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ORIGINAL ARTICLE

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Tick-borne pathogens in ticks (Acari: Ixodidae) collected from various domestic and wild hosts in Corsica (France), a Mediterranean island environment

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Abstract

Corsica is a mountainous French island in the north-west of the Mediterranean Sea presenting a large diversity of natural environments where many interactions between humans, domestic animals and wild fauna occur. Despite this favourable context, tick-borne pathogens (TBPs) have not systematically been investigated. In this study, a large number of TBPs were screened in ticks collected over a period of one year from domestic and wild hosts in Corsica. More than 1,500 ticks belonging to nine species and five genera (*Rhipicephalus*, *Hyalomma*, *Dermacentor*, *Ixodes* and *Haemaphysalis*) were analysed individually or pooled (by species, gender, host and locality). A real-time microfluidic PCR was used for high-throughput screening of TBP DNA. This advanced methodology enabled the simultaneous detection of 29 bacterial and 12 parasitic species (including *Borrelia*, *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Bartonella*, *Candidatus* Neoehrlichia, *Coxiella*, *Francisella*, *Babesia* and *Theileria*). The Crimean–Congo haemorrhagic fever (CCHF) virus was investigated individually in tick species known to be vectors or carriers of this virus. In almost half of the tick pools (48%), DNA from at least one pathogen was detected and eleven species of TBPs from six genera were reported. TBPs were found in ticks from all collected hosts and were present in more than 80% of the investigated area. The detection of DNA of certain species confirmed the previous identification of these pathogens in Corsica, such as *Rickettsia aeschlimannii* (23% of pools), *Rickettsia slovaca* (5%), *Anaplasma marginale* (4%) and *Theileria equi* (0.4%), but most TBP DNA identified had not previously been reported in Corsican ticks. This included *Anaplasma phagocytophilum* (16%), *Rickettsia helvetica* (1%), *Borrelia afzelii* (0.7%), *Borrelia miyamotoi* (1%), *Bartonella henselae* (2%), *Babesia bigemina* (2%) and *Babesia ovis* (0.5%). The high tick infection rate and the diversity of TBPs reported in this study highlight the probable role of animals as reservoir hosts of zoonotic pathogens and human exposure to TBPs in Corsica.

KEYWORDS

Corsica, domestic animals, France, tick-borne pathogens, ticks (ixodidae), wild animals

1 | INTRODUCTION

The regional importance of ticks in terms of animal and public health depends on the tick species and tick-borne pathogens (TBPs) present in an area, and to a large extent on the local climate, management and breeding of livestock, and human activities (Jongejan & Uilenberg, 2004). The role of ticks as vectors of human pathogens is second only to mosquitoes (Parola & Raoult, 2001), and ticks are the most important vectors in the veterinary field worldwide (Nicholson, Sonenshine, Lane, & Uilenberg, 2009). Ticks can transmit many varieties of pathogens, including bacteria, parasites and viruses. Moreover, human tick-borne diseases are usually zoonotic and asymptomatic for non-human vertebrate hosts which are most often the reservoirs of pathogens causing human infection (Jongejan & Uilenberg, 2004).

Corsica is a French island in the western part of the Mediterranean area, situated 15 km north of Sardinia and 90 km west of Tuscany in Italy (Figure 1). It is the fourth largest Mediterranean island and the most mountainous and forested of these islands. Corsica consists of two administrative departments (Corse-du-Sud and Haute-Corse) and 360 communes (the smallest administrative unit in France; Figure 1). Tourism (three million people annually, 320,000 permanent inhabitants), extensive farming (sheep, goats, pigs and cattle), hunting and hiking are important activities in Corsica (Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Marco, et al., 2016). Therefore, in this context, permanent interactions occur between livestock, wildlife and humans in a small area, which certainly favour the circulation of TBPs, including zoonotic ones. Corsica is also on the route of migratory birds that create a natural link between Africa and Europe and could spread ticks infected with TBPs (Hoffman et al., 2018).

Only scattered observations on the tick fauna of Corsica, mostly grouped together in a book on the ticks of France (Pérez-Eid, 2007), were available before 2014. From May 2014 to May 2015, a large tick survey (Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Marco, et al., 2016) on domestic and a few wild animals led to the identification of nine species: *Rhipicephalus bursa*, *Hyalomma marginatum*, *Dermacentor marginatus*, *Rh. sanguineus* sensu lato, *Hy. scupense*, *Ixodes ricinus*, *Haemaphysalis punctata*, *Rh. (Bo.) annulatus* and *Ha. sulcata*. The diversity of the Corsican tick species, characterized by ticks usually collected in humid environments (*I. ricinus*) and others in drier areas (*Hyalomma* spp., *Rh. bursa*), suggested potential high diversity of TBPs on the island.

Some of the TBPs occurring in Corsica have been reported more or less reliably in former and recent studies. Various species of the genera *Anaplasma*, *Rickettsia* and *Ehrlichia* (Cabezas-Cruz et al., 2019; Cicculli, Capai, et al., 2019; Cicculli, Lamballerie, Charrel, & Charrel, 2019; Cicculli, Masse, et al., 2019; Dahmani, Davoust, Tahir, et al., 2017; ICTTD, 2000; Matsumoto, Parola, Brouqui, & Raoult, 2004; Selmi, Ballardini, Salvato, & Ricci, 2017), and the genera *Babesia* and *Theileria* (ICTTD, 2000) have been identified, and *Borrelia burgdorferi* sensu lato was recently reported (Cicculli, Capai, et al., 2019). It has remained uncertain whether all of the Corsican TBPs are known. The aim of this study was to obtain a broad overview regarding TBPs carried by more than 1,500 ticks collected on different Corsican animal hosts, focusing on the main pathogens of medical and veterinary importance known in the Mediterranean area, including *Borrelia*

