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## Molecular investigation of tick-borne pathogens in ixodid ticks infesting domestic animals (cattle and sheep) and small rodents (black rats) of Corsica, France

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1 **Molecular investigation of tick-borne pathogens in ixodid ticks infesting**  
2 **domestic animals (cattle and sheep) and small rodents (black rats) of**  
3 **Corsica, France**

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39 **ABSTRACT**

40 Although livestock farming (sheep, goats, pigs, and cattle) is an important economic activity in Corsica, a  
41 French Mediterranean island, knowledge about the tick fauna and microorganisms carried by them remains  
42 scarce. This study aimed to investigate the presence and perform molecular characterization of  
43 Anaplasmataceae, *Rickettsia* spp., and *Borrelia burgdorferi* sensu lato (sl) in tick species collected in  
44 Corsica. Ticks from cattle (*Bos taurus*), sheep (*Ovis aries*), and rodents (*Rattus rattus*) were collected from  
45 May to September 2016. DNA was purified from ticks, submitted to quantitative real-time polymerase chain  
46 reaction (qPCR) and sequenced for phylogenetic analysis. In total, 660 ticks were collected from 111 animals  
47 during the study. The most abundant collected tick species from cattle was *Rhipicephalus bursa* (n = 495;  
48 84.5%), followed by *Hyalomma marginatum* (n = 91; 15.5%). *Rhipicephalus bursa* and *Ixodes ricinus* were  
49 the only tick species collected from sheep and rodents, respectively. Overall, *Rickettsia* was the most  
50 common pathogen group (n = 48; 24%) detected in ticks. Sequence analysis of partial *gltA* and *ompA* genes  
51 revealed the presence of *Ri. aeschlimannii* and *Candidatus Ri. barbariae*. Anaplasmataceae DNA was  
52 detected in eight (6%) of the 127 cattle pools and in one (2%) of the 61 *R. bursa* specimens collected from  
53 sheep. Sequence analysis of the *rpoB* gene revealed the presence of one *Anaplasma* species, *A. marginale*.  
54 *Borrelia burgdorferi* sl DNA was detected in one pool of *H. marginatum* collected from cattle and in two  
55 (15%) of the 13 *I. ricinus* pools collected from nine black rats. To our knowledge, this is the first report of  
56 the occurrence and molecular characterization of *Candidatus Ri. barbariae*, an emerging member of the  
57 *Rickettsia* group causing spotted fever, in Corsica. The detection of *B. burgdorferi* sl DNA, which was  
58 previously believed to be rare in Corsica, confirms the presence of this agent on the island.

59 **Keywords:** Ticks, Animals, Anaplasmataceae, *Rickettsia* spp., *Borrelia burgdorferi* sl, *Candidatus Ri. barbariae* and  
60 Corsica

61

**Abbreviations:** Quantitative real-time polymerase chain reaction, qPCR; Crimean-Congo hemorrhagic fever virus, CCHFV.

## 62 Introduction

63 Ticks, ubiquitous ectoparasitic arthropods that belong to the subclass Acari, order Ixodida, are  
64 considered to be the most common vectors of disease-causing pathogens in animals (domestic and  
65 wild), and are the second most common vector of human pathogens worldwide after mosquitoes  
66 (Colwell et al., 2011; Rizzoli et al., 2014). Arthropod-borne microorganisms, such as Toscana virus  
67 in dogs (Dahmani et al., 2016) and West Nile virus in domestic animals have been reported in  
68 Corsica (Maquart et al., 2017). Corsica, a French Mediterranean island, is characterized by a mild  
69 Mediterranean climate and a high variability of microclimates because of its specific geographical  
70 situation (Grech-Angelini et al., 2016). Corsican livestock farming (sheep, goats, pigs, and cattle) is  
71 extensive, so important interactions between livestock, wildlife, and human populations could favor  
72 the circulation of tick-borne diseases. A systematic survey of tick fauna on Corsican livestock  
73 reported the dominance of the typical Mediterranean species *Rhipicephalus bursa* and *Hyalomma*  
74 *marginatum* (Grech-Angelini et al., 2016). *Hyalomma marginatum* is one of the main vectors of the  
75 zoonotic Crimean–Congo hemorrhagic fever virus (CCHFV) in the Mediterranean Basin and the  
76 Afrotropical region (Estrada-Pena et al., 2012). *Rhipicephalus bursa* is recognized as a primary  
77 vector of *Babesia ovis* (Moltmann et al., 1982), but it transmits other pathogens such as *Rickettsia*  
78 spp. and *Anaplasma* spp. (Dahmani et al., 2017; Ruele et al., 2015). An epidemiological survey on  
79 Anaplasmataceae species infecting domestic animals and ticks in Corsica described the presence of  
80 six species belonging to the genus *Anaplasma* from blood samples taken from ruminants and from  
81 ticks infecting cattle (Dahmani et al., 2017). *Ehrlichia canis* was detected once in Corsica in a non-  
82 engorged *R. bursa* tick collected from a cow (Dahmani et al., 2017). *Anaplasma* spp. and *Ehrlichia*  
83 spp. are transmitted by ticks, and both genera contain obligate intracellular Gram-negative bacterial  
84 parasites. In the vertebrate host, these bacteria infect hematopoietic cells. There are four pathogens

