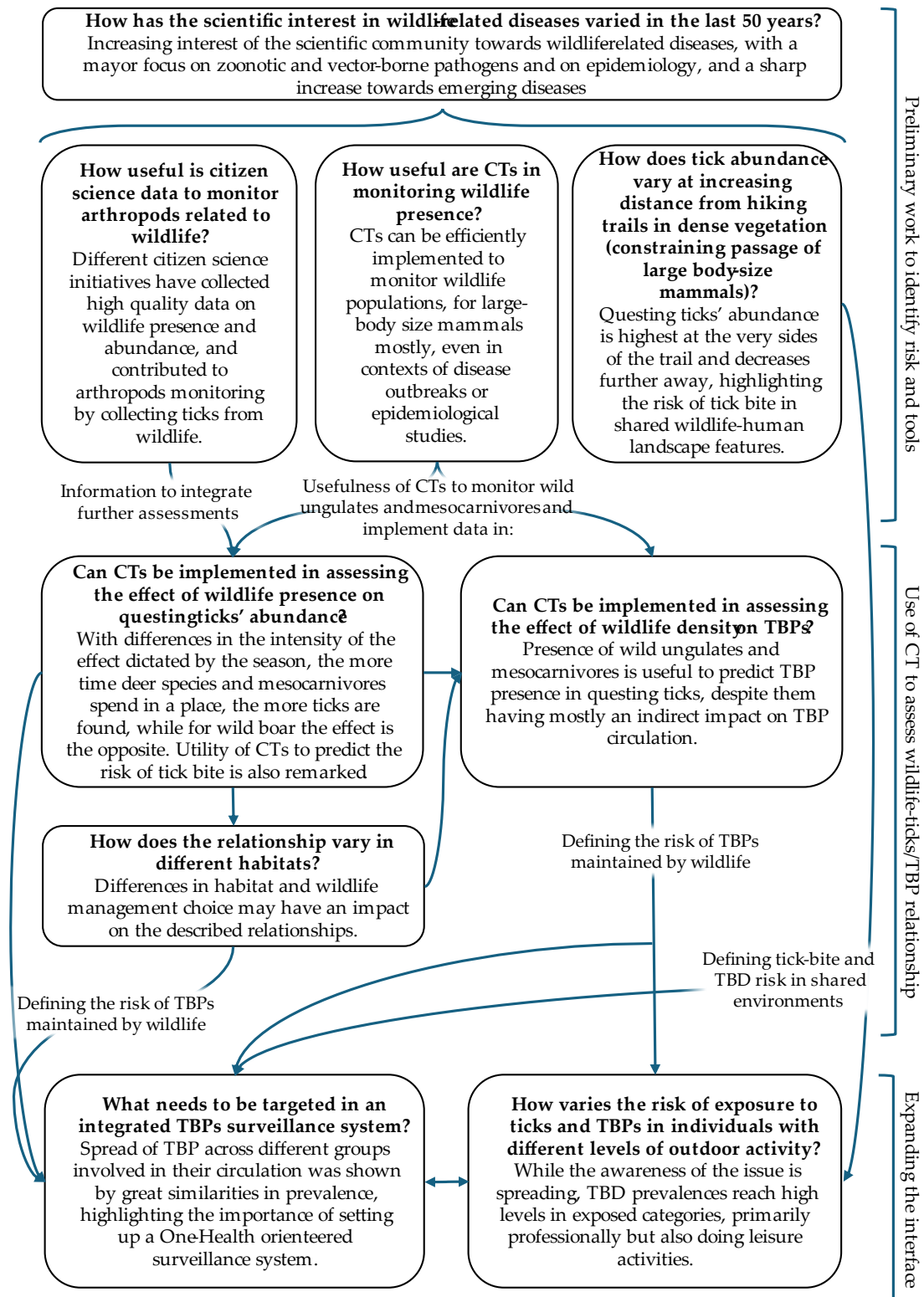
In epidemiological terms, the results of biomolecular analysis would benefit from a deeper assessment into the relationship between pathogen communities, to shed light on circulation patterns and infection origin. For example, based on the *groEL* gene classification, four ecotypes, subdivided into eight clusters, have been identified for *A. phagocytophilum*, each having their specific host range and therefore circulating among a limited range of species (Fabri et al., 2022; Grassi et al., 2021; Jahfari et al., 2014). In *Babesia canis*, targeting the 18S rRNA and Bc28.1 merozoite surface antigen genes has uncovered significant genetic diversity within populations in Germany's Berlin/Brandenburg region, indicating varied parasite origins and highlighting the method's potential for elucidating parasite circulation patterns (Helm et al., 2022), showing potentialities of this approach in providing a deeper look at the parasite circulation patterns. Moreover, transmission of *Borrelia burgdorferi* genospecies appears to be host-specific. Rodents are considered the main reservoir hosts for *B. afzelii*, *B. lusitaniae*, *B. burgdorferi* sensu stricto and *B. spielmanii*, while small birds for *B. garinii*, *B. valaisiana* as well as *B. burgdorferi* s.s. (Król et al., 2022; Kurtenbach et al., 1998a; Mysterud et al., 2019). Since for some species pathogenicity seems still uncertain, this would inform better on the risk for people of developing the disease (Hartemink & Takken, 2016).

Following the line of previous considerations, the implementation of novel detection techniques would be beneficial for a more efficient analysis. Despite their high cost, which requires careful study design to optimize the effort even in economic terms, metagenomic techniques allow for an unbiased detection of all types of tick-borne pathogens, encompassing viruses, bacteria and parasites (Rodino & Pritt, 2022). This allows the inclusion of novel variants to be detected, as well as identification at

subspecies level (Damian et al., 2020). Such methodologies also benefit from higher sensitivity, allowing more efficient detection of species that may be present at much lower frequencies (Rodino & Pritt, 2022; Thoendel, 2020) and microbiome studies (Rodino & Pritt, 2022). Apart from the cost of the methodologies, the need for contaminant background noise, and the association to the organism burden, as well as the correlation of pathogen detection with true infections should be assessed to implement the precision of this approach (Rodino & Pritt, 2022; Thoendel, 2020). Also, quantitative methods (e.g. ddPCR, (King, 2015)) could represent alternative strategies to overcome the limits of pooled sample detection (Fracasso et al., 2023). Likewise, alternative strategies to morphological tick species identification, such as barcoding and mass spectrometry, pose interesting perspective to optimize work, but assessment of their significance and a reliable and extensive bank of sequences is needed (Boucheikhchoukh et al., 2018; Estrada-Peña et al., 2013; Huynh et al., 2021; M'madi et al., 2022).

Breaking up the response variable of our tick abundance and tick-borne pathogens models returned interesting information in terms of prediction performance. Indeed, most studies performed with a similar approach usually target specific species and developmental stages, especially *I. ricinus* nymphs (e.g., Hofmeester et al., 2017a; Takumi et al., 2019). However, decomposing models would also need some more study on the ecology and ethology of specific stages and species. Relating ticks' presence to wildlife data, for instance with temporal occupancy as it was done in the present study, would require accounting for the effect of temperature and humidity on the moulting, egg deposition and hatching times. Additionally, behavioural diapause, overwintering or general survival would affect the abundance of ticks over time, without being related to host presence (Estrada-Peña et al., 2013).

Recalling the scheme interconnecting the research questions, hereafter a more complete version is presented, showing the highlights and main findings of each work and its cohesion in the comprehensive thesis. In epidemiological studies addressing diseases at the wildlife-domestic-human interface, the implementation of tools of high precision such as camera traps, allowed us to gather detailed insights into the effects of wildlife presence on tick abundance and tick-borne pathogens prevalence. This ultimately reflects on the risk for people doing outdoor activities but also remarks on the need to expand such studies to encompass more tick and host species, methods and categories of exposure.



TBP = tick-borne pathogens; CTs = camera traps

7. Concluding remarks

The findings of this study are intended to inform and enhance tick-borne disease risk management strategies. Specifically, a comprehensive and robust assessment of the association between vertebrate host presence, tick abundance, and tick-borne disease prevalence, particularly in wild species subject to active management, such as game animals, can support wildlife management and landscape planning efforts (e.g., in relation to habitat connectivity initiatives). In scenarios where ecological or political/sociological constraints limit the feasibility of wildlife or natural habitat interventions, alternative strategies can focus on modifying human behaviour. Public education and the promotion of effective preventive measures are key in these cases. A thorough assessment of human risk, including exposure factors related to outdoor behaviours and pathogen real prevalence and circulation even in a healthy population, would further support the development of targeted strategies to mitigate tick-borne disease risk.

8. References

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9. Appendix

9. 1. Appendix to chapter 3.1.

<https://www.mdpi.com/article/10.3390/vetsci9110629/s1> Table S1: link to the queries.; Table S2: number of outputs for each query; Table S3: ranking of the 100 most frequent disease-related MeSH terms per species group and decade.

9.2. Appendix to chapter 3.3.

Appendix S1. Camera trap settings.

Table 1 S1. Camera traps settings used to increase the utility of camera traps in the context of African swine fever emergence. Settings are specific for Browning Strike Force HD X Pro – model BTC-5HDPX, but note that similar options can be in other brands and/or models.

