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Detecting zoonotic and non-zoonotic pathogens in livestock and their ticks in Corsican wetlands

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Abstract

Background: Corsica is a large French island in the Mediterranean Sea with high human and animal migration rates, especially near wetlands where these migrations are particularly frequent. Among the livestock populations, cattle and sheep are widely present all across the entire Mediterranean region. Trade can be responsible for the circulation of numerous pathogens and their vectors, thereby representing a health and economic threat for the livestock industry.

Objectives: The objective of our study was to investigate the presence of pathogens in cattle and sheep farms in the wetlands of Corsica using a high-throughput screening technique.

Methods: In our study, blood samples and ticks were collected from cattle and sheep in 20 municipalities near Corsican wetlands to screen for the presence of various types of pathogens. The samples were processed using a high-throughput screening technique based on real-time microfluidic PCR: 45 pathogens were screened in 47 samples simultaneously.

Results: A total of 372 cattle and 74 sheep were sampled, and 444 ticks were collected from cattle. Out of the eight tick species detected, the main one was Rhipicephalus bursa (38.7% of the ticks collected). From cattle blood samples, one species and two genera were found: Anaplasma marginale, Trypanosoma sp. and Babesia sp. in respectively 61.5%, 58.3% and 12.2% of the cattle blood samples. From sheep blood samples, 74.3% were positive for Anaplasma sp, 2.7% for Anaplasma ovis and 1.4% for Anaplasma capra. This is the first report of A. ovis DNA in blood samples from sheep in Corsica. Out of 444 the tick samples, 114 were positive: 77.2% for Rickettsia aeschlimannii, 20.2% for Rickettsia sp., 3.5% for Babesia sp. and 1.8% for Anaplasma sp. Among them, 2.7% were co-infected with R. aeschlimannii and Babesia sp.

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Conclusions: Our results confirm the extent of possible circulation of different pathogens near Corsican wetlands, not only in ticks collected from livestock but also directly in cattle and sheep, with two (*Trypanosoma* sp. and *Babesia* sp.) being detected for the first time in cattle, one for the first time in sheep (*A. ovis*) and one for the first time in Corsica (*A. capra*)

KEYWORDS

Anaplasma, Babesia, cattle, high-throughput screening technique, *Rickettsia*, sheep, tick-borne pathogens, ticks, trypanosoma, wetlands

1 | INTRODUCTION

Livestock rearing appeared early in the Mediterranean region with cattle, goat, pig and sheep (Seder, 2008). Today, in the early 21st-century, grazing pastures represent half the agricultural pasturelands and more than 130 million ha (Pardini, 2004). Livestock are highly affected by all types of pathogens (e.g. bacteria, parasites, viruses) which can cause important economic losses. For instance, €1.660 billion was spent in 2013 in Europe just for animal diseases control including viral diseases like the avian influenza and African swine fever, bacterial diseases such as bovine tuberculosis and brucellosis, and parasite diseases such as toxoplasmosis in pig and sheep, or cryptosporidiosis in cattle(Costa & Akdeniz, 2019; Knap & Doeschl-Wilson, 2020; Mévélec et al., 2020; Thomson et al., 2017). In the Mediterranean Basin, sheep are the main livestock species, especially in North Africa and Eastern Mediterranean countries, followed by cattle and goat (Montgomery, 2014). Combined with their role in foodborne diseases and their interaction with humans, livestock can interact with wild animals, all of which may increase the potential risk of spread or creation of a disease reservoir in the animal population by these interactions, such as for boyine tuberculosis (Cowie et al., 2016). One of the main areas of interaction are feeding areas, which can be human-managed pastures or natural wetlands (Cowie et al., 2016). In 2019, the main livestock species raised in Corsica was sheep, with a total over 100,000 heads, followed by cattle, pig and goat (Chambre d'agriculture Corse, 2021).

Livestock are frequently present in wetlands and their interactions, particularly with wild ungulates (such as wild boar) can occur (Robertson, 1997). Wetlands are key areas for human activities, biodiversity and historically providing one of the main watering and resting areas for both livestock and humans and highly impact the breeding and road construction (Bacon, 1987; Fornell-Muñoz & Guerrero, 2019; Hammer, 1989). They represent 2–3% of the land area in the Mediterranean basin; nevertheless, they regroup more than 30% of the vertebrate biodiversity. However, even if wetlands represent a major importance in the biodiversity and animals and human wellbeing these various activities are disturbance factors of the wetlands ecology such as the water use due to human and agricultural activities, the impact of agriculture chemicals and the livestock grazing (Middleton, 2016; Taylor et al., 2021). Unsurprisingly, these habitats are important for public health given their role in vector proliferation and pathogen trans-

mission (Jourdain et al., 2007; Rabou et al., 2015; Rey et al., 2012). Furthermore, depending on the ecosystem, the diversity and the prevalence of ectoparasite communities and pathogens can change (Dudek, 2014).

Ticks (Acarida) are the leading vectors of veterinary pathogens and the second, after mosquitos, of human pathogens (Nicholson et al., 2019; Parola & Raoult, 2001). Furthermore, ticks are responsible of the transmission, circulation and maintenance of pathogens between different groups of animals all over the world. They allow for the maintenance and transmission of tick-borne pathogens in a natural cycle that involves feeding on the blood of their intermediate and final animal hosts and accidental human hosts (de la Fuente et al., 2008). Ticks can transmit a wide spectrum of bacteria, viruses and parasites. These tick-borne pathogens are known to be both pathogens of veterinary importance and zoonotic pathogens harmful for humans (Sonenshine, 2014). Many different animals can be reservoirs. Worldwide, one of the largest groups of reservoirs is probably ungulates (wild or domestic) as the livestock (Eldridge & Edman, 2003). The livestock are highly affect by various tick-borne pathogens (TBPs) as a target and like host of tick carrying pathogens of sanitary importance such as the bacteria responsible of the Lyme disease and the CCHF virus (Dernat Sylvain et al., 2021). In the Mediterranean Basin, more precisely, the livestock are among the most studied and reported animals host of pathogens and ectoparasites. They also belong the main targets of various ticks' genera as Rhipicephalus and Hyalomma. By these ticks' genera infestations, the livestock can by targets or reservoir of several pathogens as the causative agents of piroplasmosis, Lyme disease and Q fever (Defaye et al., 2022; Estrada-Peña, 2004). This potential role of target and reservoir is particularly important in Corsica where livestock represents more than 270,000 animals for a human population of 322,000 and coupled with both a lake of knowledge of the farmer about the ticks and TBPs; and under estimation of their risk (Chambre d'agriculture Corse, 2021; Dernat Sylvain et al., 2021). Recently, in two surveys of ticks, nine species were found in Corsica: Ixodes ricinus, Rhipicephalus bursa, Rh. sanguineus s.l., Rh. (Bo.) annulatus, Hyalomma marginatum, Hy. scupense, Haemaphysalis sulcata, Hae. punctata and Dermacentor marginatus (Grech-Angelini et al., 2016). Eight species were found on Corsican cattle. Out of the eight species, Rh. bursa was the most commonly found on cattle (>50% of the ticks collected on cattle), with only Hae. sulcata not being sampled at all on cattle. In sheep, three

species were identified: *Rh. bursa* (91% of the collected ticks), followed by *H. marginatum* and *Rh. sanguineus* s.l. (Grech-Angelini et al., 2016).