85 of ruminants in the genus *Anaplasma*: *A. marginale*, *A. centrale*, *A. bovis*, and *A. ovis*; in addition,  
86 there is *A. phagocytophilum*, which infects a variety of hosts, including humans and other animals,  
87 and *A. platys*, which infects dogs (Ndip et al., 2010). *Rickettsia aeschlimannii* was detected and  
88 isolated in *H. marginatum* in Corsica (Matsumoto et al., 2004).

89 This study aimed to investigate the presence of Anaplasmataceae, *Rickettsia* spp. and *B.*  
90 *burgdorferi* sensu lato (sl) in tick species collected from cattle, sheep, and black rats in Corsica, and  
91 to characterize them using molecular approaches.

## 92 **Materials and methods**

### 93 *Tick collection and morphological identification*

94 Ticks from cattle (*Bos taurus*), sheep (*Ovis aries*), and rodents (*Rattus rattus*) were collected  
95 from May to September 2016 in northern Corsica, France (Figure 1). Ticks were collected from  
96 June to July 2016 in the Ponte-Leccia slaughterhouse in Haute Corse. During each visit, the whole  
97 skin of slaughtered animals was inspected, and ticks were collected manually. The national cattle  
98 identification system, which uses ear tags, allowed the origin of animals to be tracked and identified  
99 three farm owners. Cattle sampled in the Ponte-Leccia slaughterhouse come from Haute Corse  
100 municipalities. Ticks were also collected from sheep (n = 60) belonging to one farm in Corte on  
101 May 2016 and from rodents (n = 9) trapped on September 2016 in Lozari, Urbino, and Terrenzana  
102 (Figure 1). Ticks were identified at species level using a pictorial guide (Estrada-Pena et al., 2004).  
103 *Ixodes* ticks were checked for being *I. inopinatus* (Estrada-Pena et al., 2014).

104 *DNA extraction*

105 Ticks were washed once in 70% ethanol for 5 min and then twice in distilled water for 5 min.  
106 Those collected from sheep and black rats were analyzed individually, whereas those collected from  
107 cattle were analyzed after monospecific pools consisting of 2–6 ticks were collected from the same  
108 animal.

109 Individual ticks or pools of ticks were crushed using the TissueLyser II (Qiagen, Hilden,  
110 Germany) in phosphate-buffered saline at 5500 rpm for 20 s. DNA extraction was performed on a  
111 QIAcube HT (Qiagen) using a QIAamp cadof Pathogen Mini kit according to the manufacturer's  
112 instructions. DNA was eluted in 150 µl of buffer and stored at –20 °C. For each PCR reaction, the  
113 template DNA had a final concentration <200 ng.

114 *Molecular analysis of ticks and bacteria*

115 Morphological identification of ticks was confirmed by polymerase chain reaction (PCR)  
116 amplification and sequencing mitochondrial 16S rDNA (Table 1) (Black and Piesman, 1994). At  
117 least two specimens from each species were randomly selected and subjected to molecular  
118 identification. The PCR assays for Anaplasmataceae (Dahmani et al., 2017), *Rickettsia* spp.  
119 (Labruna et al., 2004), *B. burgdorferi* s.l (Courtney et al., 2004) and *B. miyamotoi* (Diaz et al., 2012)  
120 were performed using the primers and probes listed in Table 1. Reactions were performed on a 96-  
121 well Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System using QuantiFast Pathogen +  
122 Internal Control Kits (Qiagen). Internal and negative controls were included in each run. Each run  
123 was repeated three times. Samples that were positive for Anaplasmataceae were tested by  
124 conventional PCR using *Anaplasma* genus-specific primers targeting the 525-bp fragment of the  
125 RNA polymerase subunit beta (*rpoB*) gene (Dahmani et al., 2017) (Table 1). Positive samples for

126 *Rickettsia* spp. were analyzed using primers that amplified the 850-bp fragment of the *gltA* gene  
127 encoding citrate synthase (Table 1) (Mediannikov et al., 2004; Roux et al., 1997) and primers to  
128 amplify the 532-bp fragment of the 190-kDa outer membrane protein (*ompA*) gene (Regnery et al.,  
129 1991). The reactions were carried out using Applied Biosystems GeneAmp PCR System 9700  
130 (Courtabouef, France). Negative and positive controls were included. The PCR products were  
131 visualized in 2% agarose gels in Tris-Acetate-EDTA (TAE Buffer) and were visualized under  
132 ultraviolet light after staining with ethidium bromide. A 100-bp DNA ladder was used as a standard  
133 marker.

