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Zoonotic tick-borne pathogens in domestic animals and associated ticks: emerging threat and One Health perspective

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Preface

The investigations described in this thesis were primarily performed at the Department of Veterinary Medicine, University of Bari. The thesis follows a "thesis by publication" format and consists of four articles published in international peer-reviewed journals. Each article reflects the original intellectual contributions of the co-authors based on their involvement in the studies.

The thesis, entitled "Zoonotic tick-borne pathogens in domestic animals and associated ticks: emerging threat and One Health perspective," is organized into two main chapters and further sections:

Chapter 1. "Grazing systems and tick infestation in livestock: zoonotic risks and control strategies"–composed of Section 1.1 and 1.2, which include two articles published in *Acta Tropica* and *Veterinary Parasitology: Regional Studies and Reports*.

Chapter 2. "Zoonotic pathogens in ticks and domestic animals: why continued surveillance matters"–composed of Section 2.1 and 2.2, which include two articles published in *Acta Tropica*.

This final dissertation represents the original intellectual property of the author, who carried out the development and writing.

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General introduction

Ticks are obligate hematophagous arthropods that parasitize a wide range of vertebrate hosts, including wild and domestic animals as well as humans, hence being of major veterinary and

medical importance. Their medical relevance is mainly linked to their ability in transmitting a variety of pathogens, many of which are of zoonotic interest (**Springer et al., 2020**). Indeed, tick-borne pathogens (TBPs) of zoonotic concern include viruses (e.g., tick-borne encephalitis virus, Crimean-Congo hemorrhagic fever virus), bacteria (e.g., *Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp.), and protozoa (e.g., *Babesia* spp.), which collectively account for a substantial burden of disease worldwide (**Springer et al., 2020**; **Rodriguez-Morales et al., 2018**). The global relevance of ticks and tick-borne diseases (TBDs) is underscored by their impact on animal health, human health, and livestock production systems, which together have important socioeconomic consequences.

Domestic animals play a central role in the epidemiology of TBPs. They can be clinically affected by a range of TBDs, leading to direct economic losses through reduced productivity, mortality, and costs of treatment and control (**Singh et al., 2022**). Beyond their veterinary significance, domestic animals may act as sentinel hosts, signaling the presence of pathogens in particular contexts, or serve as reservoirs and amplifying hosts for zoonotic agents (**Springer et al., 2020**). In some cases, they may facilitate the transmission of pathogens to humans through direct or indirect pathways; for example, viremic ruminants can transmit tick-borne encephalitis virus via the consumption of unpasteurized dairy products (**Tomassone et al., 2025**). Thus, the health of livestock, humans, and ecosystems is tightly interconnected within the context of One Health.

The risk of tick-borne infections is shaped by ecological and management factors. Livestock grazing systems and management of kennel dogs may affect tick intensity, abundance, and host–vector–pathogen cycles (**Jimale et al., 2024a**; **Zahri et al., 2025**). Silvo-pasture grazing, for instance, increases exposure to questing ticks (**Jimale et al., 2024a)**, whereas poor management practices in kenneled dogs may favor higher parasitism and increased host–tick contact (**Zahri et al., 2025**). Climate change, land-use modifications, and changes in wildlife populations further contribute to the expansion of tick habitats and the emergence of novel tick–pathogen associations (**Diuk-Wasser et al., 2021**). As a result, new zoonotic threats continue to arise in both endemic and previously unaffected regions. For instance, *Colpodella* spp., close relatives of apicomplexans with zoonotic potential, were previously reported from human patients and ticks in China, and has recently been molecularly detected in cattle ticks in Italy (**Jimale et al., 2024b**).

Addressing the threat of zoonotic TBPs (ZTBPs) requires an integrated One Health approach, recognizing that the health of humans, animals, and ecosystems are interdependent. This framework emphasizes the importance of cross-sectoral collaboration to understand the ecology of ticks, assess the role of domestic animals in pathogen circulation, and design effective surveillance and control strategies (**Johnson et al., 2022**). Sustained surveillance of tick populations and TBPs in domestic animals is crucial for early detection of emerging threats, guiding veterinary interventions, and protecting public health (**Jimale et al., 2024b**).

The aim of this thesis was to address some knowledge gaps about ZTBPs in domestic animals and associated ticks, with a focus on their epidemiology, diagnosis and control strategies within a One Health framework. Specifically, the thesis examines the impact of different management practices on tick infestation in free-grazing livestock (i.e., cattle, sheep, goats and camel) and sheltered dogs, and evaluates the zoonotic risks in these contexts.

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Abstract

This thesis presents original data on the epidemiology, diagnosis, and potential control strategies of ticks and associated pathogens of zoonotic relevance in domestic animals, with an

emphasis on the One Health perspective. The study examines the impact of different management practices on tick parasitism in free-grazing livestock and dog kennels, and assesses the risks of zoonoses in these contexts, underscoring the importance of continued surveillance for emerging pathogens.

Chapter 1 is entitled "Grazing systems and tick infestation in livestock: zoonotic risks and control strategies" and is divided into two sections that focus: (i) ticks and associated pathogens in livestock (i.e., camel, sheep, goats, and cattle) under pastoral lifestyles, and (ii) the impact of different grazing systems (i.e., open pasture vs silvopasture) on tick infestation in cattle and the consequent risk of zoonotic exposure, highlighting control strategies for sustainable management.

Chapter 2 is entitled "Zoonotic pathogens in ticks and domestic animals: why continued surveillance matters" and is divided into two sections that address: (i) zoonotic pathogens associated with ticks and livestock hosts (i.e., cattle, and goats) in southern Italy, and (ii) zoonotic pathogens associated with ticks and canine hosts in central Morocco, emphasizing the critical role of ongoing surveillance for the early detection of emerging zoonotic threats.

Finally, the general **discussion and conclusions** section underscores the dynamic nature of zoonotic tick-borne pathogens (ZTBPs) at the interface of humans, animals, and tick vectors. It demonstrates how management practices, such as grazing systems, can shape tick infestation dynamics and zoonotic risk, with silvopasture and pastoral systems being favours. Novel findings, including the first report of *Colpodella* sp. in ticks in Italy and the detection of pathogens in Moroccan kennel dogs, highlight the circulation of emerging and potentially zoonotic agents. These results stress the need for strengthened diagnostic capacity, sustained surveillance, and the integration of management-based control strategies within a One Health framework to mitigate the risks of ZTBPs in both endemic and resource-limited settings.

Chapter 1. Grazing systems and tick infestation in livestock: zoonotic risks and control strategies

Section 1.1

Ticks and tick-borne pathogens of domestic animals in Somalia and neighbouring regions of Ethiopia and Kenya

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Abstract
Ticks and tick-borne pathogens (TBPs) of domestic animals in Somalia and neighbouring regions of Ethiopia and Kenya are reviewed to identify knowledge gaps in these regions, where unrestricted livestock movements across borders are common. Major scientific databases, such

as PubMed, Web of Science, Scopus, CABI, and Google Scholar were searched, to retrieve articles based on papers published between 1960 and March 2023. Thirty-one tick species representing six genera (*Rhipicephalus, Hyalomma, Amblyomma, Haemaphysalis, Ornithodoros* and *Argas*) were reported to infest domestic animals, mainly livestock. Overall, the most represented species were *Rhipicephalus pulchellus* (up to 60% of specimens identified), followed by *Hyalomma dromedarii* (up to 57%), *Hyalomma truncatum* (up to 57%), *Amblyomma lepidum* (up to 21%), *Amblyomma variegatum* (up to 21%) and *Amblyomma gemma* (up to 19%), with morphological characterization being the principal method of tick identification. In addition, 18 TBPs, including zoonotic pathogens (e.g., Crimean-Congo haemorrhagic fever virus), were detected, with *Babesia* spp., *Theileria* spp., and *Rickettsia* spp. being the most commonly reported. Half of the pathogens documented were detected using molecular techniques, while the other half were detected by serology and microscopic techniques. Generally, ticks and TBPs in the region are under-studied, particularly, data relating to pet animals and equines is lacking. Further, the infection intensity and herd prevalence of ticks and TBPs is unclear because of insufficient data and poor approaches to quantitative analysis, making it difficult to propose management policies in the region. There is an urgent need, therefore, for more and better studies, particularly those that take a 'One Health' perspective, focusing on the prevalence and socioeconomic impact of ticks and TBPs in animals as well as in humans, so that sustainable control strategies against them can be planned.

Keywords: Horn of Africa; Zoonosis; Livestock; One Health; Prevalence; Surveillance

1. Introduction

Ticks present a major threat to livestock production in many parts of the world both directly, through their blood-feeding and, indirectly, through the transmission of a range of viral, bacterial and protozoal pathogens (McGinley-Smith and Tsao, 2003). Some of these pathogens are zoonotic and of public health concern (de la Fuente et al., 2008). The effect of ticks and tick-borne pathogens (TBPs) on the economies and livelihoods of people dependent on livestock is particularly pronounced in Africa (Sahil et al., 2004). In sub-Saharan Africa, population growth and grazing pressure, exacerbated by socioeconomic pressures and human-induced environmental changes, are resulting in increasing exposure of domestic animals and humans to ticks and TBPs (Heylen et al., 2021). To allow more effective management of ticks and TBPs, precise information about the distribution, abundance and phenology of the various species of ticks in a geographical area is of importance, as it allows more precise and regionally focused control measures to be implemented. Such information is particularly poorly reported for the Horn of Africa including the Somali peninsula, both because of the paucity of studies and the scattered nature of the published reports which, considering the suitability of the region for ticks (Pegram, 1976; Schoepf et al., 1984), the socioeconomic importance of livestock, and the unrestricted movements of animals (both pastoralism and unofficial trade) between Somalia and its neighbours (Ortiz-Pelaez et al., 2010; Teka and Azeze, 2002), makes the development of a better understanding of these issues of importance. Therefore, the aim of this study was to review published data on the occurrence and distribution of ticks and TBPs of domestic animals in Somalia and neighbouring regions of Ethiopia and Kenya, to highlight knowledge gaps and establish baseline information in designing future research directions.

2. Geography and the livestock sector of Somalia and neighbouring regions

Somalia, formerly known as the Somali Democratic Republic, is a coastal nation which occupies the tip of a region commonly referred to as the "Greater Horn of Africa" (Fig. 1), that also includes Ethiopia, Djibouti, and Eritrea. This country is bordered by the Indian Ocean to

the east, the Gulf of Aden to the north, Djibouti to the northwest, Ethiopia to the west, and Kenya to the southwest. Somalia has a surface area of about 637,700 km^2 and a coastline of approximately 3300 km (Gure, 2021). Somalia historically consists of two main regions: southern Somalia (including central and eastern regions), and northern Somalia (Somaliland). The climate and agroecology of Somalia and neighbouring regions of Ethiopia and Kenya is generally arid to semi-arid lands (ASAL) (Table 1), with a bimodal annual rainfall pattern, with major (*Gu*) rains from April to July and the minor *Dayr* rains from September to November. The rainy seasons are separated by two dry spells known locally as *Jilaal* (January-March) and *Hagaa* (July-September) (Gure, 2021). The average annual rainfall is about 300 mm in most parts of the region. Only the northern coastline of Somalia receives significantly less rainfall (up to 50 mm). Rainfall in southern Somalia is higher at approximately 400 mm and highest in the southwest with around 600 mm rainfall on average (SWALIM/FAO, 2007). Annual mean temperature is close to 30°C throughout the region (Table 1). June to September are the hottest months in the north, and December to March the hottest for the south. The vegetation in this region is generally characterized by bushy and spiny shrubs with scattered trees, predominantly acacia (Abebe et al., 2010).

Livestock production is considered economically fundamental in the region, since it accounts for at least 40% of the gross domestic product of Somalia, 80% of the foreign exchange earnings and generates 65% of the employment opportunities (Warsame et al., 2022; Too et al., 2016). As a result, most of the population is directly or indirectly involved in livestock production. The people in rural areas live a nomadic lifestyle which represents the most productive activity in the majority of Eastern Africa's rangelands (Lind et al., 2020). Yet, pastoralists in the region face myriad of challenges including recurrent droughts, flash floods, epidemics, poor infrastructure, and livestock export bans, exacerbated by climate change coupled with lack of sectoral emergency preparedness (Mtimet et al., 2021; Lwanga-Ntale and Owino, 2020). Various cross breeds of *Bos indicus* cattle and camels (*Camelus dromedarius*), sheep (*Ovis aries*), goats (*Capra aegagrus hircus*), and also horses (*Equus caballus*) and donkeys (*Equus asinus*) are kept. Pastoral movements across Somalia's borders have occurred for centuries (Ortiz-Pelaez et al., 2010), resulting in seasonal movement patterns of herds/flocks in search of water and pasture. This cross-border migration usually occurs in the seasons of spring (*Gu* rain) and in autumn (*Dayr* rain) which may result in introduction of different tick species and related pathogens into new areas (Fig. 1). The spread of vectors and vector-borne diseases in the region under examination is also influenced by the pastoralism and unofficial animal trade movements between countries in the Horn of Africa (Teka and Azeze, 2002). Since the neighboring countries of Somalia have different ecological and climatic features, which might affect tick and TBP distributions (Kaba, 2022; Herrero et al., 2010), only data from Ethiopian and Kenyan regions close to Somalia borders were included in this study.

Table 1. The agroecology, average annual rainfall (mm) and average annual temperature (°C) in Somalia and neighbouring regions of Ethiopia and Kenya.

