Version of Record: https://www.sciencedirect.com/science/article/pii/S1877959X17304685
Manuscript_09fea66e5fc78fa3c5f9c0c275e6fff9

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

Cicculli V[1], Capai L[1], Quilichini Y[2], Masse S[1], Fernández-AlvarezA[2], Minodier L[1], Bompard P[3,4], Charrel R[5], Falchi A[1*]

1. EA7310, Laboratoire de Virologie, Université de Corse-Inserm, Corte, France.
2. CNRS - Università di Corsica, UMR 6134 - SPE, Corte, France.
3. Institut Pierre Louis d'Epidémiologie et de Santé Publique, Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, 56, Boulevard Vincent Auriol, 81393-75646, Paris, France.
4. INSERM, UMR_S 1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique, 56, Boulevard Vincent Auriol, 81393-75646, Paris,
5. UMR "Emergence des Pathologies Virales" (EPV: Aix-Marseille Univ – IRD 190 – Inserm 1207 – EHESP – IHU Méditerranée Infection), Marseille, France

Cicculi V : EA7310, Laboratoire de Virologie, Université de Corse-Inserm, France, vincent.cicculli2b@gmail.com

Capai L : EA7310, Laboratoire de Virologie, Université de Corse-Inserm, France, capai@univ-corse.fr

Quilichini Y: CNRS - Università di Corsica, UMR 6134 - SPE, Corte, France, quilichini@univ-corse.fr

Masse S : EA7310, Laboratoire de Virologie, Université de Corse-Inserm, France, masse_s@univ-corse.fr

Fernandez-Alvarez A: CNRS - Università di Corsica, UMR 6134 - SPE, Corte, France, angela.fdez.alvarez@gmail.com

Minodier L : EA7310, Laboratoire de Virologie, Université de Corse-Inserm, France, minodier@univ-corse.fr

Bompard P : Institut Pierre Louis d'Epidémiologie et de Santé Publique, Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, 56, Boulevard Vincent Auriol, 81393-75646, Paris, France. INSERM, UMR_S 1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique, 56, Boulevard Vincent Auriol, 81393-75646, Paris, France. priscillia.bompard@iplesp.upmc.fr

Charrel R: UMR "Emergence des Pathologies Virales" (EPV: Aix-Marseille Univ – IRD 190 – Inserm 1207 – EHESP – IHU Méditerranée Infection), Marseille, France

Falchi A : EA7310, Laboratoire de Virologie, Université de Corse-Inserm, France, falchi@univ-corse.fr

***Corresponding Author/Corresponding Author for reprints**

Alessandra Falchi EA7310, Laboratory of Virology, University of Corsica -Inserm

20250 Corte, France Tel: + 33 495 45 06 77 Fax: + 33 4 20 20 23 92 E-mail: falchi@univ-corse.fr

(A. Falchi)

© 2019 published by Elsevier. This manuscript is made available under the CC BY NC user license
https://creativecommons.org/licenses/by-nc/4.0/

ABSTRACT

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

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

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

Introduction

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

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

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).

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

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 cador 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* sl (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

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

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 Anaplasmataceae were purified and
140 directly sequenced using an Applied Biosystems model 3730XL (Fisher Scientific S. A. S., Illkirch-
141 Graffenstaden, France). Sequences of ticks, Anaplamataceae, 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

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

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

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

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%

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

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

Detection of Anaplasmataceae

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

Detection of B. burgdorferi (sl*)*

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

Discussion

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

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

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* sl 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 Anaplamataceae 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

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

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* sl 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* sl DNA in two *I. ricinus* ticks collected from rodents
252 (15%). The detection rate of *B. burgdorferi* sl 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* sl
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* sl 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).

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

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

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

Declarations

Acknowledgements

We are grateful to the staff of the slaughterhouse of Ponte-Leccia for their help in collecting ticks.

Funding

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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

Ethics approval and consent to participate

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

References

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389-3402.

Black, W. C. t., Piesman, J. 1994. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. Proc Natl Acad Sci U S A. 91, 10034-10038.

Castella, J., Estrada-Pena, A., Almeria, S., Ferrer, D., Gutierrez, J., Ortuno, A. 2001. A survey of ticks (Acari: Ixodidae) on dairy cattle on the island of Menorca in Spain. Exp Appl Acarol. 25, 899-908.