134 The pathogens detected in pools were expressed as the percentage and minimum infection rate  
135 based on the assumption that each PCR-positive pool contained at least one positive tick (Sosa-  
136 Gutierrez et al., 2016). Detection rate of DNA bacteria were compared by using Fisher exact test  
137 ( $p < 0.05$ ). The analysis was conducted using the R statistical platform (version 3.1.2) (R, 2015).

#### 138 *Sequencing and phylogenetic analysis*

139 A selected number of positive samples for *Rickettsia* and Anaplasmatidae were purified and  
140 directly sequenced using an Applied Biosystems model 3730XL (Fisher Scientific S. A. S., Illkirch-  
141 Graffenstaden, France). Sequences of ticks, Anaplasmatidae, or *Rickettsia* spp. were aligned using  
142 MEGA X (Kumar et al., 2018). All sequences were assembled and compared with similar  
143 sequences retrieved from the GenBank nucleotide database using BLASTn (Altschul et al., 1997).  
144 Phylogenetic analyses were inferred using the maximum-likelihood method implemented on Mega  
145 X.

146

## 147 **Results**

### 148 *Tick identification*

149 In total, 660 ticks were collected during the study (Table 2). Forty-two cattle were inspected  
150 in the Ponte-Leccia slaughterhouse from June to July 2016 and 586 ticks were collected. Among the  
151 42 infested cows, 57% were infested with less than 10 ticks, 28% with 10–30 ticks, and 15% with  
152 more of 30 ticks. Two tick species were identified morphologically on cattle (Table 2). The most  
153 abundant species was *R. bursa* (n = 495; 84.5%), followed by *H. marginatum* (n = 91; 15.5%).  
154 Among the 586 ticks collected from cattle, 541 (94.1%) were adults (38.6% females and 55.5%  
155 males) and 34 (5.9%) were nymphs. Sex and life stage were not determined for 11 ticks. Sixty-one  
156 ticks were collected from 60 sheep in one farm on May 2016 (Corte, Haute-Corse) (Table 2). All  
157 these were *R. bursa*; 51 (83.6%) were adults (33% males and 51% females). Thirteen *I. ricinus* ticks  
158 (all adult females) were collected from nine rodents (black rats) captured in Lozari, Urbino, and  
159 Terrenzana (Haute-Corse) in September 2016. Among them, six were infested by one tick, two by  
160 two ticks, and one by three ticks. The sequences of mitochondrial 16S rDNA fragments of 16 ticks  
161 selected in this study after blast analysis were confirmed to be *R. bursa* (n = 7), *H. marginatum* (n =  
162 7), and *I. ricinus* (n = 2) (GenBank accession numbers MH663984-90 for *R. bursa*, MH663977-83  
163 for *H. marginatum* and MH663991-92 for *I. ricinus*). The seven 16S rDNA sequences of *H*  
164 *marginatum* had 98–100% identity with each other and 99–100% identity with *H. marginatum* from  
165 Sardinia (KT931964), Israel (KT391060), Turkey (KR870973), and Algeria (KP776645) (Figure 2).

166 The seven (MH663984-90) 16S rDNA sequences of *R. bursa* were identical to each other and  
167 showed 100% identity with *R. bursa* from the Iberian Peninsula (AJ002956), Italy (KJ814007-  
168 KJ814010), and Turkey (KR870983.1-KU664350.1) (Figure 2). The two (MH663991-92) 16S



169 rDNA sequences of *I. ricinus* were identical to each other and showed 100% identity with *I. ricinus*  
170 from Germany (JF928526), Italy (KF197116), Sweden (KX384811), Belgium (KJ414457), and  
171 Turkey (KR870982). They had 98% identity with 16S rDNA sequences from Morocco (GU074602)  
172 and France (GU074612) (Figure 2). Seventy-four individual ticks (61 collected from sheep and 13  
173 from rodents) and 127 pools prepared with cattle ticks (100 pools of *R. bursa* and 27 pools of *H.*  
174 *marginatum* in cattle) were analyzed (Tables 2 and 3).

#### 175 *Detection of Rickettsia spp.*

176 In ticks collected from cattle, *Rickettsia* spp. DNA was detected in 37 pools (29%), with the  
177 highest detection rate in *H. marginatum* (59%) compared with *R. bursa* (21%) ( $p = 0.00$ ; Table 3).  
178 Similar *Rickettsia* spp. detection rates were observed in *R. bursa* collected from cattle and sheep  
179 (21% and 18%, respectively;  $p = 0.68$ ). The 13 *I. ricinus* specimens collected from rodents were all  
180 negative for *Rickettsia* spp. Nine pools of ticks (three *R. bursa* and six *H. marginatum*) and seven  
181 individual ticks (*R. bursa*) collected from cattle and sheep, respectively, were screened for the  
182 presence of the *gltA* gene (GenBank accession numbers MH675633-MH675648). Sequence  
183 analysis of the *gltA* sequences revealed the presence of two *Rickettsia* species: *Ri. aeschlimannii*  
184 and *Candidatus Ri. barbariae*. The seven *gltA* sequences derived from five pools of *H. marginatum*  
185 and two pools of *R. bursa* collected from cattle were 100% identical to each other and clustered  
186 together with *Ri. aeschlimannii* sequences (KU961540, DQ235776, and HM50282) (Figure 3).  
187 Nine *gltA* sequences derived from eight *R. bursa* specimens collected from sheep and one pool of  
188 *H. marginatum* ticks collected from cattle showed 99–100% identity with each other and 99%  
189 identity with *Candidatus Ri. barbariae* collected in Sardinia (EU272185) (Figure 3). The analyses of  
190 the *ompA* gene (GenBank accession numbers MH797764-MH79777) confirmed these results (99%