Administrational zone	Agroecology	Average	Average annual

		annual rainfall (mm)	temperature (C°)
Southern Somalia	Semi-arid to arid	400–500	27–29
Northern Somalia (Somaliland)	Arid to Semi- arid	200–300	25–28
Somali State of Ethiopia	Arid to Semi-arid	300–400	25–28
Northeastern Kenya	Arid to Semi-arid	250–300	27–29

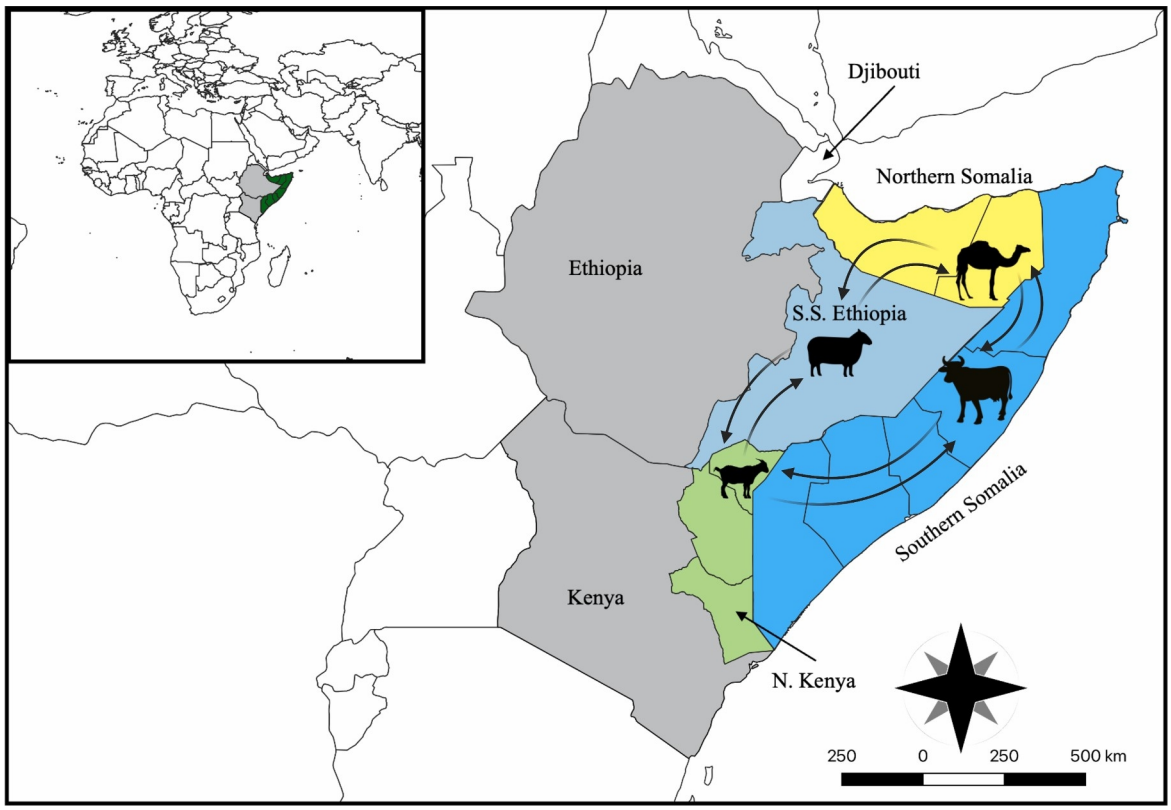

Fig. 1. Map of the study regions showing livestock crossing borders. The colours symbolize different administrative regions of the study.

3. Literature search and bibliographic analysis

Databases, including PubMed, Web of Science, Scopus, CABI, and Google Scholar, were used to search for literature on ticks and TBPs in Somalia and bordering regions using search terms related to ticks and tick-borne pathogens (i.e., ticks, ixodid or argasid ticks and TBPs of domestic animals or livestock) followed by the name of each administrational region (i.e., southern Somalia, northern Somalia, Somali State of Ethiopia or Northeastern Kenya). All accessible studies, published in English and relevant to the region in question, from 1960 to March 2023, were included in this study. Case studies, clinical reports and online posts were not included. The geographical location of each study reviewed was classified as southern or northern Somalia, Somali State of Ethiopia or Northeastern Kenya. Only tick species names given by Guglielmone et al. (2010) were considered as valid and used here. As a result, the genus *Boophilus* is replaced by *Rhipicephalus* and the species *Hyalomma erythraeum* is replaced by *Hyalomma impeltatum*.

Overall, only 20 relevant and accessible studies were identified with the first published in 1975 (Edelsten, 1975) and the most recent in December 2022 (Ferrari et al., 2022). Only two papers on ticks and TBPs were published in the 1980s, and none between 1990 and 2010 (Tables 2, 3). Over the last three decades only six articles were published in both regions of Somalia. This paucity of data is likely to be due to lack of researchers and research-oriented institutions interested in the area and the limited resources and poorly equipped laboratories. In addition, the political upheaval and social disruptions in Somalia and greater Horn of Africa in the past three decades (Ibrahim et al., 2022), probably also contributed to the lack of local and international research projects on ticks and TBPs. Of the 20 papers considered, 10 were on ticks only, five on TBPs and five on the combination of ticks and TBPs. The greatest number

of studies (n = 7) were conducted in Somali State of Ethiopia, and southern Somalia (n = 7) followed by northern Somalia (n = 4). Only two studies describing ticks and/or TBPs from Northeastern Kenya were found.

4. Tick diversity and distribution

Thirty-one tick species, belonging to six genera, were recorded from domestic animals mainly in livestock (Fig. 2). Ixodid ticks were represented by four genera (*Rhipicephalus, Amblyomma, Hyalomma* and *Haemaphysalis*) of which *Rhipicephalus pulchellus, Hyalomma rufipes, Hy. truncatum, Amblyomma gemma, Am. lepidum* and *Am. variegatum* were the most reported tick species (Fig. 2). Argasid ticks were represented by two genera (*Ornithodoros* and *Argas*) of which only two species (*Ornithodoros savignyi* and *Argas persicus*) were found; both species were reported in northern Somalia (Pegram, 1976). The most-reported tick genus was *Rhipicephalus*, which was represented by thirteen species, followed by *Hyalomma* and *Amblyomma*, which were represented by nine and six species, respectively and *Haemaphysalis* with only one species (Fig. 2). Of the four regions investigated, 25 tick species (i.e., 23 hard ticks and two soft ticks) were recorded in northern Somalia (Pegram, 1976), while in southern Somalia 14 hard tick species were documented (Schoepf et al., 1984; Scramella, 1983; Hassan et al., 2022). For the neighbouring regions of Somalia (i.e., Somali State of Ethiopia and Northeastern Kenya), ten ixodid tick species were reported in each of them (Tomassone et al., 2016; Feyera et al., 2017; Hussen and Agonafir, 2018; Lutomiah et al., 2014; Sang et al., 2011) (Table 2).

Six tick species (Fig. 3) were identified as common and widespread in the two regions of Somalia and bordered regions of Ethiopia and Kenya (i.e., *Rh. pulchellus, Hy. truncatum, Hy. dromedarii, Am. gemma, Am. lepidum* and *Am. variegatum*). In addition, it was reported that these tick species are carriers of TBPs in the region including zoonotic pathogens, such as *Rickettsia aeschlimannii* (Tomassone et al., 2016). Furthermore, in a study conducted in Northeastern Kenya, at the border with southern Somalia, Crimean-Congo haemorrhagic fever virus (CCHFV) was detected in *Hy. truncatum* (Sang, 2011), suggesting that this virus may also circulate in the other bordering regions, where its vector is common. For instance, *Hy. marginatum*, the competent vector of CCHFV (Gargili et al., 2017), is endemic in the Somali territories (Pegram, 1976; Lutomiah et al., 2014). Moreover, it was reported that *Am. gemma* collected from cattle in Somali State of Ethiopia was positive of *Rickettsia africae* (Tomassone et al., 2016).

Table 2. The species of ticks recovered from different hosts, and where reported the percentage of each species in the sample, in northern and southern Somalia and neighbouring regions of Ethiopia and Kenya, along with the appropriate reference.

Administrative region	**Host**	**Tick species (%)**	**References**
Southern Somalia	Cattle	*Amblyomma gemma, Am. lepidum, Am. variegatum, Rhipicephalus pravus, Rh. pulchellus, Rh. simus, Rh. humeralis, Hyalomma rufipes, Hy. truncatum*	Schoepf et al., 1984
Southern Somalia	Sheep, goats	*Rh. pulchellus* (64.97%), *Rh. e. evertsi* (8.45%), *Rh. pravus* (5.53%), *Am. lepidum* (5.22%), *Am. gemma* (3.38%), *Hy. truncatum, Rh. bursa* (2.46%) and *Rh. turanicus* (1.99%)	Hassan et al., 2022
Southern Somalia	Cattle	*Rh. pulchellus* (68.18%), *Am. gemma* (18.18%), *Am. lepidum* (9.09%), *Hy. marginatum* (1.51%), *Hy. rufipes* (1.51%), *Rh. pravus* (1.51%)	Ferrari et al., 2022
Southern Somalia	Goats	*Rh. pulchellus* (34.6%), *Rh. evertsi* (72.8%), *Am. lepidum* (3.7%), *Hy. rufipes* (1%)	Ahmed et al., 2013
Southern	Cattle	*Rh. pulchellus* (60.6%), *Rh. humeralis* (7.2%),	Scaramella,

Somalia		*Rh. sanguineus* (3.9%), *Am. lepidum* (21%), *Am. gemma* (2.7%), *Haemaphysalis leachii muhsami* (4.6%)	1983
Southern Somalia	Camels	*Hy. dromedarii* (56.8%), *Hy. truncatum* (43.2%)	Farah et al., 2017
Northern Somalia	Camels	*Hy. truncatum* (56.7%), *Hy. rufipes* (4.3%), *Hy. anatolicum* (2.3%), *Am. variegatum* (21%), *Am. gemma* (5.7%), *Rh. pulchellus* (5.3%), *Rh. pravus* (4.7%)	Hamza et al., 2019
Northern Somalia	Camels, cattle, sheep, goats, horses	*Am. gemma, Am. variegatum, Hy. dromedarii, Hy. rufipes, Hy. truncatum, Rh. e. evertsi, Rh. pravus, Rh. pulchellus* (60%)	Pegram, 1976
Northern Somalia	Came, cattle, sheep, goats	*Hy. impeltatum, Hy. a. anatolicum, Rh. sanguineus, Rh. simus*	Pegram, R. G. 1976
Northern Somalia	Cattle, sheep, goats	*Am. lepidum, Rh. decoloratus, Rh. longicoxatus*	Pegram, 1976
Northern Somalia	Camels, cattle	*Hy. a. excavatum, Hy. marginatum, Hy. turanicum*	Pegram, 1976
Northern Somalia	Sheep, goats	*Hy. punt, Rh. armatus*	Pegram, 1976
Northern Somalia	Mixed*	*Am. falsomarmoreum, Am. sparsum*	Pegram, 1976
Northern Somalia	Camels, cattle, sheep, goats	*Ornithodoros savignyi*	Pegram, 1976
Northern Somalia	Chicken	*Argas persicus*	Pegram, 1976
Northeastern of Kenya	Cattle, goats	*Rh. pulchellus* (57.8%), *Am. gemma, Am. lepidum, Am. hebraeum, Hy. truncatum* (27.8%), *Hy. marginatum, Rh. appendiculatus*	Lutomiah et al., 2014
Northeastern of Kenya	Sheep, goats	*Rh. pulchellus, Am. gemma, Rh. appendiculatus, Hy. truncatum, Hy. marginatum*	Lutomiah et al., 2014
Administrative region	Host	Tick species (%)	References
Northeastern of Kenya	Camels, cattle	*Rh. pulchellus, Am. gemma, Hy. truncatum, Hy. marginatum, Hy. dromedarii, Rh. annulatus*	Lutomiah et al., 2014
Northeastern of Kenya	Goats	*Am. variegatum*	Lutomiah et al., 2014
Somali State of Ethiopia	Sheep, goats	*Rh. e. evertsi* (34.9%), *Hy. truncatum* (29.7%), *Rh. pulchellus* (21.6%), *Am. variegatum*	Mohammed and

		(21.4%), *Hy. rufipes* (16.7%), *Rh. decoloratus* (11.2%), *Am. gemma* (2.1%).	Admasu, 2015
Somali State of Ethiopia	Sheep, goats	*Rh. pulchellus* (37.5%), *Rh. e. evertsi* (23.8%), *Rh. decoloratus* (21.6%), *Hy. truncatum* (17%)	Habtemichael et al., 2020
Somali State of Ethiopia	Cattle	*Am. gemma* (19%), *Am. Lepidum* (15%), *Am. variegatum* (4%), *Rh. decoloratus* (25%), *Rh. e. evertsi* (10%), *Rh. pulchellus* (25%), *Rh. pravus* (12%), *Hy. rufipes* (2%)	Abebe et al., 2010
Somali State of Ethiopia	Camels	*Rh. pulchellus* (37.5%), *Hy. dromedarii* (20.1%), *Am. gemma* (11.9%), *Hy. truncatum* (8.2%), *Hy. rufipes* (6.3%), *Am. variegatum* (6.2%), *Rh. decoloratus* (5.4%), *Am. Lepidum* (4.6%)	Hussen and Agonafir, 2018
Somali State of Ethiopia	Camels, cattle	*Hy. impeltatum* (1.2%), *Hy. dromedarii* (0.5%), *Hy. truncatum* (2.8%), *Hy. rufipes* (13.3%), *Am. gemma* (9.4%), *Rh. pulchellus* (40.1%), *Rh. pravus* (25.8%)	Tomassone et al., 2012
Somali State of Ethiopia	Goats, sheep	*Rh. pulchellus, Rh. pravus*	Tomassone et al., 2012
Somali State of Ethiopia	Camels	*Rh. pulchellus, Rh. decoloratus, Hy. dromedarii, Hy. truncatum, Hy. rufipes, Am. gemma, Am. variegatum, Am. lepidum*	Feyera et al., 2017

Mixed*=ticks were collected from all domestic animals.

Table 3. Tick-borne pathogens detected in different animal hosts or tick carrier, and where reported their prevalence, host which tick were collected from, in Somalia and neighbouring regions of Ethiopia and Kenya, along with the appropriate reference.