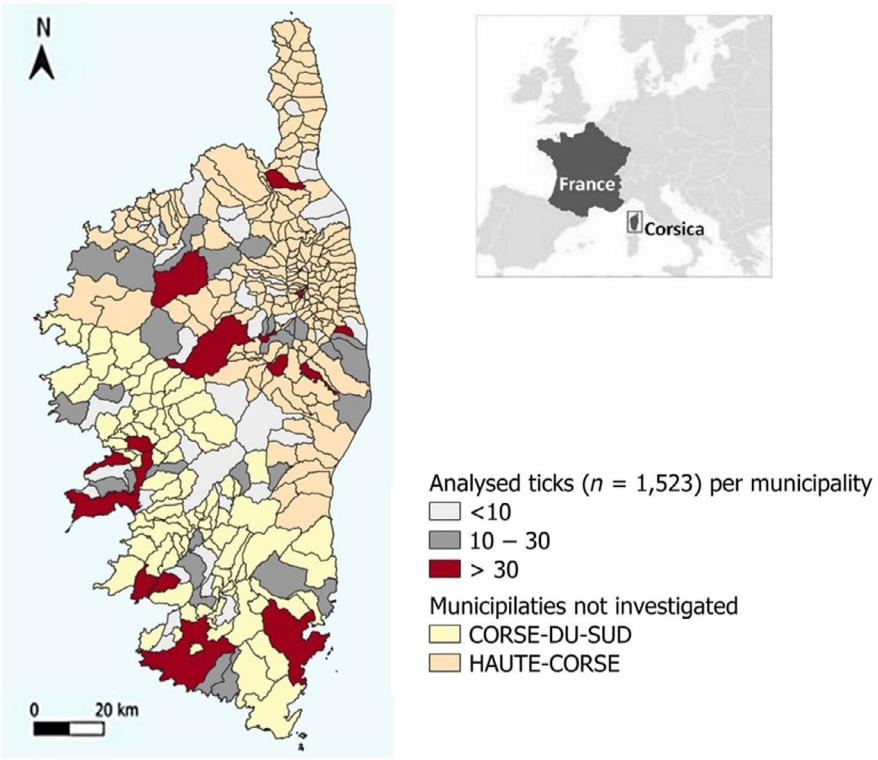

FIGURE 1 Distribution of the analysed ticks collected from animals in Corsica

spp., *Rickettsia* spp., *Anaplasma* spp., *Francisella* spp., *Ehrlichia* spp., *Coxiella* spp., *Theileria* spp., *Babesia* spp., *Bartonella* spp., *Candidatus* Neoehrlichia and the CCHF virus.

2 | MATERIAL AND METHODS

2.1 | Study area and tick collection

A large-scale tick collection was previously conducted on domestic and a few wild animals in Corsica (Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Gharbi, et al., 2016 and Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Marco, et al., 2016). Cattle were chosen as the host model because of the extensive, free-ranging livestock farming system, with a low frequency of acaricide treatments. Ticks were collected over one year (May 2014 to May 2015) in the three Corsican cattle slaughterhouses. Ticks on sheep, goats and horses were collected less systematically from May to August 2014 on three farms for each host. Ticks from domestic carnivores were provided by practising veterinarians. Ticks from wild boars (*Sus scrofa*) and hedgehog (*Erinaceus europaeus*) were collected by hunters, while ticks from mouflons (*Ovis aries musimon*), deer (*Cervus elaphus corsicanus*) and birds (European greenfinch, *Chloris chloris*) were obtained from staff from the National Office for Hunting and Wildlife (ONCFS), from staff from the Regional Natural Park of Corsica (PNRC) and from bird banders, respectively. Ticks were stored in 70% ethanol at −20°C until their identification.

Ticks were identified based on their morphology using appropriate keys and descriptions (Estrada-Peña, 2004; Pérez-Eid, 2007). For some ticks species, never reported in Corsica or impossible to distinguish morphologically, molecular identification was carried out for a few specimens by sequencing the mitochondrial *cox*1 (cytochrome *c* oxidase subunit 1) and ITS2 (internal transcribed spacer 2) for *Hy. scupense* and 16S ribosomal RNA genes for *Rh. sanguineus* s.l. and *Ha. sulcata*. All the methodology concerning the tick collection and the identification of ticks is detailed in Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Gharbi, et al., (2016) and Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Marco, et al., (2016).

2.2 | Pools of ticks

Tick was analysed individually or by pools consisting of two to five ticks. *Rhipicephalus bursa*, *Hy. marginatum*, *Ha. punctata*, *Rh. sanguineus* s.l. and *D. marginatus*, found in large numbers on their hosts, were grouped in pools by sex, host and locality. Other species found more rarely or with a special interest in terms of public and animal health were systematically analysed individually: *I. ricinus*, *Hy. scupense*, *Ha. sulcata* and *Rh.* (*Bo.*) *annulatus*.

2.3 | DNA and RNA extraction

After washing once in 70% ethanol for 5 min and twice in distilled water for 5 min, pools of one to five ticks were crushed in 300 µl of DMEM (Dulbecco's Modified Eagle Medium) with 10% foetal calf serum and six steel balls using a Precellys® 24 Dual homogenizer (Bertin, France) at 5,500 rpm for 40 s. DNA was then extracted using 100 µl of the homogenate according to the procedure recommended for the Wizard genomic DNA purification kit (Promega, France) following the manufacturer's instructions. Total DNA per sample was eluted in 50 µl of rehydration solution and stored at −20°C until further use (Michelet et al., 2014). Using 100 µl of tick homogenate for *Hyalomma* spp. and *Rh. bursa* (species known to transmit or to carry the CCHF virus), a Nucleospin RNA II kit (Macherey-Nagel, Duren, Germany) was used for total RNA extraction following the manufacturer's instructions (Gondard et al., 2018).

2.4 | Detection of tick-borne pathogen DNA or RNA

2.4.1 | Bacteria and parasites

Forty-one sets of primers and probes were used in this study to detect TBPs (29 bacterial and 12 parasitic species, Table 1) (Gondard et al., 2019; Michelet et al., 2014). A BioMark™ real-time PCR system (Fluidigm) was used for high-throughput microfluidic real-time PCR amplification using 48.48 dynamic arrays (Fluidigm). These chips dispense 48 PCR mixes and 48 samples into individual wells, after which on-chip microfluidics assemble PCR reactions in individual chambers prior to thermal cycling, resulting in 2,304 individual reactions (Michelet et al., 2014).

Conventional PCR using primers targeting genes or regions other than those in the BioMark™ system were used to confirm the presence of pathogenic DNA in the field samples. Amplicons were sequenced by Eurofins MWG Operon (Germany) and then assembled using BioEdit software (Ibis Biosciences) (Gondard et al., 2019; Michelet et al., 2014). An online BLAST (National Center for Biotechnology Information) was used to compare results with published sequences listed in GenBank sequence databases. All the target genes and primer sequences, used both for the BioMark™ real-time PCR system and individual PCR, are described in the studies of Michelet et al. (2014) and Gondard et al. (2019).

2.4.2 | Specific detections of CCHF virus and *Theileria* spp

Crimean–Congo haemorrhagic fever viral RNA was specifically investigated in tick species known to transmit, or at least able to carry this virus: *Hy. marginatum*, *Hy. scupense* and *Rh. bursa*. The presence of *Theileria* spp. DNA was also investigated by specific real-time PCR in *Hy. scupense* and *Haemaphysalis* spp. because *Hy. scupense* is an efficient vector of *T. annulata* and because *T. buffeli*, of which *Ha. punctata* is a vector, has already been reported in Corsica (ICTTD, 2000) (for primers and probes see Gondard et al., and, 2018, 2019).