The aims of this study were (i) to determine the main tick species of cattle and sheep in Corsican wetland areas and (ii) to investigate the potential occurrence of zoonotic and non-zoonotic pathogens in ticks and hosts (livestock) from Corsican wetlands. To do so, we focused our screening efforts on the detection of 34 species and 11 genera of bacteria, viruses and parasites with a high impact on animal and human health suspected to be present in Corsica.

2 | METHODS

2.1 | Sites and animal samples

Corsica is the largest French island in the Mediterranean Sea. It is located 15 km from the closest Italian land mass (the island of Sardinia) and 160 km from the nearest French coast. It is characterised by a mild Mediterranean climate and a wide diversity of landscapes, such as wetlands (mainly on the coastline), forests and mountains (elevation of up to 2706 m). Corsica is divided into two administrative *départements* (Haute-Corse and Corse-du-Sud), with a population of 322,000, which increases by a factor of 10 during the tourist season (summer) with approximately 3 million tourists. Sampling was carried out in wet-lands, which can be classified into five categories: lagoon, river and river mouth, artificial lake, altitude lake and temporary pool. In this study, all samples were collected in coastal wetlands including lagoon, artificial lake and river mouth.

A total of 372 cattle and 74 sheep were sampled across Corsica on respectively 22 and 3 farms during the winter and spring seasons in 2019(February to June) and winter in 2020(October 2019 to February 2020). The farm choose for the study were farm included in the prophylactic program of the Corsican veterinary groups and located near coastal wetlands (Figure 1). For cattle, 288 blood samples and 444 ticks were collected in 18 municipalities, and for sheep, 74 blood samples were collected in 3 municipalities. Samples were collected only from animals older than 6 months of age. Every blood sample was collected in an EDTA tube by veterinarians as part of national surveillance program for animal diseases. All samples were stored at -80° C within a few hours after sampling until further analysis.

2.2 DNA extraction and PCR pre-amplification

Ticks were washed in ethanol and distilled water and then morphologically determined to the species level using species keys (Estrada-Peña, 2004; Pérez-Eid, 2007). Prior to extraction, ticks were crushed in microtubes filled with six metal beads by using a FisherbrandTM bead mill 24 homogeniser (Thermo Fisher, USA) at 5500 rpm for 20 s. Tick DNA was extracted using a NucleoSpinTM Tissue kit (Macherey-Nagel, Germany). For blood samples, a Nucleospin Quickpure Blood kit (Macherey-Nagel, Germany) was used according to the manufacturer's instructions. All ticks from the same animal were pooled by species,

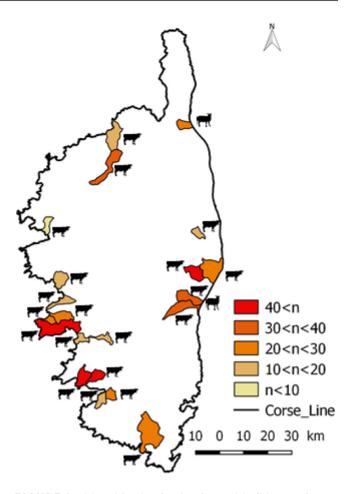


FIGURE 1 Map of Corsica showing the municipalities samples (*n* = number of animal sampled by municipalities)

stage and sex (minimum number of ticks = 1; maximum number of ticks = 8).

To improve the detection of pathogen DNA, total DNA was pre-amplified by using the PreAmp Master Mix according to the manufacturer's instructions (Fluidigm, California, USA). Prior to the experiments, primers targeting different pathogens were pooled by combining an equal volume of each primer (200 nM final each). The experiment was performed with 1 μ l of PreAmp Master Mix, 1.25 μ l of pooled primer mix, 1.5 μ l distilled water and 1.25 μ l DNA for a final volume of 5 μ l. Pre-amplification was performed as follows: 1 cycle at 95°C for 2 min, 14 cycles at 95°C for 15 s and 60°C for 4 min. Amplified samples were diluted to 1:10. The pre-amplified DNAs were stored at -20°C until further use.

2.3 Assay design

Pathogens and their targeted genes were Anaplasma spp. (16S rRNA), A. marginale (msp1b), A. phagocytophilum (msp2), Borrelia spp. (23S rRNA), Bo. burgdorferi s.s. (rpoB), Bo. afzelii (flagellin), Bo. miyamotoi (glpQ), Bo. lusitaniae (rpoB), Bo. spielmanii (flagellin), Rickettsia spp. (gltA), R. slovaca (23S-5S ITS), R. helvetica (23S-5S ITS), R. aeschlimanii

TABLE 1 List of pathogens, targets and primers/probes created for microfluidic real-time PCR screening

Pathogen	Name	Sequence	Length (nucleotides)	Gene	Gene reference
Aujeszky's disease	Aujv_gp50_F	CTTTATCGAGTACGCCGACTG	225	gp50	Y14834.1
viral agent	Av_gp50_R	AACGGGCACTCTTGCCCC			
	Av_gp50_P	CAGATCTTTGGGCGCTGCCGGC			
Chlamydia psittaci	Chl_psi_16S-23S_F	ACGCCGTGAATACGTTCCC	214	16S-23S rRNA	U68450.1
	Chl_psi_16S-23S_R	AGTCAAACCGTCCTAAGACAG			
	Chl_psi_16S-23S_P	CCTTGTACACACCGCCCGTCACATC			
Haemoproteus spp.	Hae_cytB_F	ATATGCATGCTACTGGTGCTAC	240	cytochrome B	AF465579.1
	Hae_cytB_R	CAAATCCATGAAACAAGTCCAGG			
	Hae_cytB_P	CGGTTGCACCCCAGAAACTCATTGAC			
Leptospira spp.	Lep_Lipl32_F	CTCTATGTTTGGATTCCTGCC	158	lipL32	MK541891.1
	Lep_Lipl32_R	CCAAGTATCAAACCAATGTGGC			
	Lep_Lipl32_P	ATTGATTTTTCTTCTGGGGTAGCCGCTTTG			
Leucocytozoon spp.	Leu_cytB_F	GGGTTATGTCTTACCATGGGG	177	cytochrome B	KF717066.1
	Leu_cytB_R	AATTGCTAGTGCTACGAATGGG			
	Leu_cytB_P	AAATGAGTTTTTGGGGAGCAACCGTTATTAC			
Plasmodium spp.	Pla_ssrRNA_F	ATATAGAAACTGCGAACGGCTC	339	ssrRNA	MK650620.1
	Pla_ssrRNA_R	TTTCTCAGGCTCCCTCTCC			
	Pla_ssrRNA_P	CTCTAATTCCCCGTTACCCGTCATAGC			
Rickettsia monacensis	Ric_mon_F	CTCGGTGCCGGTACTTTAAAC	192	ompB	KU961543.1
	Ric_mon_R	GAGCACCGCCAATAAGAGC			
	Ric_mon_P	AGTGCCGATGCAAATACTCCGGTGAC			
Trypanosoma spp.	Try_18SRNA_F	GTAATTCCAGCTCCAAAAGCG	178	18S rRNA	EU596263.1
	Try_18SRNA_R	TCAGGAAGGAACCACTCCC			
	Try_18SRNA_P	ACCTCAAGGGCATGGGTCACCAATCC			