Colwell, D. D., Dantas-Torres, F., Otranto, D. 2011. Vector-borne parasitic zoonoses: emerging scenarios and new perspectives. Vet Parasitol. 182, 14-21. doi:10.1016/j.vetpar.2011.07.012 S0304-4017(11)00481-X [pii]

Courtney, J. W., Kostelnik, L. M., Zeidner, N. S., Massung, R. F. 2004. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. J Clin Microbiol. 42, 3164-3168. doi:10.1128/JCM.42.7.3164-3168.2004

Dahmani, M., Alwassouf, S., Grech-Angelini, S., Marie, J. L., Davoust, B., Charrel, R. N. 2016. Seroprevalence of Toscana virus in dogs from Corsica, France. Parasit Vectors. 9, 381. doi:10.1186/s13071-016-1665-4

Dahmani, M., Davoust, B., Tahir, D., Raoult, D., Fenollar, F., Mediannikov, O. 2017. Molecular investigation and phylogeny of Anaplasmataceae species infecting domestic animals and ticks in Corsica, France. Parasit Vectors. 10, 302. doi:10.1186/s13071-017-2233-2 10.1186/s13071-017-2233-2 [pii]

Diaz, M. H., Bai, Y., Malania, L., Winchell, J. M., Kosoy, M. Y. 2012. Development of a novel genus-specific real-time PCR assay for detection and differentiation of Bartonella species and genotypes. J Clin Microbiol. 50, 1645-1649. doi:10.1128/JCM.06621-11

Estrada-Pena, A., Bouattour, A., Camicas, J. L., Walker, A. R. (2004). Ticks of veterinary and medical importance: the Mediterranean basin. A guide of identification of species. (Vol. University of Zaragoza Press, Zaragoz): University of Zaragoza Press, Zaragoza.

Estrada-Pena, A., Jameson, L., Medlock, J., Vatansever, Z., Tishkova, F. 2012. Unraveling the ecological complexities of tick-associated Crimean-Congo hemorrhagic fever virus transmission: a gap analysis for the western Palearctic. Vector Borne Zoonotic Dis. 12, 743-752. doi:10.1089/vbz.2011.0767

Estrada-Pena, A., Nava, S., Petney, T. 2014. Description of all the stages of Ixodes inopinatus n. sp. (Acari: Ixodidae). Ticks Tick Borne Dis. 5, 734-743. doi:10.1016/j.ttbdis.2014.05.003

Ferrolho, J., Antunes, S., Santos, A. S., Velez, R., Padre, L., Cabezas-Cruz, A., Santos-Silva, M. M., Domingos, A. 2016. Detection and phylogenetic characterization of Theileria spp. and Anaplasma marginale in Rhipicephalus bursa in Portugal. Ticks Tick Borne Dis. 7, 443-448. doi:10.1016/j.ttbdis.2016.01.004 S1877-959X(16)30004-8 [pii]

Gern, L. 2009. Life cycle of *Borrelia burgdorferi* sensu lato and transmission to humans. Curr Probl Dermatol. 37, 18-30. doi:10.1159/000213068