2.5 | Data analysis

The infection rate is given for all pathogens in each tick species and each host. The infection rate of individually analysed tick species

TABLE 1 List of pathogens (and endosymbionts) detectable by the real-time PCR chip (Biomark™)*

Genus	Number of target pathogens/endosymbionts	Species
Anaplasma	6	*A. bovis*, *A. centrale*, *A. marginale*, *A. ovis*, *A. phagocytophilum*, *A. platys*
Borrelia	8	*B. afzelii*, *B. bissetti*, *B. burgdorferi* sensu stricto, *B. garinii*, *B. lusitaniae*, *B. miyamotoi*, *B. spielmanii*, *B. valaisiana*
Bartonella	2	*Bar. henselae*, *Bar. quintana*
Ehrlichia	3	*E. canis*, *E. chaffeensis*, *E. ruminantium*
Francisella	2	*F. tularensis*, *Francisella-like endosymbionts*
Neoehrlichia	1	*Candidatus* Neoehrlichia mikurensis
Rickettsia	5	*R. aeschlimannii*, *R. conorii*, *R. helvetica*, *R. massiliae*, *R. slovaca*
Coxiella	2	*Coxiella burnetii*, *Coxiella*-like organisms
Babesia	10	*Ba. canis*, *Ba. bigemina*, *Ba. bovis*, *Ba. Caballi*, *Ba. divergens*, *Ba. major*, *Ba. microti*, *Ba. ovis*, *Ba. venatorum*, *Ba. vogeli*
Theileria	2	*T. annulata*, *T. equi*

*See Michelet et al. (2014) and Gondard et al. (2019) for target genes and primers/probe sets sequences.

is the actual rate detected. The infection rate of pooled tick species (see Methods, 2.4) means that in an infected pool there was at least one infected tick. To assess differences in tick species infection between development stage and by sex, a Kruskal–Wallis test was used. Differences were considered statistically significant if $p < .05$. Spatial distribution (Figures 1 and 2) was generated using Qgis ver. 3.4 software.

3 | RESULTS

3.1 | Collected ticks and pool preparation

More than three thousand ticks (3,134) from nine species and five genera were collected from May 2014 to May 2015 (Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Marco, et al., 2016). Almost

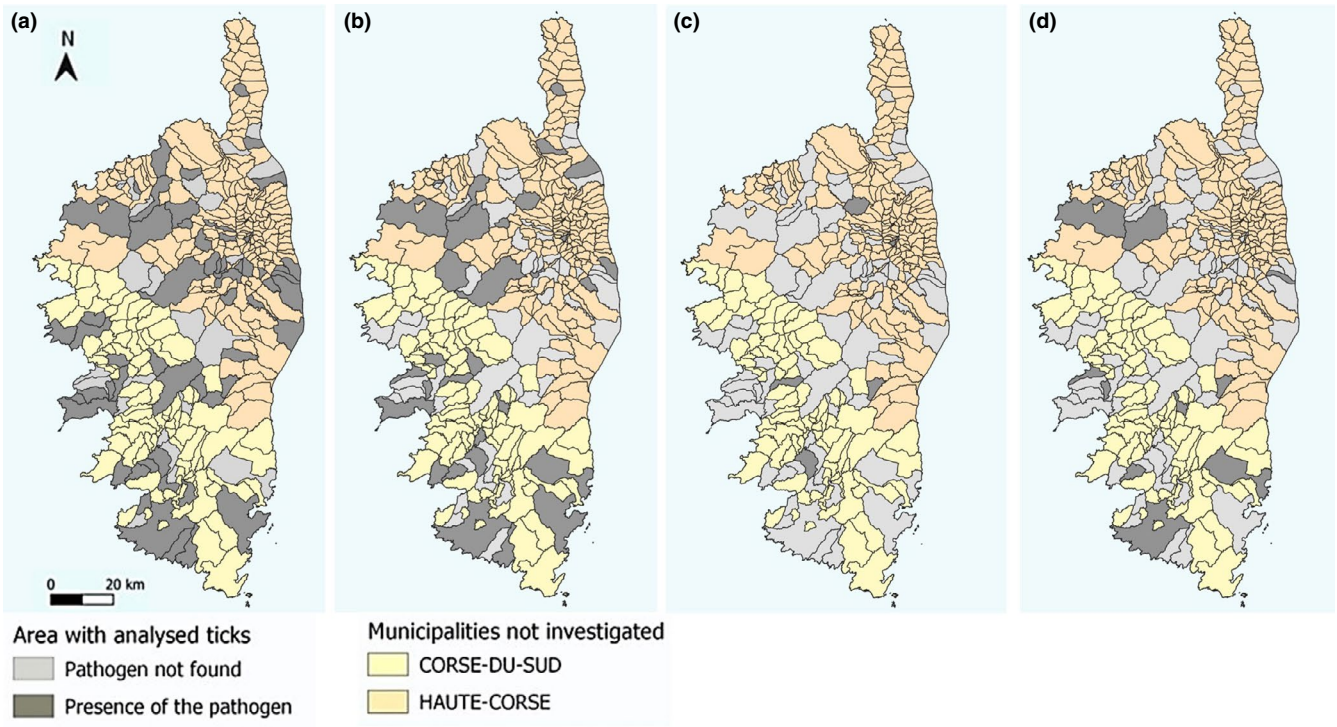

FIGURE 2 Distribution of the main pathogen DNA found in Corsican ticks. (a) *Rickettsia* spp., (b) *Anaplasma* spp., (c) *Borrelia* spp. and (d) *Babesia* spp

TABLE 2 Infection rates of individual ticks or pools of ticks collected in Corsica

Tick species	Number of pools	Stage or sex of ticks			Total ticks analysed	Infection rate %
		Female	Male	Nymph		
Rh. bursa	108	42	51	15	518	43
Hy. marginatum	89	24	65	0	362	100
Hy. scupense[*]	135	60	56	19	135	8
I. ricinus[*]	115	79	36	0	115	70
Rh. sanguineus s.l.	41	22	16	3	150	24
D. marginatus	38	16	22	0	156	66
Ha. punctata	30	18	12	0	74	30
Rh. (Bo.). annulatus[*]	11	6	3	2	11	36
Ha. sulcata[*]	2	2	0	0	2	0

*Ticks individually analysed: the infection rate is the real tick infection rate.