(23S-5S ITS), R. massiliae (23S-5S ITS), R. conorii (23S-5S ITS), C. burnetii (idc, IS1111), N. mikurensis (16S rRNA), Bartonella spp. (ssrA), Ba. henselae (pap31), Ba. quintana (bqtR), Ehrlichia spp. (16S rRNA), E. ruminatum (dsb), Hepatozoon spp. (18S rRNA), To. gondii, B. microti (CCTeta), B. bigemina (18S rRNA), B. vogeli (hsp70), B. caballi (rap1), B. bovis (CCTeta), B. ovis (18S rRNA), B. divergens (hsp70), T. equi (ema1), T. annulata (18S rRNA), Leishmania spp. (hsp70), L. infantum (ITS) and African swine fever virus (Vp72). Primers and probes were designed by Gondard et al. (2018), Grech-Angelini et al. (2020) and Michelet et al. (2014). The set of primers and probes created especially for this study is listed in the Table 1.

2.4 | DNA amplification and microfluidic real-time PCR

The detection of the vector-borne pathogen targets was performed on pre-amplified DNA by using the BioMarkTM real-time PCR system (Fluidigm, California, USA) for high-throughput microfluidic real-time PCR amplification using the 48.48 Dynamic ArrayTM (Fluidigm). Fluidigm chips can carry out 2304 real time PCR reactions simultaneously with

48 PCR mixes on 48 samples placed in individual wells prior to transfer to individual chambers for the reaction. The thermal cycling conditions were 50°C for 2 min, 95°C for 10 min and 40 cycles at 95°C for 15 s and 60°C for 1 min. One negative water control, one inhibitory molecule control (*E. coli* EDL933 strain) and one DNA extraction control (animal species target) were added to each array. *E. coli*-specific primers and probes were used.

2.5 Confirmation of pathogen detection

The confirmation of the pathogen detection was performed using nested PCR or real-time PCR targeting a gene other than the one used in the microfluidic experiment. The targets and primers were selected from the literature when available or newly designed for this study. The positive sequences after gel migration of the nested PCR product were sent to Eurofins MWG Operon (Cologne, Germany) for sequencing and assembled using BioEdit software (Ibis Biosciences, Carlsbad, CA, USA). An online BLAST (National Center for Biotechnology Information) was used to identify the sequenced organisms. All targeted genes and primer sequences from the literature are described in Gondard

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TABLE 2 List of pathogens, targets and primers/probes used for confirmation by real-time PCR

Pathogen	Name	Sequence	Length (nucleotide)	Genes	Genes references
Aujeszky's disease	Aujv_gp50_F2	AACATCCTCACCGACTTCATG	158	gp50	Y14834.1
viral agent	Av_gp50_R2	CTGGTAGAACGGCGTCAGG			
	Av_gp50_P2	AATCGCATCACGTCCACGCCCCC			
Chlamydia psittaci	Chl_psi_16S-23S_F2	AGTAATCTTCGGCGAGCTGG	177	16S-23S rRNA	U68450.1
	Chl_psi_16S-23S_R2	CGCTACTTAGGGAATCTCTTTG			
	Chl_psi_16S-23S_P2	TATAAAGCTATGACCCGGAGGTCTCCG			
Haemoproteus spp.	Hae_cytB_F2	CCTTGGGGTCAAATGAGTTTC	231	cytochrome B	AF465579.1
	Hae_cytB_R2	AAGCCGTATCATATCCTAAAGG			
	Hae_cytB_P2	CCTGGACTTGTTTCATGGATTTGTGGAGG			
Leptospira spp.	Lep_Lipl32_F2	CTCTATGTTTGGATTCCTGCC	158	lipL32	MK541891.1
	Lep_Lipl32_R2	CCAAGTATCAAACCAATGTGGC			
	Lep_Lipl32_P2	ATTGATTTTTCTTCTGGGGTAGCCGCTTTG			
Leucocytozoon spp.	Leu_cytB_F2	GAGTTTCTGGGGAGCTACTG	197	cytochrome B	KU842391.1
	Leu_cytB_R2	GGATTAGTGCTACCTTGAATATG			
	Leu_cytB_P2	TGAATAAATACAATTGCTAGTGCTACGAATGG			
Plasmodium spp.	Pla_ssrRNA_F2	TCGAGTTTCTGACCTATCAGC	264	ssrRNA	MK650620.1
	Pla_ssrRNA_R2	AGACTTGCCCTCCAATTGTTAC			
	Pla_ssrRNA_P2	TGGCCTTGCATTGTTATTTCTTGTCACTACC			
Trypanosoma spp.	Try_18SRNA_F2	CAACACGGGGAACTTTACCAG	141	18SRNA	EU596263.1
	Try_18SRNA_R2	ATCCTACTGGGCAGCTTGG			
	Try_18SRNA_P2	CAGGGTGAGGATTGACAGATTGAGTGTTC			

et al. (2018) and Michelet et al. (2014). The set of primers/probes created for this publication is listed in Table 2.

3 | RESULTS

3.1 | Tick collection

A total of 444 ticks were collected on 178 cattle in 18 municipalities close to wetlands (Table 3). Six tick species were identified: *Rhipicephalus bursa* (38.7%), *Rh. sanguineus* s.l. (22.8%), *Rhipicephalus* sp. (17.1%), *Hy. marginatum* (8.6%), *Hy. scupense* (7.2%), *D. marginatus* (0.7%) and *Rh.* (*Bo.*) annulatus (0.2%). Finally, 4.7% of the ticks could not be determined and were designated as undetermined ticks (Table 3). Regarding their life-cycle stage, 55.6% were nymphs and 44.4% were adults; of these adults 57.4% were partially or fully engorged females and 42.6% were males. No ticks were found on sheep.

3.2 | Pathogens detected in ticks

Out of the 312 tick pools from cattle, 96 pools were positive (30.5%) and three pathogen genera were detected.

The most frequently found pathogen was *Rickettsia* sp., detected in 93 pools; 15 of the pools detected positives to *Rickettsia* sp. could not be determined to the specific level. These bacteria were detected in *Rh. sanguineus* s.l. (46.6%), *Rh. bursa* (33.3%), but only 6.7% for *Rhipicephalus* sp., *Hy. scupense* and undetermined ticks. The remaining 78 pools were positive for *R. aeschlimannii*. This pathogen was mainly found in *Rh. bursa* (41%), with the remaining positive tick species being *Hy. marginatum* (29.5%), *H. scupense* (14.1%), *Rh. sanguineus* s.l. (10.2%), *Rhipicephalus* sp. (3.8%) and unknown (1.4%) (Table 3, Figure 2a). These samples were found to be related to the ompb gene of a *R. aeschlimannii* sequence detected in a *Hy. marginatum* individual in Russia (Genbank: KU961544.1) with an identity between 96% and 100%.