Setting	Chosen	Reason
Capture timer	Off	We were focused on different species with different activity patterns. Thus, the cameras should be active 24 hours per day
Capture mode	Pictures	Rapidfire bursts of pictures are useful to reconstruct the trajectory of the animals when crossing the field of view. Pictures have some practical advantages in terms of cost-effectiveness in relation to videos (e.g. optimized memory usage, less time-consuming to process).
Capture delay	1 sec	As lower as possible. Increase the probability of multiple activations in the same encounter
Picture size	4 MP	As lower as possible. Optimize memory usage and is high enough to differentiate species
Multishot mode	8 Rapid fire	Allows rapid image capture, and then increases the accuracy of animal position in the field of view
Adjustable IR flash	Power save	It is well described that the effective detection distance for wild boar and mammals is usually lower than 10 meters. “Power save” intensity illuminated properly medium-size mammals at this range of distances.

Appendix S2. Summary of species recorded.

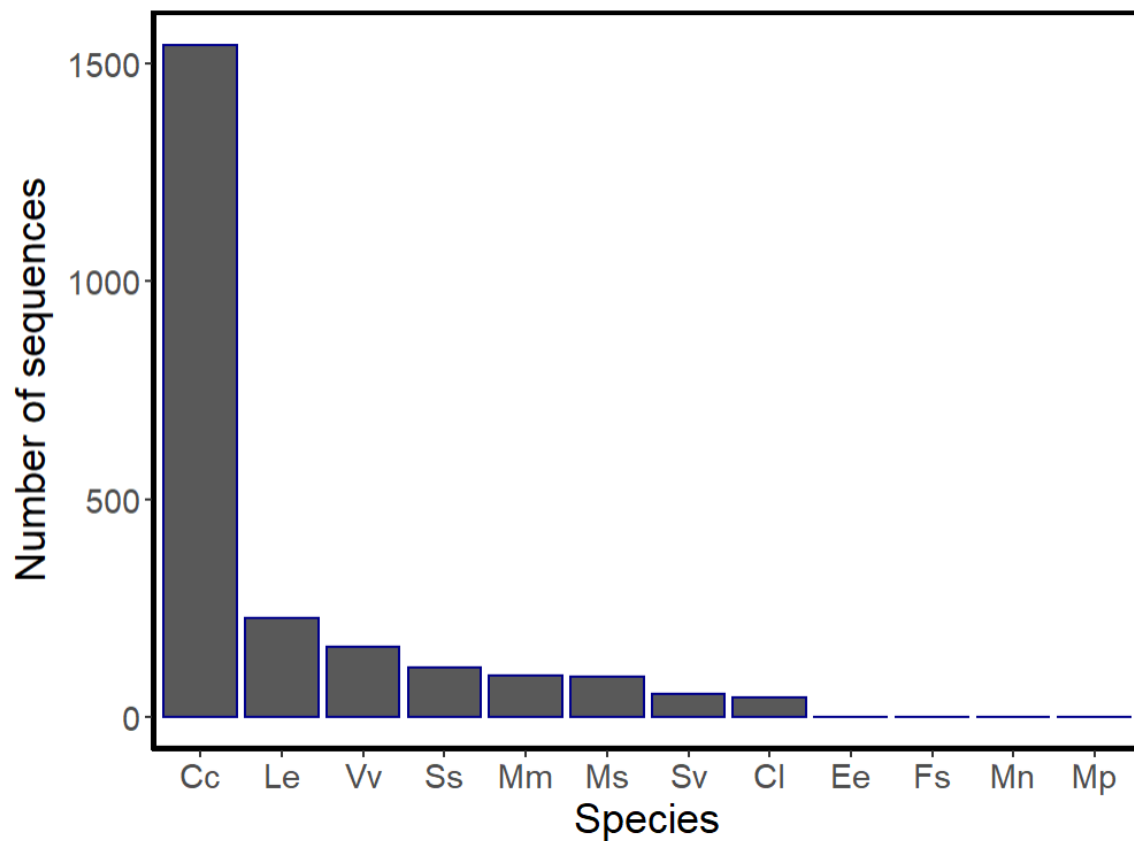


Figure 1 S2: Frequency plot of the number of medium-size mammals independent sequences recorded. Periods longer than 10 minutes were selected to consider two consecutive sequences of the same species in the same camera as an independent. Micro-mammals (mainly rodents) were not included in the plot due to the difficulties to recognize the species. A total of 402 bird sequences were also recorded. Abbreviations: Cc (*Capreolus capreolus*), Le (*Lepus europaeus*), Vv (*Vulpes vulpes*), Ss (*Sus scrofa*), Mm (*Meles meles*), Ms (*Martes martes* and *Martes foina*), Sv (*Sciurus vulgaris*), Cl (*Canis lupus*), Ee (*Erinaceus europaeus*), Fs (*Felis silvestris*), Mn (*Mustela nivalis*) and Mp (*Mustela putorius*).

Appendix S3. Raw data from camera traps.

Table 1 S3: Raw data from roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*) and wolf (*Canis lupus*) to replicate random encounter model analysis.

Point_ID	Sp	G_size	Date	H_first	Speed (m/s)	Dist_det (m)	Ang_det (°)
1	RoeDeer	1	16/03/2022	12:44:42		18	-5
1	RoeDeer	2	17/03/2022	18:33:56		4.7	23
1	RoeDeer		17/03/2022			6	23
1	RoeDeer	1	19/03/2022	11:32:32		12	-25
1	RoeDeer	1	22/03/2022	12:26:45		13	21
2	RoeDeer	1	11/03/2022	3:06:02		13	20
2	RoeDeer	1	12/03/2022	13:07:57	0.24	4.8	-26
2	RoeDeer	1	14/03/2022	13:08:35	3.50	6.5	8

2	RoeDeer	1	15/03/2022	16:58:07	0.27	12	-6
2	RoeDeer	1	17/03/2022	14:36:19	0.14	6.5	27
2	RoeDeer	1	23/03/2022	11:34:25	0.07	5.3	26
2	RoeDeer		23/03/2022	11:36:06	0.12	5.2	27
2	RoeDeer	1	25/03/2022	9:42:56	0.16	8.5	-3
2	RoeDeer	1	02/04/2022	3:50:43		12	4
2	RoeDeer	1	03/04/2022	4:26:50	0.02	6.1	18
2	RoeDeer	1	07/04/2022	6:52:50	0.22	6.6	27
2	RoeDeer	1	08/04/2022	4:06:14	0.48	7.8	14
2	RoeDeer	1	11/04/2022	4:17:03	1.07	8.5	7
2	RoeDeer	1	12/04/2022	6:55:15	0.19	9.5	8
2	RoeDeer	1	12/04/2022	9:13:05	1.28	8.3	18
2	RoeDeer	1	14/04/2022	6:40:53		8.5	22
2	RoeDeer	1	14/04/2022	9:46:49	0.10	7.3	4
2	RoeDeer	1	15/04/2022	7:07:46	0.10	5.4	21
2	RoeDeer		15/04/2022	7:09:30		8	25
2	RoeDeer		15/04/2022	7:11:38	0.36	9	22
2	RoeDeer	1	18/04/2022	7:12:14	0.05	6.9	14
2	RoeDeer	1	18/04/2022	9:53:26	0.25	8.6	27
2	RoeDeer	1	20/04/2022	0:21:48		1.2	-27
2	RoeDeer	1	22/04/2022	15:49:36	0.25	8.3	27
2	RoeDeer	1	23/04/2022	11:10:28		8.1	17
2	RoeDeer	1	24/04/2022	5:52:50	1.20	9.5	15
2	RoeDeer	1	25/04/2022	4:30:32		5.2	-16
2	RoeDeer		25/04/2022	4:31:36		6	-26
2	RoeDeer	2	25/04/2022	18:31:52	0.13	8	-18
2	RoeDeer		25/04/2022	18:31:52		12	20
2	RoeDeer	1	26/04/2022	5:19:29	0.10	5.6	-21
2	RoeDeer	1	27/04/2022	5:27:09	1.20	9.5	17
2	RoeDeer	1	27/04/2022	6:03:28	0.12	4.3	23
3	RoeDeer	1	18/03/2022	6:54:00	0.03	5.2	-25
3	RoeDeer	1	18/03/2022	18:11:53		8.8	-4
3	RoeDeer	1	19/03/2022	7:04:07		3.4	22
3	RoeDeer	1	19/03/2022	12:04:27		40	21
3	RoeDeer	1	20/03/2022	10:45:10	0.50	3.1	22
3	RoeDeer	1	21/03/2022	7:34:15		9	-23
3	RoeDeer	2	24/03/2022	7:35:17	3.00	8.5	-18
3	RoeDeer		24/03/2022	7:35:17	3.17	10.5	-25
3	RoeDeer	1	25/03/2022	11:51:10	0.17	9	-26
3	RoeDeer	1	25/03/2022	14:35:53		9	-27
3	RoeDeer	1	26/03/2022	6:24:50	0.09	4.3	27
3	RoeDeer		26/03/2022	6:26:35	0.10	8	27
3	RoeDeer	1	26/03/2022	8:30:14	2.15	8.2	-18
3	RoeDeer	1	26/03/2022	16:30:43		7.6	4
3	RoeDeer	1	29/03/2022	9:19:02	0.16	7.4	11
3	RoeDeer	1	02/04/2022	7:20:42	0.12	1.5	-27

