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Feline vector-borne haemopathogens in Türkiye: the first molecular detection of *Mycoplasma wenyonii* and ongoing *Babesia ovis* DNA presence in unspecific hosts

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Abstract

Background Cats are hosts and reservoirs for many haemopathogens such as piroplasms, *Rickettsia*, hemotropic *Mycoplasma*, *Bartonella*, *Ehrlichia*, and *Anaplasma*, which are transmitted by various vector arthropods and some of which have a zoonotic concern. Although it is noteworthy that the rate of ownership of companion animals has increased in Türkiye in recent years and that cats account for a large proportion of these animals, there is limited research on the vector-borne infectious agents carried by them. The present study aimed to provide a comprehensive molecular epidemiological data and molecular characterization of feline vector-borne haemopathogens (FVBHs), including piroplasms, anaplasmataceae, rickettsias, haemoplasmas, and *Bartonella* species in Türkiye. In total, 250 feline blood samples were collected from client-owned cats ($n=203$) and shelter cats ($n=47$) brought to the Small Animal Hospital of Selcuk University, Veterinary Faculty.

Results Overall, 40 (16%) cats were found to be infected with at least one of the investigated haemopathogens and piroplasm, *Mycoplasma* spp. and *Bartonella* spp. prevalence was 1.6%, 11.2%, and 4.8%, respectively. No *Anaplasma/Ehrlichia* spp. and *Rickettsia* spp. DNA was detected in the investigated feline samples. Sequence analysis revealed that all four piroplasms belonged to *Babesia ovis* with a 97.93–99.82% nucleotide sequence identity to *18S rRNA* gene sequences from Spain and Türkiye, while some sequenced hemoplasmas were *Mycoplasma haemofelis* (*Mhf*), *Candidatus* Mycoplasma haemominutum (CMhm) and *Mycoplasma wenyonii*, and *Bartonella* spp. were *Bartonella henselae* and *Bartonella koehlerae* species. Co-infections with *Mycoplasma* spp. and *Bartonella* spp. were also detected in 4 cats (1.6%) in this study, where single infections were predominant.

Conclusion This study provides valuable information on zoonotically important feline vector-borne hemopathogens in Türkiye, some of which have received attention under the One Health perspective, and is the first molecular

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epidemiological study to demonstrate the presence of *Babesia ovis*, the causative agent of ovine babesiosis, and *Mycoplasma wenyonii* DNA, the causative agent of bovine haemotropic mycoplasmosis, in cats. Further studies on the roles of such pathogens detected in unspecific hosts and the host specificity of the vectors that transmit them will contribute to the elucidation of this situation.

Keywords *Babesia*, Cats, *Mycoplasma wenyonii*, Türkiye, Zoonotic

Background

Due to human growing population and cat ownership habits, cats are a significant part of social life in Türkiye. Although there is no precise data on the number of stray cats in this country, it is estimated that almost one-fifth of the population owns cats. A study conducted in metropolitan Ankara indicates that there are thousands of stray cats in this province, giving an idea of the problem nationwide [1, 2]. Although cats are an essential part of human life as pets, they are hosts and reservoirs for many vector arthropod-transmitted bacterial, viral, protozoal, and helminthic infectious haemopathogens, some of which are of zoonotic concern. In different regions and socio-economic contexts, these companion animals, which share the same environment with humans, play an important role in the transmission of zoonotic agents [3].

Despite the presence of a large number of life-threatening and zoonotic infectious agents in cats, studies on this topic have not kept pace with the renewed interest in feline vector-borne diseases (VBDs), and therefore, the lack of knowledge on feline VBDs in particular dramatically hinders developing and implementing effective preventive and control measures at regional and national level [4]. The primary vector-borne pathogens (VBPs) of cats include *Babesia*, *Cytauxzoon*, *Hepatozoon*, *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Bartonella*, and *Mycoplasma* species, but this list can be further expanded [3–8].

Serological and molecular epidemiological studies contribute to increasing the recognition of feline VBPs worldwide [9, 10]. In Türkiye, data on the molecular epidemiology and phylogeny of feline VBHs are limited, although there have been studies of some FVBHs, including clinical cases of hepatozoonosis [11] and ehrlichiosis [12] in Türkiye [13–17]. Most of the investigations have been carried out on feline zoonotic *Bartonella* species [13, 17–19], but much remains to be done on other feline vector-transmitted bacterial and parasitic pathogens. The most comprehensive study on the molecular epidemiology of FVBHs was carried out in the province of Tekirdağ, Türkiye, in 2021, in which *Babesia canis canis*, *B. microti*, *Hepatozoon felis*, *Cytauxzoon felis*, *Bartonella henselae*, *Anaplasma platys*, *Anaplasma phagocytophilum*, *Rickettsia felis*, *Borrelia burgdorferi*, and *Mycoplasma* spp. were identified in symptomatic cats by species-specific conventional polymerase chain reaction (PCR) [16]. A study material representing a wider geographical area, including phylogenetic analyses, is thought to provide valuable data on FVBHs of Turkish cats. Therefore, this study aimed to molecularly investigate the vector-borne haemopathogens of owned and shelter cats from Türkiye and to perform phylogenetic characterization of the identified species.

Results

Prevalence of feline vector-borne haemopathogens

The present study molecularly screened 250 cats for FVBHs, and 40 cats (16%) were found to be positive for at least one pathogen. The study unexpectedly revealed that four cats (1.6%) tested positive for *B. ovis* deoxyribonucleic acid (DNA), the primary etiological agent of ovine babesiosis. The overall prevalences of *Mycoplasma* spp. and *Bartonella* spp. were identified as 11.2%, and 4.8%, respectively. Co-infections were also detected in four cats (1.6%) as *C*Mhm+*B. koehlerae* ($n=2$), *C*Mhm+*B. henselae* ($n=1$), and *C*Mhm+*Bartonella* spp. ($n=1$), respectively. The DNA of other piroplasma species, *Anaplasma* spp., *Ehrlichia* spp., and *Rickettsia* spp. was not detected in the study (Table 1).

Phylogenetic analysis

In the present study, the phylogenetic trees of *B. ovis* and *Mycoplasma* spp. were constructed based on their respective genes (*18S rRNA* and *16S rRNA*) by comparing them with the sequences deposited in the NCBI GenBank database. Four sequences of *B. ovis 18S rRNA* gene obtained in the study (OR984759- OR984762) established a well-supported clade with the *18S rRNA* gene sequences from Spain (sheep and goats) and Türkiye (sheep, horse) with a 97.93–99.82% nucleotide sequence identity (Fig. 1).

For *Mycoplasma* species, 11.2% of cats were detected as positive in terms of *Mycoplasma* spp., and most of the PCR-positive products were sequenced. As a result of the sequence analysis, 17 of the 21 *Mycoplasma* spp. positive PCR products were *C*Mhm, two were *M. haemofelis*, and one was uncultured *Mycoplasma* sp. On the other hand, one *Mycoplasma* isolate was confirmed as a bovine species, *M. wenyonii*. The *16S rRNA* gene sequences of *Mycoplasma* species are clustered in four separate clades. The *C*Mhm sequences OR979160- OR979168, OR979170-OR979173, OR979176, OR979177, OR979179, OR979180 formed a well-supported clade with *C*Mhm *16S rRNA* gene sequences obtained from cats in Hungary

Table 1 Prevalences of feline vector-borne pathogens

Province (*n*)	Piroplasma	Mycoplasma					Bartonella		
	Babesia ovis	*Mycoplasma haemofelis*	*Candidatus* Mycoplasma haemominutum	Uncultured *Mycoplasma* sp.	*Mycoplasma wenyonii*	*Mycoplasma* spp.	*Bartonella henselae*	*Bartonella koehlerae*	*Bartonella* spp.
Adana (3)	-	-	1	-	-	-	-	-	-
Aksaray (4)	-	-	-	-	-	-	-	-	1
Amasya (1)	-	-	1	-	-	-	-	-	-
Ankara (20)	1	-	3	-	-	-	-	2	1
Antalya (12)	-	-	1	-	-	2	-	-	-
Burdur (1)	-	-	1	-	-	-	-	-	-
Bursa (3)	-	-	-	-	-	-	-	-	1
Diyarbakır (1)	-	-	1	-	-	-	-	-	-
İstanbul (17)	-	-	2	-	-	-	-	-	-
Karaman (32)	-	-	2	-	-	1	2	-	1
Kocaeli (3)	-	-	-	-	-	1	-	-	1
Konya (87)	3	1	4	1	1	2	1	-	1
Manisa (3)	-	-	1	-	-	-	-	-	-
Niğde (2)	-	-	-	-	-	-	-	1	-
Samsun (3)	-	-	-	-	-	1	-	-	-
Siirt (1)	-	1	-	-	-	-	-	-	-
Other provinces (57)		-	-	-	-	-	-	-	-
Total (250)	4	2	17	1	1	7	3	3	6
Total positivity and prevalence (%)	**4 (1.6%)**	**28 (11.2%)**					**12 (4.8%)**		

(EU128752), Italy (KR905451), Switzerland (DQ157144), and Thailand (MK632396) with a nucleotide sequence identity ranging from 99.49 to 100%. The *M. wenyonii* isolate of the study clustered together with isolates reported from buffaloes (OM747881, OM747882) and cattle (OM468183) in Türkiye with a high level of nucleotide sequence similarity (98.0-99.46%). Although the uncultured *Mycoplasma* sp. isolate was located close to the *M. turicensis* and *M. haemofelis* clades, it formed a separate branch from the main clades. Finally, our *M. haemofelis* isolates (OR979169 and OR979175) showed 99.59–100% nucleotide sequence identity with feline *M. haemofelis* isolates from countries such as India (MN240855), Iran (KX253966), Romania (MT926038), Spain (AY150065) and Thailand (KU645929) and 99.77–99.80% nucleotide sequence identity with some *M. haemocanis* isolates from countries such as Iraq (MW784616) and Japan (AY529641) (Fig. 2).