191 of identity with *Candidatus* Ri. barbariae with KY233248-49 from Lebanon).

## 192 *Detection of Anaplasmataceae*

193 Anaplasmataceae DNA was detected in eight (6%) of the 127 tick cattle pools. Among them,  
194 DNA was detected in five (18%) of the 27 *H. marginatum* pools and in three (3%) of the 100 *R.*  
195 *bursa* pools (Table 3). Anaplamataceae DNA was detected in one (2%) of the 61 *R. bursa* ticks  
196 collected from sheep and analyzed individually. Anaplamataceae DNA was not detected in *I.*  
197 *ricinus*. Sequence analysis of the *rpoB* gene sequencing of three pools (one *R. bursa* and two *H.*  
198 *marginatum*) revealed the presence of one *Anaplasma* species, *A. marginale* (GenBank accession  
199 numbers MH651041-MH651043). The three sequences of *A. marginale* had 99–100% identity with  
200 each other and showed 99–100% identity with an *A. marginale* strain reported in Corsica  
201 (KY498343-KY498345) (Figure 4).

## 202 *Detection of B. burgdorferi* (sl)

203 *Borrelia burgdorferi* sl DNA was detected in two (15%) out of the 13 *I. ricinus* ticks collected  
204 from nine rodents and in *H. marginatum* one (4%) cattle pool. *B. miyamotoi* DNA was not detected  
205 in any of the 13 *I. ricinus* analyzed.

## 206 **Discussion**

207 Here we report the detection rate of *Rickettsia* spp., Anaplasmataceae, and *B. burgdorferi* sl in  
208 ticks collected from cattle, sheep, and black rats in Corsica, France. Although *R. bursa* was the  
209 most-represented tick species, *H. marginatum* ticks showed the highest detection rate for *Rickettsia*  
210 spp. and Anaplasmataceae. *Rickettsia* spp. DNA was present in almost 20% of the ticks collected

211 from sheep and cattle examined. To our knowledge, we report here for the first time the detection of  
212 *Candidatus* *Ri. barbariae* and *B. burgdorferi* sI DNA in ticks collected from ruminants and black  
213 rats in Corsica, respectively.

214 *Rhipicephalus bursa* was the most abundant tick species collected from cattle (>80%) and the  
215 only tick species collected from small ruminants. This was in line with previous findings from  
216 Corsica (Dahmani et al., 2017; Grech-Angelini et al., 2016). In the Mediterranean basin, this species  
217 is considered as the major ectoparasite of sheep (Yeruham et al., 2000). The majority of *R. bursa*  
218 specimens collected in this study were adults, as they were collected during the summer period  
219 (Ferrolho et al., 2016). Majority of *R. bursa* ticks analyzed were positive for *Rickettsia* spp., with a  
220 similar detection rate between cattle and sheep. Sequence analysis revealed the detection of *Ri.*  
221 *aeschlimannii* and *Candidatus* *Ri. barbariae* in *R. bursa*. *Rickettsia* spp., such as *Ri. aeschlimannii*  
222 and *Ri. massiliae*, had previously been detected in *R. bursa* in several Mediterranean countries in a  
223 wide range of animal hosts (Parola et al., 2013); however, to the best of our knowledge, this is the  
224 first detection report of *Candidatus* *Ri. barbariae* in *R. bursa* ticks collected from domestic animals  
225 in Corsica. These species were described previously in several tick species in Portugal (*R. bursa*),  
226 Cyprus (*R. turanicus*), Sardinia (*R. turanicus*), and China in the flea (*Vermipsylla alakurt*) (Zhao et  
227 al., 2016). Here, 3% of *R. bursa* tick cattle pools were positive for Anaplasmataceae DNA. We  
228 found a detection rate of Anaplasmataceae DNA of 2% among the 61 *R. bursa* collected from sheep,  
229 which is similar to the detection rate reported in other Mediterranean regions (Satta et al., 2011;  
230 Torina et al., 2010). We also detected the DNA of *A. marginale*. This is consistent with a previous  
231 study reporting that the DNA of *A. marginale* was detected in two engorged female ticks removed  
232 from cattle (2%) and from all (12/12) cattle blood samples in Corsica (Dahmani et al., 2017).