TABLE 3 Proportion (%) of infected pools (or ticks)

Tick species (pools; ticks analysed)	*Anaplasma*	*Babesia*	*Bartonella*	*Borrelia*	*Rickettsia*	*Theileria*
Rh. bursa (108;518)	15	12	5	0	27	1
Hy. marginatum (89;362)	7	0	1	0	100	0
Hy. scupense (135;135)[*]	2	0	0	0	6	0
I. ricinus (115; 115)[*]	64	0	2	5	6	0
Ha. punctata (30;74)	13	0	3	13	0	0
Ha. sulcata (2;2)[*]	0	0	0	0	0	0
Rh. sanguineus s.l. (41;150)	12	0	2	0	17	0
Rh. (B.) annulatus (11;11)[*]	18	0	9	0	0	9
D. marginatus (38;156)	3	0	3	0	66	0

*Ticks individually analysed.

half of them (1,523) collected from 82 municipalities (Figure 1) were analysed to detect DNA or RNA (Table 2) of microorganisms pathogenic to humans or animals. A total of 569 samples were analysed (consisting of one to five ticks): 269 adult females, 261 adult males and 39 nymphs. Nymphs of four species were found: *Rh. bursa*, *Hy. scupense*, *Rh. sanguineus* group and *Rh. (Bo.) annulatus* (Table 2). *Rhipicephalus bursa* ticks (4.8 ticks per pool on average), *Hy. marginatum* (4.1), *Dermacentor marginatus* (4.1), *Rh. sanguineus* sensu lato (3.7) and *Ha. punctata* (2.5) were systematically pooled whereas ticks from the other four species were analysed individually (*Rh. (Bo.) annulatus*, *Ha. sulcata*, *Hy. scupense* and *Ixodes ricinus*).

3.2 | Detection of TBP DNA or RNA in tick species

As for each tick species, the proportion of infected pools was not significantly different between development stage and sex ($p > .05$), the identified pathogens are presented by tick species, not separately for the stage of development and the sex of adults. Almost half of the samples (48%) were positive for at least one pathogen DNA (Table 2) and among them 12% were positive for two pathogen DNA or more. The most infected tick species were *Hy. marginatum* (100% of pools), *I. ricinus* (70% of the individual ticks) and *D. marginatus* (66% of pools). Pathogen DNA was found in all tick species collected in Corsica except in *Ha. sulcata*, but only two specimens were analysed (Tables 2 and 3).

3.3 | Pathogens identified

DNA of eleven pathogenic species from six genera was identified in the ticks: *Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp., *Babesia* spp., *Bartonella* spp. and *Theileria* spp.

3.3.1 | *Borrelia* spp

Borrelia spp. DNA was detected in two tick species and two different *Borrelia* were identified. DNA of *Borrelia miyamotoi* was found in *Ixodes ricinus* (2% of individual ticks) and in *Ha. punctata* (13% of the pools). A sequence was obtained from *I. ricinus* (GenBank, accession number: MK732472) and showed 100% homology with reference sequences from a *B. miyamotoi* strain isolated in Austria (AN: KP202177). DNA of *B. afzelii* was reported only in *I. ricinus* (4% of ticks). Unfortunately, the sequence could not be obtained.

3.3.2 | *Rickettsia* spp.

DNA of *Rickettsia* spp. was detected in six tick species (Table 3). Three species of *Rickettsia* were identified. *Rickettsia aeschlimannii* was found in *Rh. bursa* (27% of pools), *Hy. marginatum* (100% of pools), *Rh. sanguineus* s.l. (10% of pools) and *Hy. scupense* (5% of individual ticks). The four sequences obtained from *Hy. marginatum* and *Hy.*

scupense presented 100% identity. One sequence was deposited in GenBank (AN: MK732478) and showed 99% homology with reference sequences from a *R. aeschlimannii* strain isolated in Egypt (AN: HQ335153). *Rickettsia slovaca* was mostly identified in *D. marginatus* (66% of pools). Three pools of *Rh. sanguineus* s.l. (7%) and one *Hy. scupense* tick (0.7%) were also positive for this bacterium. The sequence obtained from *Hy. scupense* (GenBank, AN: MK732480) showed 99% homology with reference sequences from a *R. slovaca* strain isolated in the United States (AN: KR559551). *Rickettsia helvetica* was detected only in *I. ricinus* (6% of ticks) but the sequence could not be obtained.

3.3.3 | *Anaplasma* spp.

DNA of *Anaplasma* spp. was detected in eight tick species (Table 3) and two species were recorded: *A. phagocytophilum* and *A. marginale*. *Anaplasma phagocytophilum* DNA was mainly found in *I. ricinus* (63% of ticks), but it was also reported in *Rh. sanguineus* s.l. (7% of pools), *Rh. bursa* (7% of pools), *Hy. marginatum* (1% of pools), *Hy. scupense* (2% of ticks), *D. marginatus* (3% of pools) and *Ha. punctata* (7%). Unfortunately, the sequence of *A. phagocytophilum* could not be obtained. *Anaplasma marginale* DNA was frequently detected in *Rh. (Bo.) annulatus* (18% of ticks), but it was also identified in *Rh. bursa* (8% of pools), *Hy. marginatum* (6% of pools), *I. ricinus* (2% of ticks), *Ha. puncta* (7% of pools) and *Rh. sanguineus* s.l. (7% of pools). The sequence obtained from *Rh. (Bo.) annulatus* (GenBank, AN: MK732471) showed 99% homology with reference sequences from a *A. marginale* strain isolated in South Africa (AN: AF414873).

3.3.4 | *Babesia* spp.

DNA of *Babesia* spp. was detected only in *Rh. bursa* and two species were identified. *Babesia bigemina* was found in 9% of the pools of *Rh. bursa*. The two sequences obtained presented 100% identity. One sequence was deposited in GenBank (AN: MK732475) and showed 100% homology with reference sequences from a *Ba. bigemina* strain from the Virgin Islands (AN: EF458206). *Babesia ovis* was identified in 3% of *Rh. bursa* pools. The sequence obtained (GenBank, AN: MK732477) showed 92% homology with reference sequences from a *Ba. ovis* strain isolated in Sudan (AN: AY260171). As this sequence had no more than 92% homology with *Babesia* reference sequences available in GenBank, it could be an unknown species of *Babesia*.

3.3.5 | *Bartonella* spp.

Bartonella henselae was the only species of the genus *Bartonella* detected and its DNA was identified in seven tick species (Table 3) but mainly in *Rh. bursa* (5% of pools), *I. ricinus* (3% of ticks), *Rh. (Bo.) annulatus* (9% of ticks) and *Ha. punctata* (3% of pools). The five sequences obtained in *Rh. bursa* and *I. ricinus* presented 100% identity. One sequence was deposited in GenBank (AN: MK732473) and showed 100% homology with reference sequences from *Bar. henselae* strain Houston-1 (AN: BX897699).

3.3.6 | *Theileria* spp.