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

000213068 [pii]
Gray, J. S., Kirstein, F., Robertson, J. N., Stein, J., Kahl, O. 1999. *Borrelia burgdorferi* sensu lato in Ixodes ricinus ticks and rodents in a recreational park in south-western Ireland. Exp Appl Acarol. 23, 717-729.
Grech-Angelini, S., Stachurski, F., Lancelot, R., Boissier, J., Allienne, J. F., Marco, S., Maestrini, O., Uilenberg, G. 2016. Ticks (Acari: Ixodidae) infesting cattle and some other domestic and wild hosts on the French Mediterranean island of Corsica. Parasit Vectors. 9, 582. doi:10.1186/s13071-016-1876-8
10.1186/s13071-016-1876-8 [pii]
Keskin, A., Bursali, A., Tekin, S. 2016. Molecular detection of spotted fever group rickettsiae in ticks removed from humans in Turkey. Ticks Tick Borne Dis. 7, 951-953. doi:S1877-959X(16)30071-1 [pii]
10.1016/j.ttbdis.2016.04.015
Khanakah, G., Kocianova, E., Vyrostekova, V., Rehacek, J., Kundi, M., Stanek, G. 2006. Seasonal variations in detecting *Borrelia burgdorferi* sensu lato in rodents from north eastern Austria. Wien Klin Wochenschr. 118, 754-758. doi:10.1007/s00508-006-0730-y
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol. 35, 1547-1549. doi:10.1093/molbev/msy096
Labruna, M. B., Whitworth, T., Horta, M. C., Bouyer, D. H., McBride, J. W., Pinter, A., Popov, V., Gennari, S. M., Walker, D. H. 2004. Rickettsia species infecting Amblyomma cooperi ticks from an area in the state of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. J Clin Microbiol. 42, 90-98.
Maquart, M., Dahmani, M., Marie, J. L., Gravier, P., Leparc-Goffart, I., Davoust, B. 2017. First Serological Evidence of West Nile Virus in Horses and Dogs from Corsica Island, France. Vector Borne Zoonotic Dis. 17, 275-277. doi:10.1089/vbz.2016.2024
Marsot, M., Sigaud, M., Chapuis, J. L., Ferquel, E., Cornet, M., Vourc'h, G. 2011. Introduced Siberian chipmunks (*Tamias sibiricus barberi*) harbor more-diverse *Borrelia burgdorferi* sensu lato genospecies than native bank voles (*Myodes glareolus*). Appl Environ Microbiol. 77, 5716-5721. doi:10.1128/AEM.01846-10
AEM.01846-10 [pii]
Matsumoto, K., Parola, P., Brouqui, P., Raoult, D. 2004. *Rickettsia aeschlimannii* in *Hyalomma* ticks from Corsica. Eur J Clin Microbiol Infect Dis. 23, 732-734. doi:10.1007/s10096-004-1190-9
Mediannikov, O. Y., Sidelnikov, Y., Ivanov, L., Mokretsova, E., Fournier, P. E., Tarasevich, I., Raoult, D. 2004. Acute tick-borne rickettsiosis caused by *Rickettsia heilongjiangensis* in Russian Far East. Emerg Infect Dis. 10, 810-817. doi:10.3201/eid1005.030437
Moltmann, U. G., Mehlhorn, H., Friedhoff, K. T. 1982. Electron microscopic study on the development of *Babesia ovis* (Piroplasmia) in the salivary glands of the vector tick *Rhipicephalus bursa*. Acta Trop. 39, 29-40.
Ndip, L. M., Ndip, R. N., Esemu, S. N., Walker, D. H., McBride, J. W. 2010. Predominance of *Ehrlichia chaffeensis* in *Rhipicephalus sanguineus* ticks from kennel-confined dogs in Limbe, Cameroon. Exp Appl Acarol. 50, 163-168. doi:10.1007/s10493-009-9293-8
Parola, P., Paddock, C. D., Socolovschi, C., Labruna, M. B., Mediannikov, O., Kernif, T., Abdad, M. Y., Stenos, J., Bitam, I., Fournier, P. E., Raoult, D. 2013. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev. 26, 657-702. doi:10.1128/CMR.00032-13
Pascucci, I., Di Domenico, M., Dall'Acqua, F., Sozio, G., Camma, C. 2015. Detection of Lyme Disease and Q Fever Agents in Wild Rodents in Central Italy. Vector Borne Zoonotic Dis. 15, 404-411. doi:10.1089/vbz.2015.1807
Punda-Polic, V., Petrovec, M., Trilar, T., Duh, D., Bradaric, N., Klismanic, Z., Avsic-Zupanc, T. 2002. Detection and identification of spotted fever group rickettsiae in ticks collected in southern Croatia. Exp Appl Acarol. 28, 169-176.
R. (2015). R Development Core Team. A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, 2012). . Retrieved from URL: http://www.R-