233 *Hyalomma marginatum* was the second most abundant tick species collected from cattle  
234 (15.5%), as described previously (Grech-Angelini et al., 2016). *Hyalomma marginatum*, which is

235 the vector of CCHFV, is present in southeastern continental France (Vial et al., 2016), but also in  
236 Mediterranean islands such as Sicily (Italy) and Minorca (Spain) (Castella et al., 2001). More than  
237 50% of *H. marginatum* pools were positive for DNA of *Ri. aeschlimannii* and *Candidatus Ri.*  
238 *barbariae*. Such a high infection rate is in agreement with *Ri. aeschlimannii* infection rate of  
239 *Hyalomma* (>70%) previously reported in Corsica (Matsumoto et al., 2004) and Croatia (64%)  
240 (Punda-Polic et al., 2002). Lower rates of *Rickettsia* spp. were observed in Sicily (4%) (Torina et  
241 al., 2010) and Turkey (6.5%) (Keskin et al., 2016). These differences in results could be related to  
242 differences in environmental factors, animal species, the numbers of ticks collected and the analysis  
243 methods. In our study, the detection of *Candidatus Ri. barbariae* in a pool of *H. marginatum*, not a  
244 recognized vector for this *Rickettsia* species (Parola et al., 2013), could also be explained by the  
245 presence of this bacterium in the blood meal of the ticks.

246 *Borrelia burgdorferi* s.l. is the causative agent of Lyme borreliosis, which is the most prevalent  
247 tick-borne disease in Europe and North America (Raileanu et al., 2017). The French *Sentinelles*  
248 General Practitioner Network observed that the incidence of Lyme borreliosis is much lower in  
249 Corsica (incidence rate reported, 20–50 cases per 100,000 inhabitants) compared with endemic  
250 regions of France (>150 per 100,000 inhabitants) (Vandenesch et al., 2014). Here we report for the  
251 first time the detection of *B. burgdorferi* s.l. DNA in two *I. ricinus* ticks collected from rodents  
252 (15%). The detection rate of *B. burgdorferi* s.l. reported here was similar to that reported for *I.*  
253 *ricinus* collected from rodents in Italy (14.7%) (Pascucci et al., 2015) and was in the range of  
254 previous studies from France, Ireland, and Austria, which found a detection rate of *B. burgdorferi* s.l.  
255 in small mammal species ranging from 2.3% to 24% (Gray et al., 1999; Khanakah et al., 2006;  
256 Marsot et al., 2011). Although cattle are not a reservoir host, we detected *B. burgdorferi* s.l. DNA in  
257 one *H. marginatum* cattle pool. This detection could be explained by the presence of reservoir hosts  
258 of *B. burgdorferi* in the same location (Gern, 2009).

259           The limitations of our study were that first, the ticks were collected mostly in a municipal  
260 slaughterhouse in northern Corsica. We have no data about the southern part of the island. Second,  
261 ticks were analyzed mostly in pooled samples and not individually. The screening of pooled  
262 samples is complicated because it is impossible to determine whether a positive result is caused by  
263 one or more infected ticks. Third, the ticks investigated here were removed from hosts. The  
264 presence of bacterial DNA in an engorged tick could be caused by its presence in the blood meal.

265           These results contribute to the knowledge of tick-borne disease in Corsica and provide a  
266 useful contribution to understanding the epidemiology. Further research should be carried out to  
267 investigate the eco-epidemiological cycle of the pathogenic agents detected here.

## 268   **Declarations**

## 269   **Acknowledgements**

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272   Not applicable.

## 273   **Competing interests**

274   The authors declare that they have no competing interests.

275 **Ethics approval and consent to participate**

276 The inspected cattle were slaughtered for human consumption. Living sheep were examined with  
277 the assistance of their owner.