3	RoeDeer	1	05/04/2022	8:46:22	0.06	7	26
3	RoeDeer	1	15/04/2022	18:50:22	0.08	6.5	-12
3	RoeDeer	1	16/04/2022	20:14:39		6.7	16
4	RoeDeer	1	21/04/2022	5:53:19	0.06	7.7	-12
4	RoeDeer	1	21/04/2022	16:31:03		5.3	-27
4	RoeDeer	1	24/04/2022	7:46:42		12	-12
6	RoeDeer	1	15/04/2022	8:01:00		7.6	-16
6	RoeDeer	1	16/04/2022	11:29:51	0.64	7.2	-25
7	RoeDeer	1	19/03/2022	4:19:03		2	21
7	RoeDeer	1	23/03/2022	18:41:12		15	-2
7	RoeDeer	1	27/03/2022	10:08:41		1	-25
7	RoeDeer	1	30/03/2022	6:28:29		18	6
7	RoeDeer	1	11/04/2022	23:14:51	0.19	8.2	4
7	RoeDeer	2	13/04/2022	16:26:50		15	12
7	RoeDeer	1	21/04/2022	16:23:11		3	27
8	RoeDeer	1	24/03/2022	6:23:27		2.3	20
8	RoeDeer	1	17/04/2022	8:25:41		8	-18
11	RoeDeer	1	18/03/2022	6:47:08	0.07	4.2	26
11	RoeDeer	1	20/03/2022	22:56:00		6	-1
11	RoeDeer	1	26/03/2022	11:39:58	0.04	4.5	6
11	RoeDeer	1	26/03/2022	12:30:19	0.25	2	-27
11	RoeDeer	1	29/03/2022	6:51:59	0.23	4.6	-25
11	RoeDeer	1	01/04/2022	17:30:47	0.33	3.2	-27
11	RoeDeer	1	02/04/2022	21:55:27	0.22	5	-25
11	RoeDeer	1	04/04/2022	21:05:08	0.14	1.7	-16
11	RoeDeer	1	06/04/2022	0:28:30	0.25	3.6	-27.5
11	RoeDeer	1	08/04/2022	19:53:47	1.20	1.7	12
11	RoeDeer	1	16/04/2022	6:51:42	0.05	5.2	-2
11	RoeDeer		16/04/2022	6:54:24	0.18	3.7	-23
11	RoeDeer	1	19/04/2022	6:12:38	0.11	3.1	1
11	RoeDeer		19/04/2022	6:13:26		2.7	13
11	RoeDeer	2	20/04/2022	1:22:38	1.80	5.9	-17
11	RoeDeer		20/04/2022	1:22:52		3.7	-16
13	RoeDeer	1	19/04/2022	23:43:44	3.15	8	-11
13	RoeDeer	1	20/04/2022	3:24:14	1.20	3.1	-27
14	RoeDeer	1	06/04/2022	6:27:15		7.7	-7
14	RoeDeer	1	14/04/2022	18:55:31	1.00	7.7	25
14	RoeDeer	1	18/04/2022	20:06:08	2.20	9	17
14	RoeDeer	1	29/03/2022	10:44:38		6.5	-23
14	RoeDeer		29/03/2022	10:53:45		1.8	18
14	RoeDeer	1	19/04/2022	10:33:59		2.6	-27
14	RoeDeer		19/04/2022	10:35:20		1.7	27
16	RoeDeer	1	13/03/2022	13:10:21		15	12
16	RoeDeer	2	17/03/2022	6:54:50		7	-2
16	RoeDeer		17/03/2022	6:54:50		12	0
16	RoeDeer	1	18/03/2022	16:46:10		15	-9

16	RoeDeer	1	18/03/2022	18:31:49	0.25	5.8	23
16	RoeDeer	1	20/03/2022	13:16:13	0.20	2.7	27
16	RoeDeer	1	22/03/2022	7:32:37		15	15
16	RoeDeer	1	25/03/2022	9:46:34		8.5	17
16	RoeDeer	1	26/03/2022	6:32:38	0.11	-13	13
16	RoeDeer	1	28/03/2022	8:59:43	0.26	-9	9.2
16	RoeDeer	1	04/04/2022	17:09:54	0.26	11	14
16	RoeDeer	1	09/04/2022	8:49:13		8.3	-21
16	RoeDeer	1	09/04/2022	13:38:09		11.5	-3
16	RoeDeer	1	12/04/2022	11:56:00	0.05	2.1	23
16	RoeDeer	1	13/04/2022	19:58:43	0.05	3.2	27
16	RoeDeer	1	14/04/2022	19:07:09	0.14	8.5	17
16	RoeDeer	1	14/04/2022	21:40:55	0.01	3.5	27.5
16	RoeDeer	1	16/04/2022	18:31:35	0.06	7	24
16	RoeDeer	1	24/04/2022	18:36:32	0.04	5	-26
16	RoeDeer	1	24/04/2022	12:56:31	0.16	9.4	-9
16	RoeDeer	1	24/04/2022	21:17:38	0.09	1.8	27
16	RoeDeer	1	26/04/2022	17:23:49	0.16	11.5	7
18	RoeDeer	1	22/03/2022	17:07:49		5.2	-24
18	RoeDeer	1	24/03/2022	19:27:39		6.6	18
20	RoeDeer	1	25/03/2022	9:23:46		3.3	19
20	RoeDeer	1	27/03/2022	6:53:30	0.07	1.8	27.5
20	RoeDeer	1	06/04/2022	12:52:58	0.17	0.6	27.5
23	RoeDeer	1	20/03/2022	4:44:12	0.15	5.7	-22
23	RoeDeer	1	22/03/2022	22:44:18	0.07	6.8	16
23	RoeDeer	1	22/03/2022	23:56:19		3.8	16
23	RoeDeer	1	24/03/2022	19:31:55	0.01	5.2	-27.5
24	RoeDeer	2	18/03/2022	6:51:33		2.8	27
24	RoeDeer		18/03/2022	6:52:05	0.16	2.6	-3
24	RoeDeer	1	18/03/2022	9:49:37	0.01	2.1	-27.5
24	RoeDeer	1	20/03/2022	12:04:36		2.6	-12
24	RoeDeer	1	22/03/2022	15:10:46	0.05	2.9	27.5
24	RoeDeer	1	23/03/2022	23:04:14		6	23
25	RoeDeer	1	02/04/2022	21:56:33	0.40	1.2	-27.5
25	RoeDeer	1	08/04/2022	7:32:09	0.54	2.1	-19
26	RoeDeer	1	06/04/2022	2:15:06		0.5	27.5
26	RoeDeer	1	08/04/2022	14:37:59		15	-4
26	RoeDeer	1	09/04/2022	2:18:47		16	-15
26	RoeDeer	1	09/04/2022	13:00:15		12	21
26	RoeDeer	1	12/04/2022	6:51:35		20	-6
26	RoeDeer	1	13/04/2022	8:51:50		20	6
26	RoeDeer	1	20/04/2022	19:34:45		8	-7
27	RoeDeer	1	18/03/2022	10:44:07		5.3	15
27	RoeDeer	1	19/03/2022	22:03:04		3.2	15
27	RoeDeer	1	20/03/2022	1:51:50	0.44	6	25
27	RoeDeer	1	21/03/2022	4:33:59	0.15	6	24

27	RoeDeer	1	21/03/2022	6:37:16	0.08	3.1	23
27	RoeDeer	1	29/03/2022	20:12:50		5.3	-8
27	RoeDeer	1	30/03/2022	5:02:19	0.32	6.5	24
27	RoeDeer	1	31/03/2022	7:28:35	2.15	4.8	-27.5
27	RoeDeer	1	01/04/2022	6:33:22	0.40	7.6	13
27	RoeDeer	1	03/04/2022	18:31:20	0.36	4.9	14
27	RoeDeer	1	07/04/2022	13:20:35	0.13	5.4	23
27	RoeDeer	1	08/04/2022	3:10:53	0.51	2	27.5
27	RoeDeer	1	09/04/2022	16:01:09	0.37	8.5	12
27	RoeDeer	1	11/04/2022	19:33:16		11	-5
27	RoeDeer	1	13/04/2022	11:45:48		5.4	15
27	RoeDeer	1	14/04/2022	18:32:13	0.21	5.5	27.5
27	RoeDeer	1	19/04/2022	18:21:21	0.35	14	14
27	RoeDeer	1	19/04/2022	18:48:20	0.24	4.8	-27.5
27	RoeDeer	1	20/04/2022	18:46:09	0.20	2.3	-19
27	RoeDeer		20/04/2022	18:47:12		9.5	21
27	RoeDeer	1	21/04/2022	17:56:28	0.37	8.4	17
27	RoeDeer	1	21/04/2022	10:26:56		12	-21
27	RoeDeer	1	21/04/2022	10:45:18	0.75	2	0
27	RoeDeer	1	22/04/2022	5:32:10	0.90	3.2	-27.5
27	RoeDeer	1	22/04/2022	11:51:35		5.8	11
28	RoeDeer	1	13/03/2022	18:13:55		1.3	-27.5
28	RoeDeer	1	18/03/2022	18:11:17		5.2	-13
28	RoeDeer	1	22/03/2022	13:26:53		15	27
28	RoeDeer	1	23/03/2022	20:21:44		1	-27.5
30	RoeDeer	1	24/03/2022	19:09:22		7	3
30	RoeDeer	1	28/03/2022	9:37:48		3.1	-24
30	RoeDeer	1	29/03/2022	22:05:51		8	4
30	RoeDeer	1	10/04/2022	6:12:47	1.02	3.3	-14
30	RoeDeer	1	12/04/2022	11:17:10		1.8	27.5
30	RoeDeer	1	12/04/2022	17:39:37		8.5	6
30	RoeDeer	1	18/04/2022	6:18:22	0.58	4	-1
32	RoeDeer	1	12/03/2022	8:53:19		7	-13
32	RoeDeer	1	18/03/2022	12:50:09	0.30	3.7	-25
32	RoeDeer	1	18/03/2022	17:27:46		7	-15
32	RoeDeer	1	22/03/2022	18:26:08	0.12	4.5	-14
33	RoeDeer	1	19/03/2022	13:20:07	0.26	7.6	-12
33	RoeDeer	1	27/04/2022	1:32:26	0.04	5.4	-17
35	RoeDeer	1	11/03/2022	22:39:08		7.8	-14
35	RoeDeer	1	14/03/2022	21:30:16		3.2	12
39	RoeDeer	1	20/03/2022	7:23:54		5.2	-26
39	RoeDeer	1	21/03/2022	18:23:04		11	23
39	RoeDeer		21/03/2022	18:23:42		15	27
39	RoeDeer	1	15/04/2022	14:43:31	0.30	2.6	-19
39	RoeDeer	1	20/04/2022	13:06:22		7	24
39	RoeDeer	1	22/04/2022	7:42:01		3.8	24