Administrative region	**TBPs**	**Host/Tick (%)**	**Host from which ticks were collected**	**Technique of detection**	**Reference**
Southern Somalia	*Mycoplama wenyonii, Candidatus Mycoplasma haematobovis*'	Cattle (45.94%)	–	Molecular	Ferrari et al., 2022
Southern Somalia	*Coxiella burnetii*	*Hyalomma, Amblyomma*	Camels	Molecular	Frangoulidis et al., 2021
Northern Somalia	*Coxiella burnetii*	Cattle (0.2%)	–	Serology	Schmatz et al., 1978
Northern Somalia	NSDV	Sheep, goats (90%)	–	Virus isolation and Serology	Edelsten, 1975
Northern Somalia	*Babesia ovis*	Sheep (25%), goats (18%)		Parasitological (microscopical) technique	Edelsten, 1975
Northern Somalia	*B. motasi*	Sheep, goats		Parasitological (microscopical) technique	Edelsten, 1975
Northern Somalia	*Ehrlichia* (Cowdria)	Sheep, goats		Bacteriology	Edelsten, 1975

	ruminantium				
Southern Somalia	*Theileria mutans*	Cattle (23%), sheep, goats	–	Serology	Schoepf et at.,1984
Southern Somalia	*Anaplasma marginale*	Cattle, sheep, goats	–	Serology	Schoepf et at.,1984
Southern Somalia	*Babesia* spp. and *Theileria* spp.	Goats (100%)	–	Microscopical (blood film) technique	Ahmed et al., 2013
Somali State of Ethiopia	*Th. mutans*	Cattle, camels (10%)		Molecular	Tomassone et al., 2012
Somali State of Ethiopia	*T. velifera*	Cattle (4%), *Am. gemma* (0.96%)	Cattle	Molecular	Tomassone et al., 2012
Somali State of Ethiopia	*E. ruminantium*	*Am. gemma* (3.4%)	Cattle	Molecular	Tomassone et al., 2012
Somali State of Ethiopia	*R. africae*	*Am. gemma* (28.5%)	Cattle	Molecular	Tomassone et al., 2016
Somali State of Ethiopia	*R. aeschlimannii*	*Hy. rufipes* (88.3%), *Hy. truncatum* (27%)	Cattle	Molecular	Tomassone et al., 2016
Northeastern Kenya	Dugbe virus, Dhori virus	*Rh. pulchellus, Am. hebraeum, Hy. truncatum*	Cattle	Molecular	Lutomiah et al., 2014
Northeastern Kenya	Ngari virus	*Am. gemma*	Cattle	Molecular	Lutomiah et al., 2014
Northeastern Kenya	CCHFV	Hy. rufipes *Hy.* truncatum	Cattle, camels	Molecular	Sang et al., 2011

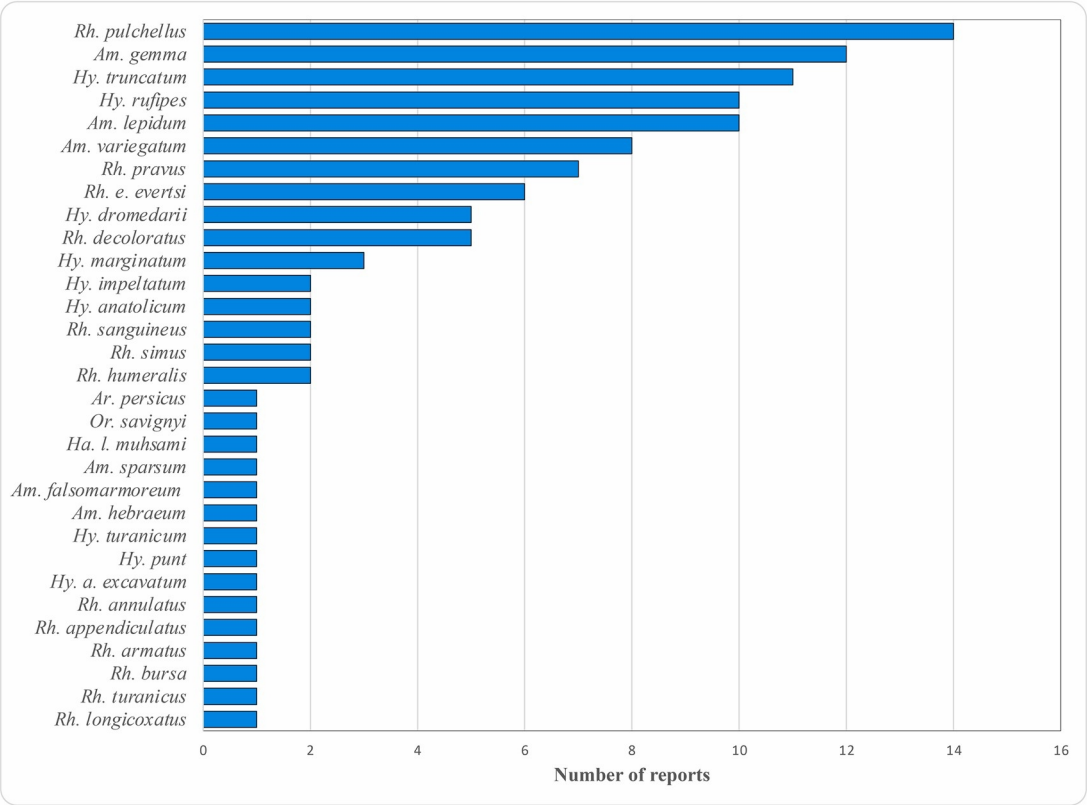

Fig. 2. Tick species frequency by report.

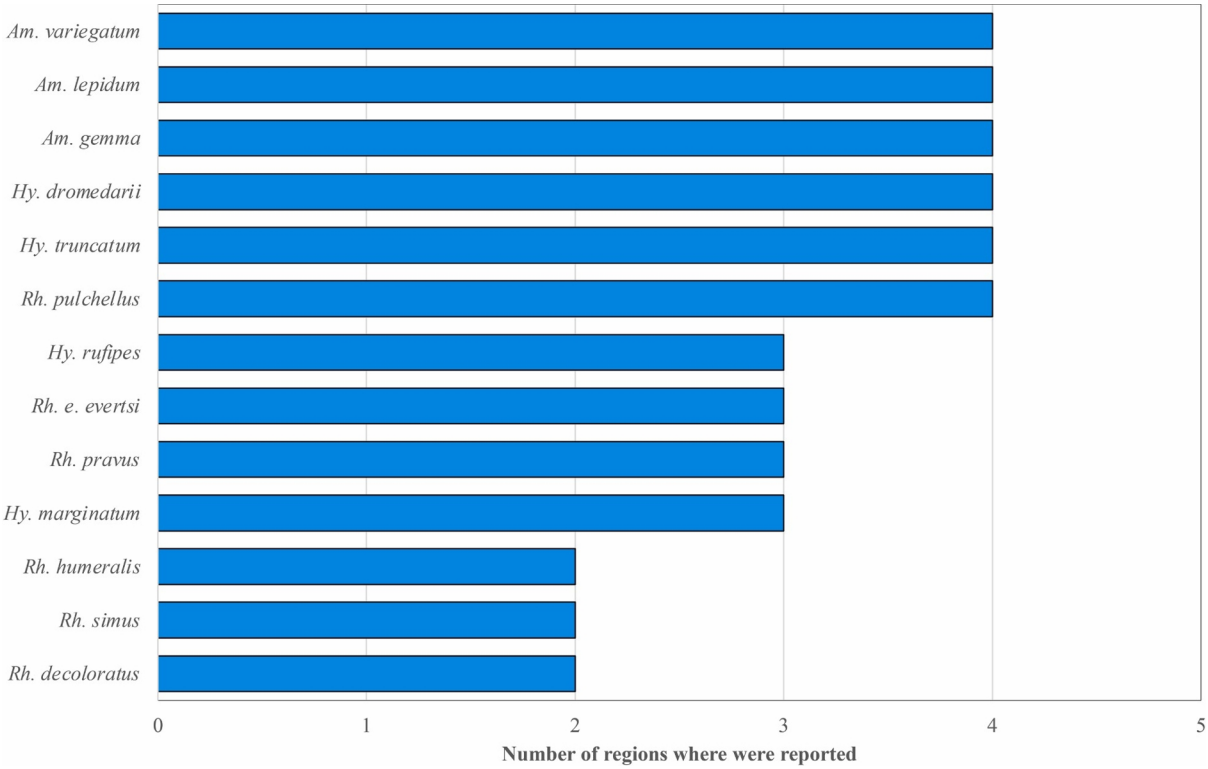

Fig. 3. Tick species reported in more than two regions.

5. Tick borne pathogens

Of the 18 TBPs reported from domestic animals and/or ticks, about 50% were zoonotic pathogens (e.g., *Rickettsia* spp. and CCHFV) and were detected in ticks (n = 6), in animals (n = 9) and in both ticks and animals (n = 3). All the four administrative regions considered in this review had one or more records of TBPs. Among these, *Coxiella burnetii, Theileria* spp., and *Babesia* spp. were the most widespread, followed by *Anaplasma* spp., *E. ruminantium*, and CCHFV of the genus Orthonairovirus (Table 3). The latter is known to cause serious diseases in humans globally (except Australia and the Americas), with high fatality rate of up to 40% (Shahhosseini et al., 2021). In Somalia, there are no data showing whether this virus is circulating in the country despite the presence of its vectors (i.e., *Hy. marginatum* and *Hy. rufipes*). In terms of parasite and pathogen detection, about 50% of the articles reviewed used molecular techniques while the remaining 50% used methods such as serological analysis, microscopy and bacteriological approaches (Table 3). In both regions of Somalia and Somali region of Ethiopia, only four studies used advanced molecular methodology to detect TBPs (Frangoulidis et al., 2021; Tomassone et al., 2016, 2012; Ferrari et al., 2022), and these were studies where the analysis was undertaken in laboratories outside of the region, therefore highlighting the lack of modern laboratories in the regions where the samples were collected.
The data presented here concerns only livestock and information on ticks and TBPs in dogs and cats in the area is completely missing. This gap can be attributed to the fact that the communities of these regions do not routinely keep companion animals, although there is large population of stray dogs and cats in the urban and peri-urban areas (Ghanem et al., 2010). A study conducted in the Oromia region of Ethiopia, which shares a border to the current study area, reported the infestation of ticks (*Ha. leachi*, *Am. variegatum*, *Rh. sanguineus* and *Rh. pulchellus*) on dogs and cats with a 97% of prevalence (Kumsa et al., 2019). On the other hand, despite the large population of equines in the area (Mohamed et al., 2021), there are no reports on ticks or TBPs of these animals in the region.

6. Ways forward, knowledge gaps and general recommendations

Research on ticks and TBPs in the area is clearly under-represented, with only 20 papers published from 1960 through to March 2023. To fill this research gap, existing local institutions and concerned authorities (e.g., universities and ministries) should prioritize research in this area. Key gaps include a detailed understanding of tick intensity of infestation, infection pressure or the true prevalence of infestation or infection, socioeconomic impact and zoonotic burden. One key problem in many existing studies is the report of prevalence based on pooled individual animals from multiple sites with different herd sizes, rather than the appropriate calculation of farm, flock or herd prevalence. Without this basic epidemiological information, it is difficult to develop strategies to help livestock keepers to manage TBDs. In addition, most of the existing research laboratories have relatively poor infrastructure which does not allow cutting-edge research. For instance, there is no veterinary reference laboratory in Somalia. To overcome this, government and university laboratories should be modernized by allocating adequate research budget. As ticks are among the most important vectors of pathogens, in both veterinary and medical contexts, it is important to prioritize research through a One Health approach (Dantas-Torres and Otranto, 2016). To do this, all stakeholders such as veterinarians, medical doctors, environmental scientists, and government authorities should collaborate closely. Finally, given the degree of movement of animals across borders, the establishment intergovernmental tick and TBD surveillance programs are of paramount importance to obtain accurate tick distribution data in the region.

7. Conclusion

Overall, this review has shown that ticks and TBPs of domestic animals in Somalia and neighbouring regions of Ethiopia and Kenya are under-studied. Nonetheless, a relatively high diversity of tick species (n = 31) and many TBPs (n = 18) circulate in the region compared to a recent review article conducted in Ethiopia (Kaba, 2022), which reported 19 tick species and 16 TBPs. In addition, published studies often lack reliable statistical or analytical rigor, and as a result potentially there are unidentified ticks and TBPs circulating in the area, including possible zoonotic pathogens. More studies focusing on domestic animals including dogs, cats and equines and on tick systematics, their biology and associated pathogens, as well as their socioeconomic impact, are warranted.

Author contributions

KAJ, DO, RW: contributed to design and write the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Section 1.2

Grazing system and *Hyalomma marginatum* tick infestation in cattle with high prevalence of SFG *Rickettsia* spp.

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Abstract
Tick-borne diseases (TBDs) represent a significant portion of infectious diseases of global public health interest. In Italy, knowledge about the occurrence of tick-borne pathogens (TBPs) in ticks parasitizing cattle is scarce. In this research, we focused on ticks infesting Maremmana cattle grazing in open pasture and silvopasture systems. After being morphologically identified, ticks were molecularly tested for the presence of pathogens of the genus *Rickettsia*. Of the 794 ticks detected, 117 were collected, being the majority *Hyalomma marginatum* (72.6%) followed by other *Hyalomma* species (23%), *Rhipicephalus turanicus* (1.7%), *Rh. bursa* (0.9%), *Hy. lusitanicum* (0.9%) and *Dermatocentor marginatus* (0.9%). All ticks were adults, 58.1% males and 41.8% females. The highest tick prevalence was noted in April for silvopasture system cattle (90%), and in May for open pasture ones (85%). TBPs were detected only in *Hy. marginatum*, and all belong to *Rickettsia* spp. of zoonotic interest. In particular, 21/40 (52.5%) ticks scored positive for *Rickettsia* spp. by *glt*A gene and of these 15/21 (71.4%) also to spotted fever group (SFG) rickettsiae by *omp*A gene. Of the total positive specimens, 19 were successfully sequenced and scored *Rickettsia aeschilimannii* (17/19, 89.5%), *R. slovaca* (1/19, 5%), and *R. massiliae* (1/19, 5%). This research highlights the potential impact of grazing systems on cattle parasitization by hard ticks. The molecular investigation of TBPs in ticks collected from Maremmana cattle shed light on the presence of pathogenic bacteria of SFG *Rickettsia* spp., pointing out the potential risk of TBPs transmission between livestock and humans.

Keywords: Rickettsioses; Distribution; Livestock; Hard ticks**;** *Hyalomma marginatum;* One Health

1. Introduction

Tick-borne diseases (TBDs) represent an increasing global public health concern. Hard ticks (Acarina: Ixodidae) are hematophagous arthropods that have significant veterinary and medical importance due to their ability to transmit pathogens responsible for diseases in animals and humans (Otranto et al., 2014; Benelli, 2020). They are micro- predators representing the most diverse group with a worldwide distribution and high adaptation to different environments, climates, and hosts (Otranto et al., 2014; Di Giovanni et al., 2021). Several species are implicated in transmitting pathogenic agents, tick-borne pathogens (TBPs), to humans and animal hosts, including cattle (Guccione et al., 2023). Beyond transmitting pathogens, ticks represent an issue for animal productivity in terms of milk and meat, leading in turn to significant economic losses (Jonsson, 2006). In this context, the characteristics of grazing system adopted in livestock farming may lead to differences in tick infestation dynamics on animals, a subject poorly investigated till now. The incidence of TBDs is growing globally; some of these illnesses, such as Lyme and tick-borne encephalitis (TBE), are a major public health concern, while others, such as Crimean-Congo haemorrhagic fever (CCHF), are expanding their spectrum of distribution and threatening to become endemic in new areas (Messina et al., 2015; Stone et al., 2017; Van Heuverswyn et al., 2023). Several *Rickettsia* species are pathogenic to humans causing rickettsioses, a global zoonosis that is the second most frequent non-malarial fever sickness in Southeast Asia, following dengue infection (Robinson et al., 2019). The pathogenic *Rickettsia* spp. can be categorized into two main groups: the spotted fever group (SFG) and the typhus group (TG) (Bhengsri et al., 2016).