No phylogenetic tree was constructed for *Bartonella* spp. due to the short amplified fragments of *16–23S rRNA* intergenic space region. Direct sequence analysis of half of the PCR amplicons of 12 *Bartonella* spp. positive samples identified in the study revealed *B. henselae* and *B. koehlerae*. Blast analysis of our *B. henselae* sequences (OR978401, OR978402, and OR978403) showed 99.42–100% nucleotide sequence identity to a rodent (*Mus macedonius*) isolate from Türkiye (OR198887), human isolates from Korea (JQ638927), Australia (AJ439687), France (AF312496), and a dog isolate from China (JQ009430). On the other hand, the *B. koehlerae* isolates from this study (OR978398, OR978399, and OR978400) have 99.33–100% nucleotide sequence similarity with *Bartonella* isolates from cat, lions (*Panthera leo*), and hard ticks reported from the USA, South Africa, and Palestine, respectively.

Statistical analysis results

The distribution of the species detected in the study in relation to cat breeds, age, sex, and status was statistically analyzed and the findings are shown in Table 2. The study findings revealed that *Mycoplasma* spp. had a statistically significant high prevalence in males ($p=0.031$) and cats older than one year ($p=0.022$), and piroplasms had a statistically significant high prevalence in shelter cats ($p=0.015$). Although statistically insignificant, it is noteworthy that all *Bartonella* infections were detected in owned cats older than one year.

Discussion

Cats, especially those living outdoors, are exposed to various arthropods, including fleas, ticks, blood-sucking flies, and the pathogens they harbor. Cats may pose a risk of infection to humans or other animals that share the same habitat with vector arthropods or may act as reservoirs of

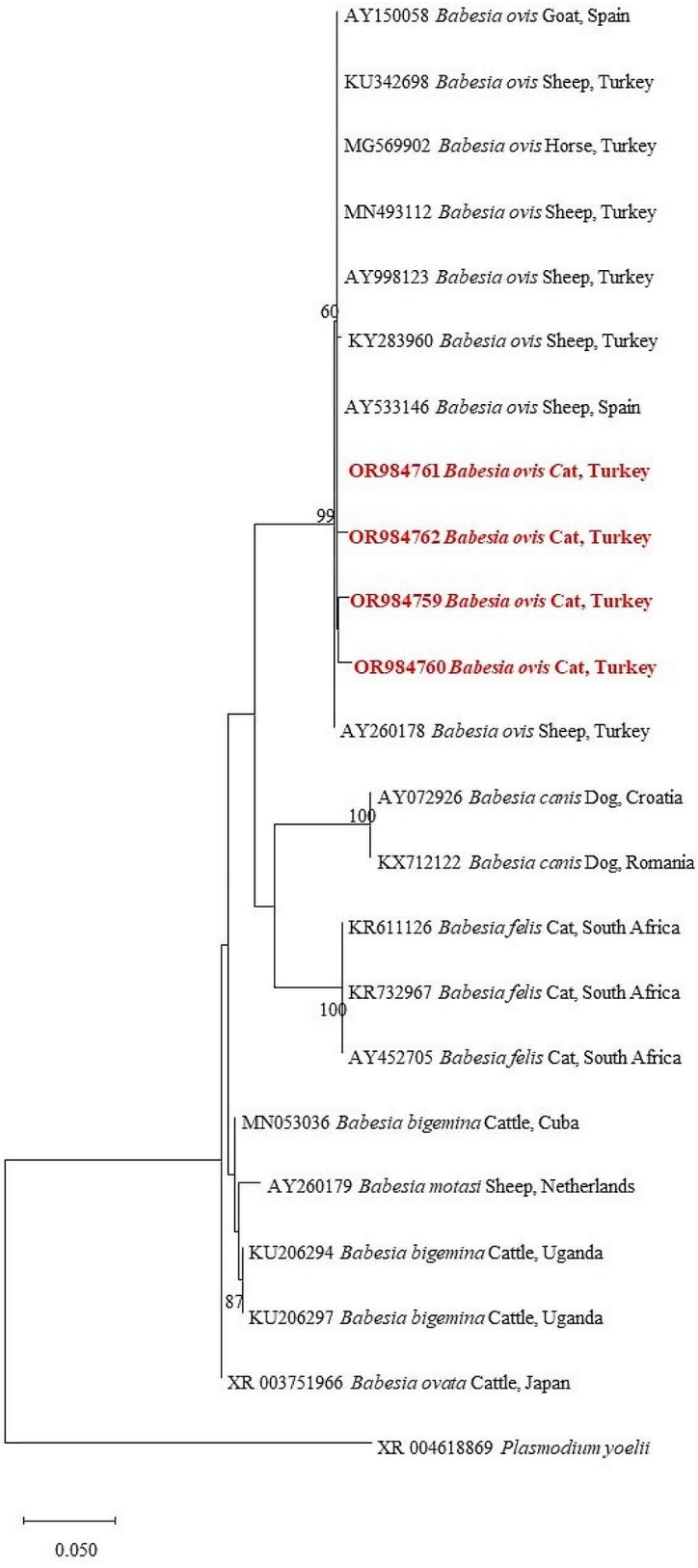

Fig. 1 A maximum likelihood phylogram of *Babesia ovis* inferred from the *18S rRNA* gene. The tree was constructed by MEGA version X software using the Kimura 2-parameter and evolutionary rates among sites (K2 + G). Bootstrap analysis with 1000 replications was employed to ascertain the confidence of the nodes and branches within the tree. The sequences of this study are shown in boldface. The *18S rRNA* gene sequence of *Plasmodium yoelii* (XR004618869) was used as an outgroup

Fig. 2 A maximum likelihood phylogram of *Mycoplasma* sp. inferred from the *16S rRNA* gene. The tree was constructed by MEGA version X software using the Kimura 2-parameter and evolutionary rates among sites (K2 + G). Bootstrap analysis with 1000 replications was employed to ascertain the confidence of the nodes and branches within the tree. The sequences of this study are shown in boldface. The *16S rRNA* gene sequence of *Anaplasma bovis* (KM114613) was used as an outgroup

VBPs, thereby posing a risk of infection for living organisms in these environments [20, 21]. Notably, studies on the VBPs' epidemiology in pet animals have increased in recent years. However, feline VBPs have yet to be investigated as much as canine VBPs [21]. This situation is similar both on a large scale and on a Turkish scale, where there are serious gaps in the molecular epidemiology and phylogeny of feline VBPs. One of the priorities in keeping diseases under control is to analyze the situation, identify the presence of pathogens and risk factors, and try to keep this information as up-to-date as possible. Conducting large-scale studies increases the quality of the results to be obtained due to varying climatic conditions, structure, and density of vector arthropod populations [22]. Therefore, VBPs were investigated by molecular methods in cats brought to Konya, Selçuk University, Faculty of Veterinary Medicine, Animal Hospital from many provinces of Türkiye, and unique epidemiological data were obtained in this study. The study revealed the presence of *Babesia* spp. (1.6%), *Mycoplasma* spp. (11.2%), and *Bartonella* spp. (4.8%) in cats, respectively. Sequencing and phylogenetic analyses also revealed the presence of ovine babesiosis agent *B. ovis* and bovine severe anemia-associated *M. wenyonii* DNA in cats for the first time. Moreover, significant feline hemotropic *Mycoplasma* species such as *C*Mhm, *M. haemofelis*, uncultured *Mycoplasma* sp., and *Bartonella* species, including *B. henselae* responsible for cat-scratch disease (CSD) in humans and *B. koehlerae* causing human endocarditis were identified by sequencing.