One pool was positive for *Anaplasma* sp. It was composed of *Rh. sanguineus* s.l. ticks collected on a farm in Viggianello (Table 3, Figure 2b). However, sequencing was unsuccessful for *Anaplasma* sp.

Two pools were positive for *Babesia* sp. They were one pool of *Rh. sanguineus* s.l. and one of *Hy. marginatum* both collected on a farm in Figari (Table 3, Figure 2c). Unfortunately, the sequencing of the *Babesia* sp. positive samples was unsuccessful.

Only one pool of *Rh. sanguineus* s.l. ticks was positive for more than one pathogen: *R. aeschlimannii* and *Babesia* sp.

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Municipality (n°)	sheep/no. positives (A/Ao/Ac)	rvo. sampreu cattle/no. positives (Am/B/T)	D. marginatus (A/B/R/Ra)	Hy. marginatum (A/B/R/Ra)	Hy. scupense (A/B/R/Ra)	Rhipicephalus spp.(A/B/R/Ra)	Rh. annulatus (A/B/R/Ra)	Rh. bursa (A/B/R/Ra)	мн. sanguineus s.l. (A/B/R/Ra)	Undetermined tick species (A/B/R/Ra)	Total ticks from cattle (A/B/R/Ra)
Aghione (1)	ı	28/9 (Am), 1 (B), 15 (T)		2/1 (Ra)	1/1 (R)	2/2 (Ra)	1	2/2 (Ra)	1/1 (R)	0/6	17/2 (R), 5 (Ra)
Ajaccio (2)	1	38/14 (Am), 1 (B), 22 (T)			1	16/0	1/0	26/3 (Ra)	14/0		57/3 (Ra)
Alata (3)	1	6/6 (Am), 3 (B), 2 (T)	1/0	1/1 (Ra)	1/0	1/0	1	1/0	4/4 (R)		9/4 (R), 1 (Ra)
Aleria (4)	I	22/0		3/3 (Ra)	3/0	2/0	ı	22/ 3 (Ra)	2/2 (Ra)	2/0	34/8 (Ra)
Calcatoggio (5)	ı	10/9 (Am), 4 (T)	ı	1/0	ı	ı	ı	ı	ı	1/0	2/0
Coggia (6)	1	10/10 (Am), 4 (B), 9 (T)	1	1/0	8/0	1	1	ı	ı	2/1 (R)	11/1 (R)
Figari (7)	ı	14/14 (Am), 2 (B), 11 (T)	1/0	4/1 (B), 2 (Ra)	1	6/0		9/2 (R), 1 (Ra)	12/3 (B), 3 (R), 6 (Ra)	1/0	33/4 (B), 5 (R), 9 (Ra)
Furiani (8)	25/22 (A), 2 (Ao)		1			,	1	1	1		
Grosseto- Prugna (9)	ı	11/1 (Am), 6 (T)	1	6/3 (Ra)	3/3 (Ra)	1		1/0	ı	1	10/6 (Ra)
Olmi-Capella (10)	1	19/19 (Am), 4 (B), 13 (T)	1	4/3 (Ra)	3/1 (Ra)	12/1 (Ra)	1	2/1 (Ra)	3/0	2/1 (Ra)	26/7 (Ra)
Palasca (11)	ı	12/10 (Am), 10 (T)	ı	12/9 (Ra)	8/3 (Ra)	ı	ı	54/3 (R), 26 (Ra)	ı		74/3 (R), 38 (Ra)
Partinello (12)	1	8/5 (Am), 4 (B), 7 (T)				1	ı	ı	1		
Propriano (13)	ı	10/7 (Am), 3 (B), 4 (T)	ı	ı	ı	1/0	ı	8/0	3/0	2/0	14/0
Prunelli-Di- Fiumorbo (14)	25/0	8/6 (Am), 4 (T)			I		1	1	1		
											(Continues)

 TABLE 3
 Tick-borne pathogens and tick distribution on livestock in Corsican wetlands

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Municipality (n°)	No. sampled sheep/no. positives (A/Ao/Ac)	No. sampled cattle/no. positives (Am/B/T)	D. marginatus (A/B/R/Ra)	Hy. marginatum (A/B/R/Ra)	Hy. scupense (A/B/R/Ra)	Rh. Rhipicephalus annulatus spp.(A/B/R/Ra) (A/B/R/Ra)	Rh. annulatus (A/B/R/Ra)	Rh. bursa (A/B/R/Ra)	Rh. sanguineus s.l. (A/B/R/Ra)	Undetermined tick species (A/B/R/Ra)	Total ticks from cattle (A/B/R/Ra)
Serra-Di- Ferro (15)	1	34/29 (Am), 5 (B), 20 (T)	ı	ı		2/2 (Ra)	T	25/2(R)	14/3 (R)	1	41/5 (R), 2 (Ra)
Serra-Di- Fiumorbo (16)	34/33 (A), 1 (Ac)	1	1	1	1	1	1		1	1	
Sollacaro (17)	ı	28/18 (Am), 4 (B), 20 (T)	1/0	ı	ı	17/0	ı	15/1 (Ra)	28/1 (Ra)	1/0	62/2 (Ra)
Tox (18)	1	9/9 (Am), 2 (B), 9 (T)	ı	,	I	1		1/0		1	1/0
Viggianello (19)		15/7 (Am), 3 (T)	ı	1/0	ı	16/0		ı	8/2 (A)	1/0	26/2 (A)
Villanova (20)	ı	16/4 (Am), 2 (B), 9 (T)		3/1 (Ra)	5/4 (Ra)	1/1 (R)		0/9	12/2 (R), 2 (Ra)	1	27/3 (R), 7 (Ra)
Total sampled/ positives	74/55 (A), 1 (Ac), 2 (Ao)	288/177 (Am), 35 (B), 168 (T)	3/0	38/1 (B), 23 (Ra)	32/1 (R), 11 (Ra)	76/1 (R), 5 (Ra)	1/0	172/7 (R), 37 (Ra)	101/2 (A), 3 (B), 13 (R), 11 (Ra)	21/1 (R), 1 (Ra)	444/2 (A), 4 (B), 23 (R), 88 (Ra)
Abbreviations: A,	Abbreviations: A, Anaplasma spp.; Ac, A. capra; Am, A. marginale; Ao, A. ovis	A. capra; Am, A. marg		B, Babesia spp.; R,	, Rickettsia spp.;	B, Babesia spp.; R, Rickettsia spp.; Ra, R. aeschlimannii; T, Trypanosoma spp.	ii; T, Trypanoson	ла spp.			