Rickettsia spp. may be transmitted by ticks of different genera (e.g., *Dermacentor, Rhipicephalus, Haemaphysalis, Ixodes, Amblyomma* and *Hyalomma*), as well as through other arthropod vectors, including fleas and mites (Guccione et al., 2023). Though the occurrence of TBPs in ticks infesting different host species has been well documented in Italy (Scarpulla et al., 2016; Sgroi et al., 2021), data on those parasitizing cattle is still scant.
To address this research gap, the present study molecularly investigates tick species parasitizing Maremmana cattle in the Tuscany region (central Italy), and the associated pathogens, with an emphasis on rickettsiae. Furthermore, we investigated if Maremmana cattle grazing in an open pasture or in a silvopastoral system were differently parasitized by hard ticks.

2. Material and methods

2.1. Study area

The research was carried out in the Tenuta di Paganico farm located in Grosseto province (Tuscany, central Italy), from April to September 2022, where cattle breeds graze freely on open pasture and silvopasture areas (42° 57′ 10.9″ N 11° 14′ 25.4″ E, 87 a.s.l.) (Fig. 1). Forty growing cattle of the Maremmana breed were randomly allotted to the two grazing systems at the beginning of the trial. The open pasture grazing system consists of about 1.96 ha of grassland where cattle were managed under rotational and continuous grazing during spring and summer respectively. Whereas the silvopastoral grazing system consist of about 1.86 ha of grassland and 3.31 ha of Turkey-oak forest with no understory vegetation. Within silvopastoral grazing system, the cattle were allowed to access freely the forest for the whole spring and summer while grassland was managed as in the open pasture system. Full details on the study area and on the characteristics of the animals are detailed in Ripamonti et al. (2023).

2.2. Tick collection and identification

Ticks were collected directly from Maremmana cattle contemporaneously during the periodical management controls of the cattle (e.g., blood sampling, weighting). Cattle was checked visually, with special attention paid to the tail and perianal region, which are considered as primary sites of infestation (Theuret and Trout Fryxell, 2018), considering that further inspections would be dangerous for the investigator. The number of ticks were noted according to the position on the cattle body. The half-body tick counts of cattle were doubled to obtain the whole-body tick burdens (Kemal et al., 2016). Ticks were removed manually from the animal by a rotating manner. Ticks were stored in vials with ethanol 70%, and subsequently transported to the parasitology laboratory of the Department of Veterinary Medicine of University of Bari for morphological identification at species level using taxonomic keys (Estrada-Pena et al., 2004˜), and for molecular investigation of TBPs.

2.3. Molecular procedures

Genomic DNA was extracted from individual ticks collected from Maremmana cattle (n = 40), one specimen per animal, using in-house method called guanidine isothiocyanate-phenol technique, as described elsewhere (Sangioni et al., 2005). Briefly, ticks were removed from alcohol and dried at room temperature. Thereafter ticks were cut into two equal halves using sterile blade and petri dish; one part was extracted, and the other was stored for later use. After extraction, DNA samples were tested by conventional PCR (cPCR) for the detection of pathogens using pair of primers (CS-78F and CS-323R) targeting the gene citrate synthase (*gltA*, 401 bp) of *Rickettsia* spp. Positive samples were tested by a second cPCR using the primers (Rr190.70F/ Rr190.701R) targeting a fragment of the outer membrane protein A gene (*ompA*, 632 bp).

Amplicons were then purified and sequenced in both directions using the same primers as for PCRs. The assembly of consensus sequences was performed by the Geneious software and then compared with those available in the GenBank database by the BLAST tool (https://blast.ncbi. nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch &LINK_LOC=blasthome). For phylogenetic inferences, sequences from the present study were aligned with those retrieved from GenBank using MAFFT software version 7 (Katoh et al., 2019). The best evolutionary model was chosen under the Akaike Information Criterion (AIC) and Bayesian analysis was performed using MrBayes available on CIPRES Science Gateway (https://www.phylo.org/). The phylogenetic tree edition and rooting (outgroup) were performed using TreeGraph 2.0 beta software (Stover and Muller, 2010). Homologous sequence from *Rickettsia australis* was used as out-group (accession number: AF149108).

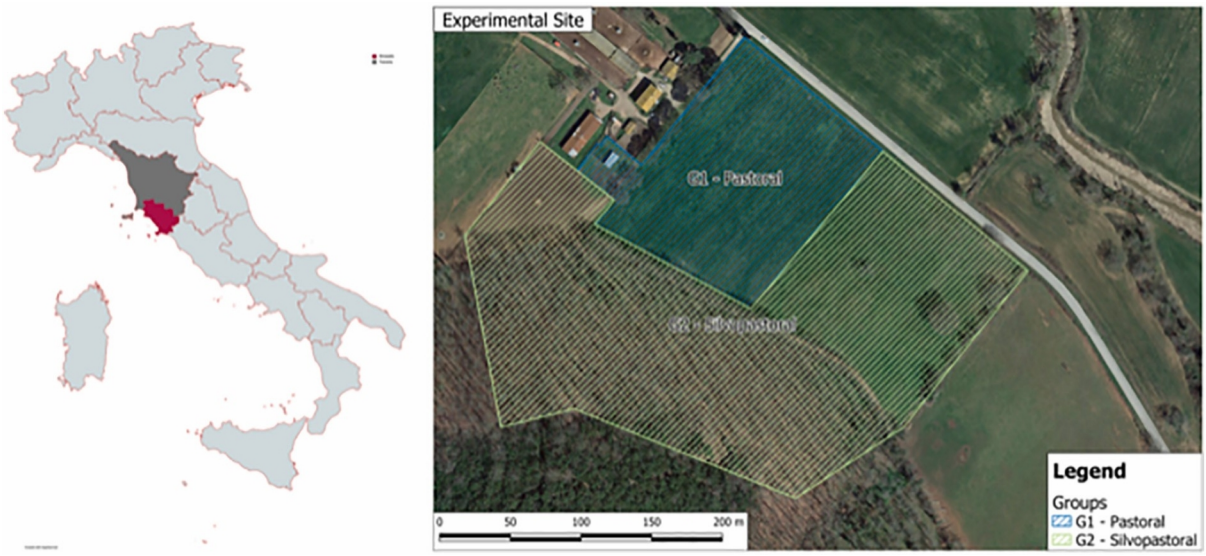

Fig. 1. (A) Map of Italy showing the study area, located in Tuscany (central Italy). (B) Field map of the pasture area. In blue the open pasture area (G1). In green the silvopastoral area (G2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.4. Statistical analysis

Descriptive statistics was used to determine the prevalence of tick infestation in cattle. The overall prevalence of ticks was determined by dividing the number of positive animals by total sample size and was expressed as percentage. The mean intensity was calculated by dividing total numbers of ticks by total number of infested animals, and standard deviation was inferred from this mean. Estimated parameters were not normally distributed, and the variance was not homogeneous (Shapiro–Wilk test, goodness of fit $p < 0.05$; Levene's test, goodness of fit $p < 0.05$). Therefore, differences between open pasture and silvopastoral grazing systems were tested using non-parametric statistics, i.e., the Wilcoxon test. To assess whether there was a difference in tick infestation among cattle sexes, we performed a contingency analysis. A *p*-value of 0.05 was used as the threshold to assess significant differences. Statistical analysis was performed using RStudio (2023.09.1 + 494) and graphic representation was performed using GraphPad (9.4.3.2).

3. Results

Of the 794 ticks counted during the survey period on 40 cattle, 117 were manually collected and identified as *Hy. marginatum* (n = 85, 72.6%), *Hyalomma* spp. (n = 27, 23%), *Rhipicephalus turanicus* (n = 2, 1.7%), *Rh. bursa* (n = 1, 0.9%), *Hy. lusitanicum* (n = 1, 0.9%) and *Dermatocentor marginatus* (n = 1, 0.9%). All ticks were adults, being 68 (58.1%) males and 49 (41.8%) females (Supplementary Data Table S1). The highest prevalence of ticks was observed in April for silvopasture (n = 196, 90%) and in mid-May survey for open pasture (*n* = 124, 85%); the lowest one was observed in late summer for both (Table 1).

The number of ticks significantly differed between the groups ($\chi^2 = 3.9173$; *d.f.* = 1; $p = 0.0478$) (Fig. 2). However, removing the first sampling (i.e., the ticks collected in April), no significant differences were detected between them ($\chi^2 = 0.1690$; *d.f.* = 1; $p = 0.6810$). Contingency analysis showed no differences among tick abundance and cattle sex in both grazing systems ($\chi^2 = 0.778$; $p = 0.3778$) (Fig. 3).

Among the examined tick species, only *Hy. marginatum* scored positive for TBPs. In total, 21/40 (52.5%) ticks (12 from silvopasture and 9 from open pasture) scored positive for *Rickettsia* spp. DNA by *glt*A gene and of these 15/21 (71.4%) tested positive to spotted fever group (SFG) rickettsiae by *omp*A gene. Of the total positive specimens, 19 of them were successfully sequenced, which showed 99–100% identity with *R. aeschilimannii* (17/19, 89.5%), *R. slovaca* (1/19, 5%), and *R. massiliae* (1/19, 5%) when compared with the corresponding sequences in the GenBank. The *omp*A partial sequences of *R. aeschilimannii* from *Hy. margunatum* clustered with high bootstrap value (100%) together with those of the same tick species from Turkey (MG920561), while *R. slovaca* clustered with those of *D. margintus* (MT680021) from Italy and *Rh. turanicus* (MW779473) from Iran (Fig. 4). *Rickettsia massiliae* sequences clustered with a bootstrap value of 100% together with those of *Amblyomma sylvaticum* (OP329175) from South Africa and humans from China (MH549236). All sequences obtained herein were deposited in the GenBank under accession numbers: OR820147 - OR820155 and OR828925 - OR828929 (*ompA*), and OR828918 - OR828924 (*gltA*) (Fig. 4).

Table 1. Seasonal distribution of ticks on Maremmana cattle from April to September 2022 in open and silvopasture grazing sytems, their prevalence, mean intensity and standard deviation.

	Variable	**Months**						
		April	Mid May	End May	June	July	Augost	September
Open pasture (G1)	No. ticks	34	124	120	8	0	6	0
	Prevalence	6/20 (30%)	17/20 (85%)	9/20 (45%)	2/20 (10%)	0	1/20 (5%)	0
	Mean intensity (SD)	5.6 (4.45)	7.29 (4.9)	13.3 (9.89)	4 (0)	0	6 (0)	0
Silvopasture (G2)	No. ticks	196	90	174	14	12	16	0
	Prevalence	18/20 (90%)	14/20 (70%)	12/20 (60%)	2/20 (10%)	2/20 (10%)	3/20 (15%)	0
	Mean intensity (SD)	10.8 (9.97)	6.4 (6.13)	14.5 (13.46)	7 (7.07)	6 (2)	5.3 (3.05)	0

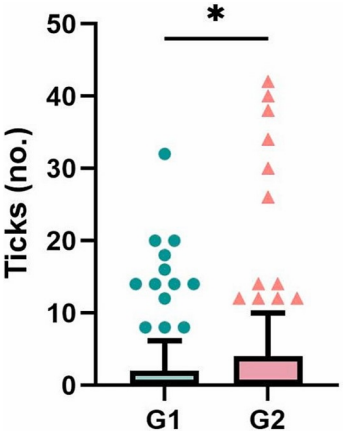

Fig. 2. Number of ticks per grazing system (open pasture, G1; silvopasture, G2) during April–September 2022. Box plots indicate the median (line) within each box and the range of dispersion (upper quartiles and outliers). * = significant difference (Wilcoxon test, $p < 0.05$).

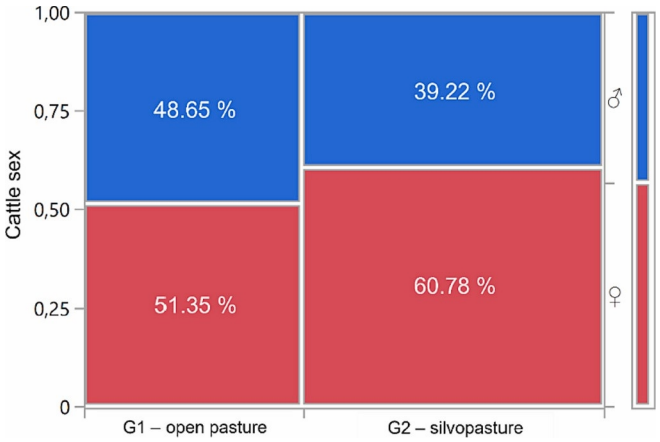

Fig. 3. Contingency analysis between the number of ticks infesting cattle according to the host sex and grazing system (open pasture, G1; silvopasture, G2). The right bar represents the relative abundance (%) of ticks found on cattle males and females over the total number of infested individuals per group. No significant differences were detected between grazing systems ($p > 0.05$).

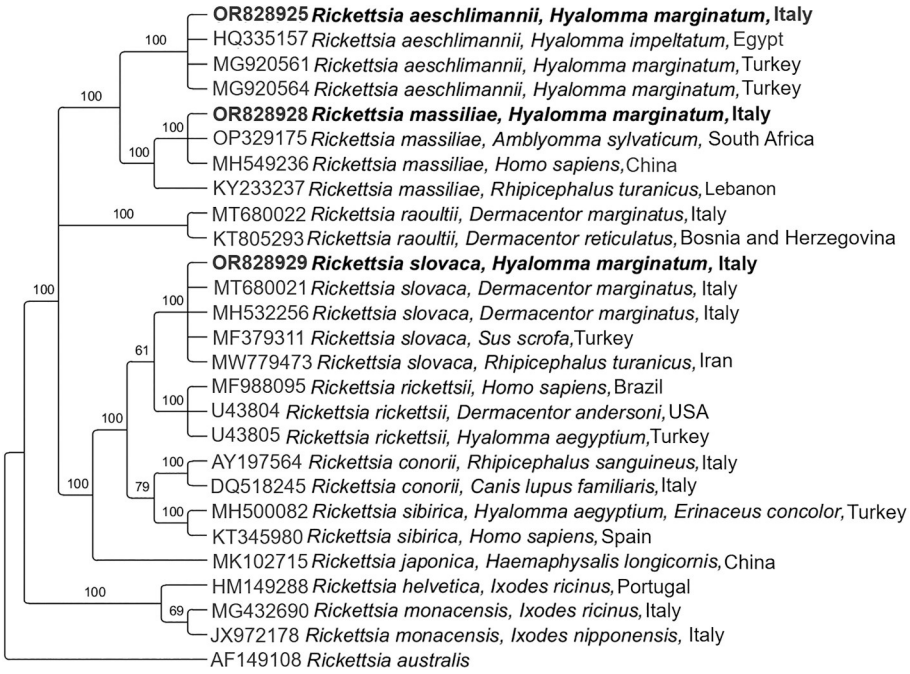

Fig. 4. Phylogenetic relationship of gltA and ompA gene fragments of SFG rickettsiae (*Rickettsia aeschlimannii*, *R. slovaca* and R. *massilliae*) detected in this study and other *Rickettsia* spp. inferred by Bayesian analysis, TPM2 + F + G4 evolutionary model and gamma shape of 0.9702. Posterior probabilities are shown as numbers at the nodes. Sequences detected at the present study are highlighted in bold.