Babesia is one of the most common haemoprotozoan parasites in mammalian hosts and infects the erythrocytes of many vertebrate hosts, including cats [23]. Although babesiosis transmitted by ticks is one of the main problems for livestock farming, infections in pet animals can cause serious economic losses and deaths [7, 23]. Feline babesiosis cases with severe clinical symptoms associated with erythrocyte destruction are particularly prevalent in South Africa, sporadic clinical cases have also been reported in Europe and Asia [9, 24, 25]. To date, various *Babesia* species such as *B. felis*, *B. cati*, *B. canis canis*, *B. gibsoni*, *B. canis vogeli*, *B. honkongensis*, *B. lengau*, *B. leo*, *B. microti*, *B. presentii*, and unidentified *Babesia* spp. have been detected in cats, and it is noteworthy that especially canine *Babesia* species cause clinical infections in cats and are frequently encountered [24, 26–29]. However, the number of new species is increasing in parallel with advances in molecular technology. Genetic diversity among *Babesia* species also indicates that new *Babesia* species can emerge [26, 30]. Epidemiological data on feline babesiosis in Türkiye are limited and no molecular data on feline-specific *Babesia* species have been detected. However, a rodent species *B. microti* (2.4%) and a canine species *B. canis canis*

Table 2 Statistical findings on the distribution of infections according to different variables

		Piroplasm positive (%)	*Mycoplasma* spp. positive (%)	*Bartonella* spp. positive (%)
Age	< 1 year (*n*: 35)	0 (0%)	0 (0%)	0 (0%)
	≥ 1 year (*n*: 215)	4 (1.86%)	28 (13.02%)	12 (5.58%)
	Total (*n*: 250)	4 (1.59%)	28 (11.16%)	12 (4.78%)
	p value	0.409	**0.022**	0.146
Gender	Male (*n*: 121)	2 (1.65%)	19 (15.70%)	7 (5.79%)
	Female (*n*: 129)	2 (1.55%)	9 (6.98%)	5 (3.88%)
	Total (*n*: 250)	4 (1.59%)	28 (11.16%)	12 (4.78%)
	p value	0.955	**0.031**	0.490
Status	Owned (*n*: 203)	1 (0.49%)	23 (11.33%)	12 (5.91%)
	Shelter (*n*: 47)	3 (6.38%)	5 (10.64%)	0 (0%)
	Total (*n*: 250)	4 (1.6%)	28 (11.2%)	12 (4.8%)
	p value	**0.015**	0.930	0.225
Breed	Crosbreed	3 (3.06%)	12 (12.24%)	4 (4.08%)
	Tabby	0 (0%)	12 (15.38%)	6 (7.69%)
	Bombay	0 (0%)	0 (0%)	0 (0%)
	British Shorthair	0 (0%)	0 (0%)	1 (6.25%)
	British Longhair	0 (0%)	0 (0%)	0 (0%)
	Blue Point Siamese	0 (0%)	0 (0%)	0 (0%)
	Chinchilla	0 (0%)	0 (0%)	0 (0%)
	Exotic Shorthair	0 (0%)	0 (0%)	0 (0%)
	Orange Tabby	1 (14.29%)	1 (14.29%)	0 (0%)
	Himalayan	0 (0%)	0 (0%)	0 (0%)
	Scottish Fold	0 (0%)	1 (7.69%)	1 (7.69%)
	Tuxedo	0 (0%)	2 (28.57%)	0 (0%)
	Siamese	0 (0%)	0 (0%)	0 (0%)
	Norwegian Forest Cat	0 (0%)	0 (0%)	0 (0%)
	Turkish Angora	0 (0%)	0 (0%)	0 (0%)
	Turkish Van	0 (0%)	0 (0%)	0 (0%)
	Persian	0 (0%)	0 (0%)	0 (0%)
	Total (*n*: 250)	4 (1.6%)	28 (11.2%)	12 (4.8%)
	p value	0.862	0.907	0.999

(24%) have recently been reported molecularly in cats with symptoms such as weight loss, fever, haematological abnormalities, and lymphadenopathy in Tekirdağ province of Türkiye [16]. Furthermore, a study investigating the microscopic prevalence of *Babesia* in apparently healthy cats from Van province of Türkiye about 20 years ago revealed a prevalence of 10.8% for *B. felis* [31]. Unlike previous studies, the current study reports for the first time the presence of *B. ovis* DNA, the most pathogenic ovine babesiosis agent, in the four cats without any signs of clinical infection. The higher molecular prevalence of *B. ovis* DNA detected in shelter cats with statistical significance may be attributed to the different implementations applied to owned cats and shelter cats in terms of vector control. Our *B. ovis* isolates were also phylogenetically analyzed, and the four sequences constructed a well-supported clade with other Turkish isolates from sheep with the nucleotide sequence identity 97.93–99.82% and horse with the identity of 99.19–99.57%. There are many studies indicating that haemoprotozoa can be found in different hosts other than the original host [30, 32, 33], and some of these studies have recently reported that *B. ovis* has been detected in unspecific hosts such as donkeys and horses in Türkiye [34, 35]. This study provides valuable molecular epidemiological data, including cats among the unspecific hosts in which *B. ovis* DNA was detected, and the detection of *B. ovis* in cats opens new avenues for research into the epidemiology and pathogenicity of this and related species in unspecific host populations.

Feline haemotropic mycoplasmosis, which had been diagnosed cytologically before the 2000s in Türkiye [36], is now being investigated using PCR in parallel with advances in molecular diagnostic methods. It has been noted that the disease can cause mortality in domestic cats in this country [37, 38], and molecular prevalences have reached high levels in cats suspected of having the disease [39, 40]. When the studies on the subject were compiled, few studies regarding the molecular epidemiology of hemotropic mycoplasmas in domestic cats (Felis

catus) have been encountered in Türkiye. In these studies, haemoplasmas were molecularly detected in Ankara (23.1%), Antalya (17.6%), Bursa (7.7%), İzmir (17.5%) [40], Kayseri (9.5%) [41], İstanbul (19.3%) [15] and Tekirdağ (11.4%) [16] provinces. In most of these studies, *C*Mhm was found to be the main species responsible for infections [37, 39, 40, 42], and in some studies, the species has not been identified [16]. Species-specific PCR analysis revealed that the haemotropic *Mycoplasma* species molecularly detected in cats in Türkiye consisted of *Mhf*, *C*Mhm, and *Candidatus* Mycoplasma turicensis (*C*Mt). Cetinkaya et al. [15] reported molecular prevalences of 9.9%, 17.7%, and 0.8% for each species in their study conducted in İstanbul province, respectively. Although some molecular epidemiological studies have been carried out at the provincial level in Türkiye [15, 16, 40], there is a scientific research gap in the molecular phylogenetic characterization of the identified *Mycoplasma* species. The present study revealed that the molecular prevalence of *Mycoplasma* spp. in Turkish cats was 11.2% by *Mycoplasma* genus-specific PCR analyses targeting the *16S rRNA* gene fragment and revealed remarkable findings to fulfill the scientific gap on the subject in Türkiye. The study findings also revealed that *Mycoplasma* spp. infections were statistically more common in males and cats older than one year. This is thought to be due to a higher chance of exposure to vector arthropods with increasing age and to aggressive interactions between male cats.

Direct sequencing of most of the PCR amplicons (n=21) of positive *Mycoplasma* spp. samples confirmed that two samples belonged to *Mhf* from Konya and Siirt provinces, 17 samples to *C*Mhm from Adana, Amasya, Ankara, Antalya, Burdur, Diyarbakır, İstanbul, Karaman, Konya, and Manisa provinces, one sample to uncultured *Mycoplasma* sp. from Konya province and one sample to *M. wenyonii* from Konya province, one of the bovine haemotropic *Mycoplasma* species. Sequence analysis could not performed on the remaining seven PCR-positive samples. The specific identification of *Mhf*, *C*Mhm, uncultured *Mycoplasma* sp., and *M. wenyonii* in our study provides insight into the diversity of *Mycoplasma* infections affecting cats in Türkiye and calls for a deeper understanding of their clinical and epidemiological significance. The findings of the study showed that *C*Mhm is the main species playing a role in feline haemoplasmosis, and this is consistent with previous studies conducted in Türkiye [37, 39, 40]. The fact that *M. wenyonii* DNA, which is one of the remarkable findings of the study, was detected for the first time in a female Tabby cat owned in Konya province points to the need for further studies on the transmission dynamics of feline haemotropic *Mycoplasma* species, the vector arthropods involved in the transmission, and whether this species is important in terms of clinical infection outside its original host. Detecting *M. wenyonii* in a domestic cat also highlights potential cross-species transmission and underscores the complex ecosystem interactions facilitating the spread of vector-borne diseases. To the best of the authors' knowledge, this study is also the most comprehensive study on the phylogenetic analysis of feline haemotropic *Mycoplasma* species in Türkiye to date, showing that feline-specific *Mycoplasma* species cluster together with isolates reported from different countries with high nucleotide sequence similarities (99.49–100%). The *M. wenyonii* isolate reported for the first time in cats clustered with Turkish buffalo (OM747881, OM747882) [43] and cattle (OM468183) [43] isolates with 98.00-99.46% nucleotide sequence similarity. The diversity of *Mycoplasma* species, identified in this study ranging from well-documented feline pathogens like *Mhf* and *C*Mhm to the less common *M. wenyonii*, emphasizes the complex nature of feline haemotropic mycoplasmosis. Further investigations are essential to elucidate the clinical relevance and transmission dynamics of *M. wenyonii* infections in cats.