40	RoeDeer	1	18/03/2022	20:09:44		2.7	15
2	WildBoar	1	15/03/2022	5:53:54	0.12	5.8	24
2	WildBoar	1	07/04/2022	8:10:51	0.43	5.3	22
2	WildBoar	1	07/04/2022	5:22:39	0.8	2.2	-13
27	WildBoar	1	23/03/2022	18:22:14	2.2	6.4	-22
27	WildBoar	1	27/03/2022	5:44:25		5.2	-3
27	WildBoar	1	28/03/2022	17:05:26	2.27	6.8	-23
27	WildBoar	1	30/03/2022	17:49:31	1.4	7.8	-26
27	WildBoar	1	02/04/2022	8:15:51		0.5	10
27	WildBoar	1	22/04/2022	6:25:02	0.35	1.7	-22
27	WildBoar	1	27/04/2022	4:57:44	0.85	1.1	-27
27	WildBoar	1	24/03/2022	23:04:29		6.5	0
5	WildBoar	1	11/03/2022	21:14:35	0.58	1	-27
30	WildBoar	1	27/03/2022	4:48:39		6.8	8
25	WildBoar	1	21/03/2022	12:40:56		0.5	0
25	WildBoar	1	15/04/2022	9:57:05	0.35	2.4	22
9	WildBoar	1	20/03/2020	18:47:26	0.13	6.3	17
9	WildBoar		20/03/2020	18:49:15		8	22
39	WildBoar	1	15/04/2022	1:03:12	0.28	2.2	27
33	WildBoar	1	12/04/2022	1:29:26	0.7	7.6	-16
26	WildBoar	1	21/03/2022	10:31:56	0.27	9.7	-2
37	WildBoar	1	18/03/2022	7:13:31	0.43	7.8	-4
5	WildBoar	2	04/05/2022	5:02:13		25	10
5	WildBoar		04/05/2022	5:02:31	0.11	5.3	-27
5	WildBoar	1	19/05/2022	21:53:07	0.85	1.7	0
11	WildBoar	1	27/04/2022	23:19:41	0.85	5.3	6
11	WildBoar	1	28/04/2022	20:51:26	0.6	5.4	-24
11	WildBoar	1	06/05/2022	16:25:34	0.1	7	-2
11	WildBoar	1	19/05/2022	20:19:46	0.08	4.7	21
11	WildBoar		19/05/2022	20:24:32	0.05	3.8	-22
11	WildBoar		19/05/2022	20:28:00	0.25	4	26
11	WildBoar	1	21/05/2022	18:58:30	1	7.6	8
11	WildBoar	1	26/05/2022	12:39:08	0.1	5.2	-23
20	WildBoar	1	30/05/2022	10:15:26	3.25	12	18
27	WildBoar	1	28/04/2022	7:09:59		2.3	13
27	WildBoar	1	02/05/2022	10:50:42		1	0
27	WildBoar	1	04/05/2022	6:42:19		4.7	25
27	WildBoar		04/05/2022	6:43:06		3.2	9
27	WildBoar	1	12/05/2022	7:18:46	1.25	5.8	-17
27	WildBoar	1	29/05/2022	18:19:33	1.2	5.2	18
35	WildBoar	1	26/05/2022	5:56:32		1	-10
1	WildBoar	1	27/04/2022	20:16:03		0.5	0
1	WildBoar		27/04/2022			3	27
1	WildBoar		27/04/2022	20:16:44	0.45	6	-18
1	WildBoar	1	06/05/2022	14:52:30	0.01	9.5	-17
1	WildBoar	2	06/05/2022	15:23:21	0.03	2.3	-21

1	WildBoar		06/05/2022	15:24:21	0.24	1.5	-18
1	WildBoar	2	06/05/2022	15:36:45		12	-17
14	WildBoar	1	14/05/2022	6:22:48	3	9.8	12
14	WildBoar	1	28/05/2022	6:06:12	3.8	4.7	-9
14	WildBoar	1	23/04/2022	5:25:02		16	18
14	WildBoar	1	24/05/2022	20:42:25		22	-2
24	WildBoar	1	05/05/2022	18:52:26	1.3	8.5	24
11	WildBoar	1	22/06/2022	3:07:03	0.9	5.81	-19
11	WildBoar	1	01/07/2022	10:46:51	1.07	9.77	6
18	WildBoar	1	30/06/2022	17:33:19	0.21	7.74	-7
2	WildBoar	1	17/06/2022	21:19:38		4.07	14
20	WildBoar	1	10/06/2022	3:58:00	1.5	2.8	0
24	WildBoar	1	01/07/2022	0:33:53	0.58	6.05	23
27	WildBoar	2	07/06/2022	7:40:22	1.61	6.88	-5
27	WildBoar		07/06/2022	7:41:13	0.94	5.59	11
27	WildBoar	1	27/06/2022	15:20:50	0.8	8.56	-18
29	WildBoar	1	12/06/2022	4:24:01		4.7	22
3	WildBoar	1	10/06/2022	0:54:02	0.84	12.55	4
3	WildBoar	1	14/06/2022	21:50:05	0.25	12.45	19
32	WildBoar	1	29/06/2022	21:11:22	2.31	14.76	-3
35	WildBoar	1	01/06/2022	20:45:36	1.17	9.36	1
35	WildBoar	1	27/06/2022	20:03:59	0.69	8.61	19
5	WildBoar	1	19/06/2022	2:41:27	0.22	3.23	18
5	WildBoar	1	23/06/2022	21:07:04	0.26	7.65	18
5	WildBoar		23/06/2022	21:07:29	1	3.81	21
1	WildBoar	2	28/07/2022	20:10:12	0.21	12	-15.21
1	WildBoar		28/07/2022	20:10:16		12.5	-17.9
1	WildBoar	2	28/07/2022	17:41:08		12	-20
1	WildBoar		28/07/2022	17:41:08		12.5	-19
18	WildBoar	1	24/07/2022	20:26:32	0.53	2.8	20
20	WildBoar	1	11/07/2022	20:46:19	0.49	2.7	-0.24
24	WildBoar	1	08/07/2022	1:53:57	1.03	4.8	22.88
24	WildBoar	1	21/07/2022	20:59:27	0.81	6.2	-19.53
27	WildBoar	1	09/07/2022	6:43:19	0.37	10.16	-2.91
27	WildBoar	1	31/07/2022	12:17:26	1.1	9.79	-8.93
3	WildBoar	1	11/07/2022	3:07:30	0.85	4.7	21.01
3	WildBoar	1	23/07/2022	22:47:05	1.28	12.86	17.45
32	WildBoar	1	28/07/2022	4:54:22	0.64	1.13	28.25
35	WildBoar	1	12/07/2022	6:05:50	1.04	15.84	13.06
35	WildBoar	1	12/07/2022	6:27:58	2.83	6.99	18.8
35	WildBoar	1	19/07/2022	3:49:57	3.16	16.78	12.43
5	WildBoar	2	08/07/2022	20:29:11	0.18	7.91	3.98
5	WildBoar		08/07/2022	20:30:42		1.34	14.84
5	WildBoar		08/07/2022	20:32:19	0.1	4.24	-22.01
5	WildBoar		08/07/2022	20:33:34	0.31	11.32	18.73
5	WildBoar	1	21/07/2022	6:06:26	0.62	6.83	6.33