4. Discussion

Our results show a difference in tick abundance between cattle having access to silvopastoral grazing and open pasture. The inclusion of April sampling in data analysis is the primary contributor. There is no significant variation in the number of specimens taken between the two grazing systems when these data are eliminated. This phenomenon might be caused by a variety of circumstances such as a more favorable habitat to tick development in humid woodland environment. Indeed, *Hy. marginatum* prefers damp and rainy conditions, and mature ticks actively seek a host when humidity levels range from 75% to 100% (Valcarcel et al., 2020´). The earliest stages of *Hy. marginatum*, larvae and nymphs, are carried by small animals, generally ground feeding birds, whereas the adults mostly feed on big ungulates, including cattle (Valcarcel et al., 2020´). The major ecological complexity of the woodland, including a higher number of small-sized hosts potentially exploitable by hard ticks, can partially explain the higher tick infestation on cattle in silvopastoral grazing system if compared to the ones grazing in the open pasture system (Dantas-Torres and Otranto, 2013). In addition, the growth of wildlife populations (i.e., small and medium- sized vertebrates) in protected lands, presumably due to predator-prey cycle imbalance, may lead to increase of hard tick populations, including *Hy. marginatum.* Accordingly, the abundance of *Hy. marginatum* in grazing lands increases the risk of human-tick contact, in particular for livestock farmers, and hence TBPs exposure.

The summer drop might be attributed to an increase in daily average temperatures, where ticks prefer to hide (Valcarcel et al., 2020´). Cattle are one of the main targets of various tick species including hunting ticks, and among them *Hy. marginatum*, commonly found in the Mediterranean basin (Chisu et al., 2020), is the main vector and reservoir of CCHF virus as well as many *Rickettsia* spp. (Hekimoglu et al., 2023; Bonnet et al., 2022; Gargili et al., 2017). The predominance of *Hy. marginatum* among other species on cattle is not uncommon as this tick species feeds on ruminant animals as primary hosts in their adult stage (Valcarcel et al., 2020´). Moreover, Italy's Tyrrhenian coast, that is close to the present study area, is a common location for the presence of migratory birds carrying ixodid ticks such as *Ixodes ricinus* (Falchi et al., 2012) and *Hyalomma* spp. from CCHF endemic areas (Guccione et al., 2021). Indeed, one cannot say all about *Hy. maginatum* without discussing CCHF virus, and this is what makes this tick species so important in public health point of view. Furthermore, it is noteworthy to mention that Italy is the only CCHF-free country with a widely distributed *Hy. marginatum* ticks, thus the establishment of the virus is highly possible if introduced (Fanelli and Buonavoglia, 2021).

Interestingly, we detected a high prevalence (>52%) of *Rickettsia* spp. DNA in *Hy. marginatum* ticks, all of them being SFG (i.e., *R. aeschilimannii, R. slovaca* and *R. massiliae)*. *Rickettsia aeschlimannii* is detected mainly in *Hyalomma* and *Amblyomma* ticks (Chisu et al., 2017; Parola et al., 2013) from Africa and several European countries, including Italy (Scarpulla et al., 2016; Cicculli et al., 2019; Abdelkadir et al., 2019). In particular, *R. aeschlimannii* has been isolated from *Hy. marginatum* imported into Germany, Russia, Hungary, and Cyprus (European Centre for Disease Prevention and Control ECDC, 2023); however, its vectorial capacity is yet to be determined.

Although *R. slovaca* is commonly detected in *Dermacentor marginatus* of wild boars in Italy (Grassi et al., 2022; Sgroi et al., 2021) and *R. massiliae* in *Rhipicephalus sanguineus* (Milhano et al., 2014), we detected them in *Hy. marginatum*. These SFG rickettsiae are the causative agents of non-pathogen-specific illness of SENLAT (scalp eschar and neck lymphadenopathy after tick bite), a zoonotic TBDs transmitted by various tick species (Barlozzari et al., 2021; Vitale et al., 2006). To the best of our knowledge, this is the first time the DNA of these pathogens has been detected in *Hy. marginatum.*

In recent years, knowledge about TBDs as well as people's awareness of the risk of tick bites has increased. Many people who find ticks on their pets or attached on themselves seek for a specialized laboratory able to analyze whether ticks are infected with pathogens (Scarpulla et al., 2016). It is noteworthy to mention that cattle hosts sampled in present study were apparently healthy, and detection of pathogens in ticks collected from them does not necessarily mean transmission. Nevertheless, these ruminants may subclinically serve as amplifiers of pathogenic agents, including those of zoonotic concern, such as SFG rickettsiae, hence maintain the enzootic cycle of these agents (Ortuno ˜ et al., 2012). In addition, since these ruminants carry large numbers of ticks, they could be exploited as sentinels of priority TBDs (e.g., CCHF) when planning monitoring and surveillance, particularly in more prone areas such as Italy. Nonetheless, as this study was limited to the screening of *Rickettsia* spp. only, a larger panel of pathogens should be screened.

5. Conclusions

Our study pointed out that Maremmana cattle grazing in the silvopasture system were parasitized by a higher number of ticks. The molecular investigation of TBPs in ticks collected from Maremmana cattle showed presence of pathogenic bacteria of SFG *Rickettsia* spp., highlighting the potential risk of TBPs transmission between livestock and humans. In the One Health perspective, knowledge about occurrence of zoonotic pathogens in ticks, including those parasitize livestock species, can inform the development of effective control strategies to mitigate the impact of TBDs on livestock. Further large-scale studies (including animal blood/sera screening) are however needed to determine the vectorial role of cattle ticks in the transmission of pathogenic agents in both domestic and wild cycles, so that effective control measures could be planned.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CRediT authorship contribution statement

Kassim Abdullahi Jimale: Conceptualization, Formal analysis, Investigation, Methodology, Writing – review & editing. **Valeria Zeni:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Alice Ripamonti:** Investigation, Writing – review & editing. **Angelo Canale:** Supervision, Writing – review & editing. **Marcello Mele:** Supervision, Writing – review & editing. **Giovanni Benelli:** Conceptualization, Supervision, Writing – review & editing. **Domenico Otranto:** Conceptualization, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.vprsr.2024.100994.

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Chapter 2. Zoonotic pathogens in ticks and domestic animals: why continued surveillance matters

Section 2.1

Molecular detection of *Colpodella* sp. and other tick-borne pathogens in ticks of ruminants, Italy

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Abstract
Colpodella species are close relatives of Apicomplexan protozoa. Although most species of this genus are free- living organisms that feed on other protists and algae, reports indicate their occurence in ticks and human patients, including an individual with a history of tick bite manifesting neurological symptoms. During an investigation of tick-borne pathogens (TBPs) in blood samples of cattle, goats, and in ticks collected on them, *Colpodella* sp. DNA was detected in a *Rhipicephalus bursa* tick collected from cattle, while of *Theileria sergenti/ buffeli/orientalis, Babesia bigemina, Sarcocystis cruzi, Babesia* spp., and *Rickettsia* spp. were molecularly detected in cattle, goats, and ticks in southern Italy. Data herein reported highlight the unprecedented presence of *Colpodella* sp. in ticks in Italy, raising concern due to the potential pathogenic role of this less known protozoan. This finding advocates for performing routine epidemiological surveys to monitor potential emerging vector-borne pathogens.

Keywords: Zoonosis; Emerging pathogens; One health; Survailance

1. Introduction

Ticks infest mammals, birds, reptiles and amphibians, in different biomes and climates, and are commonly found in areas of dense vegetation such as forests, woodlands, meadows, and grasslands (Otranto et al., 2014). Ticks may transmit tick-borne pathogens (TBPs), including some of zoonotic concern (e.g., *Rickettsia* spp., *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato), which can cause serious illnesses and, if untreated, may be potentially fatal to people worldwide (Dantas-Torres et al., 2012). Species of the genus *Colpodella* are close relatives of apicomplexans such as *Babesia* spp. and *Plasmodium* spp., and they are mostly free-living organisms feeding on other protists and algae (Kuvardina et al., 2002). However, *Colpodella* are not well-known in terms of their biology, epidemiology, and pathogenicity, although they are of increasing importance in specific ecological contexts, such as some mountainous areas of China (Qi et al., 2024). In addition, *Colpodella* spp. have been suggested to be potential TBPs (Jiang et al., 2018), being molecularly detected in tick species collected from cattle, goats, dogs, and the environment (Matsimbe et al., 2017; Jiang et al. 2018; Qi et al., 2024). Importantly, *Colpodella* spp. have also been associated with human diseases in China where it was molecularly diagnosed in hospitalized patients, including detection of the parasite DNA in the cerebrospinal fluid (CSF) of an individual manifesting neurological symptoms who had a tick bite history (Jiang et al., 2018), and in another case of erythrocyte infection in a patient with babesiosis-like relapsing fever (Yuan et al., 2012). In a further case, *Colpodella* sp. was detected in a urine sample from an immunocompromised patient in Romania (Neculicioiu et al., 2021). Despite the molecular detection of organisms from this genus in several vertebrate host species and ticks, limited information is available on their pathogenicity as well as their occurrence in tick vectors. In the present study, we report the occurrence of TBPs in endemic populations of cattle, goats, and ticks, highlighting the detection of *Colpodella* sp. DNA in a tick collected from cattle in southern Italy.

2. Materials and methods

2.1. Tick and blood collection

Blood and tick samples were collected from cattle (n = 98) and goats (n = 104) grazing freely on pasture grasslands in the Puglia region (southern Italy) from February to May 2023. Observed ticks were removed from animals using tweezers and individually stored in ethanol 70 %, transported to the parasitology laboratory of the Department of Veterinary Medicine of

the University of Bari for morphological identification (Estrada-Pena et al., 2004˜), and molecular investigation.

2.2. Molecular procedures and phylogenetic analysis

Genomic DNA was extracted from blood samples using a commercial kit (GenUPBlood DNA Kit, Biotechrabbit GmbH, Hennigsdorf, Germany), according to the manufacturer's instructions, whilst a guanidine isothiocyanate in-house protocol (Sangioni et al., 2005) was used to extract DNA from unfed ticks. Briefly, individual tick specimens were cut into two equal halves using sterile blades and petri dish; one part was extracted, and the other was stored for later use. Different conventional PCR (cPCR) assays were thereafter performed for detection of TBPs such as *Babesia* spp., *Theileria* spp., and *Rickettsia* spp., using primers targeting a partial region of the 18S rRNA gene for piroplasm parasites and the citrate synthase (*gltA*) gene for *Rickettsia* spp. Another cPCR targeting the 16S rRNA gene was also performed for species confirmation of ticks positive for pathogens using a procotol previously described (Latrofa et al., 2013). Details regarding molecular protocols according to the different pathogens investigated are reported in Table 1. Amplified products were examined in 2 % agarose gel stained with GelRed (VWR International PBI, Milan, Italy) and visualized on a Gel Logic 100 gel documentation system (Kodak, NY, USA). Positive amplicons were purified and sequenced in both directions using the same primers as for PCRs, employing the Big Dye Terminator v.3.1 chemistry in a 3130 Genetic analyzer (Applied Biosystems, CA, USA) in an automated sequencer (ABI-PRISM 377). The assembly of consensus sequences was edited, aligned, and analyzed by the BioEdit software and compared with those available in the GenBank by the BLASTn (https://blast.ncbi. nlm.nih.gov/Blast.cgi). For phylogenetic analysis the reference sequences of 18S rRNA of *Theileria* spp., *Babesia* spp., *Colpodella* spp., *Cytauxzoon* spp., and *Cryptosporidium* spp. were selected and aligned with the sequence obtained in this study using the ClustalW multiple alignment tool of MEGAX software. The phylogenetic tree was inferred based on Maximum Likelihood method, with the Tamura 3-parameter model and Gamma Distributed (G) as rates among sites for 5000 bootstrap replications using MEGAX software. *Plasmodium vivax* sequence (JQ627158) was used as outgroup.

Table 1. Details of molecular protocols employed in the present study.

Primer name	Fragment size (bp)	Sequences (5′–3′)	Target gene	Target organisms
RLB-F/RLB-R	460-520	GAGGTAGTGACAAGAAATAACAAT A TCTTCGATCCCCTAACITTC	18S rRNA	Piroplasms/ *Colpodella*
PiroA/ PiroB	408	AATACCCAATCCTGACACAGGG TTAAATACGAATGCCCCCAAC	18S rRNA	Piroplasms/ *Colpodella*
CS-78F/ CS-323R	401	GCAAGTATCGGTGAGGATGTAAT GCTTCCTTAAAATTCAATAAATCAGGAT	gltA	*Rickettsia*
Rh16sf/ Rh16sr	~300 bp	TTACGCTGTTATCCC CTGCTCAATGATTTT	16S rRNA	Ticks

3. Results

Overall, 36/98 (36.7 %) of the bovine blood DNA samples scored positive for piroplasms, with 18S rRNA sequences nucleotide identity of 99–100 % to *Babesia bigemina* (*n* =7; accession number KP745623.1), 99 % with the *Theileria sergenti/buffeli/orientalis* group (*n* = 29; accession numbers MH208641.1, FJ225391.1), and two samples (2 %) showed 99 % nucleotide identity to *Sarcocystis cruzi* (accession number MT254612.1). Of the 104 goats tested 39 (37.5 %) resulted positive for *Babesia* spp. (100 % nucleotide identity; accession number KU714605.1) (Table 2). All blood samples collected from both cattle and goats tested negative for *Rickettsia* spp.

Ticks (*n* = 42) were collected on 33/98 (33.7 %) free grazing cattle, and morphologically identified as *Rhipicephalus bursa* (*n* = 31) and *Rh. secundus* (*n* = 11; based on the recent reclassification of *Rh. turanicus,* Mumcuoglu et al., 2022). Similarly, 36/104 (34.6 %) goats were infested by ticks (*n* = 36) which were identified as *Rh. bursa* (*n* = 25) and *Rh. secundus* (*n* = 11). All ticks were adults, 32 (41 %) males and 46 (59 %) females. A female of *Rh. bursa* from cattle scored positive for *Colpodella* sp. DNA, with 100 % nucleotide identity (99 % nucleotide query coverage) with a sequence available in the GenBank (accession number OQ540588.1). In addition, one *Rh. bursa* female from a goat tested positive for *Rickettsia* spp., which presented 100 % nucleotide identity with *R. massiliae* (accession number KY640405.1) and *Candidatus* Rickettsia kulagini (accession number DQ365806.1) (Table 2). Ticks positive for pathogens were molecularly confirmed as *Rh. bursa* (100 % nucleotide identity; accession number KX553962.1). The phylogenetic analysis of 18S rRNA sequences confirmed the molecular identification of *Colpodella* sp. by clustering the sequence herein obtained within the clade including those from China and as a paraphyletic clade of other apicomplexan protozoa such as *Theileria* spp*., Babesia* spp. and *Cytauxzoon* spp. (Fig. 1). All nucleotide sequences obtained in the present study were deposited in the GeneBank database under accession numbers PP658184 - PP658191.