Theileria equi DNA was reported in one pool of *Rh. bursa* (1%) and in one *Rh. (Bo.) annulatus* (9% of ticks) (Table 3). The sequence obtained from *Rh. bursa* (GenBank, AN: MK732476) showed 99% homology with reference sequences from a *T. equi* strain isolated in Brazil (AN: KJ573370). No *Theileria annulata* DNA was detected by the BioMark™ real-time PCR system in the analysed ticks. Individual PCR for *Theileria* spp. did not show the presence of any other species of *Theileria* in the tick species analysed (*Hy. scupense* and *Haemaphysalis* spp.).

3.3.7 | Others pathogens and microorganisms

Francisella tularensis was not identified in this study but *Francisella*-like endosymbionts were detected in five tick species: *Hy. marginatum* (90% of pools), *Hy. scupense* (7% of ticks), *I. ricinus* (4% of ticks), *Rh. sanguineus* s.l. (10% of pools) and *Rh. (Bo.) annulatus* (27% of ticks). Neither DNA of *Coxiella* spp. nor DNA of *Candidatus* Neoehrlichia mikurensis were identified in this study. RNA of the CCHF virus was not found in *Hy. marginatum* (89 pools; 362 ticks), *Rh. bursa* (108; 518) and *Hy. scupense* (135 ticks).

For some *Rickettsiae*, the exact species could not be determined. These *Rickettsiae* were reported in seven tick species: *Rh. bursa* (8% of pools), *Hy. scupense* (2% of ticks), *I. ricinus* (9% of ticks), *Rh. sanguineus* s.l. (12% of pools), *D. marginatus* (11% of pools), *Ha. punctata* (3% of pools) and *Rh. (Bo.) annulatus* (9% of ticks). One sequence was obtained from *Rh. bursa* (GenBank, AN: MK732479) and showed 99% homology with reference sequences from *Rickettsia*-like endosymbiont strain 162 citrate synthase (gltA) gene (AN: JQ925589).

3.4 | Presence of pathogens in ticks collected on the different hosts

Ticks were collected from different hosts: 404 samples (consisting of one to five ticks) originating from cattle, 19 from goats, 12 from sheep, 24 from horses, 22 from dogs, four from cats (Table 4), 41 from wild boars, 26 from mouflons, 13 from deer and two from hedgehog and birds (Table 5). For birds and deer, as the collected ticks were all *I. ricinus*, they were analysed individually.

Borrelia spp. DNA was found only in ticks from cattle; *Borrelia miyamotoi* and *B. afzelii* infected 2% and 1% of pools from this host, respectively.

Rickettsia spp. DNA was found in ticks collected from almost all animal hosts. *Rickettsia aeschlimannii* was the most common pathogen infecting ticks from domestic ruminants and horses, reported in 23% of pools from cattle, 37% from goats, 58% from sheep and 75% from horses. It was rarely found in pools from dogs (5%), wild boars (7%) and mouflons (4%). *Rickettsia slovaca* DNA was mainly found in pools from wild boars (59%) but it was also reported in pools from cattle (0.5%), horses (4%), dogs (5%) and hedgehogs (one positive pool). *Rickettsia helvetica* DNA was identified only in ticks collected from cattle (2%).

TABLE 4 Pathogen DNA detected in ticks collected from domestic animals

Host	Analysed pools (ticks)	Tick species pools (or individual ticks*)	% infected pools (or ticks*)	Pathogens identified (% of infected pools or ticks*)
Cattle	404 (894)	*Rh. bursa* (54), *Hy. marginatum* (67), *Hy. scupense* (135)*, *I. ricinus* (98)*, *Rh. sanguineus* s.l. (9), *D. marginatus* (4), *Ha. punctata* (26), *Rh. (B.) annulatus* (11)*	47	*Anaplasma spp. (22%)*, *Babesia* spp. (3%), *Bartonella* spp. (2%), *Borrelia* spp. (3%), *Rickettsia* spp. (25%), *Theileria* spp. (0.3%)
Goat	19 (91)	*Rh. bursa* (18), *Ha. punctata* (1)	47	*Anaplasma* spp. (2%), *Babesia* spp. (5%), *Bartonella* spp. (11%), *Rickettsia* spp. (37%),
Sheep	12 (45)	*Rh. bursa* (9) *Hy. marginatum* (2), *Rh. sanguineus* s.l. (1)	58	*Rickettsia* spp. (58%)
Dogs	22 (86)	*Hy. marginatum* (1), *Rh. sanguineus* s.l. (21)	14	*Anaplasma* spp. (4%), *Rickettsia* spp. (9%)
Horses	24 (106)	*Rh. bursa* (6), *Hy. marginatum* (16), *Rh. sanguineus* s.l. (1), *D. marginatus* (1)	83	*Bartonella* spp. (4%), *Rickettsia* spp. (79%), *Theileria* spp. (4%)
Cats	4 (6)	*I. ricinus* (1)*, *Rh. sanguineus* s.l. (3)	25	*Anaplasma* spp. (25%)

*Ticks individually analysed.

TABLE 5 Pathogen DNA detected in ticks collected from wild animals

Host	Analysed pools	Total ticks	Tick species (pools or individual ticks*)	% infected pools (or ticks*)	Pathogens (% of infected pools or ticks*)
Wild boars	41	177	*Rh. bursa* (2), *Hy. marginatum* (3), *I. ricinus* (1)*, *Rh. sanguineus* s.l. (3), *D. marginatus* (32),	66	*Anaplasma* spp. (2%), *Bartonella* spp. (2%), *Rickettsia* spp. (66%)
Mouflons	26	98	*Rh. bursa* (19), *Rh. sanguineus* s.l. (1), *D. marginatus* (1), *Ha. punctata* (3), *Ha. sulcata* (2)*	12	*Anaplasma spp. (4%)*, *Babesia spp. (8%)*, *Rickettsia* spp. (4%),
Deer	13	13	*I. ricinus* (13)*	92	*Anaplasma* spp. (92%*)
Hedgehogs	2	5	*Rh. sanguineus* s.l. (2)	50	*Rickettsia* spp. (50%)
Birds	2	2	*I. ricinus* (2)*	100	*Anaplasma* spp. (100%*)

*Ticks individually analysed.

Anaplasma phagocytophilum DNA was reported in ticks from cattle (18% of pools), goats (11% of pools), cats (25% of pools), wild boars (2% of pools), deer (92% of individual ticks) and birds (two positive ticks), whereas *A. marginale* DNA was found in pools from cattle (6%) and mouflons (4%).

Babesia bigemina DNA was detected mainly in ticks sampled on cattle (2% of pools), but one pool of ticks from goats was also positive for this pathogen. *Babesia ovis* DNA was reported in two hosts; one pool of ticks collected on cattle and two pools from mouflons were positive.

Bartonella henselae DNA was reported in pools from cattle (2%), goats (11%), horses (4%) and wild boars (2%). *Theileria equi* DNA was found in one pool from cattle and one from wild boars.