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Species	Target	Name	Sequence	Annealing temperature (°C)	References
<b>qPCR</b>					413 414 415
<i>B. burgdorferi</i> sl	23S RNA	<i>Bb23Sf</i>	CGAGTCTTAAAAGGGCGATTTAGT	60	Courtney et al., 2004
		<i>Bb23Sfr</i>	GCTTCAGCCTGGCCATAAATAG		
		<i>Bb23Sp</i>	AGATGTGGTAGACCCGAAGCCGAGTG		
<i>B. miyamotoi</i>	<i>glpQ</i>	<i>Bmi-F</i>	CACGACCCAGAAATTGACACA	60	Diaz et al., 2012
		<i>Bmi-R</i>	GTGTGAAGTCAGTGGCGTAAT		
		<i>Bmi-P</i>	TCGTCCGTTTTCTCTAGCTCGATTGGG		
<i>Rickettsia</i> spp.	<i>gltA</i>	<i>RspP-F</i>	GAGAGAAAATTATATCCAAATGTTGAT	60	Labruna et al., 2004
		<i>RspP-R</i>	AGGGTCTTCGTGCATTTCTT		
		<i>RspP-P</i>	CATTGTGCCATCCAGCCTACGGT		
Anaplasmataceae	23S rRNA	<i>Tt-Ana-F</i>	TGACAGCGTACCTTTTGCAT	60	Dahmaniet al., 2017
		<i>Tt-Ana-R</i>	GTAACAGGTCGGTCCTCCA		
		<i>Tt-Ana-P</i>	GGATTAGACCCGAAACCAAG		
<b>Conventional PCR (sequencing)</b>					
<i>Rickettsia</i> spp.	<i>gltA</i>	<i>CS2D</i>	ATGACCAATGAAAATAATAAT	54	Rouxet al., 1997, Mediannikov et al., 2004
		<i>CSEndR</i>	CTTATACTCTCTATGTACA		
		<i>409D</i>	CCTATGGCTATTATGCTTGC	54	
		<i>1258R</i>	ATTGCAAAAAGTACAGTGAACA		
	<i>ompA</i>	<i>Rr190.70p</i>	ATGGCGAATATTTCTCCAAAA	48	Regnery et al., 1991
		<i>Rr190.602n</i>	AGTGCAGCATTCGCTCCCCCT		
<i>Anaplasma</i> spp.	<i>rpoB</i>	<i>Ana-rpoBF</i>	GCTGTCCTAGGCTYTCTTACGCGA	55	Dahmaniet al., 2017
		<i>Ana-rpoBR</i>	AATCRAGCCAVGAGCCCCTRTAWGG		
Ticks	16S rDNA	<i>16S+1</i>	CTGCTCAATGATTTTTTAAATTGCTGTGG	48 and 54	Black and Piesman, 1994
		<i>16S-1</i>	CCGGTCTGAACTCAGATCAAGT		

416 **Table 2.** Total tick species collected from hosts sampled.

Number of ticks per pool (n)	Number of pools with n ticks <i>H. marginatum</i>	Number of pools with n ticks <i>R. bursa</i>	Positive <i>Rickettsia</i> spp. n (%)	Positive Anaplasmataceae n (%)	Positive <i>Borrelia burgdorferi</i> (sl) n (%)
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Host (n)	Tick species	n ticks (%)	Male n (%)	Female n (%)	Nymph n (%)
Cattle (n = 42)	<i>R. bursa</i>	495 (84.5)	251 (51.1)	207 (42.2)	33 (6.7)
	<i>H. marginatum</i>	91 (15.5)	68 (81.0)	15 (18.0)	1 (1.0)
	Total*	586	319 (55.5)	222 (38.6)	34 (5.9)
Sheep (n = 60)	<i>R. bursa</i>	61 (100)	20 (33.0)	31 (51.0)	10 (16.0)
Rodents (n = 9)	<i>I. ricinus</i>	13 (100)	0 (0)	13 (100)	0 (0)
Total (n = 111)	Total tick species	660	339 (52.2)	266 (41.0)	34 (6.8)

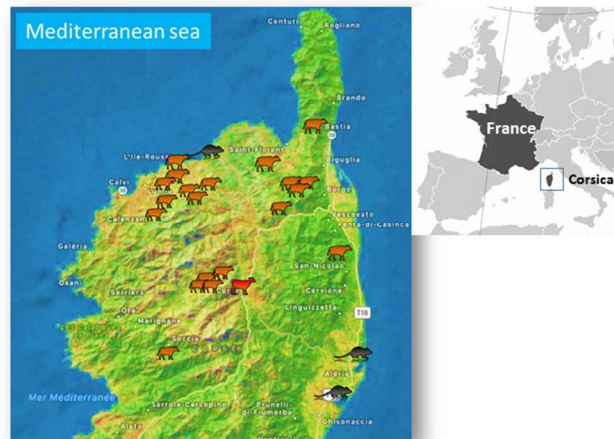
417 \*Sex and life were not determined for 11 ticks

418 **Tableau 3.** Infection rate of bacteria in ticks collected from cattle.

**Abbreviations:** Quantitative real-time polymerase chain reaction, qPCR; Crimean-Congo hemorrhagic fever virus, CCHFV.

			<i>H. marginatum</i>	<i>R.bursa</i>	Total	<i>H. marginatum</i>	<i>R. bursa</i>	Total	<i>H. marginatum</i>	<i>R. bursa</i>	Total
1	8	1	2 (25)	0 (0)	2 (22)	1(12)	0 (0)	1 (11)	0(0)	0 (0)	0 (0)
2	5	7	4 (80)	1 (14)	5 (42)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	1 (8)
3	2	9	1 (50)	1 (11)	2 (18)	2 (100)	0 (0)	2 (18)	0 (0)	0 (0)	0 (0)
4	3	15	2 (66)	3 (20)	5(28)	0 (0)	1 (33)	1 (5)	0 (0)	0 (0)	0 (0)
5	3	12	2 (66)	2 (17)	4 (27)	1 (33)	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)
6	6	16	5 (83)	14 (87)	19 (86)	1 (17)	2 (12)	3 (14)	0 (0)	0 (0)	0 (0)
Total pools	27	100	16 (59)	21 (21)	37 (29)	5 (18)	3 (3)	8 (6)	1 (4)	0 (0)	1 (1)