Bartonella species are emerging opportunistic bacterial haemopathogens transmitted by blood-sucking arthropods, have a wide range of hosts, including cats, and can colonize endothelial cells, migrate into the bloodstream, and infect erythrocytes [8]. Cats are the main reservoirs of public health-relevant *Bartonella* species such as *B. henselae* and *B. clarridgeiae* causing CSD and *B. koehlerae* causing human endocarditis [3, 44]. While the transmission of the infection is mainly through fleas of the species *Ctenocephalides felis felis*, known as cat fleas [45], it has been scientifically confirmed that ticks of the species *Ixodes ricinus* can transmit *B. henselae* transtadially [46]. There are several serological and molecular studies investigating *Bartonella* spp. in humans and cats in Türkiye [13, 14, 16, 18, 47, 48]. Atıcı et al. [49] reported a systemic CSD characterized with hepatic mass in an immunocompromised child. Kaynar et al. [50] reported CSD in a patient with hypotension, pancytopenia, and acute kidney injury who had a history of being scratched more than once by stray cats and pointed out that this infection, for which there is limited data, should be more widely recognized among Turkish clinicians to prevent delays in diagnosis. In Türkiye, some other case studies emphasize the importance of *Bartonella* spp. infection and show that it is a pathogen that needs attention [51, 52]. Moreover, Sayın-Kutlu et al. [47] detected high levels of *B. henselae* seroprevalence among cattle breeders (19%) and veterinarians (29.6%) and therefore pointed out that bartonellosis should be considered in the differential diagnosis list of fever of unknown origin in humans in rural areas of Aydın and Denizli provinces of Türkiye, where the study was conducted. A recent study has reported that the prevalence of *B. henselae* is statistically

high in people working in agriculture and animal husbandry and exposed to arthropods such as ticks and sandflies [53]. Although most of the studies are on human medicine, clinical infection with *B. henselae* characterized by pyogranulomatous myocarditis, and diaphragmatic myositis has also been reported in a flea-infested cat in Türkiye [54]. In light of the literature above, studies indicate the significance of feline-associated *B. henselae* infections in both human and veterinary medicine in Türkiye. The fact that most of the case reports, serologic and molecular epidemiological studies have been conducted in the last ten years reflects the emerging public awareness about bartonellosis in the country. However, further scientific studies are still needed to increase the data on this emerging vector-borne infection. This study reports a molecular prevalence of 4.8% for *Bartonella* spp. in a sample representing a wide geographical area of Türkiye, for which a seroprevalence of 18.6–36.2% [13, 14, 18, 48] and a molecular prevalence of 12.1–40.1% [16, 17, 19, 55] have previously been detected at provincial levels in cats. Sequence analysis of PCR amplicons from half of the positive samples ($n=12$) confirmed that apparently healthy cats are reservoirs for *B. henselae* ($n=3$) and *B. koehlerae* ($n=3$) species. The presence of *B. henselae* and *B. koehlerae* in the feline population in Türkiye underscores the critical role of companion animals in the epidemiology of zoonotic diseases. While *B. henselae* is widely recognized for its zoonotic potential, particularly concerning CSD, our findings on *B. koehlerae* highlights an expanding horizon of concerns and interests in the veterinary and medical communities. Although less documented, the implications for human health necessitate a vigilant approach to understanding and managing *B. koehlerae* infections. This underscores the complexity of *Bartonella* as a genus, with multiple species impacting animal and potentially human health, and stresses the importance of a One Health approach to address these zoonotic threats effectively.

In addition to single infections, a total of four cats (1.6%) were co-infected with three different combinations of *C*Mhm+*B. koehlerae*, *C*Mhm+*B. henselae*, and *C*Mhm+*Bartonella* spp. in the study. Pennisi et al. [56] stated that co-infections caused by multiple vector-borne haemopathogens can occur in various animals including cats, but there are uncertainties about their clinical outcomes and further studies are needed to clarify this situation, especially in cats. Although there are studies reporting that *Bartonella* spp. and haemotropic *Mycoplasma* spp. coinfection is common in cats, the predisposing factors are not known [57]. No clinical signs were observed in cats co-infected with *Mycoplasma* spp. and *Bartonella* spp. in this study. However, it should be noted that different types of co-infection may have a negative impact on the prognosis of VBDs in animals, especially when compared to animals infected with a single species, co-infected animals may be adversely affected under stressful conditions [58–60]. Qurollo et al. [61] suggested that co-infection in a cat with *Anaplasma platys*, *B. henselae*, *B. koehlerae*, and *C*Mhm may be associated with immunosuppressive conditions such as myeloma-related disorders. Such an inference cannot be made about the co-infection status in this study, because the cats were apparently healthy and further clinical diagnostic laboratory analyses were not performed. However, co-infections may be attributed to infections following previous ectoparasite infestations and carrier animals status due to preimunition.

It is noteworthy that although the detection of *B. ovis* DNA in cats indicates a history of tick infestation, no *Rickettsia* spp. or *Anaplasma*/*Ehrlichia* spp. DNA was detected in this study. A study on the subject reported that this may be related to transient rickettsemia [62]. In addition, the fact that anaplasmosis, ehrlichiosis, and rickettsiosis are not common in cats supports the findings of the study [57, 63–65].

The highly conserved *18S rRNA* gene was utilized for the identification of piroplasms, as it is widely used in molecular epidemiological studies as a marker for the primary screening [66–68]. However, it is important to note that the *18S rRNA* gene has limited sequence variation among different species within the same genus. Consequently, this gene may not provide sufficient resolution to effectively distinguish piroplasm species [69, 70]. Previous studies showed that *Babesia duncani* n.sp. is phylogenetically indistinguishable from other *Babesia* and *Theileria* species when analyzed using *18S rRNA* sequencing. However, it can be distinctly identified through internal transcribed spacer 2 (*ITS2*) sequencing [70]. Moreover, Niu et al. [71] successfully described the differentiation of *B. motasi* and *B. sp. Xinjiang* based on *ITS* region sequences. Mitochondrial genes evolve faster, resulting in higher sequence divergence compared to *18S rRNA* [72]. Based on the cytochrome c oxidase subunit 1 (*COI*) gene, *B. canis* and *B. vogeli* infections can be diagnosed faster and more reliably [73]. Furthermore, Sivakumar et al. [69] successfully used the mitochondrial *cytochrome b* gene to distinguish between *Theileria annulata* and *Theileria* sp. *yokoyama*. Thus, future research should consider targeting other faster evolving genes of *B. ovis* for approach confirming the presence of *B. ovis* in unexpected hosts.

Conclusion

Our study is one of the most comprehensive epidemiological surveys conducted for molecular screening and characterization of multiple feline VBPs from a wide geographical area of Türkiye and revealed that the cat population harbors a wide range of VBPs. In the study,

Babesia ovis, *Mycoplasma* spp. including *Mycoplasma haemofelis*, *Candidatus* Mycoplasma haemominutum, uncultured *Mycoplasma* spp., *Mycoplasma wenyonii*, and *Bartonella* spp., including *Bartonella henselae* and *Bartonella koehlerae* were detected as single infections and co-infections. This study also reported *Babesia ovis* and *Mycoplasma wenyonii* for the first time in cats. Considering the zoonotic concern of some of the haemopathogens detected in the current study, further epidemiological studies should be advocated in humans and pets to determine the distribution status of VBPs, identify risk factors, and implement effective vector arthropod control strategies for prevention.

Methods

Cats and blood samples

The study material consisted of 250 randomly selected cats (Owned=203, Shelter=47) brought to Selcuk University, Faculty of Veterinary Medicine, Animal Hospital for routine procedures from 44 different provinces of Türkiye. Selcuk University is located in the province of Konya (37° 53′ N, 32° 31′ E), which is localized in the central part of Türkiye. Konya province is close to some of the tourism resorts bordering the Mediterranean Sea and is located on the transit route of domestic tourists going to tourism regions, especially during the summer seasons. Therefore, tourists from many different provinces and their pet cats visit the animal hospital for various reasons such as chipping, shaving, vaccinations, surgical procedures or various diseases. Comprehensive details regarding the sampling sites and sample numbers are shown in Fig. 3; Table 3. The body surface of cats with no skin lesions was visually inspected for relatively large ectoparasites such as ticks, fleas, and lice. No ectoparasite infestation was recorded in cats enrolled in the study. Blood samples were collected from clinically healthy cats without symptoms of infectious diseases. Blood samples (0.5–1.5 mL) were collected into ethylenediamine tetraacetic acid (EDTA) coated vacuum vials from each cat through the cephalic or saphenous veins. All collected samples were centrifuged (3000 rpm for 15 min) to separate red blood cells and plasma and kept at -20℃ until use.

Genomic DNA isolation

High Pure PCR Template Preparation Kit (Roche, Germany) was used for genomic DNA extraction from feline blood samples following the manufacturer's instructions. Until the molecular analysis, the genomic DNAs were held at −20ºC. The extracted genomic DNA concentrations were quantified using a NanoDrop 2000 spectrophotometer (Colibri TITERTEK BERTHOLD).

PCR assays for detection of feline haemopathogens

Each feline genomic DNA was screened by genus- or species-specific PCR/nPCR targeting to amplify *Babesia/Theileria/Hepatozoon* spp. *18S rRNA*, *Babesia/Theileria* spp. *18S rRNA* (*V4*), *Anaplasma/Ehrlichia* spp. *16S rRNA*, *Mycoplasma* spp. *16S rRNA*, *Rickettsia* spp. and *Rickettsia felis gltA* gene fragments, and *Bartonella* spp. *16–23S rRNA* intergenic region. The molecular screening was carried out following the references, correspondingly listed in Table 4. The final volume of the PCR reaction mixture was prepared as 10 µL containing 6.35 µL of double-distilled water (Invitrogen, Ultrapure™ Distilled Water, DNAse, RNAse, Free), 2 µL of 5X One Taq Standard Reaction buffer (BioLabs, New England), 0.2 µL of dNTP mix (Deoxynucleotide solution mix, BioLabs, New England), 0.05 µL One Taq DNA polymerase (BioLabs, New England), 0.2 µM of each primer, and 1 µL of DNA