3.5 | Geographical distribution of TBPs found in ticks

Ticks from 82 municipalities were analysed, and among them, pathogen DNA was found in 68 (Figure 2). *Rickettsia* spp. were widespread (Figure 2a) as they were reported in more than 80% of the municipalities investigated. *Rickettsia slovaca* DNA was found in ticks collected from 13 municipalities, *R. aeschlimannii* DNA from 49, and *R. helvetica* DNA was reported from a single municipality in the centre of Corsica (Haute-Corse). *Anaplasma* spp. (Figure 2b) were also distributed over a large part of the investigated area, found in 37% of the municipalities. *Anaplasma phagocytophilum* DNA was reported in 19 municipalities and *A. marginale* DNA in 16. *Borrelia* spp. were found in five municipalities (Figure 2c). *Borrelia miyamotoi* DNA was identified in all these municipalities, whereas *B. afzelii* DNA was reported only in one from Haute-Corse. *Babesia* spp. were identified in 10 municipalities, both in Corse-du-Sud and Haute-Corse (Figure 2d). *Bartonella henselae* DNA was identified in 12 municipalities, whereas *T. equi* DNA was found in two municipalities near the eastern coast in Haute-Corse (results not shown).

4 | DISCUSSION

In this study, a method using multiple primer/probe sets was implemented to perform high-throughput detection of TBP DNA. This

large-scale investigation (a) enabled the detection of rare pathogens and (b) generated prevalence estimates for pathogens, thus creating a comprehensive overview of the epidemiological situation for 29 bacteria and 12 parasites present or not found in ticks from Corsica. A positive tick or a positive pool means it contains DNA (or RNA) sequences similar to those of corresponding genes of TBPs and does not necessarily mean that the TBP is present in the tick. For the sake of commodity, the term 'infection rate' is often used in this study, but it in fact means 'positive for pathogen DNA (or RNA)'. As all collected ticks were removed from their hosts, potentially infected by pathogens, the identification of pathogen DNA in a tick suggests its presence in Corsica, but the tick species is not necessarily a biological vector of this pathogen. The microorganism or its DNA may simply be present in the ingested blood meal, the host having been infected by an actual tick vector species (Estrada-Peña, Gray, Kahl, Lane, & Nijhoff, 2013). However, it should be noted that in this survey, the main pathogens were most often identified in their natural vector suggesting a stronger link between one pathogen and its natural vector than with other tick species. Almost 70% of the *R. aeschlimannii* were detected in *Hy. marginatum*, 86% of the *R. slovaca* in *D. marginatus*, 80% of *A. phagocytophilum* in *I. ricinus*, more than 60% of *A. marginale* in ticks from the genus *Rhipicephalus*, and all the *Babesia* spp. were identified in *Rh. bursa*.

4.1 | Tick-borne pathogens reported for the first time in Corsican ticks

In almost half of the pools, DNA of at least one pathogen was reported, and 11 species of pathogens from six genera were identified. Among them, seven were reported for the first time in Corsican ticks: *B. afzelii*, *B. miyamotoi*, *R. helvetica*, *A. phagocytophilum*, *Ba. ovis*, *Ba. bigemina* and *Bar. henselae*.

Borrelia afzelii DNA was found in 4% of the Corsican *I. ricinus*; this species belongs to the *Borrelia burgdorferi* s.l. group, the causative agents of Lyme disease, the most important zoonotic infection in Europe. The presence of *B. burgdorferi* s.l. is linked to its natural vectors, ticks of the genus *Ixodes*, mainly *I. ricinus* in the Mediterranean area. This pathogenic agent was believed to be rare or even absent from Corsica, but a few cases of Lyme disease have been reported in recent years by the French National Centre for *Borrelia* and Santé Publique France (Vandenesch et al., 2014). *Borrelia burgdorferi* s.l. has been reported in mainland France, continental Italy, Spain (Hubálek & Halouzka, 1997), and also in the cooler areas of North Africa (Tunisia, Algeria and Morocco; Benredjem, Leulmi, Bitam, Raoult, & Parola, 2014), but has not been found in ticks from Sardinia and Sicily, two Mediterranean islands with a drier climate than Corsica. Recently, it was reported in *I. ricinus* collected from rats in Corsica (Cicculli, Capai, et al., 2019), but without determining the exact species. The identification of *B. afzelii* DNA in ticks confirms the exposure of the Corsican population to Lyme disease.

DNA of *Borrelia miyamotoi*, first identified in 1995 in ticks from Japan (Fukunaga et al., 1995), was also detected for the first time in Corsica. This species belongs to the relapsing fever group of *Borrelia* and its natural vectors are *Ixodes* ticks. *Borrelia miyamotoi* is currently considered an emerging pathogen affecting humans. Relapsing fever borreliosis is characterised by influenza-like illness and one or more relapse episodes of bacteraemia and fever (Platonov et al., 2011). *Borrelia miyamotoi* was reported in 3% of ticks from mainland France (Cosson et al., 2014) and 0.7% of ticks from northern Italy (Ravagean et al., 2018). Although no human case of *B. miyamotoi* has been identified in Corsica and in the Mediterranean area, the bacterium has been found in humans throughout Europe (Siński, Welc-Falęciak, & Zajkowska, 2016).

Rickettsia helvetica DNA was only found in *I. ricinus*, a natural vector of this bacterium. It was initially reported in 1979 in Switzerland from *I. ricinus* (Beati, Péter, Burgdorfer, Aeschlimann, & Raoult, 1993) and the species has now been identified in ticks from many countries worldwide. It is especially widespread in the Mediterranean area including in mainland France (Parola, Beati, Cambon, & Raoult, 1998), Italy (Beninati et al., 2002), Spain (Fernández-Soto, Pérez-Sánchez, Encinas-Grandes, & Sanz, 2004), Croatia (Dobec, Golubic, Punda-Polic, Kaeppeli, & Sievers, 2009), Algeria (Kernif et al., 2012) and Tunisia (Sfar et al., 2008). Human cases of rickettsiosis caused by *R. helvetica* have been reported in Italy and continental France (Portillo, Santibáñez, García-Álvarez, Palomar, & Oteo, 2015). *Rickettsia helvetica* is a member of the spotted fever group (SFG) and reportedly causes a self-limiting illness associated with headache and myalgias (Parola et al., 2013).