Table 2. Screened animals and ticks, amplified genes, detected pathogens, positivity rate, GenBank matching identification and accession numbers.

Screened animal/ tick	Amplified gene	Detected pathogens	Positivity rate (%)	GenBank matching spp. (% identity)	Accession numbers
Bovine	18S rRNA	*Babesia*	7/98 (7)	*B. bigemina* (99.5 %)	KP745623.1
		Theileria	29/98 (29.6)	*T. sergenti/buffeli/orientalis* (99.15 %)	MH208641.1, FJ225391.1
		Sarcocystis	2/98 (2)	*S. cruzi* (99 %)	MT254612.1
Caprine	18S rRNA	*Babesia*	39/104 (37.5)	*Babesia* spp. (100 %)	KU714605.1
Rh. bursa	18S rRNA	*Colpodella*	1/18 (5.5)	*Colpodella* spp. (100 %)	OQ540588.1
Rh. bursa	*gltA*	*Rickettsia*	1/51(2)	*R. massiliae* (100 %), *Candidatus* Rickettsia kulagini (100 %)	KY640405.1, DQ365806.1

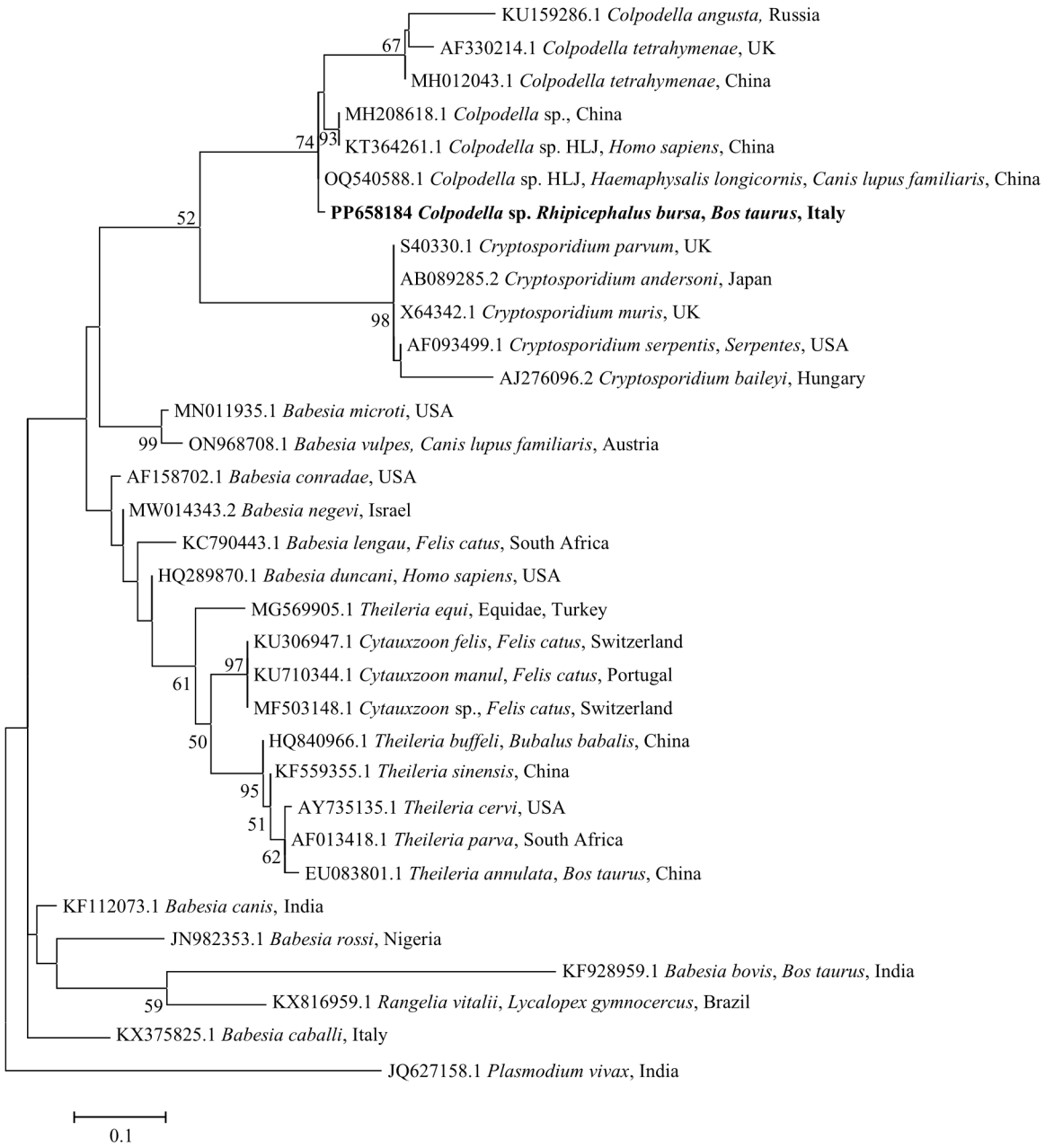

Fig. 1. Phylogenetic analysis of 18S rRNA of *Colpodella* spp. and other apicomplexan protozoa. Sequence obtained in the present study is highlighted in bold. The phylogenetic tree was constructed based on Maximum Likelihood Phylogeny using the Tamura 3-parameter model by MEGAX software. The scale bar indicates nucleotide substitutions per site.

4. Discussion

Along with the commonly detected TBPs of cattle and goats (i.e., *Babesia* spp., *Theileria* spp., *Rickettsia* spp.) this study reports the unprecedent detection of *Colpodella* sp. in ticks in Italy. Case reports from other countries have emphasized the potential concern for this protozoan, which was implicated to be associated with disease in humans (Yuan et al., 2012; Jiang et al., 2018; Neculicioiu et al., 2021). Indeed, *Colpodella* spp. was molecularly detected in *Rh. microplus* infesting cattle in Mozambique (Matsimbe et al., 2017), in questing *Ixodes persulcatus* in China (Jiang et al. 2018), in *Haemaphysalis longicornis* from goats and dogs in China (Qi et al., 2024), and in blood of horses from the same country (Xu et al., 2022). The DNA sequence of *Colpodella* spp. obtained in the present study is identical to that detected in *Ha. longicornis* from a dog in China (Qi et al., 2024), which was previously associated with a human patient manifesting neurological symptoms and designated as *Colpodella* sp. HLJ (Jiang et al. 2018). The finding above suggests that the same *Colpodella* species or strains may infect different tick genera from diverse animal hosts and geographic areas, providing further insights on the epidemiology and biology of this rather enigmatic protozoan. Furthermore, the finding of *Theileria* spp. and *Babesia* spp. confirm the presence of these protozoa in livestock species in Italy (Cassini et al., 2012). In particular, the detection of *T. sergenti/buffeli/orientalis* in cattle by 18S rRNA gene amplification is in line with the findings of a study on cattle in Sardinia (Chisu et al., 2023), as well as in central and northern regions of Italy (Cassini et al., 2012).

Spotted fever group (SFG) *Rickettsia* spp. DNA identical to *R. massiliae* and *Candidatus* Rickettsia kulagini were herein detected in *Rh. bursa*. *Rickettsia massiliae* has previously been reported in Italy in *Hyalomma marginatum* from cattle and *Rhipicephalus sanguineus* sensu lato from a fox (Jimale et al., 2024; Chisu et al., 2017). Data above underscores the importance of continued TBPs surveillance for the early detection of potentially zoonotic pathogens transmitted by ticks to humans and animals.

The finding of *Colpodella* sp. DNA in ticks highlight the importance of routine epidemiological studies in the monitoring of potential emerging vector borne pathogens. Altogether, considering the increasing reports of this flagellate in different vertebrate hosts, and ticks (Xu et al., 2022; Qi et al., 2024; Yuan et al., 2012), as well as its speculated pathogenicity to humans (Jiang et al. 2018;), *Colpodella* spp. could be considered as a potential emerging TBP of medical and veterinary concern. Further studies are advocated in order to establish complete baseline knowledge of this pathogen.

CRediT authorship contribution statement

Kassim Abdullahi Jimale: Methodology, Data curation, Writing – original draft. **Marcos Antonio Bezerra-Santos:** Conceptualization, Methodology, Data curation, Writing – review & editing, Visualization. **Jairo Alfonso Mendoza-Roldan:** Writing – review & editing, Visualization. **Maria Stefania Latrofa:** Conceptualization, Methodology, Data curation, Writing – review & editing, Visualization. **Gad Baneth:** Writing – review & editing, Visualization. **Domenico Otranto:** Conceptualization, Methodology, Data curation, Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Section 2.2

Vector-borne pathogens in dogs and in *Rhipicephalus sanguineus* sensu stricto ticks in Morocco

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Abstract
Canine vector-borne diseases (CVBDs) are of major concern in veterinary medicine worldwide. Amongst the arthropods transmitting CVBD-causing pathogens, the brown dog tick (*Rhipicephalus sanguineus* sensu lato) is an important vector of agents, such as *Babesia vogeli, Cercopithifilaria* spp., *Ehrlichia canis, Hepatozoon canis*, and *Anaplasma platys*. While data on CVBDs transmitted by *Rh. sanguineus* s.l. are limited in Morocco, *Leishmania* spp., transmitted by phlebotomine sand flies, are known to be endemic in several regions of the country. In this study, we investigated the occurrence of tick-borne pathogens (TBPs) (i.e., *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Hepatozoon* spp., *Rickettsia* spp.), *Leishmania* spp. and filarioids in shelter dogs and their ticks in central Morocco. Blood samples were collected from 144 dogs, and 5,363 ticks were removed from 314 dogs of the same population. DNA samples extracted from blood and from 276 ticks (divided into 55 pools) were screened for selected pathogens by PCR and DNA sequencing. Ticks were morphologically identified as *Rh. sanguineus* s.l., and molecular analysis of 10 representative specimens confirmed them as *Rh. sanguineus* s.s. Out of 144 dogs tested, 78 (54.1 %) scored positive for at least one pathogen, with 15 (10.4 %) being co-infected. *H. canis* was the most prevalent pathogen (38.2 %, 55/144), followed by *L. infantum* (15.3 %; 22/144), *A. platys* (5.6 %; 8/144), *B. vogeli* and *E. canis* (2.8 %; 4/144). Tick DNA pools scored positive for *H. canis* (36.4 %; n = 20/55). All dogs tested negative for filarioids and *Rickettsia* spp. Data herein reported demonstrate a high overall prevalence of CVBD-causing pathogens in dogs from central Morocco, with the unprecedented report of *H. canis* in dogs and ticks in this country.

Keywords: Surveillance; Brown dog tick; *Hepatozoon canis*; *Anaplasma platys*; *Ehrlichia canis*; *Leishmania infantum*; Dog shelters

1. Introduction

Canine vector-borne diseases (CVBDs) are of major concern in veterinary medicine worldwide, chiefly caused by pathogens associated with ticks, mosquitoes, and phlebotomine sand flies (Otranto et al., 2009a). Among arthropod vectors, brown dog ticks (*Rhipicephalus sanguineus* sensu lato) are widely distributed and known to transmit many pathogens to dogs (e.g., *Anaplasma platys, Babesia vogeli, Cercopithifilaria* spp., *Ehrlichia canis* and *Hepatozoon canis*), including some of zoonotic concern, such as *Rickettsia* spp. (Dantas-Torres, 2010; Parola et al., 2013; Dantas-Torres and Otranto, 2015). In northern Africa, data on the epidemiology of tick-borne diseases (TBDs) in dogs are scant, with most published studies being case reports or performed at regional level, such as in Algeria, where *A. platys, E. canis, B. vogeli*, and *H. canis* were molecularly detected in canine blood samples and in *Rh. sanguineus* s.l. (Dahmani et al., 2015; Medkour et al., 2020; Laatamna et al., 2022). In addition, *Rickettsia conorii* and *R. massiliae* were detected in *Rh. sanguineus* s.l. collected on dogs in northern and northeastern Algeria (Bessas et al., 2016; Leulmi et al., 2016). In Tunisia, DNA of *H. canis, B. vogeli, A. phagocytophilum*, and *E. canis* were detected in dogs (M'ghirbi et al., 2009; Bouattour et al., 2021a); whilst *B. vogeli, A. platys, Dirofilaria repens*, and *Acanthocheilonema reconditum* were reported in dogs from different regions of Egypt (Abdullah et al., 2021; Selim et al., 2021). Moreover, *H. canis, B. vogeli,* and Anaplasmataceae were detected in *Rh. sanguineus* s.l. ticks collected from dogs in Egypt (Hegab et al., 2022). On the other hand, both sand flies and canine leishmaniosis (CanL) are endemic in the Mediterranean basin, including Morocco (El-Mouhdi et al., 2022; Morales-Yuste et al., 2022), with a 17 % seropositivity reported in dogs in a study conducted in Tunisia (Zribi et al., 2023). Since the first report of CanL (Jeaume, 1932), *Leishmania infantum* is known to be endemic in dogs in most regions of Morocco, with prevalence up to 42 % in dogs from Rabat province (El-Mouhdi et al., 2022). Moreover, *Leishmania tropica* and *Leishmania major*, both causative

agents of cutaneous leishmaniasis, are endemic in Morocco (Rioux et al., 1982; Rhajaoui, 2011; Kahim et al., 2014; Echchakery et al., 2015), with reports of L. *tropica* in dogs (Dereure et al., 1991; Pratlong et al., 1991; Lemrani et al., 2002). However, data on CVBDs in Morocco are still scant, with most published studies focused on CanL (Guessous-Idrissi et al., 1997; Nejjar et al., 1998; Natami et al., 2000; Sahibi et al., 2001; Rami et al., 2003; Fellah et al., 2014; Idrissi et al., 2021), and a single study on *A. platys* in dogs (El Hamiani Khatat et al., 2017a). To bridge this knowledge gap, this study aimed to molecularly investigate the occurrence of vector-borne pathogens (VBPs) in dogs and *Rh. sanguineus* s.l. ticks in central Morocco.

2. Material and methods

2.1. Study area

The study was conducted from April to October 2023 in three legally operated private dog shelters (DS) located in urban, peri-urban, and rural areas in central Morocco (Fig. 1), which features a Mediterranean climate, with intense rainfall in winter and hot summers (Morocco's climate, 2023). The surveyed DS were as follows: DS1 (n = 100 dogs) in Rabat, Rabat-Salè-Kènitra region (34°0′47.7″N, 6°49′57.18″W); DS2 (n = 300 dogs) in a peri-urban area at the outskirt of Fes city, Fès-Meknès region (34°2′13.74″N, 4°59′59.28″W); and DS3 (n = 50 dogs) in the Shoul rural areas (34◦3′11.16″N, 6◦47′54.46″W) belonging to the Salè prefecture, Rabat-Salè-ènitra region. All dogs were properly vaccinated against rabies and sterilized immediately after their admission to the shelters. However, these dogs had never been dewormed or subjected to ectoparasiticide treatments.