5	WildBoar		21/07/2022	6:07:07	1.28	1.95	-17.36
5	WildBoar	2	05/08/2022	4:35:19	0.69	8.95	-12.35
2	Wolf	1	12/03/2022	5:08:14	0.33	5.8	26
4	Wolf	1	26/04/2022	19:02:09	0.31	12	6
9	Wolf	1	20/03/2022	22:32:20		6.9	22
24	Wolf	1	24/03/2022	7:44:52		2	0
24	Wolf	1	02/04/2022	5:37:00	2.90	3.2	-15
27	Wolf	3	21/03/2022	9:28:25	0.78	4.6	20
27	Wolf		21/03/2022	9:28:36			
27	Wolf		21/03/2022	9:28:52	2.50	6.2	20
39	Wolf	1	24/03/2022	19:52:34		2.6	-21
39	Wolf	1	27/03/2022	22:39:08	2.10	2.5	-21
39	Wolf	1	28/03/2022	1:16:06	0.50	2.6	-27
39	Wolf	1	30/03/2022	21:10:06	1.67	2.7	-27
39	Wolf	1	22/04/2022	3:38:50	0.93	2.8	-18
4	Wolf	1	05/05/2022	4:19:46		2.6	28
10	Wolf	1	29/04/2022	7:00:00	0.33	5.8	8
26	Wolf	2	09/05/2022	0:04:56	0.40	3.8	-26
26	Wolf		09/05/2022	0:05:15	1.00	4	-22
26	Wolf		09/05/2022	0:07:04	0.43	5.3	22
26	Wolf		09/05/2022	0:07:11	0.59	5.6	27
26	Wolf	2	09/05/2022	6:41:23	1.50	14	-12
26	Wolf		09/05/2022	6:42:09	0.93	16	-15
26	Wolf	1	21/05/2022	19:58:39	0.78	15	-6
14	Wolf	1	28/04/2022	19:30:10		3.8	-20
24	Wolf	1	14/05/2022	18:18:23	2.10	4.3	21
10	Wolf	2	22/06/2022	1:28:28		1.47	-1
10	Wolf		22/06/2022	1:28:45	0.93	7.5	4
10	Wolf	1	23/06/2022	5:25:41		1.67	10
10	Wolf	1	02/06/2022	0:20:59	0.29	2.37	14
10	Wolf	2	04/06/2022	5:02:14	0.85	1	15
10	Wolf		04/06/2022	5:02:14		6.5	20
10	Wolf		04/06/2022	5:02:25		10.3	-18
10	Wolf	1	04/06/2022	6:03:43		7.63	9
10	Wolf	1	04/06/2022	8:51:01	2.81	6.83	1
10	Wolf	1	04/06/2022	23:11:09	1.28	4.2	-9
11	Wolf	1	14/06/2022	12:41:03	0.89	5.37	-18
24	Wolf	1	07/06/2022	22:46:02		4.78	-7
26	Wolf	1	28/06/2022	3:38:41	0.89	2.83	-24
10	Wolf	1	09/07/2022	2:58:46	1.20	1.55	-25.8
27	Wolf	1	31/07/2022	16:35:16	0.67	6.32	12.04
32	Wolf	1	13/07/2022	12:01:36		1.39	-25.8
37	Wolf	2	01/08/2022	2:00:37	2.63	1.15	-1.15
37	Wolf		01/08/2022	2:00:46		1	-15

Table 2 S3: Operativity matrix for wild boar and wolf.

CAM	Operative days
1	73
2	149
3	131
4	71
5	101
6	117
7	136
8	129
9	70
10	58
11	136
12	70
13	142
14	72
15	131
16	82
17	97
18	131
19	13
20	134
21	123
22	104
23	45
24	117
25	144
26	136
27	144
28	63
29	82
30	130
31	2
32	115
33	129
34	101
35	106
36	90
37	79
38	79
39	38
40	67
41	100

Table 3 S3: Operativity matrix for roe deer.

CAM	Operative days
1	8
2	49
3	36
4	23
5	2
6	18
7	38
8	38
9	5
10	6
11	37
12	0
13	44
14	38
15	36
16	48
17	2
18	36
19	2
20	36
21	23
22	23
23	11
24	19
25	45
26	38
27	45
28	19
29	2
30	32
31	2
32	16
33	41
34	6
35	7
36	2
37	5
38	26
39	38
40	9
41	8

Table 4 S3: Recruitment data.

SEX	PIGLETS
Unknown	0
Female	3
Female	3
Alone	1
Female	3
Female	1
Male	0
Male	0
Unknown	0
Female	0
Unknown	0
Unknown	0
Male	0
Unknown	0
Unknown	0
Female	0
Female	0
Alone	1
Alone	2
Female	4
Male	0
Male	0
Alone	2
Male	0
Male	0
Male	0
Male	0
Unknown	0
Unknown	0
Female	2
Male	0
Unknown	0
Female	0
Alone	1
Alone	1
Alone	1
Alone	2
Female	0
Alone	2
Alone	1
Alone	2
Alone	1
Unknown	0
Unknown	0

Female	4
Male	0
Male	0
Male	0
Female	0
Male	0
Female	0
Unknown	0
Male	0
Male	0
Male	0
Unknown	0
Unknown	0
Alone	1
Alone	1
Alone	1
Alone	2
Female	1
Alone	2
Alone	1
Alone	1
Alone	2
Alone	1
Female	0
Female	0
Male	0
Alone	2
Alone	1
Alone	2

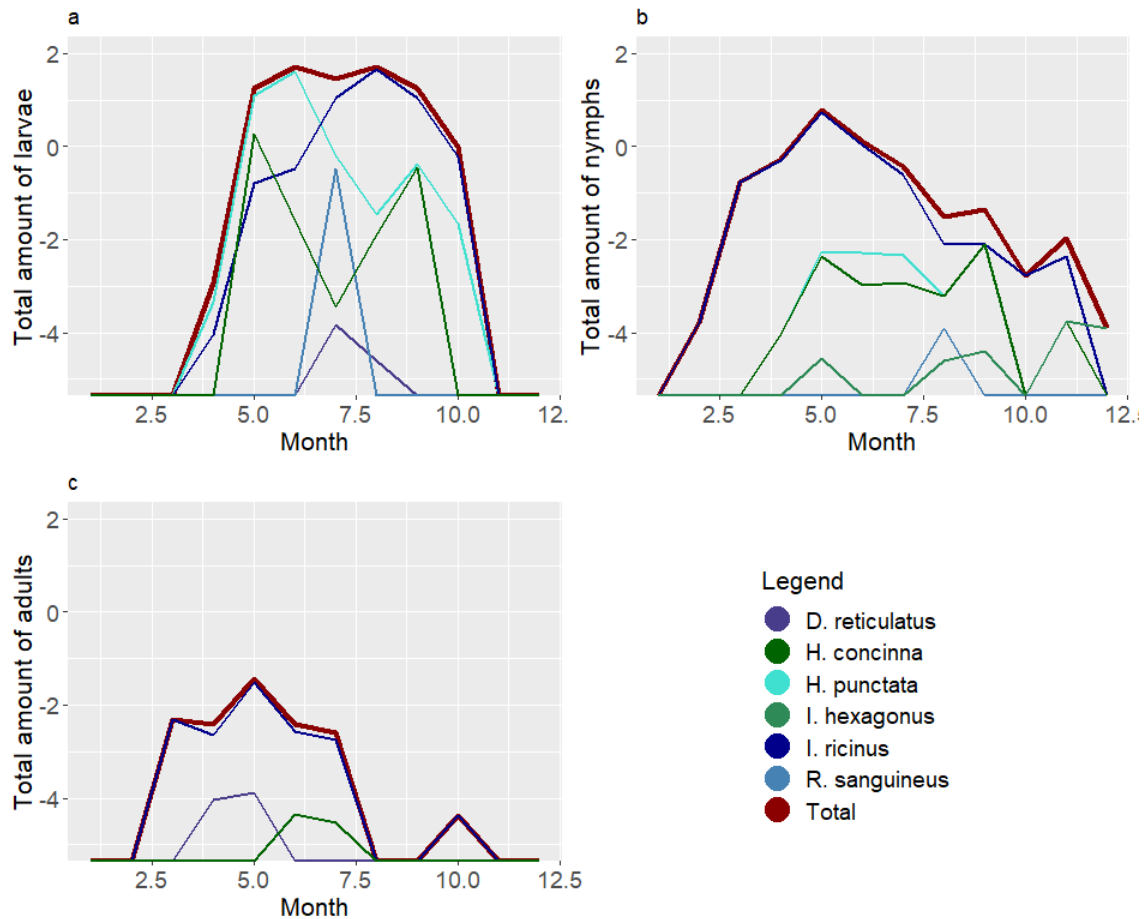
Table 5 S3: Biosafety human restriction data.

CAM	Specie	Dog	N_people
4	Human	no	1
16	Human	yes	1
14	Human	yes	1
14	Human	yes	1
12a	Human		stolen
14	Human		stolen
16	Human		stolen
19	Human		stolen
23	Human		stolen
39a	Human		stolen
39b	Human		stolen
4	Human	no	1

5	Human	yes	1
5	Human	yes	0
6	Human	no	1
10	Human	no	2
10	Human	yes	1
13	Human	yes	2
13	Human	no	2
16	Human	yes	1
16	Human	yes	1
18	Human	no	2
29	Human	no	1
38	Human	no	1
3	Human	no	1
6	Human	no	1
13	Human	no	3
13	Human	no	4
13	Human	no	1
32	Human	no	1
7	Human	yes	0
3	Human	yes	0
30	Human	yes	0
5	Human	yes	1
18	Human	no	1
3	Human	no	1
13	Human	no	2

9.3. Appendix to chapter 4.1.

Supplementary Figure 1. Temporal trend of ticks collected in La Mandria. The graph shows the trends per developmental stage and species, plotting the logarithmic mean number over the month of collection. The red line defines all species together, while single species are plotted with thinner lines.