Anaplasma phagocytophilum DNA was mostly found in *I. ricinus*, an important vector of this zoonotic agent responsible for human granulocytic anaplasmosis (HGA), tick-borne fever (or pasture fever) in ruminants and equine anaplasmosis. In Corsica, this pathogen was relatively unknown and was previously only detected in a single sample of bovine blood (ICTTD, 2000). There have been no HGA cases reported in Corsica, but in mainland France the first case was identified in 2003, and others have been reported infrequently (Edouard et al., 2012). Human cases have also occurred in continental Italy (Ruscio & Cinco, 2003). A recent study showed that, in the French Basque Country, 22.4% of collected ticks contained *A. phagocytophilum* DNA (Dahmani, Davoust, Tahir, et al., 2017). This species has also been reported from Sardinia (Alberti et al., 2005), Sicily (Torina et al., 2010) and North Africa (Dahmani et al., 2015).

Babesia spp. are protozoan blood parasites with more than 100 described species. In this study, *Ba. bigemina* and *Ba. ovis* DNA were found for the first time in ticks collected in Corsica. *Babesia bigemina* is a causative agent for bovine babesiosis and it occurs in most areas of the world (Uilenberg, 2006). *Babesia ovis* is a causative agent of ovine babesiosis, with strains varying in pathogenicity and is also widespread throughout the world (Uilenberg, 2006).

Ticks (especially *I. ricinus)* are highly suspected of being among the vectors of *Bartonella* species. *Bartonella henselae* DNA is reported here for the first time in ticks from Corsica and was found in seven tick species. It causes an infection commonly encountered in cats (cat scratch disease) and potentially in dogs and humans worldwide (Álvarez-Fernández, Breitschwerdt, & Solano-Gallego, 2018). In Sardinia, it was found in at least 0.2% of collected ticks (Chisu, Foxi, Mannu, Satta, & Masala, 2018).

4.2 | Other TBPs identified in Corsica

Rickettsia species were the pathogens with the highest infection rate found in the Corsican pools. They are known to be zoonotic agents and, although their pathogenicity and the reservoir role for animals are debated, most species of this genus cause serious human diseases (Davoust et al., 2010). The real impact of rickettsial diseases in Corsica is unknown, but some cases of Mediterranean spotted fever (MSF, caused by *R. conorii*) have been reported by local medical doctors and the French National Reference Centre (CNR) for *Rickettsia* species. A former sero-epidemiological study showed in 1985 that 4.8% of people were exposed to theses pathogens in Corse-du-Sud (Raoult, Nicolas, De Micco, Gallais, & Casanova, 1985). *Rickettsia aeschlimannii* DNA was the most frequently detected, infecting 100% of pools of *Hy. Marginatum*, one of its main natural vectors (Matsumoto et al., 2004). This confirmed the high presence of this bacterium in Corsica, already reported in 74% of *Hy. marginatum* collected by Matsumoto et al. (2004). *Rickettsia aeschlimannii* was first isolated from *Hy. marginatum* in 1997 from Morocco (Beati, Meskini, Thiers, & Raoult, 1997) and is now reported in the whole Mediterranean area (Parola et al., 2013). *Rickettsia aeschlimannii* infections in humans cause spotted fever; this has previously been confirmed in North Africa and South Africa, and in 2010 a first case occurred in Southern Europe, in a Greek patient (Germanakis, Chochlakis, Angelakis, Tselentis, & Psaroulaki, 2013). Given the important infestation rate of this pathogen in Corsican ticks, human exposure to *R. aeschlimannii* infection is high and human cases of tick-borne spotted fever acquired in Corsica could often be due to *R. aeschlimannii*.

Rickettsia slovaca DNA was identified mainly in *D. marginatus* which is an important natural vector of this bacterium. This report confirmed the results of Selmi et al. (2017) who identified *R. slovaca* in *D. marginatus* collected on Corsican vegetation. *Rickettsia slovaca* was first isolated in 1968 in a *D. marginatus* specimen collected in Slovakia and is largely spread in the Mediterranean area. It was found in Sardinia and continental Italy (Chisu et al., 2017 and Selmi et al., 2017), mainland France (Beati, Finidori, & Raoult, 1993) and Spain (Fernández-Soto, Pérez-Sánchez, Encinas-Grandes, & Sanz, 2006). The first proven human case of *R. slovaca* infection was reported in 1997 in mainland France and this microorganism is now known as a cause of disease in various Mediterranean European countries (de Sousa et al., 2013). It is associated with TIBOLA (tick-borne lymphadenopathy) syndrome, characterized by lymph node enlargement and scalp eschars (Parola et al., 2013). So far, TIBOLA syndrome has not been reported in Corsica.

This study did not identify DNA of other *Rickettsia* spp. previously reported in Corsica as *Rickettsia felis*, an SFG *Rickettsia*, found in a flea (*Archaeopsylla erinacei*) collected from a fox (Marié et al., 2012). Matsumoto, Ogawa, Brouqui, Raoult, and Parola (2005) reported the species *R. massiliae* in *Rh. sanguineus* s.l. collected from dogs in Corse-du-Sud and a recent study identified *R. africae* DNA in an *Amblyomma variegatum*, a tick species not established in Corsica but whose one adult was collected, certainly following introduction of a nymph by a migrating bird (Cicculli, Lamballerie, et al., 2019). DNA of another species of *Rickettsia*, Candidatus *Ri. barbariae*, was recently detected in ticks collected from Corsican cattle (Cicculli, Capai, et al., 2019), but its pathogenicity remains unknown.

This study also showed that *Anaplasma* species are widespread in Corsican ticks. *Anaplasma marginale* DNA was found in ticks from the genus *Rhipicephalus* which are its natural vectors, but also in *Hy. marginatum*, *I. ricinus* and *Ha. punctata*. *Anaplasma marginale* DNA was formerly reported in blood from Corsican cattle (ICTTD, 2000) and two recent studies detected it in *Rh. bursa* collected from cattle confirming its presence on the island (Dahmani, Davoust, Tahir, et al., 2017 and Cicculli, Capai, et al., 2019). *Anaplasma marginale* is distributed worldwide in tropical and subtropical regions. It is the causative agent of erythrocytic bovine anaplasmosis that can affect various species of domestic and wild ruminants (Aubry & Geale, 2011). Regarding islands near to Corsica, it has been identified in Sardinia (Zobba et al., 2014) and Sicily (Torina et al., 2010), but there are no reports of its presence in continental France. *Anaplasma ovis* DNA, not found in this study, was recently reported in blood (52%) and ticks (*Rh. bursa*) collected from Corsican goats (Cabezas-Cruz et al., 2019). *Anaplasma bovis* and *A. omatjenne* were formerly reported in cattle blood (ICTTD, 2000), and other potential new species of *Anaplasma* were described from Corsican sheep blood (Dahmani, Davoust, Tahir, et al., 2017).

Concerning *Babesia* species, *B. bovis*, not found in this study, was reported earlier from Corsican cattle blood (ICTTD, 2000). *Babesia bovis* occurs in most subtropical and tropical regions of the world (Uilenberg, 2006), and two of its proven vectors occur in Corsica (*Rh. bursa* and *Rh. (B.) annulatus*). Bovine babesiosis affecting cattle in Corsica is probably due either to *B. bigemina* or to *B. bovis*.