2.2. Blood and tick sampling

Blood samples were collected only from dogs that could be handled (n = 144), placed in EDTA tubes, and stored at -20 ◦C until molecularly processed. Individual data such as age (in months), sex, breed, and potential clinical signs were recorded from each sampled dog. Ticks (n = 5,363) were collected from 314 dogs (i.e., 230 from DS2, 50 from DS3, and 34 from DS1), including those enrolled for blood sampling, using forceps twice a month from different body sites (i.e., head, ears, breast, neck, back and interdigital areas) (Lorusso et al., 2010). All tick specimens sampled were placed in labelled tubes, preserved in 70 % ethanol and grouped according to the date and shelter of collection. Ticks were then classified by their developmental stage (i.e., larvae, nymphs, adults), sex (female and male), feeding status (engorged and non-engorged females), and identified according to morphological keys and species description (Walker et al., 2000; Nava et al., 2018). All national and international protocols pertaining to handling, transporting, and processing of biological materials were strictly followed (European Union Directive 2010/63/EU and Animal Research: Reporting of In Vivo Experiments guidelines). The study was performed following guidelines provided by the Hassan II Institute of Agronomy and Veterinary Medicine of Rabat and the Moroccan Ministry of Agriculture, which are in accordance with international ethical standards (European Union Directive 2010/63/EU and Animal Research: Reporting of In Vivo Experiments guidelines).

2.3. Molecular procedures

DNA was extracted from whole blood samples using GenUP Blood DNA Kit (Biotechrabbit GmbH, Hennigsdorf, Germany), according to the manufacturer's instructions, while a guanidine isothiocyanate in-house protocol (Sangioni et al., 2005) was used to extract DNA from ticks. Briefly, a total of 276 tick specimens (i.e., 196 males, 74 females and six nymphs), representing 10 % of the total ticks, were selected for DNA extraction. Visibly engorged females and nymphs were excluded from ticks molecularly analyzed to minimize the possibility of amplifying DNA from the blood of an infected animal. Ticks were removed from alcohol, dried at room temperature, and then individual specimens were placed on petri dishes and cut

into two equal halves using a sterile blade; one part was used for DNA extraction, and the other was stored for later use. Ten tick specimens were analyzed to confirm the species using molecular and sequencing approaches according to Latrofa et al. (2013). DNA samples from blood were individually processed, while DNA from ticks were pooled (five tick DNA samples each pool; n = 55 pools), according to site and date of collection, sex and developmental stage. A pool size of five tick specimens was established to minimize processing time and diagnostic costs (Fracasso et al., 2023), given that the purpose of the screening was to confirm that the dogs tested have been infested by ticks, and those ticks were infected by pathogens. Conventional PCRs (cPCR) or real-time PCR (qPCR) assays targeting selected VBPs were used for the molecular analysis as reported in Table 1. Amplified products were examined on 2 % agarose gels stained with GelRed (VWR International PBI, Milan, Italy) and visualized on a Gel Logic 100 documentation system (Kodak, NY, USA). Positive samples were purified and sequenced in both directions using the same primers as for PCRs, employing the Big Dye Terminator v.3.1 chemistry in a 3130 Genetic analyzer (Applied Biosystems, CA, USA) in an automated sequencer (ABI-PRISM 377). Sequences were aligned and edited using the BioEdit software (version 7.1.1) and consensus sequences were compared with those available in the GenBank database by the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/ Blast.cgi).

2.4. Data analyses

Statistical analyses were performed using R (version 4.2.0) (R Core Team, 2022). The relationship between risk factors (i.e., sex, age class and sampling sites) and PCR outcome (i.e., presence/absence of *Ehrlichia* spp./*Anaplasma* spp., *Babesia* spp./*Hepatozoon* spp., and *Leishmania* spp. DNA) were assessed using separate binomial generalized linear models (GLMs) for each of these three pathogen taxa. The statistical analyses were conducted on this higher taxonomic level as only a subsample of the pathogens was identified to species level. The association between variables was assessed using odds ratio (OR). Analyses were considered significant if *P*-value <0.05 and the confidence interval (CI) for all analyses was set at 95 %.

3. Results

Data on sex, age class, breed, and location of sampled dogs are summarized in Table 2 along with number and percentage of dogs positive for CVBD-causing pathogens. Overall, 54.1 % (78/144) of the dogs tested positive for at least one pathogen. The most prevalent pathogen was *H. canis*, which was molecularly detected in 38.2 % (*n* = 55), followed by *L. infantum* in 15.3 % (*n* = 22). Sequence analyses of the positive samples showed 99–100 % nucleotide identity with other sequences of *H. canis* (accession number: MK645966) and *L. infantum* (accession number: MF137828) deposited at GenBank database. Additionally, eight sequences presented 99–100 % nucleotide identity with *A. platys* (accession number: CP046391), four with *E. canis* (accession number: MN922610), and four with *B. vogeli* (accession number: OQ996773). Co-infections were detected in 10.4 % (*n* = 15) of the dogs (Table 3). All blood samples scored negative for *Rickettsia* spp. and filarioids.

Of the 450 dogs checked, 69.7 % (*n* = 314) were infested by ticks, with a mean intensity of 17.1. All ticks (*n* = 5,363) were morphologically identified as *Rh. sanguineus* s.l., being 77.3 % (*n* = 4,145) adults (i.e., 47.8 % males and 52.2 % females) and 22.7 % (*n* = 1,218) nymphs. Sequences from 10 representative specimens were identified as *Rh. sanguineus* sensu stricto (nucleotide identity: 99.2–100 % with MN685289). Overall, 36.4 % (*n* = 20/55) of the tick DNA pools scored positive for protozoa, with sequences showing 99–100 % nucleotide identity with *H. canis* (accession number: MK673834) (Table 3). All DNA pools from ticks were negative for Anaplasmataceae.

Sequences obtained in this study were deposited in the GenBank database under the accession numbers: PQ856862-PQ856874 (*H. canis* from blood) and PQ897115-PQ897118 (*H. canis* from ticks); PQ877402- PQ877409 for *A. platys*; PQ877410-PQ877413 for *E. canis*; PQ877387- PQ877390 for *B. vogeli*; and PQ899741-PQ899745 for *Rh. sanguineus* sensu stricto.

Table 1. Primers and targeted pathogens investigated in this study.

Pathogen/Arthropod vector	Primers	Target gene	Fragment length (bp)	Reference
Piroplasmida/ *Hepatozoon* spp.	RLBF: 5′-GAGGTAGTGACAAGAAATAACAATA-3′ RLBR: 5′-biotin TCTTCGATCCCCTAACTTTC-3′	18S rRNA	460	Gubbels et al. (1999)
Babesia spp.	PIRO-A: 5′-AATACCCAATCCTGACACAGGG-3 PIRO-B 5′-TTAAATACGAATGCCCCCAAC-3′	18S rRNA	408	Olmeda et al. (1997)
Ehrlichia spp./*Anaplasma* spp.	EHR16SD: 5′-GGTACCYACAGAAGAAGTCC-3′ EHR16SR: 5′-TAGCACTCATCGTTTACA GC-3′	16S rRNA	345	Martin et al. (2005)
Filarioids	NTF: 5′-TGATTGGTGGTTTTGGTAA-3′ NTR: 5′-ATAAGTACGAGTATCAATATC-3′	*cox*1	648	Casiraghi et al. (2001)
Rickettsia spp.	CS-78F: 5′-GCAAGTATCGGTGAGGATGTAAT-3′ CS-323R: 5′-GCTTCCTTAAAATTCAATAAATCAGGAT-3′	*gltA*	401	Labruna et al. (2004)
Leishmania spp.	LEISH1: 5′-AACTTTTCTGGTCCTCCGGGTAG-3′ LEISH2: 5′-ACCCCCAGTTTCCCGCC-3′ Probe: TaqMan-MGB, FAM-5′-AAAAATGGGTGCAGAAAT-3′	kDNA minicircle	120	Francino et al. (2006)
	F25: 5′-GGACGCCGGCACGATTKCT-3′ R617: 5′-CGAAGAAGTCCGATACGAGGGA-3′	*Hsp*70	590	Fraga et al. (2010)
Ticks	Rh16sf: 5′-CTGCTCAATGATTTTTTAAATTGCTGT-3′ Rh16sr: 5′-TTACGCTGTTATCCCTAGAG-3′	16S rRNA	~300 bp	Black and Piesman (1994)

Table 2. Population characteristics of examined dogs and distribution of vector-borne pathogens in three dog shelters in central Morocco.

Variable	Total no. of dogs positive for VBPs (n =78)	Pathogens detected (%)				
		A. platys	*B. vogeli*	*E. canis*	*H. canis*	L. *infantu*
Dog sex	No. (%)	Pos/Total (%)	Pos/Total (%)	Pos/Total (%)	Pos/Total (%)	Pos/Total (%)
Male	39 (50.0)	4/69 (5.8)	2/69 (2.9)	2/69 (2.9)	23/69 (33.3)	14/69 (20.3)
Female	39 (50.0)	4/75 (5.3)	2/75 (2.7)	2/75 (2.7)	32/75 (42.7)	8/75 (10.7)
Age class						
Adult	76 (97.5)	8/138 (5.8)	3/138 (2.1)	4/138 (2.9)	54/138 (39.1)	22/138 (16.0)
Young	2 (2.5)	0	1/6 (16.7)	0	1/6 (16.7)	0
Breed						
Mongrel	75 (96.1)	8/144 (5.6)	3/144 (2.0)	4/144 (2.8)	54/144 (37.5)	21/144 (14.6)
Other breed	3 (3.8)		1 (1.3)		1 (1.3)	1 (1.3)
Sampled shelters						
DS1	31 (39.8)	3/64 (4.7)	3/64 (4.7)	4/64 (6.2)	15/64 (23.4)	11/64 (17.1)
DS2	35 (44.9)	5/40 (12.5)	1/40 (2.5)	0	33/40 (82.5)	6/40 (15)
DS3	12 (15.4)	0	0	0	7/40 (17.5)	5/40 (12.5)

Adults: > 12 months, Youngs: 0 – 12 months; DS = Dog Shelter; *A. platys = Anaplasma platys; B. vogeli = Babesia vogeli; E. canis = Ehrlichia canis; H. canis = Hepatozoon canis*; *L. infantum = Leishmania infantum*; VBPs = canine vector-borne pathogens

Table 3. Single and co-infection distribution of vector-borne pathogens molecularly detected in sheltered dogs in central Morocco.

Infections	**Dog shelters (DS)**				**Accession number (% of nucleotide identity in the GenBank)**
	DS1; *n* = 63	**DS2; *n* = 40**	**DS3; *n* = 41**	**Total; *n* = 144**	
	No. Positive (%)	No. Positive (%)	No. Positive (%)	No. Positive (%)	
Single infection	**23 (36.5)**	**28 (70)**	**10 (24.4)**	**61 (42.4)**	
A. platys	2 (3.2)	0 (0)	0 (0)	2 (1.4)	CP046391 (100%)
B. vogeli	3 (4.7) 0	0	0	3 (2.1)	OQ996773 (100 %)
E. canis	2 (3.2)	0 (0)	0 (0)	2 (1.4)	MN922610 (100%)
H. canis	12 (18.7)	23 (57.5)	7 (17.5)	42 (29.2)	MK645966 (100%),
L. infantum	7 (10.9)	0 (0)	5 (12.5)	12 (8.4)	MF137828 (99.8%)
Co-infection	**4 (6.2)**	**11 (27.5)**	**0 (0)**	**16 (11.1)**	
A. platys + *H. canis*	0 (0)	5 (12.5)	0 (0)	5 (3.5)	-
H. canis + *L. infantum*	2 (3.1)	5 (12.5)	0 (0)	7 (4.8)	-
H. canis + *E. canis*	2 (3.1) 0	0 (0)	0 (0)	2 (1.4)	-
B. vogeli + *L. infantum*	0	1 (2.5)	0 (0)	1 (0.7)	-
B. vogeli + *L. infantum* + *H. canis*	1 (1.6)	0 (0)	0 (0)	1 (0.7)	-

A. platys = Anaplasma platys; B. vogeli = Babesia vogeli ; *E. canis = Ehrlichia canis; H. canis = Hepatozoon canis; L. infantum = Leishmania infantum*

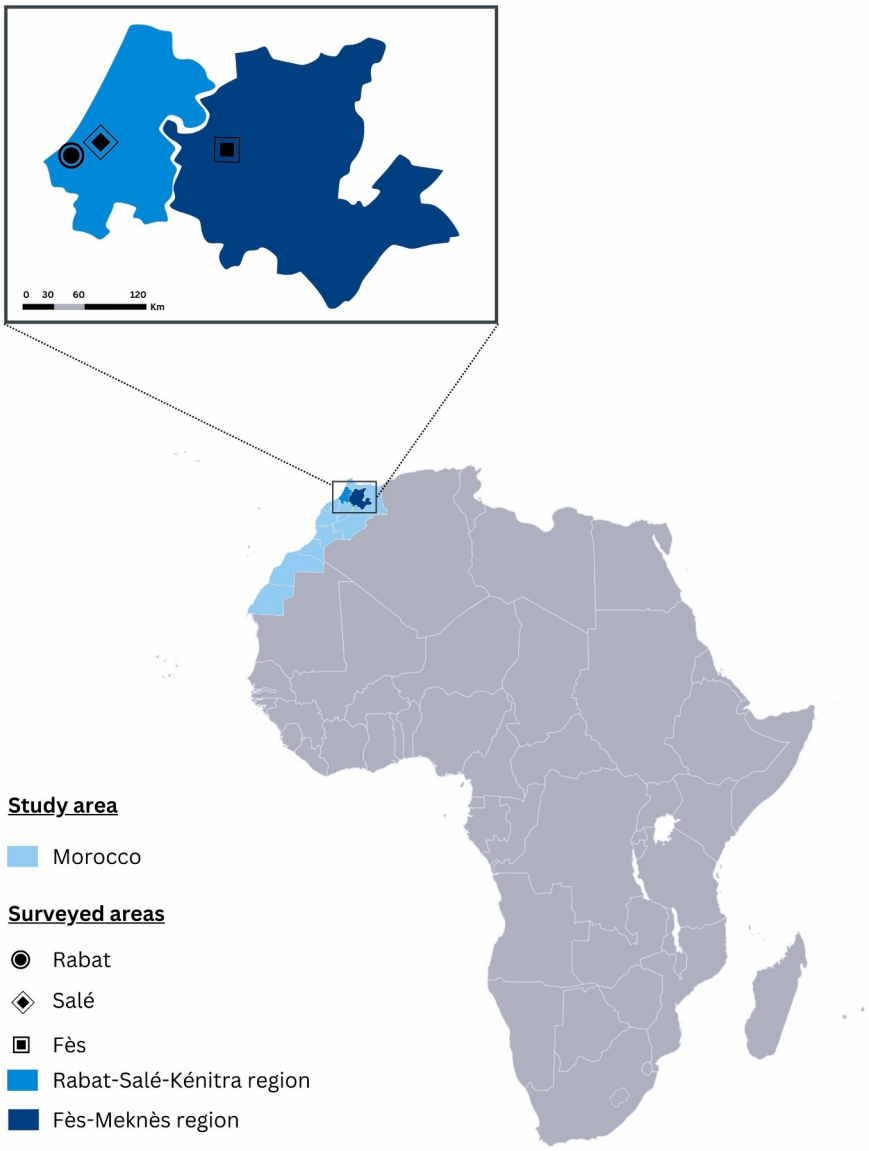

Fig. 1. Map showing the location of dog shelters studied in central Morocco.