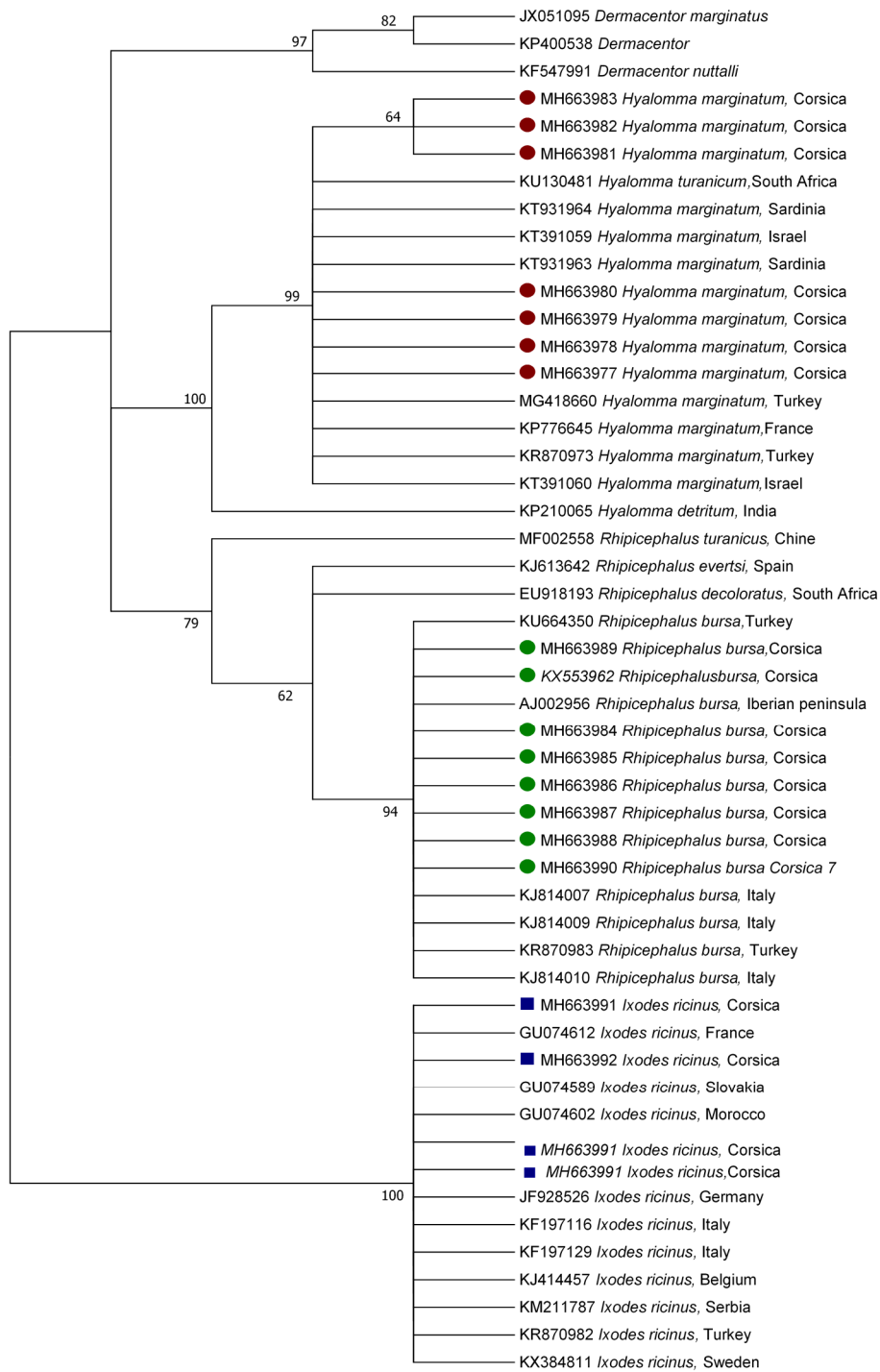
**Abbreviations:** Quantitative real-time polymerase chain reaction, qPCR; Crimean-Congo hemorrhagic fever virus, CCHFV.