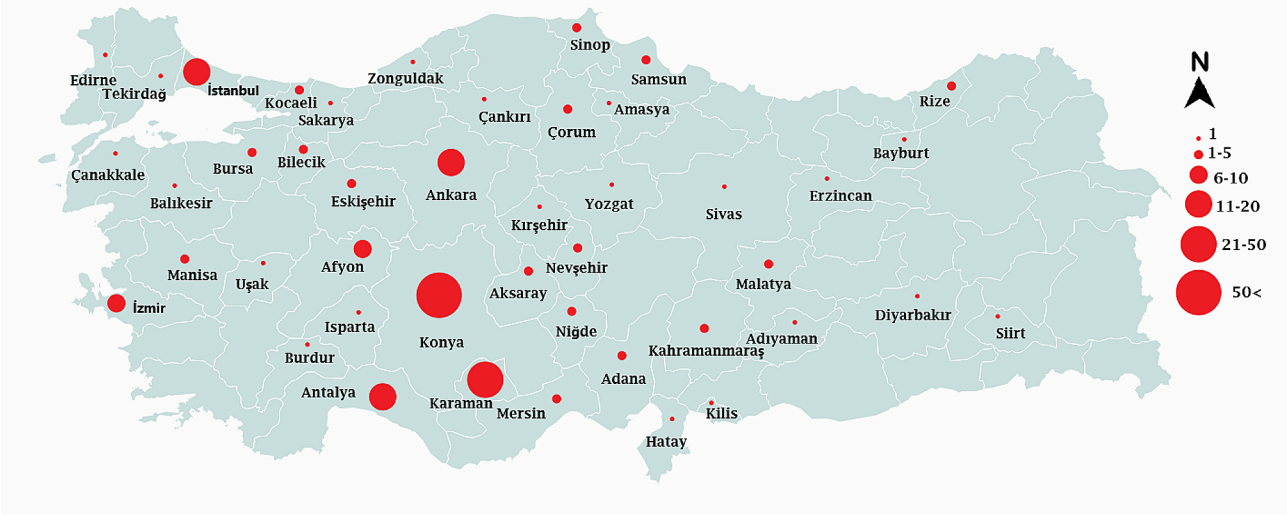

Fig. 3 Provinces where the sampled cats are registered

Table 3 Detailed information regarding the sampled cats

Province	*n*	Breed (*n*)	Sex	Status
Antalya	12	British shorthair (*n* = 1), Crossbreed (*n* = 3), Scottish fold (*n* = 1), Tabby (*n* = 6), Orange Tabby (*n* = 1)	6 ♂, 6 ♀	Owned (*n* = 12)
Adana	3	British shorthair (*n* = 1), Crossbreed (*n* = 1), Tabby (*n* = 1)	3 ♀	Owned (*n* = 3)
Adıyaman	1	Crossbreed (*n* = 1)	1 ♂	Owned (*n* = 1)
Amasya	1	Crossbreed (*n* = 1)	1 ♂	Owned (*n* = 1)
Ankara	20	Crossbreed (*n* = 7), Sarman (*n* = 2), Scottish fold (*n* = 1), Tuxedo (*n* = 1), Tabby (*n* = 9)	10 ♂, 10 ♀	Owned (*n* = 20)
Afyon	6	Chinchilla (*n* = 1), Crossbreed (*n* = 2), Tabby (*n* = 2), Turkish Angora (*n* = 1)	6 ♂	Owned (*n* = 6)
Aksaray	4	Crossbreed (*n* = 4)	1 ♂, 3 ♀	Owned (*n* = 4)
Balıkesir	1	British shorthair (*n* = 1)	1 ♂	Owned (*n* = 1)
Bilecik	3	Crossbreed (*n* = 2), Scottish fold (*n* = 1)	3 ♀	Owned (*n* = 3)
Burdur	1	Crossbreed (*n* = 1)	1 ♀	Owned (*n* = 1)
Bursa	3	Chinchilla (*n* = 1), Crossbreed (*n* = 1), Tabby (*n* = 1)	1 ♂, 2 ♀	Owned (*n* = 3)
Bayburt	1	Tabby (*n* = 1)	1 ♂	Owned (*n* = 1)
Çanakkale	1	Tabby (*n* = 1)	1 ♀	Owned (*n* = 1)
Çorum	2	Tabby (*n* = 2)	2 ♀	Owned (*n* = 2)
Diyarbakır	1	Orange Tabby (*n* = 1)	1 ♂	Owned (*n* = 1)
Çankırı	1	Tabby (*n* = 1)	1 ♂	Owned (*n* = 1)
Edirne	1	Tabby (*n* = 1)	1 ♂	Owned (*n* = 1)
Erzincan	1	Crossbreed (*n* = 1)	1 ♂	Owned (*n* = 1)
Eskişehir	3	Tuxedo (*n* = 1), Tabby (*n* = 2)	1 ♂, 2 ♀	Owned (*n* = 3)
Hatay	1	Norwegian forest cat (*n* = 1)	1 ♀	Owned (*n* = 1)
Isparta	1	Tabby (*n* = 1)	1 ♀	Owned (*n* = 1)
İstanbul	17	British longhair (*n* = 1), British shorthair (*n* = 1), Crossbreed (*n* = 10), Scottish fold (*n* = 1), Tabby (*n* = 3), Tuxedo (*n* = 1)	11 ♂, 6 ♀	Owned (*n* = 17)
İzmir	10	Bombay (*n* = 1), British shorthair (*n* = 2), Chinchilla (*n* = 1), Crossbreed (*n* = 1), Persian (*n* = 2), Orange Tabby (*n* = 1), Tabby (*n* = 1), Turkish Angora (*n* = 1)	4 ♂, 6 ♀	Owned (*n* = 10)
Karaman	32	Blue Point Siamese (*n* = 1), British longhair (*n* = 2), British shorthair (*n* = 3), Crossbreed (*n* = 7), Persian (*n* = 2), Scottish fold (*n* = 3), Tuxedo (*n* = 2), Tabby (*n* = 9), Turkish Angora (*n* = 2), Turkish Van (*n* = 1)	15 ♂, 17 ♀	Owned (*n* = 32)
Kahramanmaraş	2	Persian (*n* = 2)	2 ♀	Owned (*n* = 2)
Kırşehir	1	Orange Tabby (*n* = 1)	1 ♂	Owned (*n* = 1)
Kilis	1	Tabby (*n* = 1)	1 ♀	Owned (*n* = 1)
Kocaeli	3	British shorthair (*n* = 1), Scottish fold (*n* = 1), Tabby (*n* = 1)	1 ♂, 2 ♀	Owned (*n* = 3)
Konya	87	British shorthair (*n* = 4), Crossbreed (*n* = 49), Exotic shorthair (*n* = 1), Scottish fold (*n* = 4), Siamese (*n* = 2), Tuxedo (*n* = 1), Tabby (*n* = 23), Turkish Angora (*n* = 3)	39 ♂, 48 ♀	Owned (*n* = 40) Shelter (*n* = 47)
Malatya	4	Crossbreed (*n* = 2), Siamese (*n* = 1), Tabby (*n* = 1)	2 ♂, 2 ♀	Owned (*n* = 4)
Manisa	3	Crossbreed (*n* = 1), Tuxedo (*n* = 1), Tabby (*n* = 1)	2 ♂, 1 ♀	Owned (*n* = 3)
Mersin	4	Crossbreed (*n* = 2), Himalayan (*n* = 1), Tabby (*n* = 1)	1 ♂, 3 ♀	Owned (*n* = 4)
Nevşehir	2	Turkish Angora (*n* = 1), Turkish Van (*n* = 1)	1 ♂, 1 ♀	Owned (*n* = 2)
Niğde	2	Tabby (*n* = 2)	2 ♂	Owned (*n* = 2)
Rize	2	British shorthair (*n* = 1), Persian (*n* = 1)	1 ♂, 1 ♀	Owned (*n* = 2)
Sakarya	1	Scottish fold (*n* = 1)	1 ♂	Owned (*n* = 1)

Table 3 (continued)

Province	*n*	Breed (*n*)	Sex	Status
Samsun	3	Tabby (*n* = 3)	3 ♂	Owned (*n* = 3)
Siirt	1	Tabby (*n* = 1)	1 ♂	Owned (*n* = 1)
Sinop	2	Tabby (*n* = 2)	1 ♂, 1 ♀	Owned (*n* = 2)
Sivas	1	Tabby (*n* = 1)	1 ♀	Owned (*n* = 1)
Tekirdağ	1	Tabby (*n* = 1)	1 ♀	Owned (*n* = 1)
Uşak	1	British shorthair (*n* = 1)	1 ♀	Owned (*n* = 1)
Yozgat	1	Tabby (*n* = 1)	1 ♂	Owned (*n* = 1)
Zonguldak	1	Orange Tabby (*n* = 1)	1 ♂	Owned (*n* = 1)
Total	250			

♂, male; ♀, female

template. Previously sequence-confirmed genomic DNAs obtained from the National Research Center for Protozoan Diseases (Obihiro, Japan) and double-distilled water (Invitrogen, Ultrapure™ Distilled Water, DNAse, RNAse, Free) served as positive and negative controls, respectively. The PCR amplicons were run on a 1.5% agarose gel. The gels were stained with ethidium bromide after electrophoresis and visualized under a UV transilluminator.