TABLE 3 (Continued)

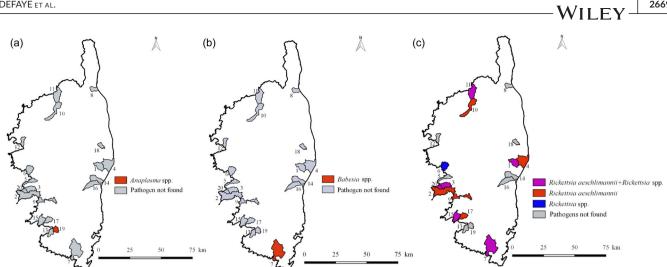


FIGURE 2 Map of Corsica showing the locations of tick samples from cattle positive for Anaplasma spp. (a), Babesia spp. (b) and Rickettsia spp. and R. aecshlimannii (c) (1. Aghione, 2. Ajaccio, 3. Alata, 4. Aleria, 5. Calcatoggio, 6. Coggia, 7. Figari, 8. Furiani, 9. Grosseto-Prugna, 10. Olmi-Capella, 11. Palasca, 12. Partinello, 13. Propriano, 14. Prunelli-Di-Fiumurbo, 15. Serra-Di-Ferro, 16. Serra-Di-Fiumorbo, 17. Sollocaro, 18. Tox, 19. Viggianello, 20. Villanova)

3.3 Pathogens detected in blood samples

From the 362 blood samples (288 cattle and 74 sheep), 79.9% of cattle and 78.4% of sheep were positive for at least one pathogen (Table 3). Bacteria from the genus Anaplasma were found in both livestock species (Table 3). In sheep blood samples, two Anaplasma species were found: A. capra in Serra-Di-Fiumorbo and A. ovis in Furiani (Table 3, Figure 3a). The A. capra sequence reported was found to be related to one 16S rRNA sequence from a blood sample collected from a Korean water deer in South Korea (GenBank: LC432126.1) with an identity of 98.69%. The A. ovis sequence reported was found to be related to a 16S rRNA sequence found in sheep blood in China (GenBank: KX579073.1) with an identity of 99.71%.

The Anaplasma species detected in cattle was A. marginale (Table 3, Figure 3b) in 61.4% of the cattle blood samples. These samples were related to a 16S rRNA sequence detected in cattle blood samples from Iran (GenBank: MK016525.1) and from Cuba (GenBank: MK804764.1) with an identity between 99% and 100% for both.

The parasite genus Babesia sp. was found in 35 cattle blood samples in 12 municipalities (Table 3, Figure 3c). The sequencing of Babesia sp. was unsuccessful and did not allow the identification of the species.

Out of the 288 cattle blood samples, 168 were positive for Trypanosoma sp. in 19 of the 20 sampled municipalities (Table 3, Figure 3d). Unfortunately, the sequencing of Trypanosoma sp. was unsuccessful in these samples and the species could not be identified.

From the 230 positive cattle blood samples, 42.7% were positive for one pathogen, 46% for two pathogens and 11.3% for three pathogens (Table 4).

DISCUSSION 4

Here, we report 1 year of sampling (2019-2020) on farms located near wetlands in 20 municipalities on the French island of Corsica.

TABLE 4 Number of cattle blood samples positives for Anaplasma
 marginale, Babesia spp. and/or Trypanosoma spp. DNA

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Pathogen	Number of positive samples (%)
Anaplasma marginale (only)	55 (19.1%)
Babesia spp. (only)	5 (1.7%)
Trypanosoma spp. (only)	38 (13.2%)
Anaplasma marginale + Babesia spp.	5 (1.7%)
Anaplasma marginale + Trypanosoma spp.	101 (35.1%)
Babesia spp. + Trypanosoma spp.	0 (0%)
Anaplasma marginale + Babesia spp. + Trypanosoma spp.	26 (9.1%)
Number of positive samples	230 (79.9%)
Number of negative samples	58 (20.1%)
Total samples	288 (100%)

The main objective of our study was to screen cattle and sheep blood samples and their ticks for tick-borne pathogens and non-vectorised pathogens (but with a high risk of presence in wetlands) involved in animal and human health threats. In the literature, many pathogens have been found all around the Mediterranean Basin in ticks collected on cattle and sheep or in cattle and sheep blood (Table S1).

4.1 | Tick species collected on cattle

In the Mediterranean region, ticks are present in 14 countries and are highly diverse. About 27 species from six genera carrying tick-borne pathogens were collected on cattle and sheep (Table S1). These genera are widely distributed in the four areas of the Mediterranean Basin: the Balkans, the Maghreb or southern border, the Middle East and Western Europe. In all four areas, cattle and sheep are host to four of the

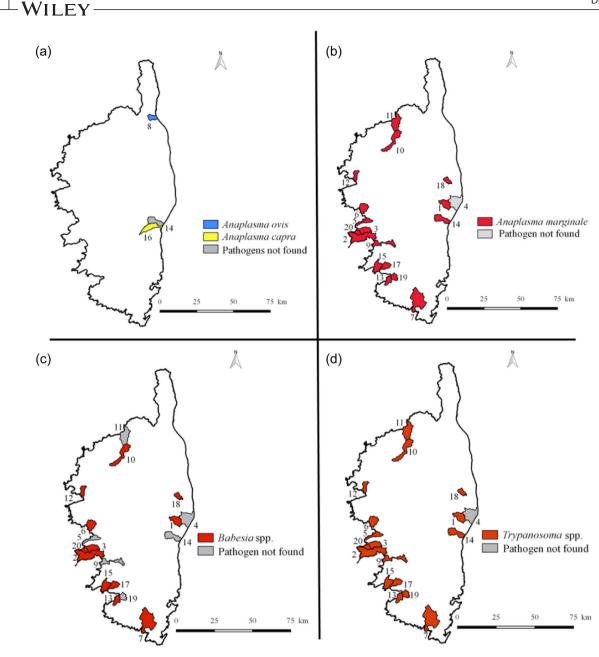


FIGURE 3 Map of Corsica showing locations of sheep blood samples positive for *Anaplasma capra* and *Anaplasma ovis* (a), and cattle blood samples positive for *Anaplasma marginale* (b), *Babesia* spp. (c) and/or *Trypanosoma* spp. (1. Aghione, 2. Ajaccio, 3. Alata, 4. Aleria, 5. Calcatoggio, 6. Coggia, 7. Figari, 8. Furiani, 9. Grosseto-Prugna, 10. Olmi-Capella, 11. Palasca, 12. Partinello, 13. Propriano, 14. Prunelli-Di-Fiumurbo, 15. Serra-Di-Ferro, 16. Serra-Di-Fiumorbo, 17. Sollocaro, 18. Tox, 19. Viggianello, 20. Villanova)

six genera: Haemaphysalis, Hyalomma, Ixodes and Rhipicephalus. Dermacentor is found in the Balkans, the Middle East and Western Europe on cattle and sheep. Finally, the last genus, *Amblyomma* has only been collected sporadically in Western Europe on cattle. In our study, four of these six genera were found on cattle: *Dermacentor*, *Hyalomma*, *Ixodes* and *Rhipicephalus* (Table S1).