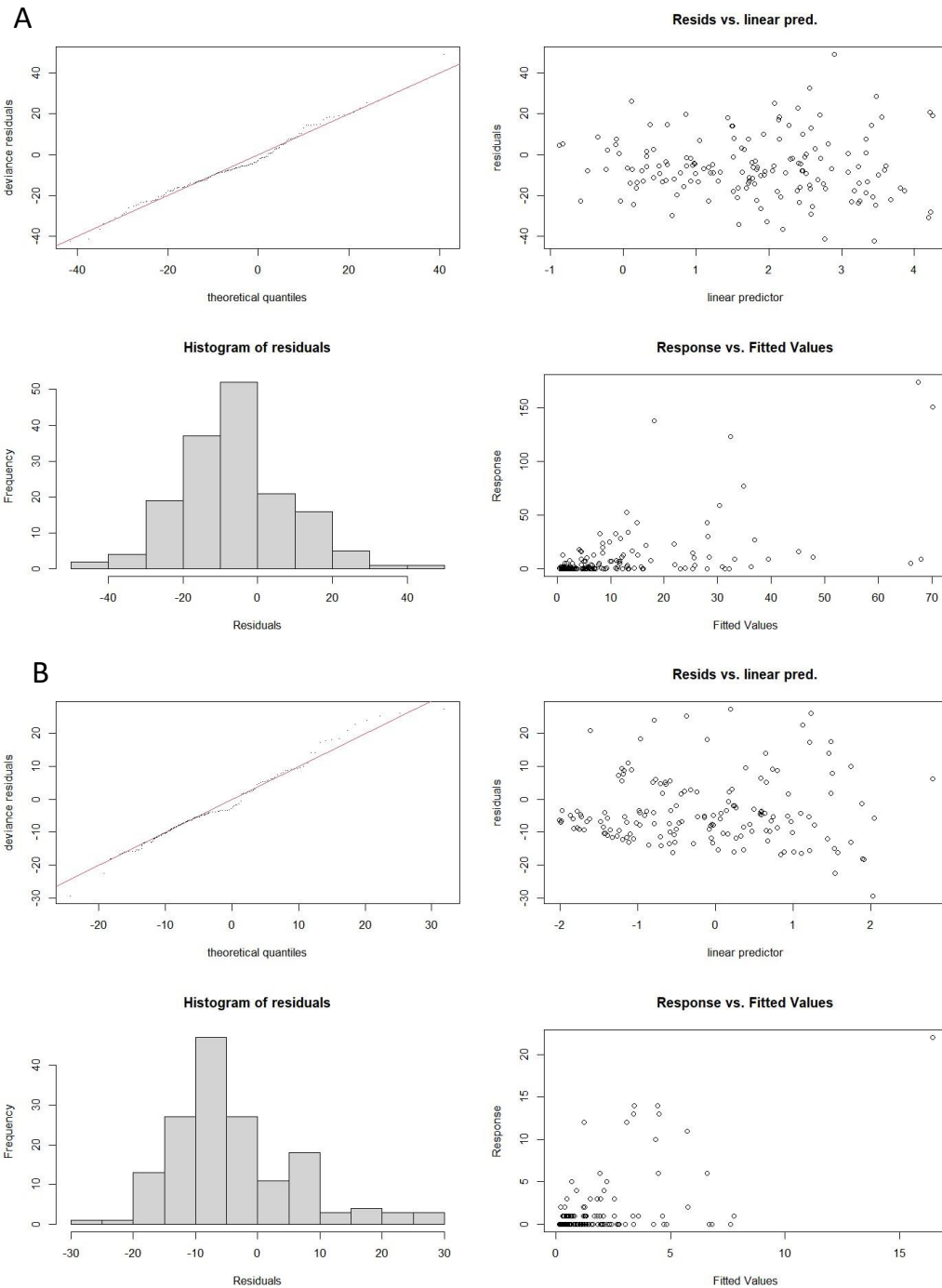


Supplementary Table 1. AIC model selection. Significance codes codes: $p = 0$ '***', $p = 0.001$ '***', $p = 0.01$ '**', $p = 0.05$ '.'. k value is the smoothing parameters and determines how many basis functions are used to approximate the smooth function for each predictor. The cubic regression splines ("cc") was used as basis function (bs).

Model	AIC
Mesocarnivores_TO+ WildBoar_TO + WildRuminants_TO	30830.71
SmaxSD1+NDVI + Month + Mesocarnivores_TO + WildBoar_TO + WildRuminants_TO	30487.80
SmaxSD1*NDVI + s(Month, bs="cc", k=6) + Mesocarnivores_TO + WildBoar_TO + WildRuminants_TO	25653.16
SmaxSD1 + NDVI + s(Month, bs="cc", k=6) + s(Mesocarnivores_TO, k=3) +	25335.71

$s(\text{WildBoar_TO}, k=3) + s(\text{WildRuminants_TO}, k=3)$
 $\text{SmaxSD1} + \text{NDVI} + s(\text{Month}, \text{bs}="cc", k=6) + s(\text{Mesocarnivores_TO},$
 $\text{Season}, k=3) + s(\text{WildBoar_TO}, \text{Season}, k=3) + s(\text{WildRuminants_TO},$ 25322.41
 $\text{Season}, k=3)$

Supplementary Figure 2. residual analysis of the final GAM global model (A) and *I. ricinus* nymphs model (B). We plot: (top-left) deviance residuals plotted against theoretical quantiles (Q-Q plot), (top-right) residuals plotted against linear predictors, (bottom-left) histogram with frequency of residuals), (bottom right) response plotted against fitted values.



Supplementary Table 2. Coefficients of parametric and smooth terms, for global model and *I. ricinus* nymphs model. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Global model

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1.80786	0.01918	94.252	<2e-16 ***

maxSD	-0.04212	0.01909	-2.207	0.0289	*
NDVI_scaled	-0.62611	0.02208	-28.353	<2e-16	***

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Month)	3.991	4	1088.5	<2e-16	***
s(Mesocarnivores_TO,Season)	2.972	2.999	103.2	<2e-16	***
s(Wildboar_TO,Season)	1.994	2	330	<2e-16	***
s(WildRuminants_TO,Season)	1.959	1.998	236.8	<2e-16	***

***I. ricinus* nymphs model**

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	-0.13786	0.02084	-6.615	7E-10	***
maxSD	0.13478	0.0229	5.885	3E-08	***
NDVI_scaled	-0.45545	0.02376	-19.169	<0	***

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Month)	3.969	4	645.52	<2e-16	***
s(Mesocarnivores_TO,Season)	2.002	2.004	75.34	<2e-16	***
s(Wildboar_TO,Season)	1.994	2	214.35	<2e-16	***
s(WildRuminants_TO,Season)	1.005	1.009	71.57	<2e-16	***

9.4. Appendix to chapter 4.2.

Table S1 – AIC model selection (Val Maira and Capanne di Marcarolo)

SD + NDVI + Altitude + WildRuminants_TO + WildBoar_TO + Mesocarnivores_TO	204
SD + NDVI + Altitude + s(WildRuminants_TO) + s(WildBoar_TO) + s(sMesocarnivores1)	198
SD + NDVI + Altitude + s(WildRuminants_TO, Altitude, k=4) + s(WildBoar_TO, Altitude, k=4) + sMesocarnivores1*Altitude	202
SD + NDVI + Altitude + s(WildRuminants_TO, Altitude, by=as.factor(Site), k=4) + s(WildBoar_TO, Altitude, by=as.factor(Site), k=4) + sMesocarnivores1*as.factor(Site)	187

Table S2 – Model output

Family: Tweedie(p=1.512)
Link function: log

Formula:
Numero1 ~ SD1 + NDVI1 + Altitudine + s(sRoe1, Altitudine, by = as.factor(Site),
k = 4) + s(sBoar1, Altitudine, by = as.factor(Site), k = 4) +
sMesocarnivores1

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.0000000	0.0000000	NaN	NaN
SD1	-0.0660683	0.3100705	-0.213	0.8322
NDVI1	0.1788460	0.2148964	0.832	0.4096
Altitudine	-0.0008294	0.0004866	-1.705	0.0951
sMesocarnivores1	-0.0245347	0.1954541	-0.126	0.9007

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:

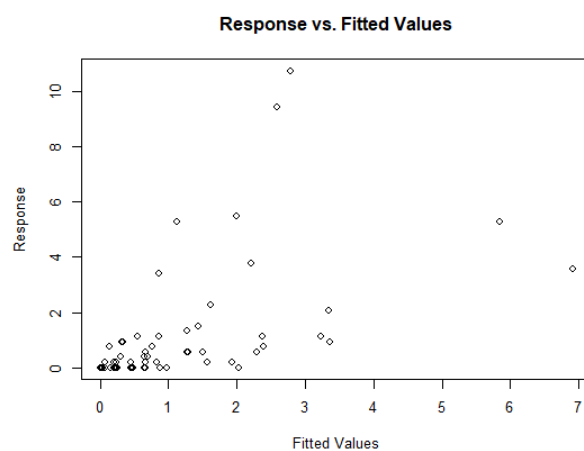
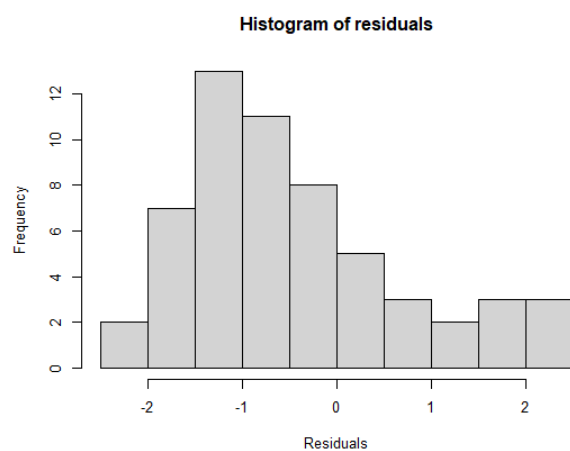
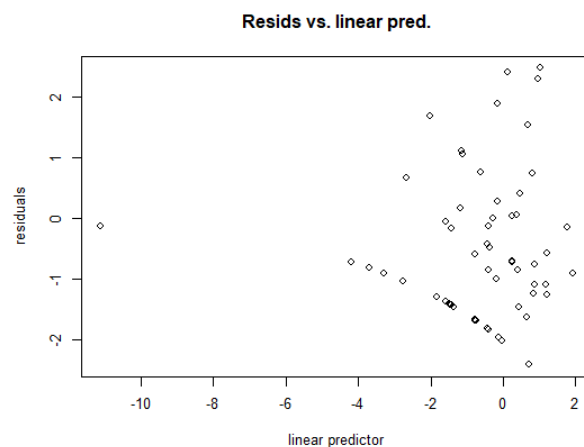
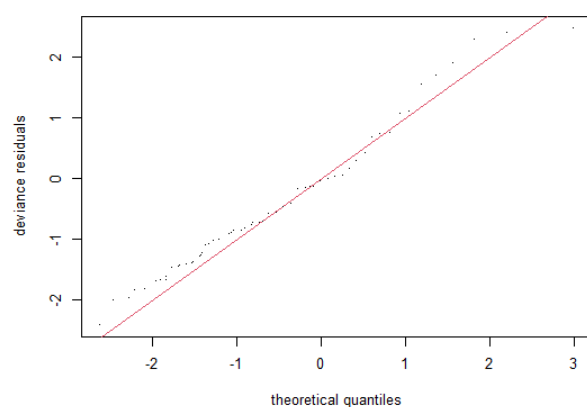
	edf	Ref.df	F	p-value
s(sRoe1,Altitudine):as.factor(Site)1	2.000	2.000	4.486	0.0167 *
s(sRoe1,Altitudine):as.factor(Site)2	2.923	2.992	4.438	0.0104 *
s(sBoar1,Altitudine):as.factor(Site)1	1.793	1.955	2.281	0.0852 .
s(sBoar1,Altitudine):as.factor(Site)2	1.000	1.000	6.364	0.0152 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Rank: 14/15

R-sq.(adj) = 0.101 Deviance explained = 43.4%
-REML = 91.609 Scale est. = 1.6992 n = 57

Figure S1 – Gam model check



9.5. Appendix to chapter 4.3.

Supplementary Table 1. Protocols and primers implemented in the study

Target pathogen	Primers (5' – 3')	Thermic profile	Reaction mixture (25 ul total volume)	Original reference
<i>B. divergens</i>	Gene: <i>18s</i> Forward: GTTTCTGMCCCATCAGCTTGAC Reverse: CAATATTAACACCACGCAAAAATTC	40 cycles: 94°C - 30" 55°C - 20" 72°C - 30"	PCR Master Mix: 1X Primers: 10 pmol DNA: 1 µl	(Hilpertshauser et al., 2006)
<i>B. microti</i>	Gene: <i>18s</i> Bab1: CTTAGTATAAGCTTTTATACAGC Bab4: ATAGGTCAGAACTTGAATGATACA	40 cycles: 94°C - 30" 51°C - 20" 72°C - 30"	PCR Master Mix: 1X Primers: 10 pmol DNA: 1 µl	(Persing et al., 1992)
<i>A. phagocytophilum</i>	Gene: <i>groEL</i> EphplgroEL(569)F: ATGGTATGCAGTTTGATCGC EphplgroEL(1193)R: TCTACTCTGTCTTTGCGTTC	40 cycles: 94°C - 30" 55°C - 30" 72°C - 45"	HotStarTaq DNA Polymerase: 2.5 U Primers: 25 pmol DNA: 2.5 µl dNTPs mix: 0.2 mM PCR buffer: 1X	(Alberti et al., 2005)

<i>B. burgdorferi</i> s.l.	Gene: spacer region between 5S and 23S <i>rRNA</i> genes 23SN1: ACCATAGACTCTTATTACTTTGAC 23SC1: TAAGCTGACTAATACTAATTACCC	Touch down: 94°C - 20" 60°C - 30" (-1°C every 2 cycles) 72°C - 30" 25 cycles: 94°C - 20" 50°C - 30" 72°C - 30"	HotStarTaq DNA Polymerase: 2.5 U Primers: 20 pmol DNA: 2.5 µl dNTPs mix: 0.2 mM PCR buffer: 1X	(Rijpkema et al., 1995)
SFG <i>Rickettsia</i>	Gene: surface protein <i>rOmpA</i> 190-70: ATGGCGAATATTTCTCCAAAA 90-701: GTTCCGTTAATGGCAGCATCT	40 cycles: 94°C - 30" 55°C - 30" 72°C - 1'	HotStarTaq DNA Polymerase: 2.5 U Primers: 7 pmol DNA: 5 µl dNTPs mix: 0.2 mM PCR buffer: 1X	(Roux et al., 1996)
<i>T. capreoli</i>	Gene: 18s Forward: TGTGGCTTATTTCCGTTATAAAAT Reverse: AAAAGCTTATCCCGTACCCTA	40 cycles: 94°C - 30" 55°C - 30" 72°C - 1'	PCR Master Mix: 1X Primers: 25 pmol DNA: 1 µl	This paper. Reference sequence accession number: AY726011.1

9.6. Appendix to chapter 5.1.

Supplementary Table 1. PCR protocols implemented in the present study, with primers and literature sources from where they were taken, and thermic profile and mix adapted to the present study.

Pathogen	Primers (5' – 3')	Thermic profile	Reaction mixture (25 ul)
<i>B. divergens</i>	18s (Hilpertshauser et al., 2006) Forward: GTTTCTGMCCCATCAGCTTGAC Reverse: CAATATTAACACCACGCAAAAATTC	95°C - 5' 40 cycles: 94°C - 30" 55°C - 20" 72°C - 30" 72°C - 10'	PCR Master Mix: 1X (Promega Corporation, WI, USA) Primers: 10 pmol DNA: 1 µl
<i>B. microti</i>	18s (Persing et al., 1992) Bab1: CTTAGTATAAGCTTTTATACAGC Bab4: ATAGGTCAGAACTTGAATGATACA	95°C - 5' 40 cycles: 94°C - 30" 51°C - 20" 72°C - 30"	PCR Master Mix: 1X (Promega Corporation, WI, USA) Primers: 10 pmol DNA: 1 µl

		72°C - 10'	
<i>B. venatorum</i>	18s (Michelet et al., 2014) Bab_EU_RNA18S_F: GCGCGCTACACTGATGCATT Bab_EU_RNA18S_R: CAAAAATCAATCCCCGTCACG Bab_EU_RNA18S_P: CATCGAGTTTAATCCTGTCCCGAAAG G	Preamplification: 95°C - 5' 20 cycles: 94°C - 30" 57°C - 1' 72°C - 10'	PCR Master Mix: 1X (Promega Corporation, WI, USA) Primers: 22.5 pmol DNA: 5 µl
		95°C - 10' 45 cycles: 95°C - 15" 60°C - 1'	Taqman Universal: 1X (ThermoFisher, MA, USA) Primers: 22.5 pmol Probe: 6.25 pmol Template: 5 µl
Anaplasmataceae	16S rRNA (Battisti et al., 2020; Goodman et al., 1996) PER1: TTTATCGCTATTAGATGAGCCTATG PER2: CTCTACACTAGGAATTCCGCTAT	95°C - 15' 40 cycles: 94°C - 1" 52.4°C - 45" 72°C - 1"	HotStarTaq DNA Polymerase: 2.5 U (Qiagen, Hilden, Germany) Primers: 25 pmol MgCl₂: 0.5 mM DNA: 1 µl

		72°C - 10'	dNTPs mix: 0.2 mM (Sigma-Aldrich, St. Louis, MO, USA) PCR buffer: 10X
<i>A. phagocytophilum</i>	Gene: <i>groEL</i> (Alberti et al., 2005) EphplgroEL(569)F: ATGGTATGCAGTTTGATCGC EphplgroEL(1193)R: TCTACTCTGTCTTTGCGTTC	40 cycles: 94°C - 30" 55°C - 30" 72°C - 45"	HotStarTaq DNA Polymerase: 2.5 U (Qiagen, Hilden, Germany) Primers: 25 pmol DNA: 2.5 µl dNTPs mix: 0.2 mM (Sigma-Aldrich, St. Louis, MO, USA) PCR buffer: 10X
<i>B. burgdorferi</i> s.l.	<i>spacer region between 5S and 23S rRNA genes</i> (Rijpkema et al., 1995) 23SN1: ACCATAGACTCTTATTACTTTGAC 23SC1: TAAGCTGACTAATACTAATTACCC 23SN2: ACCATAGACTCTTATTACTTTGACCA	95°C - 15' Touch down: 94°C - 20" 60°C - 30" (-1°C every cycle) 72°C - 30" 25 cycles: 94°C - 20"	HotStarTaq DNA Polymerase: 2.5 U (Qiagen, Hilden, Germany) Primers: 20 pmol DNA: 2.5 µl dNTPs mix: 0.2 mM (Sigma-Aldrich, St. Louis, MO, USA) PCR buffer: 10X