Cloning and sequencing procedures

After performing PCR analysis, we selected some of the PCR amplicons giving clear and strong bands on the agarose gel for direct sequencing. To ensure that the concentrations were suitable for direct sequencing, we repeated PCR analysis at higher volumes and purified the DNA from the gel. A gel extraction kit (NucleoSpin™ Gel and PCR Clean Up, Macherey-Nagel, Germany) was utilized to purify amplified PCR products from agarose gel, and the eluted DNA concentrations were quantified using a NanoDrop 2000 spectrophotometer. All PCR amplicons with sufficient DNA concentration were directly sequenced, while samples with low concentration were cloned. For this aim, the commercial protocol of pGEM®-T Easy Vector System (Promega, USA) to clone the extracts into a pGEM vector was followed. The template DNA (6 µL) was ligated into pGEM-T easy vector (2 µL) using restriction buffer and T4 DNA ligase. The mixture was then incubated at 16 °C for 2.5–3 h and kept at 4 °C overnight. Next, plasmid was transformed into *Escherichia coli* DH5α competent cells and incubated them in Luria-Bertani (LB) broth at 37 °C for at least 1 h. While the cells were incubating, LB agar plates were heated to 37 °C. After centrifugation (2500 rpm for 3 min) and removal of the supernatant, we spread the remaining mixture on LB agar plates using a spreader and incubated them overnight at 37 °C. Recombinant clones were then selected to be sequenced. These clones were transferred to LB broth with 50 µg/mL ampicillin (Wako, Saitama, Japan), and incubated overnight at 37 °C. By using the Nucleospin® Plasmid QuickPure Kit (Macherey-Nagel-German), the plasmid was purified from this culture, and the samples were sequenced with the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic analysis

The comparison of the nucleotide sequences obtained from the present study with previously deposited GenBank sequences to determine identities and similarities was performed using the BLASTn Analysis tool. Phylogenetic trees for *Babesia ovis* (*18S rRNA*) and *Mycoplasma* spp. (*16S rRNA*) were constructed by employing the maximum likelihood (ML) method of the MEGA version

Table 4 Primer pairs used in the standard and nPCR assays

Genus or species	Target gene	Primer	Primer sequence (5′ to 3′)	Size (bp)	References
Anaplasma/Ehrlichia spp.	*16S rRNA*	EHR16SD	GGTACCTACAGAAGAAGTCC	345 bp	[74]
		EHR16SR	TAGCACTCATCGTTTACAGC		
Babesia/Theileria/ Hepatozoon spp.	*18S rRNA*	BTH 18 S 1stF	GTGAAACTGCGAATGGCTCATTAC	1.4–1.6 kbp	[75]
		BTH 18 S 1stR	AAGTGATAAGGTTCACAAAACTTCCC		
		BTH 18 S 2ndF	GGCTCATTACAACAGTTATAGTTTATTTG		
		BTH 18 S 2ndR	CGGTCCGAATAATTCACCGGAT		
Babesia/Theileria spp.	*18S rRNA (V4)*	RLB-F2	GACACAGGGAGGTAGTGACAAG	403 bp	[76]
		RLB-R2	CTAAGAATTTCACCTCTGACAGT		
Mycoplasma spp.	*16S rRNA*	HBT-F	ATACGGCCCATATTCCTACG	595 bp	[32]
		HBT-R	TGCTCCACCACTTGTTCA		
Rickettsia spp.	*glt* A	F *Rick* spp. gltA	GCAAGTATCGGTGAGGATGTAAT	401 bp	[77]
		R *Rick* spp. gltA	GCTTCCTTAAAATTCAATAAATCAGGAT		
Rickettsia felis	*glt* A	gltA-F1	GCAAGTATTGGTGAGGATGTAATC	654 bp	[78]
		gltA-R1	CTGCGGCACGTGGGTCATAG		
		gltA-F2	GCGACATCGAGGATATGACAT		
		gltA-R2	GGAATATTCTCAGAACTACCG		
Bartonella spp.	*16–23S rRNA*	*Bartonella* spp. F	(C/T)CTTCGTTTCTCTTTCTTCA	154–260 bp	[79]
		Bartonella spp. R	AACCAACTGAGCTACAAGCC		

11 software [80]. To estimate the confidence in the nodes and branches of the phylogenetic trees, bootstrap analysis with 1000 replications was used. Finally, the sequences obtained in this study were registered to the National Center for Biotechnology Information's GenBank database using BankIt.

Accession numbers for FVBHs

The sequences have been deposited to GenBank under the following accession numbers: OR984759, OR984760, OR9847561, and OR984762 for *B. ovis*; OR979169 and OR979175 for *Mycoplasma haemofelis*; OR979160, OR979161, OR979162, OR979163, OR979164, OR979165, OR979166, OR979167, OR979168, OR979170, OR979171, OR979172, OR979173, OR979176, OR979177, OR979179, and OR979180 for *Candidatus* Mycoplasma haemominutum; OR979174 for uncultured *Mycoplasma* sp.; OR979178 for *Mycoplasma wenyonii*; OR978398, OR978399, and OR978400 for *Bartonella koehlerae*; OR978401, OR978402, and OR978403 for *Bartonella henselae*.

Further analysis for confirmation of *Babesia ovis* presence in cats and endogenous control of feline DNA

All blood samples underwent initial screening using a universal primer that targets a 403 bp region of the piroplasmid *18S rRNA* genes. Out of these, four samples tested positive. All PCR-positive amplicons with sufficient DNA concentration were directly sequenced, revealing the presence of *Babesia ovis*. A nested PCR amplifying approximately 1600 bp was performed on the positive samples to corroborate these findings. Subsequently, one sample was selected for direct sequencing, while three samples underwent cloning procedures. In addition, all four piroplasm-positive DNAs were molecularly tested using cat's SSR primers for endogenous control following the protocol described by Abdel-Rahman et al. [81].

Statistical analysis

A cross-tabulation evaluation was conducted using categorical data, numbers, and percentages. In cases where the expected cells were less than 20%, the data were analyzed using the Monte Carlo Simulation Method. The data were analyzed using the SPSS 25 statistical package program (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Pearson Chi-square *p*-values were calculated to determine the statistical significance of the relationship among cat breed, age, gender, and status in the distribution of FVBHs. Statistical significance was considered as $p<0.05$.

Abbreviations

CMhm	Candidatus Mycoplasma haemominutum
CMt	Candidatus Mycoplasma turicensis
CSD	Cat-scratch disease
EDTA	Ethylenediamine tetraacetic acid
FVBHs	Feline vector-borne haemopathogens
Mhf	Mycoplasma haemofelis
VBDs	Vector-borne diseases
VBPs	Vector-borne pathogens

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Author contributions

O.C., X.X., and F.S. conceptualized the study. O.C., C.C., and M.I. collected the study materials. O.C., Z.M., C.C., M.I., A.E, and A.M. conducted laboratory analysis. Z.M. performed phylogenetic analysis. O.C. and Z.M wrote the original draft of the manuscript. All authors have agreed to this final version of the manuscript and give their consent for its publication.