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Among these genera, different species are frequently found on cattle or sheep. In the continental areas of the Balkans, the Maghreb, the Middle East and Western Europe, a total of 10 main tick species have been found: the term main species defines the species found with the highest number of tick individuals on the cattle or sheep per study. Among the genera found in our study, in the *Dermacentor* genus, we identified one species in our study, *D. marginatus*, which has also been found on cattle in Italy and Turkey, and on sheep in Greece, Italy, Spain and Turkey, and can be responsible for the circulation of pathogens such as rickettsiae from the spotted fever group (Buczek et al., 2020). The second one is the *Hyalomma* genus, in which we found two species: *Hy. marginatum* and *Hy. scupense. Hyalomma marginatum*, also reported on cattle in Algeria, Spain, France, Greece, Italy and Turkey, and on sheep from Algeria, Cyprus, Greece, Italy and Turkey, is responsible for the transmission of *Babesia* spp., *R. aeschlimannii* and the CCHF virus (Table S1). *Hyalomma scupense* has been found on cattle in Corsica and

sheep in Turkey, and it mainly transmits *Theileria* parasites, but can also transmit the CCHF virus (Gharbi & Aziz Darghouth, 2014). The third tick genus and probably the most frequently encountered tick genus on cattle and sheep was *Rhipicephalus*, with: *Rh* (*Bo*). *annulatus*, a species known to have a tropism for cattle and responsible for the transmission of *Babesia*. It has been found on cattle in Algeria, Egypt, France, Italy and Lebanon and on sheep in Lebanon and Tunisia (Table S1). *Rhipicephalus bursa*, which has a tropism for domestic ungulates and transmits pathogens/parasites from the *Anaplasma*, *Babesia* and *Theileria* genera, has been found on cattle in nearly every Mediterranean country and on sheep in Algeria, France, Greece, Italy, Lebanon and Tunisia (Table S1). *Rhipicephalus sanguineus* s.I., which targets both dogs and domestic ungulates, has been found in nearly every Mediterranean country on both cattle and sheep (Table S1).

To our knowledge, nine tick species have been found on cattle and sheep in Corsica: Amblyomma variegatum, responsible for the transmission of Rickettsia spp. and Ehrlichia ruminatum, which is an African livestock tick and has been sporadically collected on cattle in Corsica (Cicculli et al., 2019b; Yonow, 1995); D. marginatus, Hae. punctata, Hy. scupense, Hy. marginatum, I. ricinus, Rh.(Bo.) annulatus, Rh. Bursa and Rh. sanguineus s.l. were previously found in exhaustive survey (Grech-Angelini et al., 2016). However, this survey covered the entire island and all seasons. In our study, we focused on wetlands during January-February; the main tick species found belong to the Rhipicephalus genus: Rh. annulatus, Rh bursa and Rh sanguineus s.l., representing respectively 0.2%, 38.7% and 22.7% of the ticks collected. Prevalence of Rh. annulatus was same as previously found and Rh. bursa was previously described as the main tick species found on cattle in Corsica (more than 50% of the ticks collected) (Grech-Angelini et al., 2016). However, Rh. sanguineus s.l. was represented by only 2.3% of the specimens collected in this previous survey (Grech-Angelini et al., 2016). This genus was followed by the Hyalomma genus with two species: Hy. marginatum and Hy. scupense represented respectively 8.6% and 7.2% of the ticks collected on cattle, which is lower than the Hy. marginatum collected (21.5%) and nearly equal to the Hy. scupense collected in a previous study (8.7%) (Grech-Angelini et al., 2016). The last species found was D. marginatus found in a low proportion (0.7%), which is similar to the one found in the previous study (Grech-Angelini et al., 2016). The tick species found in our study are similar to those reported from an exhaustive survey carried out by Grech-Angelini et al. However, we collected no Haemaphysalis and Ixodes, which can be attributed to season and altitude. Indeed, most of the Haemaphysalis ticks found by Grech-Angelini et al. (2016) were collected in November-December between 400 and 600 m altitude and *Ixodes* ticks in September-October at above 600 m of altitude, whereas we mainly collected our ticks in January-February in wetlands close to sea level. The seasonality of some species such as Hy. scupense in January-February and the absence of others such as Hae. punctata and I. ricinus during these months - which was observed in Grech-Angelini et al. (2016) - may explain the presence of the former species and the absence of the latter two during our sampling period. In addition, specific targeting of the wetlands in our study may also explain the difference between our study and the 2016 survey.

4.2 | Detected pathogens

4.2.1 | Anaplasma sp

In our study, the most frequently detected pathogen genus in livestock animal samples was *Anaplasma* sp. in 68.6% of the cattle blood samples and 77.3% of the sheep blood samples. *Anaplasma* is common in the Mediterranean region where it has been detected in cattle and sheep and their ticks in more than 10 countries. Among the six species and two Candidatus species reported, we detected *A. ovis* and *A. capra* in sheep blood samples and *A. marginale* in cattle blood samples.

Anaplasma ovis is responsible for anaplasmosis in small ruminants which can lead to severe anaemia, lower milk production and abortion (Yasini et al., 2012). It is mainly transmitted by Rhipicephalus ticks (Cabezas-Cruz et al., 2019; Friedhoff, 1997). In the Mediterranean region, A. ovis has already been detected in Algeria, France, Italy and Turkey, in both cattle and sheep, and in sheep in Tunisia. In ticks, it has been detected in Algeria, France, Italy and Morocco (Table S1). Anaplasma ovis seems to be mainly distributed in the western part of the Mediterranean Basin and is relatively common in cattle and sheep (Table S1). On Mediterranean islands, it has been detected in Sicily in cattle and sheep, and in Corsica where it has been found in goat flocks with a prevalence of 52.0% (Cabezas-Cruz et al., 2019). In our study, the presence of A. ovis DNA was detected in Corsican sheep blood (2.7%) for the first time, but at a lower prevalence (2.7%) than reported in goats (Cabezas-Cruz et al., 2019). The second Anaplasma species detected in blood samples was A. capra, an emerging zoonotic pathogen targeting small ruminants and humans. Anaplasma capra is known to be mainly asymptomatic, but sometimes it can cause fever, mvalgia, thrombocytopenia and lymphadenopathy (Li et al., 2015; Peng et al., 2018). Actually, it was mainly reported in Asia (Amer et al., 2019; Peng et al., 2021). However, in the Mediterranean region, it has already been detected in deer in France (Jouglin et al., 2019). The detection of A. capra in sheep in our study is the first report of A. capra DNA in sheep in the Mediterranean area and in Corsica (Table S1).