4. Discussion

Data herein presented show a high overall positivity rate (i.e., 54.1 %) of VBPs in sheltered dogs highly infested by ticks, demonstrating that animals from the study area are highly exposed to VBPs, particularly those transmitted by ticks and sand flies. Similar results were obtained in a study conducted in Algeria, where the overall prevalence of VBPs in dogs was 62.1 % (Medkour et al., 2020). In the present study, *H. canis* was the most frequent pathogen molecularly detected (i.e., 38.2 % of dog blood DNA samples and 36.4 % of tick DNA pools), as observed in Egypt (i.e., 10.0 % of dogs; Mahdy et al., 2024) and Algeria (i.e., 3.1 % of *Rh. sanguineus* s.s. ticks; Laatamna et al., 2022). On the other hand, the low prevalence *of B. vogeli* in our study (i.e., 2.8 %) aligns with findings from Egypt and Tunisia, where this species was detected in 8.0 % (Mahmoud et al., 2024) and 3.0 % (Bouattour et al., 2021) of tested animals, respectively.

The high tick infestation on the dogs from this study, suggests a high level of environmental infestation in the studied shelters, favoring the ingestion of ticks harboring *H. canis*, as previously demonstrated (Otranto et al., 2011; Ramos et al., 2014). Indeed, the detection of *H. canis* in sheltered dogs indicates that the shelter environment plays an important role in exposing these animals to ticks and TBPs, thus advocating for ectoparasite control on dogs and in the environment (Pacifico et al., 2020; Iatta et al., 2021; Otranto et al., 2024).

The detection of *L. infantum* in the present study was higher (i.e., 15.3 %) than previously reported in Tunisia (i.e., 1.7 %; Zribi et al., 2023). This is also surprising considering that we molecularly tested blood (as compared to lymph node samples; Zribi et al., 2023), which is not considered the most reliable sample for detecting this parasite (Maia et al., 2009; Otranto et al., 2009b). This discrepancy may be related to local epidemiological factors (e.g., vector abundance in the study areas), which may favor the transmission. Indeed, similar high molecular positivity of *Leishmania* spp. infection has been recently recorded in sheltered dogs (24.5 %) from the cities of Rabat and F`es in Morocco (Lima et al., 2024). Conversely, *L. infantum* was detected in few privately owned dogs in Rabat (i.e., 2.1 %; Idrissi et al., 2021). The above picture has also been observed in other surveys in Algeria (i.e., from 3.2 % to 4.8 %; Medkour et al., 2020; Bellatreche et al., 2021). In spite of the limited data on the occurrence of CanL in dogs from shelter and domestic settings, it may be assumed that sheltered dogs are at a higher risk of *Leishmania* spp. infection, possibly due to the absence of effective control strategies (Estevam et al., 2022), and this may represent a risk from a public health perspective. CanL is endemic in the Mediterranean region, where different sand fly vectors are known to occur (Mhaidi et al., 2018; Zarrouk et al., 2022). However, the prevalence of infection may vary widely (Mendoza-Roldan et al., 2020; Galvez et al., 2020´ ; Bouattour et al., 2021b; Touhami et al., 2023).

Dog shelters may be important local foci for VBPs, including those of zoonotic concern. In addition, the relocation of sheltered dogs to other countries may favor the spread of VBPs into non endemic regions (Otranto et al., 2017). This was the case for a sheltered dog infected by *L. i nfantum* that was imported from Morocco into Canada (Wagner et al., 2020). The introduction of infected dogs into non endemic regions may complicate the diagnosis and treatment of the disease by local veterinary practitioners, as they may not suspect such a disease.

The finding of *E. canis* in the tested dog population (4/144; 2.8 %) was expected, considering the *Ehrlichia* spp. seropositivity of 34.6 % (75/217) recorded in a previous survey of owned and military dogs from seven locations in Morocco (El Hamiani Khatat et al., 2017b). This pathogen is commonly found in dogs and ticks worldwide (Bezerra-Santos et al., 2021) and has also been detected in humans (Silva et al., 2014; Bouza-Mora et al., 2017). Similarly, *A. platys* was herein detected in eight dogs, agreeing with similar reports from Morocco (7.5 %; El Hamiani Khatat et al., 2017a), Algeria (5.4 %; Dahmani et al., 2015), and Egypt (6.4 %; Selim et al. 2021). The common occurrence of *E. canis* and *A. platys* in these countries is related

to the presence of brown dog ticks, which are the main vectors involved in their transmission (Sarih et al., 2005; Ben Said et al., 2018; Laatamna et al., 2022). In this regard, the low prevalence of *E. canis* found herein could be related to the predominance of *R. sanguineus* s.s. in the study area. This tick species is not as competent as *Rhipicephalus linnaei* in transmitting *E. canis* (Moraes-Filho et al., 2011; Dantas-Torres et al., 2024). Further studies in different regions of Morocco would be valuable to investigate whether *E. canis* is more prevalent in areas where *R. linnaei* is present.

None of the examined dogs scored positive to *Rickettsia* spp., as in Algeria (Bessas et al., 2016) and Egypt (Izenour et al., 2022). This result might be due to the short rickettsemia reported in dogs, which makes blood a poor source for diagnosing rickettsial infections (Solano-Gallego et al., 2008; Campos et al., 2020). Similarly, none of the dogs were positive for mosquito-borne filarioids, which was somewhat unexpected, given the high abundance of culicid vectors (e.g., *Culex pipiens*, the recognized vector for these infections; Otranto et al., 2013) among sheltered dogs in Morocco (Zahri et al., 2024). *Dirofilaria immitis* have already been detected in stray dogs from Rabat (i.e., 12.3 %; Pandey et al., 1987) and was serologically diagnosed in dogs living in some Moroccan regions (i.e., 16.1 %; El Hamiani Khatat et al., 2017b). This pathogen was also identified in owned dogs from Algeria (i.e., 1.4 %; Tahir et al., 2017) and Tunisia (i.e., 14.5 %; Rjeibi et al., 2017). Nonetheless, the negative results herein recorded suggest a low prevalence or absence of *D. immitis* in the studied canine population.

5. Conclusion

This study confirms the occurrence of various VBPs among sheltered dogs in central Morocco, which is probably related to the high exposure of these dogs to arthropod vectors, such as ticks and sand flies. The implementation of regular preventive strategies may be impaired by the limited availability of financial resources but could be facilitated by public-private partnerships to mitigate the risk of VBPs transmission, especially those of zoonotic concern.

CRediT authorship contribution statement

Abderrahmane Zahri: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Kassim Abdullahi Jimale:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Marcos Antonio Bezerra-Santos:** ˆ Writing – review & editing, Visualization, Supervision, Methodology, Data curation, Conceptualization. **Renata Fagundes-Moreira:** Writing – review & editing, Visualization, Methodology. **Felix Gregor Sauer:** Writing – review & editing, Formal analysis. **Salma El Allali:** Writing – review & editing, Investigation. **Abdelwahed Allouch:** Writing – review & editing, Visualization. **Filipe Dantas-Torres:** Writing – review & editing, Visualization. **Maria Bourquia:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation, Conceptualization. **Domenico Otranto:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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General discussion and conclusions

This doctoral thesis provides updated data on the epidemiology, diagnosis, and control strategies of ZTBPs in ticks and domestic animal hosts, with particular emphasis on the One Health perspective. By integrating data from different management systems and host contexts in livestock and dog kennels, the work underscores the dynamic nature of ZTBPs at the interface of humans, animals, and vectors, highlighting their critical relevance to both veterinary and public health.

In **Chapter 1**, the two studies highlight the influence of management practices on tick parasitism and the consequent risks of ZTBP circulation. In particular, grazing systems such as silvopasture and open pasture were shown to modulate tick infestation dynamics in livestock, underscoring that husbandry decisions can either exacerbate or mitigate exposure to vectors.

Section 1.1: Transhumant grazing system poses a significant risk of tick infestation in livestock within pastoral communities, subsequently increasing ZTBP exposure in both animals and farmers (**Chepkwony et al., 2021; Jimale et al., 2023**). Several ZTBPs were recorded in livestock species and their associated ticks, including *Rickettsia* spp., *Ehrlichia* spp., *Coxiella burnetii*, and Crimean-Congo haemorrhagic fever virus (CCHFV) (**Defaye et al., 2022; Akuffo et al., 2016**).

In this doctoral thesis, we began with a literature review of ticks and tick-borne pathogens (TBPs) in domestic animals in Somalia and the neighboring regions of Ethiopia and Kenya, where unrestricted livestock movements across borders are common. We identified zoonotic pathogens circulating in livestock and their ticks, including those with epidemic potential (e.g., CCHFV), a disease prioritized by the WHO (**World Health Organization, 2025**) due to its outbreak risk and lack of effective treatments. Limited regional diagnostic capacity underscores the need for investment in facilities and human resources to enable more robust surveillance. Further studies adopting a One Health perspective are needed to assess the prevalence and socioeconomic impact of ticks and ZTBPs in both animals and humans (**Jimale et al., 2023)**. Such evidence is critical for developing sustainable control strategies and mitigating zoonotic risks in resource-limited settings.

Section 1.2: The characteristics of grazing systems adopted in livestock farming can significantly shape tick infestation dynamics, thereby influencing the epidemiological risk of exposure to pathogenic agents for both animals and farmers (**Rehman et al., 2017; Zapata et al., 2024**). Several *Rickettsia* species are pathogenic to humans causing rickettsioses (e.g., spotted fever), a global zoonosis transmitted by ticks of different genera (e.g., *Rhipicephalus*, *Ixodes*, *Hyalomma*) (**Parola et al., 2013; Jimale et al., 2024**). In this doctoral thesis, molecular investigation revealed high prevalence (52.5%) of spotted fever group rickettsiae in *Hyalomma marginatum* ticks collected from Maremmana cattle grazing in open pasture and silvopasture systems. In addition, we found that Maremmana cattle grazing in the silvopasture system were parasitized by a higher number of hard ticks, mainly *Hy. marginatum*. This may be partly explained by the woodland's greater ecological complexity and the availability of small hosts exploitable by hard ticks (**Dantas-Torres and Otranto, 2013**). Given the large number of ticks carried by cattle, they can serve as effective sentinels for ZTBPs in surveillance programs.

Data underscores the importance of considering management practices in tick control strategies, particularly in free-grazing systems, as part of a broader One Health approach to ZTBPs management.

In **Chapter 2**, the two studies focused on zoonotic pathogens in ticks and livestock hosts (i.e., cattle and goats) in southern Italy, as well as in kennel dogs in central Morocco. These epidemiological investigations revealed the presence of novel pathogens with zoonotic

potential in the studied ecological settings, underscoring the importance of continued surveillance for the detection of emerging pathogens of public health concern.

Section 2.1: In this study we investigated ticks, cattle and goats in Puglia Region of Italy, and along with other common TBPs (i.e., *Babesia* spp., *Theileria* spp., and *Rickettsia* spp.), we reported for the first time the protozoan *Colpodella* sp. in ticks in Italy. This potentially emerging tick-borne protozoan has previously been implicated in human infections in China, where it was associated with neurological signs (**Yuan et al., 2012; Jiang et al., 2018; Neculicioiu et al., 2021**). The DNA sequence of *Colpodella* sp. obtained in this study was identical to that detected in *Haemaphysalis longicornis* tick from a dog in China (**Qi et al., 2024**), which had previously been associated with a human patient presenting neurological symptoms and was designated *Colpodella* sp. HLJ (**Jiang et al., 2018**). This finding suggests that the same *Colpodella* species or strains may infect different tick genera across diverse animal hosts and geographic regions, offering new insights into the epidemiology and biology of this protozoan. Data herein reported underscore the importance of ongoing surveillance for the early detection of emerging ZTBPs, utilizing PCR and sequencing technologies.

Section 2.2: Dog shelters may be important local foci for TBPs, including those of zoonotic concern. In addition, the relocation of sheltered dogs to other countries may favor the spread of ZTBPs into non endemic regions (**Otranto et al., 2017**). In this study we assessed the occurrence of TBPs in sheltered dogs and their ticks (*Rhipicephalus sanguineus* sensu stricto) to evaluate the potential risk of zoonoses in highly populated, and poorly managed dog kennels. Among the detected pathogens include *Ehrlichia canis* (2.8%) and was not unexpected given the previously reported *Ehrlichia* spp. seropositivity of 34.6% among owned and military dogs from seven Moroccan locations (**Elhamiani et al., 2017**). Although *E. canis* is primarily a canine pathogen and widespread in dogs and ticks globally (**Bezerra-Santos et al., 2021**), it has occasionally been detected in humans, suggesting a potential zoonotic role that warrants further investigation (**Silva et al., 2014; Bouza-Mora et al., 2017**).

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About the author

The author of this thesis, Dr. Jimale, was born in Mahas, Somalia, on June 26, 1994. He began his studies in Veterinary Medicine at the Somali National University in 2015, earning his degree with honors in 2021. His undergraduate thesis focused on the identification of ticks of small ruminants in Benadir region of Somalia.

Dr. Jimale then moved to Italy to pursue a Ph.D. in 'Animal Health and Zoonoses' at the Department of Veterinary Medicine, University of Bari "Aldo Moro." His research focuses mainly on the biology, epidemiology, and control of vectors and vector-borne diseases of zoonotic concern.

He has co-authored nine publications in international peer-reviewed journals, with a focus on zoonotic parasites, vector-borne diseases, and One Health approaches. He has also been an invited speaker at national and international conferences, actively participates in workshops, and contributes to the peer-reviewing process of scientific works. Dr. Jimale is a member of several scientific societies, including the World Association for the Advancement of Veterinary Parasitology (WAAVP).

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