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Data availability
The datasets generated and/or analyzed during the present study are available in the National Center for Biotechnology Information GenBank database (https://www.ncbi.nlm.nih.gov/) under the accession numbers OR984759, OR984760, OR9847561, OR984762, OR979169, OR979175, OR979160, OR979161, OR979162, OR979163, OR979164, OR979165, OR979166, OR979167, OR979168, OR979170, OR979171, OR979172, OR979173, OR979176, OR979177, OR979179, OR979180, OR979174, OR979178, OR978398, OR978399, OR978400, OR978401, OR978402 and OR978403.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Experimental Animal Production and Research Center Ethics Committee of the Veterinary Faculty of Selcuk University, Türkiye (Approval ID: 2024/88). Moreover, the ethical guidelines for the use of animal samples were approved by Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan (Approval ID: 18–41).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. BBC News (in Turkish). World cat day. (2024). https://www.bbc.com/turkce/haberler-dunya-56099552. Accessed 30 Jan 2024.
2. Özen D, Böhning D, Gürcan İS. Estimation of stray dog and cat populations in metropolitan Ankara, Turkey. Turk J Vet Anim Sci. 2016;40:7–12.
3. Nguyen V, Dantas-Torres F, Otranto D. Canine and feline vector-borne diseases of zoonotic concern in Southeast Asia. Curr Res Parasitol Vector Borne Dis. 2021;10(9):1114.
4. Otranto D, Dantas-Torres F. Canine and feline vector-borne diseases in Italy: current situation and perspectives. Parasit Vectors. 2010;3:2.
5. Sykes JE. Feline hemotropic mycoplasmas. The Veterinary clinics of North America. Small Anim Pract. 2010;40(6):1157–70.
6. Inci A, Yildirim A, Duzlu O, Doganay M, Aksoy S. Tick-borne diseases in Turkey: a review based on one health perspective. PLoS Negl Trop Dis. 2016;10(12):e0005021.
7. Ceylan O, Xuan X, Sevinc F. Primary tick-borne protozoan and rickettsial infections of animals in Turkey. Pathogens. 2021;10(2):231.
8. Jin X, Gou Y, Xin Y, Li J, Sun J, Li T, Feng J. Advancements in understanding the molecular and immune mechanisms of *Bartonella* pathogenicity. Front Microbiol. 2023;14:1196700.
9. Vilhena H, Martinez-Díaz VL, Cardoso L, Vieira L, Altet L, Francino O, Pastor J, Silvestre-Ferreira AC. Feline vector-borne pathogens in the north and centre of Portugal. Parasite Vectors. 2013;6:99.
10. Chatzis MK, Leontides L, Athanasiou LV, Papadopoulos E, Kasabalis D, Mylonakis M, Rallis T, Koutinas AF, Andreadou M, Ikonomopoulos J, Saridomichelakis MN. Evaluation of indirect immunofluorescence antibody test and enzyme-linked immunosorbent assay for the diagnosis of infection by *Leishmania infantum* in clinically normal and sick cats. Exp Parasitol. 2014;147:54–9.
11. Emek Tuna G, Bakırcı S, Dinler C, Battal G, Ulutaş B. Molecular identification and clinicopathological findings of *Hepatozoon* sp. infection in a cat: first report from Turkey. Turkiye Parazitol Der. 2018;42(4):286–9.
12. Albay MK, Sevgisunar NS, Şahinduran S, Özmen Ö. The first report of ehrlichiosis in a cat in Turkey. Ankara Üniv Vet Fak Derg. 2016;63(3):329–31.
13. Celebi B, Kilic S, Aydin N, Tarhan G, Carhan A, Babur C. Investigation of *Bartonella henselae* in cats in Ankara, Turkey. Zoonoses Public Health. 2009;56(4):169–75.
14. Maden M, Dogan M, Altintas G, Yildiz EE, Ekik M, Ince ME, Kose SI. Prevalence of *Bartonella henselae* in Pet and stray cats from the aspect of public health: a research sample in the concept of one medicine – one health. Kafkas Univ Vet Fak Derg. 2015;21(3):213–7.
15. Cetinkaya H, Haktanir D, Arun S, Vurusaner C. Molecular detection and prevalence of feline hemotropic mycoplasmas in Istanbul, Turkey. Acta Parasitol. 2016;61(1):165–71.
16. Muz MN, Erat S, Mumcuoglu KY. Protozoan and microbial pathogens of house cats in the province of Tekirdag in western Turkey. Pathogens. 2021;10:1114.
17. Köseoğlu AE, Can H, Güvendi M, Karakavuk M, Manyatsi P, Alak SE, Değirmenci Döşkaya A, Gül A, Döşkaya M, Gürüz AY, Ün C. Molecular prevalence and genetic diversity of *Bartonella* spp. in stray cats of İzmir, Turkey. Parasites Vectors. 2021;15(1):305.
18. Guzel M, Celebi B, Yalcin E, Koenhemsi L, Mamak N, Pasa S, Aslan O. A serological investigation of *Bartonella henselae* infection in cats in Turkey. J Vet Med Sci. 2011;73(11):1513–6.
19. Diren Sığırcı B, Ilgaz A. Detection of the presence of *Bartonella henselae* in cats in Istanbul. J Fac Vet Med Istanbul Univ. 2013;39(2):209–17.
20. Otranto D, Napoli E, Latfora MS, Annoscia G, Tarallo VD, Greco G, Lorusso E, Gulotta L, Falsone L, Basano FS, Pennisi MG, Deuster K, Capelli G, Dantas-Torres F, Brianti E. Feline and canine leishmaniosis and other vector-borne diseases in the aeolian islands: Pathogen and vector circulation in a confined environment. Vet Parasitol. 2017;236:144–51.
21. Morganti G, Veronesi F, Stefanetti F, Di Muccio T, Fiorentino E, Diaferia M, Santoro A, Passamonti F, Gramiccia M. Emerging feline vector-borne pathogens in Italy. Parasites Vectors. 2019;12(1):193.
22. Ringo AE, Moumouni PFA, Taioe M, Jirapattharasate C, Liu M, Wang G, Gao Y, Guo H, Lee S, Zheng W, Efstratiou A, Li J, Inoue N, Suzuki H, Thekisoe O, Xuan X. Molecular analysis of tick-borne protozoan and rickettsial pathogens in small ruminants from two South African provinces. Parasitol Int. 2018;67(2):144–9.
23. Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA. Babesia: a world emerging. Infect Genet Evol. 2012;12(8):1788–809.
24. Penzhorn BL, Oosthuizen MC. *Babesia* species of domestic cats: molecular characterization has opened Pandora's Box. Front Vet Sci. 2020;7:134.
25. Remesar S, Arnal JS, Gomez A, Prieto A, Garcia-Dios D, Benito A, Panadero R, Morrondo P, Diaz P. A case report of fatal feline babesiosis caused by *Babesia canis* in north western Spain. BMC Vet Res. 2022;18(1):177.
26. Hartmann K, Addie D, Belak S, Boucraut-Baralon C, Egberink H, Frymus T, Gruffydd-Jones T, Hosie MJ, Lloret A, Lutz H, Marsilio F, Möstl K, Pennisi MG, Radford AD, Thiry E, Truyen U, Horzinek MC. Babesiosis in cats: ABCD guidelines on prevention and management. J Feline Med Surg. 2013;15(7):643–6.
27. Kelly PJ, Koster L, Li J, Zhang J, Huang K, Brandford GC, Marchi S, Vandenplas M, Wang C. Survey of vector-borne agents in feral cats and first report of *Babesia Gibsoni* in cats on St Kitts, West Indies. BMC Vet Res. 2017;13(1):331.
28. Zhang XL, Li XW, Li WJ, Huang HL, Huang SJ, Shao JW. Molecular evidence of *Babesia* in pet cats in mainland China. BMC Vet Res. 2019;15(1):476.
29. Yin F, Mu D, Tian Z, Li D, Ma X, Wang J, Guan G, Yin H, Li F. Molecular detection of *Babesia Gibsoni* in cats in China. Anim (Basel). 2022;12(22):3066.
30. Criado-Fornelio A, Martinez-Marcos A, Buling-Sarana A, Barba-Carretero JC. Molecular studies on *Babesia*, *Theileria* and *Hepatozoon* in southern Europe Part I. Epizootiological aspects. Vet Parasitol. 2003;113(3–4):189–201.
31. Karaca M, Tütüncü M, Akkan HA, Özdal N, Değer S, Ağaoğlu ZT. Van Kedilerinde babesiosis. YYÜ Vet Fak Derg. 2005;16(2):87–8.
32. Criado-Fornelio A, Martinez-Marcos A, Buling-Sarana A, Barba-Carretero JC. Presence of *Mycoplasma haemofelis*, *Mycoplasma Haemominutum* and piroplasmids in cats from southern Europe: a molecular study. Vet Microbiol. 2003;93(4):307–17.