The Anaplasma species reported in cattle blood samples was Anaplasma marginale. A. marginale is a bacterium responsible for bovine anaplasmosis occurring across nearly the entire continent of Europe, transmitted by Rhipicephalus ticks (Ferrolho et al., 2016; Khodadadi et al., 2021; Martins et al., 2020) and mainly targeting cattle and sheep (Atif, 2015). This bacterial species can cause symptoms such as fever, anaemia, temporal infertility and anorexia (Kocan et al., 2015). Anaplasma marginale has been detected in cattle in six Mediterranean countries: Algeria, Egypt, France, Italy, Tunisia and Turkey (Acici et al., 2016; Al-Hosary et al., 2020; Belkahia et al., 2017; Boucheikhchoukh et al., 2018; Ceci et al., 2014; Dahmani et al., 2017). In Italy, A. marginale has also been detected in sheep (Torina et al., 2007). In ticks, A. marginale has been detected in Egypt, France, Italy and Morocco (Table S1). Anaplasma marginale seems to be present in both ticks and domestic animals, especially in western Mediterranean countries. On the Mediterranean islands, Anaplasma species has only been detected on French and Italian islands. Anaplasma marginale appears to be widespread in Sicily, where it has been detected in cattle, sheep and

ticks. On other islands, this bacterial species has only been detected in Corsica in *Rh. bursa* ticks collected on cattle (Dahmani et al., 2017) (Table S1). In our study, we detected *A. marginale* DNA in cattle at a prevalence of 61.4%, which is lower than the 100% detected in a previous study in cattle (Dahmani et al., 2017). This difference could be explained by difference in the methodology as the number of farm and cattle blood samples which is higher in our study (288 blood samples and 22 farms) compare to Dahmani *et al.* which was a study on multiple animal (12 samples and one farm). Our result along with the previous discovery of *A. marginale* DNA in ticks collected on cattle indicate the high prevalence of *A. marginale* DNA in Corsican cattle (Cicculli et al., 2019a; Dahmani et al., 2017; Grech-Angelini et al., 2020).

As in the cattle and sheep samples, the Anaplasma genus was detected in ticks sampled on cattle. In the Mediterranean region, a total of six species have been detected in a total of nine countries. Among these species, the most frequently detected was A. phagocytophilum, found in six countries. It is a pathogen that mainly infects domestic ruminants and lead to symptoms such as fever, anorexia, reduced milk production. It can also be responsible for human granulocytic anaplasmosis with flu-like symptoms or more severe symptoms such as anorexia and depression (Stuen et al., 2013). On Mediterranean islands, A. phagocytophilum and A. marginale have been detected in Sicily and Corsica. Anaplasma marginale has also been detected in Sardinia. In Corsica, this species was detected in 2% of the Rh. sanguineus s.l. collected, which is lower than the prevalence found (22%); however, it has been detected at a higher level in I. ricinus ticks (Grech-Angelini et al., 2020). In our study, the positive ticks were collected in Viggianello, where A. marginale was also found in cattle blood samples.

4.2.2 | Babesia sp

The second genus found in cattle blood samples was Babesia at a prevalence of 12.2%. This genus is an intracellular protozoan parasite that belongs to the piroplasmid taxon, along with the genus Theileria. They mainly infect livestock, pets and rodents in which they can cause piroplasmosis (Beuglet & Moreau, 2015). In humans, Babesia parasites can cause moderate symptoms such as myalgia, headache, anorexia as well as severe symptoms with pulmonary oedema, organ dysfunction syndrome and coma, (Vannier et al., 2015). Their primary reservoir is often rodents, which contaminate immature tick stages and the pathogens can be transmitted to domestic ungulates during the adult tick stage (Vannier et al., 2015). The zoonotic species are principally transmitted via Ixodes ticks, vector to mainly B. divergens, B. duncani, B. microti and B. venatorum. The Babesia genus is common in the Mediterranean region where it has been detected in cattle and sheep blood samples in seven countries: Algeria, Egypt, France, Italy, Morocco, Spain and Turkey, with a predominance of B. bigemina, the parasite responsible for bovine piroplasmosis (Table S1). On Mediterranean islands, only two species have been detected in cattle: B. bigemina and B. bovis, both responsible for bovine babesiosis at a prevalence of 26.3% and 20%, respectively in Sicily. From ticks collected on cattle and sheep, this genus has been

detected in Algeria, France, Italy, Palestine and Turkey with a predominance of *B. bigemina* and *B. ovis* (Table S1). In our study, *Babesia* sp. were identified in 3% of the *Rh. sanguineus* s.l. and in 2.6% of the *Hy. marginatum* ticks collected. In Figari, *Babesia* sp. was also found in one cattle blood sample, which indicates that *Babesia* sp. is possibly present in ticks and in cattle in this municipality. Our results demonstrate the presence of *Babesia* sp. in cattle for the first time in Corsica. The presence of *B. bigemina* DNA in ticks on Corsican cattle had already been shown in *Rh. bursa* ticks (Grech-Angelini et al., 2020). The species may be *B. bigemina* or *B. bovis*, which are highly present in the Mediterranean region. However, in our study, we could only identify *Babesia* to the genus level due to unsuccessful sequencing and species determination is still needed.

4.2.3 | Rickettsia aeschlimannii

The genus Rickettsia was only found in ticks, and it was the most frequently detected pathogen genus. It was detected in 25% of the collected ticks. It is composed of many zoonotic agents with diverse pathogenicity, but most of these species can be a threat for human health with symptoms ranging from cutaneous rashes to lymphadenopathy, for example (Blanton, 2019; Davoust et al., 2010). In Corsica, even if the exact impact of Rickettsia species is still unknown, diverse species have already been found in animals (R. aeschlimannii, R. africae, R. felis, R. helvetica, R. massiliae and R. slovaca; Cicculli et al., 2019b; Cicculli et al., 2019c; Grech-Angelini et al., 2020; Matsumoto et al., 2004) and a previous sero-epidemiological study showed exposure of 4.8% of the people to these pathogens in Corse-du-Sud (Raoult et al., 1985). The rickettsiae detected belong to the spotted fever group (SFG) rickettsiae. SFG is transmitted exclusively by ticks, which play the roles of both vector and reservoir owing to the trans-stadial and transovarial transmission of these pathogens (Blanton, 2019). Out of the positive ticks, 79.3% were R. aeschlimannii and the rest could not be determined to the species level. Rickettsia aeschlimannii was first found in Hy. marginatum from Morocco. The disease in humans was documented in a man who had travelled to Morocco in 2000 (Beati et al., 1997; Raoult et al., 2002). Given its circulation in Hy. marginatum, R. aeschlimannii is distributed throughout Africa. In the Mediterranean region, R. aeschlimannii is widely distributed in ticks. It has been found in Algeria, Egypt, France, Greece, Lebanon, Spain and Turkey (Table S1). On Mediterranean islands, it has been found in Cyprus with a prevalence of 12% from Hy. marginatum collected on goats and sheep (Chochlakis et al., 2012), and on the Greek island of Cephalonia in Hy. anatolicum excavatum collected on sheep (Psaroulaki et al., 2006) (Table S1). In Corsica, R. aeschlimannii has previously been reported with a prevalence of 23% in ticks collected on cattle and 58% in ticks collected on sheep (Grech-Angelini et al., 2020). It was also detected in Hy. marginatum collected on cattle in 2019 (Cicculli et al., 2019c). In our study, we confirmed the presence of R. aeschlimanii in ticks collected from cattle at a prevalence similar to that previously found (19.8%), but in a wide diversity of tick species (Table S1). It seems that R. aeschlimannii DNA is present in ticks on cattle regardless of the sampling