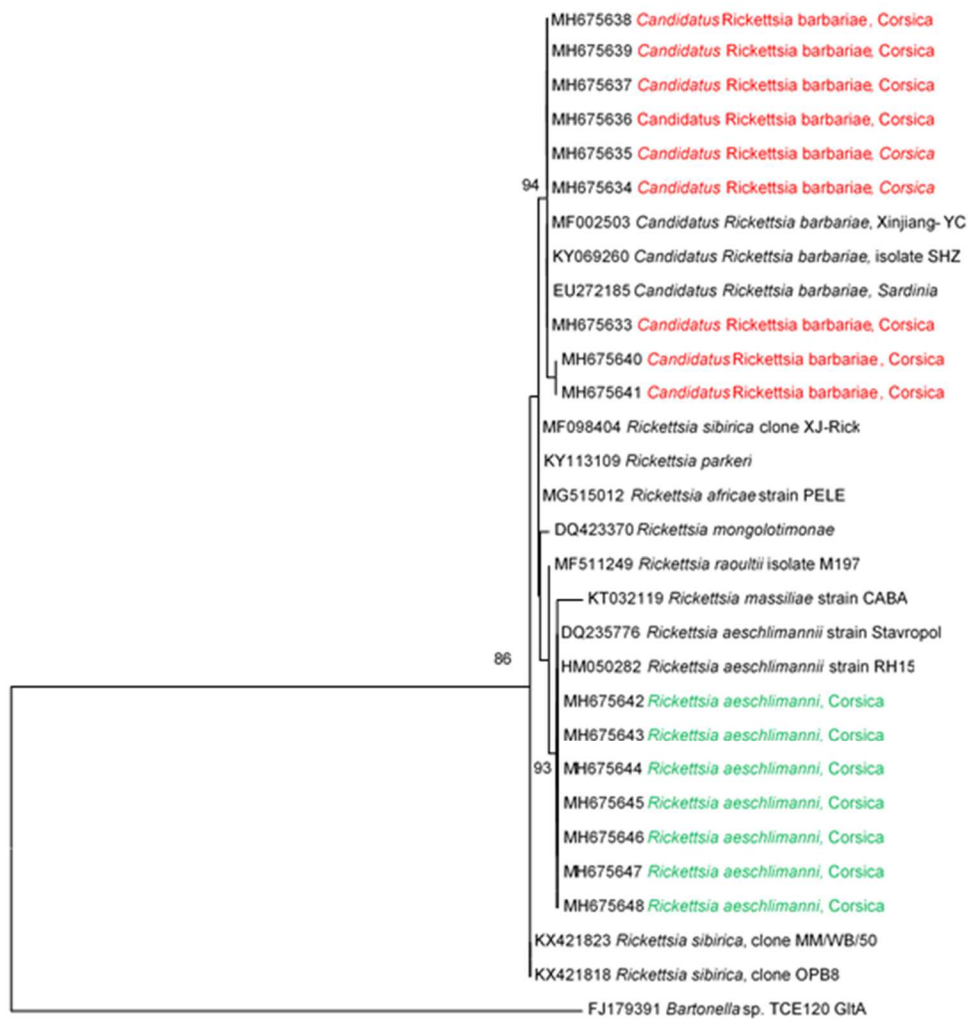
**Figure 1:** Map of Corsica showing the study area where the animals lived and the GPS coordinates.

**Figure 1 legend**

For cattle: Occhiatana (42° 34' 30" N, 9° 00' 33" E), Pighjolu (42° 10' 40" N, 8° 54' 37" E), Calacuccia (42° 20' 12"N, 9° 01' 05" E), Casamaccioli (42° 19' 06"N, 9° 00' 07" E), Olmi-Capella (42° 31' 38" N, 9° 01' 09" E), Lozzi (42° 20' 45"N, 9° 00' 14" E), Speluncatu (42° 33' 46" N, 8° 58' 54" E), Muratu (42° 34' 41" N, 9° 19' 36" E), Soriu (42° 35' 02"N, 9° 16' 28" E) Lentu (42° 31' 22"N, 9° 16' 57" E), Albertacce (42° 19' 41" N, 8° 59' 04" E), Zilia (42° 31' 52"N, 8° 54' 06" E), Barbaghju (42° 41' 26"N, 9° 22' 42" E), Calinzana (42° 30' 31" N, 8° 51' 21" E), Valone-Orneto (42° 24' 06" N, 9° 28' 18" E), Pieve (42° 34' 51"N, 9° 17' 18" E), Santu Petru di Tenda (42° 36' 22" N, 9° 15' 30" E), Lavatoghju (42° 34' 29" N, 8° 52' 42" E), Lisula (42° 38' 08" N, 8° 56' 17" E) and Santa Reperata Di Balagna (42° 36' 16"N, 8° 55' 45" E). For sheep: Corti (42° 20' 16"N, 9° 15' 45" E) and for rodents: Lozari (42°38'28N, 9°00'56E), Urbinu (42° 02' 53" N, 9° 28' 22" E), and Terrenzana (42° 9' 28"N, 9° 32' 58"E).



**Figure 2.** Phylogenetic tree showing the position of *Rhipicephalus bursa* (green circles), *Hyalomma marginatum* (red circles) and *Ixodes ricinus* (blue squares) compared to other tick species based on 16S rDNA sequences. The evolutionary history was inferred by using the Maximum Likelihood method. The analysis involved 50 nucleotide sequences. There were a total of 291 positions in the final dataset.



**Figure 3.** Phylogenetic analysis of *Rickettsia* spp. identified in Corsica. The evolutionary history was inferred by using the Maximum Likelihood method based on *Rickettsia gltA* sequences. The analysis involved 30 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 331 positions in the final dataset.