	5SCB: GAGAGTAGGTTATTGCCAGGG	55°C - 30'' 72°C - 30'' 72°C - 10'	
SFG Rickettsia	<i>surface protein rOmpA</i> (Roux et al., 1996) 190-70: ATGGCGAATATTCTCCAAAA 90-701: GTTCCGTTAATGGCAGCATCT	95°C - 15' 40 cycles: 94°C - 30'' 55°C - 30'' 72°C - 1' 72°C - 10'	HotStarTaq DNA Polymerase: 2.5 U (Qiagen, Hilden, Germany) Primers: 7 pmol DNA: 5 µl dNTPs mix: 0.2 mM (Sigma- Aldrich, St. Louis, MO, USA) PCR buffer: 10X

Supplementary Figure 1. Barplot of prevalences per sampled group for *B. divergens* (a), *B. venatorum* (b) and *B. microti*-like (c). For wildlife, a detailed barplot was added. Icons represent, in order: dogs (only in c), humans, questing ticks and wildlife; chamois (*Rupicapra rupicapra*, only in a and b), red deer (*Cervus elaphus*, only in a and b) and wild boar (*Sus scrofa*).

9.7. Appendix to chapter 5.2.

Supplementary Table 1. Translated version of the survey submitted to the participants to the study. The original survey was submitted in Italian.

Survey									
<i>Demographic data</i>									
Gender:									
Age	<20	20-30	31-40	41-50	51-60	>60			
Municipality of residence									
<i>Exposure to tick bite</i>									
Employment:									
Do you engage in any of the following activities that expose you to tick bites:									
☐	Access to a farm with livestock								
☐	House near the woods								
☐	Garden with wildlife access								
☐	Leisure outdoor activity: hunt								
☐	Leisure outdoor activity: fishing								
☐	Leisure outdoor activity: camping, trekking, cycling								
☐	Leisure outdoor activity: mushroom/other products picking								
Weekly frequency:	<1	1	2	3	4	5	6	7	
Mean of daily hours spent outdoor:	<10					>10			
Do you have any medical conditions?									
Long clothing while outdoor			Always	Sometimes			Never		
Use of spray repellents while outdoor			Always	Sometimes			Never		
Tick check once back home			Always	Sometimes			Never		
<i>Ticks bite</i>									
Annual frequency:									
Region:									
Environment:	Wood	Meadow	Cultivated field		Urban/periurban park				
Symptoms after tick bite:									
Further medical analysis:									
Antibiotic therapy:									

Supplementary Table 2. PCR protocols implemented in the study. We present the primers and the publication they were taken from and the thermic profile and reaction mixture adapted to the current study.

Pathogen	Primers (5' – 3')	Thermic profile		Reaction mixture (25 ul)	Reference
Piroplasmids	18s RLB-F2: GACACAGGGAGGTAGTGACAAG RLB-R2: CTAAGAATTCACCTCTGACAGT RLB-FINT: GACAAGAAATAACAATACRGGGC	1 step: 95°C - 5' 25 cycles 95°C - 30" 55°C - 15" 72°C - 90" 72°C - 10'	2 step: 95°C - 5' 40 cycles 95°C - 30" 58°C - 15" 72°C - 90" 72°C - 10'	PCR Master Mix: 1X (Promega Corporation, WI, USA) Primers: 10 pmol DNA: 1 µl	(Centeno-Lima et al., 2003; Gubbels et al., 1999; Schnittger et al., 2004; Teyseyre, 2005; Zanet et al., 2014)
<i>B. divergens</i>	18s Forward: GTTTCTGMCCCATCAGCTTGAC Reverse: CAATATTAACACCACGCAAAAATTC	95°C - 5' 40 cycles: 94°C - 30" 55°C - 20" 72°C - 30"		PCR Master Mix: 1X (Promega Corporation, WI, USA) Primers: 10 pmol DNA: 1 µl	(Hilpertshauser et al., 2006)

		72°C - 10'		
<i>B. microti</i>	18s Bab1: CTTAGTATAAGCTTTTATACAGC Bab4: ATAGGTCAGAACTTGAATGATACA	95°C - 5' 40 cycles: 94°C - 30" 51°C - 20" 72°C - 30" 72°C - 10'	PCR Master Mix: 1X (Promega Corporation, WI, USA) Primers: 10 pmol DNA: 1 µl	(Persing et al., 1992)
<i>B. venatorum</i>	18s Bab_EU_RNA18S_F: GCGCGCTACACTGATGCATT Bab_EU_RNA18S_R: CAAAAATCAATCCCCGTCACG Bab_EU_RNA18S_P: CATCGAGTTTAATCCTGTCCCGAAAGG	Preamplification: 95°C - 5' 20 cycles: 94°C - 30" 57°C - 1' 72°C - 10'	PCR Master Mix: 1X (Promega Corporation, WI, USA) Primers: 22.5 pmol DNA: 5 µl	(Michelet et al., 2014)
		95°C - 10' 45 cycles: 95°C - 15" 60°C - 1'	Taqman Universal: 1X (ThermoFisher, MA, USA) Primers: 22.5 pmol Probe: 6.25 pmol Template: 5 µl	

Anaplasmataceae	16S rRNA PER1: TTTATCGCTATTAGATGAGCCTATG PER2: CTCTACACTAGGAATTCCGCTAT	95°C - 15' 40 cycles: 94°C - 1'' 52.4°C - 45'' 72°C - 1'' 72°C - 10'	HotStarTaq DNA Polymerase: 2.5 U (Qiagen, Hilden, Germany) Primers: 25 pmol MgCl₂: 0.5 mM DNA: 1 µl dNTPs mix: 0.2 mM (Sigma-Aldrich, St. Louis, MO, USA) PCR buffer: 10X	(Battisti et al., 2020; Goodman et al., 1996)
<i>B. burgdorferi</i> s.l.	spacer region between 5S and 23S rRNA genes 23SN1: ACCATAGACTCTTATTACTTTGAC 23SC1: TAAGCTGACTAATACTAATTACCC 23SN2: ACCATAGACTCTTATTACTTTGACCA 5SCB: GAGAGTAGGTTATTGCCAGGG	95°C - 15' Touch down: 94°C - 20'' 60°C - 30'' (-1°C every cycle) 72°C - 30'' 25 cycles: 94°C - 20'' 55°C - 30''	HotStarTaq DNA Polymerase: 2.5 U (Qiagen, Hilden, Germany) Primers: 20 pmol DNA: 2.5 µl dNTPs mix: 0.2 mM (Sigma-Aldrich, St. Louis, MO, USA)	(Rijpkema et al., 1995)

		72°C - 30'' 72°C - 10'	PCR buffer: 10X	
SFG Rickettsia	<i>surface protein rOmpA</i> 190-70: ATGGCGAATATTTCTCCAAAA 90-701: GTTCCGTTAATGGCAGCATCT	95°C - 15' 40 cycles: 94°C - 30'' 55°C - 30'' 72°C - 1' 72°C - 10'	HotStarTaq DNA Polymerase: 2.5 U (Qiagen, Hilden, Germany) Primers: 7 pmol DNA: 5 µl dNTPs mix: 0.2 mM (Sigma- Aldrich, St. Louis, MO, USA) PCR buffer: 10X	(Roux et al., 1996)

Supplementary Table 3. Commercial IFAT or ELISA kit implemented to detect IgG antibodies against the pathogens targeted in the study.

Target	Product implemented	Cross-reactivity detected
Determination by Indirect Fluorescent Antibody Test (IFAT)		
<i>B. divergens</i>	Babesia divergens IgG IFA kit, DVG-120 Fuller Laboratories, Fullerton, California, USA	<i>Plasmodium</i> spp. and <i>Babesia</i> sp.
<i>B. microti</i>	Babesia microti IgG IFA Kit, BMG-120 Fuller Laboratories, Fullerton, California, USA	No
<i>A. phagocytophilum</i>	HGE IgG IFA kit, EEG-120 Fuller Laboratories, Fullerton, California, USA	<i>A. platys</i>

Determination by immunoenzimatic essay (ELISA)		
<i>Rickettsia conorii</i>	Rickettsia conorii ELISA IgG IgM, VL027 Vircell, Granada, Spain	Spotted Fever Group
TBE virus	TBE/FSME IgG ELISA, DETICG0440 Demeditec, Kiel, Germany	Flavivirus
<i>B. burgdorferi</i> s.l.	BORRELIA IgG (LYME DISEASE) EIA, AVO ESR121G Institut Virion\Serion GmbH, Wurzburg, Germany	<i>Leptospira</i> , <i>Treponema pallidum</i> , Rheumatoid Factor