No species from the genus *Ehrlichia* were found in this study although *E. minasensis*, a species unknown so far from the Mediterranean area, has been detected recently in a *Hy. marginatum* specimen (Cicculli, Masse, et al., 2019).

Theileria equi DNA was identified in two pools. It is a causative agent of equine piroplasmosis, which can also be due to *Babesia caballi* (not detected in this study). As the disease is widespread on the island, it is well known by local veterinary practitioners and horse owners. *Theileria annulata* DNA has not been reported so far in the Corsican tick population. The high occurrence in Corsica of *Hy. scupense*, one of its main natural vectors, highlights however the risk of transmission of *T. annulata* to the local cattle population (Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Gharbi, et al., 2016). *Theileria buffeli*, a benign cattle pathogen, already detected in Corsica (ICTTD, 2000), was not found in this study.

A total of 332 pools (1,015 ticks) of three species (*Hy. marginatum*, *Hy. scupense* and *Rh. bursa*) were tested for CCHF virus RNA and all were negative. The CCHF virus is mainly transmitted by ticks of the genus *Hyalomma* and *Hy. marginatum* is its main natural vector in Europe. Crimean–Congo haemorrhagic fever is now the most worldwide tick-borne viral infection in humans, and it can lead to haemorrhagic manifestations and high mortality. It is also an important emerging zoonotic disease in Turkey and south-eastern Europe

(Dreshaj et al., 2016). Moreover, in 2016, the first autochthonous human cases were reported in Spain showing that the disease can occur in western Europe (Negredo et al., 2017). Recently, CCHF RNA was reported in a nymph of *Hy. rufipes* on a migrating bird on the small Italian island of Ventotene, pointing to migrating birds as a possible introduction pathway for the CCHF virus into western Europe (Mancuso et al., 2019). *Hyalomma rufipes*, a significant natural vector of CCHF virus in sub-Saharan Africa, was ever found on migrating birds in Corsica (Pérez-Eid, 2007). It is possible that the CCHF virus was not detected in this study due to an insufficient sample size of analysed ticks or a deterioration of the genetic material due to the storage method. As CCHF virus is an RNA virus, it would certainly have been better to store ticks alive at −80°C to detect it, but in this study different people (hunters, breeders, staff of PNRC and ONCF) from different locations have collected ticks, preventing the storage of alive ticks at −80°C. However, as indicated above, CCHF RNA was detected recently in one *Hyalomma* tick collected on a migratory bird in Italy, and all the collected ticks of this study was conserved in 70° ethanol at −20°C (Mancuso et al., 2019) showing there is apparently no difficulties to detect CCHF RNA with this storage method. Because of the high frequency of *Hy. marginatum* in Corsica (the second most abundant tick species collected on animal hosts, Grech-Angelini, Stachurski, Lancelot, Boissier, Allienne, Marco, et al., (2016), it appears necessary to closely monitor the possible emergence of the CCHF virus. More ticks, mainly *Hy. marginatum*, should be analysed and animal sera should be collected to test for the presence of antibodies against CCHF virus in domestic ruminants which are known to be asymptomatic reservoir of the CCHF virus (Bente et al., 2013).

4.3 | Potential Corsican animal reservoirs for zoonotic pathogens and human exposure

These data concerning the presence of TBP DNA on Corsica Island will be useful for studying possible future emerging diseases and the role of animals as reservoirs of TBPs. This study demonstrated the presence of expected pathogen DNA and unexpected pathogens. Among the eleven pathogens reported in ticks collected from animal hosts, seven are zoonotic: *B. miyamotoi*, *B. afzelii*, *R. aeschlimannii*, *R. slovaca*, *R. helvetica*, *A. phagocytophilum* and *Bar. henselae*. Most of them are asymptomatic, or their effects on animal health are unknown. *Rickettsia aeschlimannii* DNA was reported in a high proportion of ticks from domestic ruminants and horses whereas *R. slovaca* DNA was mostly identified in ticks from wild boars; *R. helvetica* DNA only in ticks from cattle. All positive pools for *Borrelia* spp. consisted of ticks collected from cattle. These domestic or wild animals could be specific reservoirs for the respective pathogens. *Anaplasma phagocytophilum* was mainly found in *I. ricinus* on various hosts including migrating birds that showed the potential to introduce pathogens to Corsica via this pathway. Even though no human cases caused by these pathogens have been diagnosed in Corsica to date, the significant interactions that occur between domestic animals, wild fauna and humans highlight the risk for the human population. Some of these zoonotic pathogens could circulate undetected in the human population in Corsica. Such studies may help to draw attention to the need to monitor animal reservoirs and human exposure. Detection of pathogen DNA is not sufficient to be able to confirm the presence and the circulation of these pathogens in Corsica. Nevertheless, this first step will make it possible to carry out in-depth research on these pathogens of interest to better characterise their epidemiological cycle. Clearly, serological surveys in domestic and wild animals, and in humans, need to be carried out. Additionally, efforts should be made to isolate these pathogens from infected ticks and/or infected animals to be able to draw conclusions regarding the risk to human and animal health.

5 | CONCLUSION

In this study, 569 tick pools (1,523 ticks) collected from animals on Corsica Island (France) were analysed to investigate the presence of 27 bacteria and 12 parasites by a high-throughput real-time microfluidic PCR system. The CCHF virus and *Theileria* spp. were specifically investigated in their respective potential vectors. DNA of eleven pathogens from six genera was identified in Corsican ticks, and among them seven were reported for the first time in Corsican ticks: *B. miyamotoi*, *B. afzelii*, *R. helvetica*, *A. phagocytophilum*, *Bar. henselae*, *Ba. bigemina* and *Ba. ovis*. These results also confirmed the presence of four other TBPs in Corsica: *R. aeschlimannii*, *R. slovaca*, *A. marginale* and *T. equi*. Many of the pathogens found in this survey are mostly asymptomatic or benign in animals, showing that domestic and wild Corsican animals are probably an important epidemiological reservoir, increasing the human exposure to these zoonotic pathogens. These findings highlight the importance of more in-depth studies into the epidemiological picture concerning TBPs in Corsica in the near future.

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ETHICS APPROVAL

The authors obtained the agreement of all owners to collect ticks from their domestic animals. The cattle inspected were slaughtered for human consumption and the wild boars collected were legally hunted during the hunting season. The collected deer, mouflons and birds were captured by the PNRC and ONCFS, and they were all released. This study was approved by the veterinary Institutes (DDCSPP of Corse-du-Sud and Haute-Corse) and the French Ministry of Agriculture, General Directorate for Food (DGAL).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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