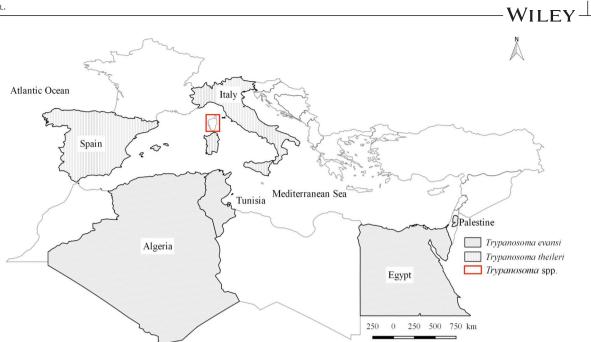


FIGURE 4 Distribution of Trypanosoma spp. in cattle in the Mediterranean region (data from the literature) and in our study (red square)

area. Nevertheless, R. aeschlimannii was not detected in cattle or sheep blood.

4.2.4 | Trypanosoma sp

Trypanosoma sp. was detected in 58.3% of the cattle blood samples. The Trypanosoma genus is mainly known as the pathogen of sleeping sickness in humans, caused by Tr. brucei, and Chagas disease, caused by Tr. cruzi, in Africa and South America, respectively. It is a ubiguitous genus, infecting a wide range of vertebrates and are mainly transmitted by biting flies, bugs and occasionally ticks (Kaufer et al., 2017). In domestic animals (cattle, horses, sheep, carnivores, etc.), these pathogens are transmitted mechanically by a wide range of biting midges. This protozoan parasite can be transmitted horizontally and vertically (Desquesnes et al., 2013). In the Mediterranean region, two species have been found: Tr. evansi responsible for 'surra' a trypanosomiasis with high morbidity and mortality rates in animals (Eregat et al., 2020). According to the infected animal host, it can be lethal (horses), cause acute disease (dogs) and variable symptoms including lethal symptoms (cattle). However, although it is morphologically similar to Tr. brucei, Tr. evansi is rarely detected in humans and is still not considered as zoonotic (Desquesnes et al., 2013). It has been found in Algeria, Egypt, Palestine and Tunisia (Figure 3). Trypanosoma theileri is a non-pathogenic haemoparasite except for cattle in which it can cause anaemia, abortion, fever and weight loss (Villa et al., 2008). It can be transmitted by tabanid flies and Hy. anatolicum anatolicum ticks (Lee et al., 2013). It has been found in Italy and in Spain (Figure 4). To our knowledge, this is the first report of Trypanosoma sp. DNA in cattle in Corsica. Unfortunately, the DNA sequence was not readable, so the sequence failed which could be due to divers factors as the quality or a degradation of the DNA extract, the presence of inhibitor or the

efficiency of the Sanger sequencing method used; therefore, parasite isolation or serological surveys are required to confirm its presence.

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A total of 44% of the cattle blood samples collected in our study were positive for DNA of more than one pathogen. Co-infection of cattle by multiple pathogens has already been widely observed in different countries, but generally involve pathogens from the same group, genus or species (variants). In our study, co-infection involved three pathogens of different genera: A. *marginale*, *Babesia* sp. and *Trypanosoma* sp. The highest prevalence of co-infection was found for *A. marginale* and *Trypanosoma* sp. which do not belong to the same groups of pathogens and are transmitted by different vectors. This finding shows the variability of exposure of Corsican cattle to different pathogens. However, the presence of *A. marginale* DNA has been confirmed by sequencing, but the specific identity of *Babesia* sp. and *Trypanosoma* sp. could not be determined in our study. Therefore, the isolation of these three pathogens in mono- and co-infected animals is still required to confirm our findings.

4.3 | Potential role of the livestock in the pathogens circulation

As these data shown, a variety of pathogens can be found in Corsican livestock. Some of these pathogens can have a direct impact in the livestock industry in Corsican wetlands as the bacteria of the Anaplasma genus reported in cattle and sheep flocks (A. marginale, A. capra and A. ovis) and the Babesia genus meanwhile other pathogens as Rickettsia aeschlimannii and the genus Trypanosoma could be a threat for human health. This show both the role of target and reservoir of the livestock in Corsican wetlands. With the detection of the genera Anaplasma and Babesia in both tick and animal shown also a potential circulation of those genera between ticks and animals in some municipalities. These different information support the importance of the veterinary activities near Corsican wetlands in order to prevent livestock to harmful pathogens, monitor the role of sentinels of the livestock to pathogens as *R. aeschlimannii* and *Trypanosoma* sp. However, in order to confirmed our results, further experimentations are require. Among them, the collect of ticks during different season will allow to have a better overview of the pathogens circulating in livestock near Corsican wetlands. In addition, our methodology was based on PCR and it was not possible to confirm the actual presence and circulation of these pathogens. Obviously, serological surveys and pathogen isolations are needed to draw conclusions regarding the actual presence and potential circulation of these pathogens in the livestock population in Corsican wetlands.

5 | CONCLUSION

In our study, we confirmed the presence of pathogen DNA in Corsican cattle, including A. marginale and in their ticks, such as R. aeschlimannii, Anaplasma sp. and Babesia sp. In addition, we detected the DNA of Babesia sp. and Trypanosoma sp. in cattle blood for the first time in Corsica with potential co-infection. The DNA of two different pathogen genera were found in both animals and ticks in the same municipalities: Babesia sp. in Figari and Anaplasma sp. in Viggianello which suggests the circulation of these pathogens in these municipalities. We also detected for the first time the presence of Anaplasma capra and A. ovis DNA in sheep flocks in Corsica. Our results are a first step in the characterisation of potential threats regarding these pathogens and farming activities near Corsican wetlands.

AUTHOR CONTRIBUTIONS

Baptiste Defaye: Conceptualisation; data curation; formal analysis; investigation; methodology; visualisation; writing – original draft. Sara Moutailler: Conceptualisation; methodology; resources; validation; writing – review & editing. Sébastien Grech-Angelini: Conceptualszation; investigation; methodology; writing – review & editing. Clémence Galon: Formal analysis; methodology. Sandrine Ferrandi: Conceptualisation; investigation; methodology; writing – review & editing. Vanina Pasqualini: Project administration; resources; supervision; validation; writing – review & editing. Yann Quilichini: Conceptualisation; methodology; project administration; resources; supervision; validation; writing – review & editing.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary material. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

ETHICAL STATEMENT

Ethical review and approval were waived for this study. The samples were collected by veterinarians during tuberculosis prevention campaigns and brucellosis monitoring and not specifically for this study. The study complies with the current French laws and followed conservation guidelines. Therefore, approval of the ethics committee was not required.

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PEER REVIEW

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