33. Fritz D. A PCR study of piroplasms in 166 dogs and 111 horses in France (march 2006 to march 2008). Parasitol Res. 2010;106(6):1339–42.
34. Ozubek S, Aktas M. Genetic diversity and prevalence of piroplasm species in equids from Turkey. Comp Immunol Microbiol Infect Dis. 2018;59:47–51.
35. Ceylan O, Benedicto B, Ceylan C, Tumwebaze M, Galon EM, Liu M, Xuan X, Sevinc F. A survey on equine tick-borne diseases: the molecular detection of *Babesia ovis* DNA in Turkish racehorses. Ticks Tick Borne Dis. 2021;12(5):101784.
36. Tuzer E, Goksu K, Bilal T, Yeşildere T. A case of haemobartonellosis in a cat in Istanbul. J Protozoology Res. 1993;3(2):69–70.
37. Ural K, Suer C, Vatansever Z, Kar S, Eker K, Cıngı CC. *Candidatus* Mycoplasma haemominutum infection in a cat in Antalya. Fırat Univ Vet J Health Sci. 2008;22(1):27–30.
38. Ider M, Ceylan C, Naseri A, Ceylan O, Durgut MK, Ok M, Iyigun SS, Erol BB, Sahin HB, Kilickaya MC. Evaluation of endothelial glycocalyx injury biomarkers in feline hemotropic mycoplasmosis. Sci Rep. 2024;14:12931.
39. Ural K, Kurtdede A. Feline haemoplasmosis in Ankara, Turkey: pathological, haematological and biochemical findings, diagnosis and mycoplasma identification by PCR and enrofloxacin treatment efficiency. Rev Med Vet. 2008;159(7):376–84.
40. Ural K, Kurtdede A, Ulutas B. Prevalence of haemoplasma infection in pet cats from 4 different provinces in Turkey. Rev Med Vet. 2009;160(5):226–30.
41. Atalay T, Aslan Ö, Karaca Bekdik I. Diagnosis of *Mycoplasma haemofelis* and *Candidatus* Mycoplasma haemominutum using PCR assay in cats. Erciyes Univ JHS. 2015;24(1):1–6.
42. Hoseinpoor E, Goudarztalejerdi A, Sazmand A. Molecular prevalence and phylogenetic analysis of hemotropic *Mycoplasma* species in cats in different regions of Iran. BMC Microbiol. 2024;24:198.
43. Erol U, Sahin OF, Altay K. Molecular prevalence of bovine hemoplasmosis in Turkey with first detection of *Mycoplasma wenyonii* and *Candidatus* Mycoplasma haemobos in cattle and water buffalo. Vet Res Commun. 2023;47:207–15.
44. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB. Bartonella spp. in pets and effect on human health. Emerg Infect Dis. 2006;12(3):389–94.
45. Inci A, Doğanay M, Özdarendeli A, Düzlü Ö, Yıldırım A. Overview of zoonotic diseases in Turkey: the one health concept and future threats. Turkiye Parazitol Derg. 2018;42(1):39–80.
46. Cotte V, Bonnet S, Le Rhun D, Le Naour E, Chauvin A, Boulouis HJ, Lecuelle B, Lilin T, Vayssier-Taussat M. Transmission of *Bartonella henselae* by *Ixodes ricinus*. Emerg Infect Dis. 2008;14(7):1074–80.
47. Sayın-Kutlu S, Ergin C, Kutlu M, Akkaya Y, Akalin S. *Bartonella henselae* seroprevalence in cattle breeders and veterinarians in the rural areas of Aydin and Denizli, Turkey. Zoonoses Public Health. 2012;59(6):445–9.
48. Aydın N, Korkmazgil B, Kırkan Ş, Telli M, Eyigör M, Aksoy AM, Parın U, Tekbıyık S. Seropositivity of *Bartonella henselae* in risky human population, cats and dogs. Meandros Med Dent J. 2019;20(1):51–6.
49. Atıcı S, Kepenekli Kadayıfcı E, Karaaslan A, Toper MH, Ataizi Celikel C, Soysal A, Bakır M. Atypical presentation of cat-scratch disease in an immunocompetent child with serological and pathological evidence. Case Rep Pediatr. 2014;2014:397437.
50. Kaynar K, Emekçi BN, Özdemir A, Arslan N, Töngel A, Bayramova A. Bartonellosis: the Achilles's heel of cat love. Turkiye Klinikleri J Case Rep. 2023;31(3):118–21.
51. Türker K, Çelebi B, Andaç Ş, Bulut P, Yalçın Ş, Dolhan S. A neglected bacteria with a case: *Bartonella henselae*. Mikrobiyol Bul. 2017;51(3):286–92.
52. Çil B, Kurt İ, Doğan S, Aygun FD. Two cases of systemic cat scratch disease presenting with different complaints. İKSSTD. 2022;14(1):104–7.
53. Akkaya Y, Ergin C. Investigation of *Bartonella henselae* seroprevalence in the northern countryside of Denizli province. Kafkas J Med Sci. 2023;13(1):11–7.
54. Gülbahar MY, Karaca E, Çiftci A, İnal S, Ahmed I, Balakrishnan N, Breitschwerdt EB. Pyogranulomatous myocarditis and diaphragmatic myositis associated with *Bartonella henselae* in a cat from Turkey. Ankara Univ Vet Fak Derg. 2019;66:103–7.
55. Kırkan Ş, Parın U, Yüksel HT, Erinç SM. Investigation of *Bartonella henselae* and *Bartonella clarridgeiae* prevalence in cats. Ann Rev Resear. 2019;5(3):555661.
56. Pennisi MG, Hofmann-Lehmann R, Radford AD, Tasker S, Belak S, Addie DD, Boucraut-Baralon C, Egberink H, Frymus T, Gruffydd-Jones T, Hartmann K, Horzinek MC, Hosie MJ, Lloret A, Lutz H, Marsilio F, Thiry E, Truyen U, Möstl K. Anaplasma, Ehrlichia and Rickettsia species infections in cats: European guidelines from the ABCD on prevention and management. J Feline Med Surg. 2017;19(5):542–8.
57. Álvarez-Fernández A, Maggi R, Martín-Valls GE, Baxarias M, Breitschwerdt EB, Solano-Gallego L. Prospective serological and molecular cross-sectional study focusing on *Bartonella* and other blood-borne organisms in cats from Catalonia (Spain). Parasit Vectors. 2022;15(1):6.
58. Sevinc F, Zhou M, Cao S, Ceylan O, Aydin MF, Sevinc M, Xuan X. Haemoparasitic agents associated with ovine babesiosis: a possible negative interaction between *Babesia ovis* and *Theileria ovis*. Vet Parasitol. 2018;252:143–7.
59. Ceylan O, Byamukama B, Ceylan C, Galon EM, Liu M, Masatani T, Xuan X, Sevinc F. Tick-borne haemoparasites of sheep: a molecular research in Turkey. Pathogens. 2021;10:162.
60. Schäfer I, Kohn B, Volkmann M, Müller E. Retrospective evaluation of vector-borne pathogens in cats living in Germany (2012–2020). Parasites Vectors. 2021;14:123.
61. Qurollo BA, Balakrishnan N, Cannon CZ, Maggi RG, Breitschwerdt EB. Co-infection with Anaplasma platys, Bartonella henselae, Bartonella koehlerae and 'Candidatus Mycoplasma Haemominutum' in a cat diagnosed with splenic plasmacytosis and multiple myeloma. J Feline Med Surg. 2014;16(8):713–20.
62. Zarea AAK, Tempesta M, Fouad EA, Ndiana LA, Mahmoud MS, Mrenoshki D, Martella V, Decaro N, Chomel B, Greco G. Prevalence of *Bartonella* spp., haemotropic *Mycoplasma* spp. and others vector-borne pathogens in private-owned dogs and cats, Egypt. Acta Trop. 2023;240:106857.
63. Guimarães A, Raimundo JM, Rodrigues RB, Peixoto MP, Santos HA, André MR, Machado RZ, Baldani CD. *Ehrlichia* spp. infection in domestic cats from Rio De Janeiro State, Southeast Brazil. Rev Bras Parasitol Vet. 2019;28(1):180–5.
64. Latrofa MS, Iatta R, Toniolo F, Furlanello T, Ravagnan S, Capelli G, Schunack B, Chomel B, Zatelli A, Mendoza-Roldan J, Dantas-Torres. Otranto D. A molecular survey of vector-borne pathogens and haemoplasmas in owned cats across Italy. Parasit Vectors. 2020;13(1):116.
65. Schafer I, Kohn B. *Anaplasma phagocytophilum* infection in cats: a literature review to raise clinical awareness. J Feline Med Surg. 2020;22(5):428–41.
66. Ji S, Ceylan O, Ma Z, Galon EM, Zafar I, Li H, Hasegawa Y, Sevinc M, Masatani T, Iguchi A, Kawase O, Umemiya-Shirafuji R, Asada M, Sevinc F, Xuan X. Protozoan and rickettsial pathogens in ticks collected from infested cattle from Turkey. Pathogens. 2022;11(5):500.
67. Ma Z, Ceylan O, Galon EM, Mohanta UK, Ji S, Li H, Do TT, Umemiya-Shirafuji R, El-Sayed SAE-S, Zafar I, Liu M, Sevinc F, Xuan X. Molecular identification of piroplasmids in ticks from infested small ruminants in Konya province, Turkey. Pathogens. 2023;12(9):1123.
68. Ceylan O, Ma Z, Ceylan C, Culha M, Galon M, Ji S, Li H, Zafar I, Mohanta U, Xuan X, Sevinc F. Wide bovine tick-borne pathogen spectrum: Predominancy of *Theileria annulata* and the first molecular detection of *Ehrlichia Minasensis* in Turkey. Vet Res Commun. 2024;48:1037–59.
69. Sivakumar T, Fujita S, Tuvshintulga B, Kothalawala H, Silva S, Yokoyama N. Discovery of a new *Theileria* sp. closely related to *Theileria annulata* in cattle from Sri Lanka. Sci Rep. 2019;9;16132.
70. Conrad PA, Kjemtrup AM, Carreno RA, Thomford J, Wainwright K, Eberhard M, Quick R, Telford SR III, Herwaldt BL. Description of *Babesia Duncani* n.sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms. Int J Parasitol. 2006;36(7):779–89.
71. Niu Q, Luo J, Guan G, Liu Z, Ma M, Liu A, Gao J, Ren Q, Li Y, Qiu J, Yin H. Differentiation of two ovine *Babesia* based on the ribosomal DNA internal transcribed spacer (ITS) sequences. Exp Parasitol. 2009;121(1):64–8.
72. Gray MW. Evolution of organellar genomes. Curr Opin Genet Dev. 1999;9(6):678–87.
73. Annoscia G, Latrofa MS, Cantacessi C, Olivieri E, Manfredi MT, Dantas-Torres F, Otranto D. A new PCR assay for the detection and differentiation of *Babesia canis* and *Babesia Vogeli*. Ticks Tick Borne Dis. 2017;8(6):862–5.
74. Harrus S, Perlman-Avrahami A, Mumcuoglu KY, Morick D, Eyal O, Baneth G. Molecular detection of Ehrlichia canis, Anaplasma bovis, Anaplasma platys, Candidatus midichloria mitochondrii and Babesia canis vogeli in ticks from Israel. Clin Microbiol Infect. 2011;17(3):459–63.
75. Masatani T, Hayashi K, Andoh M, Tateno M, Endo Y, Asada M, Kusakisako K, Tanaka K, Gokuden M, Hozumi N, Nakadohzono F, Matsuo T. Detection and molecular characterization of *Babesia*, *Theileria*, and *Hepatozoon* species in hard ticks collected from Kagoshima, the southern region in Japan. Ticks Tick Borne Dis. 2017;8(4):581–7.
76. Georges K, Loria GR, Riili S, Greco A, Caracappa S, Jongejan F, Sparagano O. Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. Vet Parasitol. 2001;99(4):273–86.
77. Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A, Popov V, Gennari SM, Walker DH. *Rickettsia* species infecting *Amblyomma cooperi*

ticks from an area in the Sao Paulo, Brazil, where Brazilian spotted fever is endemic. J Clin Microbiol. 2004;42(1):90–8.
78. Hii S, Kopp SR, Thompson MF, O'Leary CA, Rees RL, Traub RJ. Molecular evidence of *Rickettsia felis* infection in dogs from northern territory, Australia. Parasites Vectors. 2011;4:198.
79. Jensen WA, Fall MZ, Rooney J, Kordick DL, Breitschwerdt EB. Rapid identification and differentiation of *Bartonella* species using a single-step PCR assay. J Clin Microbiol. 2000;38(5):1717–22.
80. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol. 2021;38(7):3022–27.
81. Abdel-Rahman SM, El-Saadani MA, Ashry KM, Haggag AS. Detection of adulteration and identification of cat's, dog's, donkey's and horse's meat using species-specific PCR and PCR-RFLP techniques. Aust J Basic Appl Sci. 2009;3(3):1716–9.

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