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

project.org.
Raele, D. A., Galante, D., Pugliese, N., De Simone, E., Cafiero, M. A. 2015. Coxiella-like endosymbiont associated to the "Anatolian brown tick" Rhipicephalus bursa in Southern Italy. Microbes Infect. 17, 799-805. doi:10.1016/j.micinf.2015.09.011
Raileanu, C., Moutailler, S., Pavel, I., Porea, D., Mihalca, A. D., Savuta, G., Vayssier-Taussat, M. 2017. Borrelia Diversity and Co-infection with Other Tick Borne Pathogens in Ticks. Front Cell Infect Microbiol. 7, 36. doi:10.3389/fcimb.2017.00036
Regnery, R. L., Spruill, C. L., Plikaytis, B. D. 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol. 173, 1576-1589.
Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubalek, Z., Foldvari, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Spitalska, E., Kazimirova, M. 2014. *Ixodes ricinus* and Its Transmitted Pathogens in Urban and Peri-Urban Areas in Europe: New Hazards and Relevance for Public Health. Front Public Health. 2, 251. doi:10.3389/fpubh.2014.00251
Roux, V., Rydkina, E., Eremeeva, M., Raoult, D. 1997. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int J Syst Bacteriol. 47, 252-261. doi:10.1099/00207713-47-2-252
Satta, G., Chisu, V., Cabras, P., Fois, F., Masala, G. 2011. Pathogens and symbionts in ticks: a survey on tick species distribution and presence of tick-transmitted micro-organisms in Sardinia, Italy. J Med Microbiol. 60, 63-68. doi:10.1099/jmm.0.021543-0
jmm.0.021543-0 [pii]
Sosa-Gutierrez, C. G., Vargas-Sandoval, M., Torres, J., Gordillo-Perez, G. 2016. Tick-borne rickettsial pathogens in questing ticks, removed from humans and animals in Mexico. J Vet Sci. 17, 353-360. doi:10.4142/jvs.2016.17.3.353
jvs.2015.086 [pii]
Torina, A., Alongi, A., Scimeca, S., Vicente, J., Caracappa, S., de la Fuente, J. 2010. Prevalence of tick-borne pathogens in ticks in Sicily. Transbound Emerg Dis. 57, 46-48. doi:10.1111/j.1865-1682.2010.01101.x
JVA1101 [pii]
Vandenesch, A., Turbelin, C., Couturier, E., Arena, C., Jaulhac, B., Ferquel, E., Choumet, V., Saugeon, C., Coffinieres, E., Blanchon, T., Vaillant, V., Hanslik, T. 2014. Incidence and hospitalisation rates of Lyme borreliosis, France, 2004 to 2012. Euro Surveill. 19. doi:20883 [pii]
Vial, L., Stachurski, F., Leblond, A., Huber, K., Vourc'h, G., Rene-Martellet, M., Desjardins, I., Balanca, G., Grosbois, V., Pradier, S., Gely, M., Appelgren, A., Estrada-Pena, A. 2016. Strong evidence for the presence of the tick *Hyalomma marginatum* Koch, 1844 in southern continental France. Ticks Tick Borne Dis. 7, 1162-1167. doi:S1877-959X(16)30125-X [pii]
10.1016/j.ttbdis.2016.08.002
Yeruham, I., Hadani, A., Galker, F. 2000. The life cycle of *Rhipicephalus bursa* Canestrini and Fanzago, 1877 (Acarina: ixodidae) under laboratory conditions. Vet Parasitol. 89, 109-116. doi:S0304-4017(99)00225-3 [pii]
Zhao, S. S., Li, H. Y., Yin, X. P., Liu, Z. Q., Chen, C. F., Wang, Y. Z. 2016. First detection of *Candidatus Rickettsia barbariae* in the flea *Vermipsylla alakurt* from north-western China. Parasit Vectors. 9, 325. doi:10.1186/s13071-016-1614-2

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

412 Table 1: Primers and probes used in this study and PCR conditions (annealing temperature).

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

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

Number of ticks per pool (n)	Number of pools with n ticks *H. marginatum*	Number of pools with n ticks *R. bursa*	Positive *Rickettsia* spp. n (%)	Positive Anaplasmataceae n (%)	Positive *Borrelia burgdorferi* (sl) n (%)

Host (n)	Tick species	n ticks (%)	Male n (%)	Female n (%)	Nymph n (%)
Cattle (n = 42)	*R. bursa*	495 (84.5)	251 (51.1)	207 (42.2)	33 (6.7)
	H. marginatum	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)	*R. bursa*	61 (100)	20 (33.0)	31 (51.0)	10 (16.0)
Rodents (n = 9)	*I. ricinus*	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.

			H. marginatum	*R.bursa*	Total	*H. marginatum*	*R. bursa*	Total	*H. marginatum*	*R. bursa*	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.

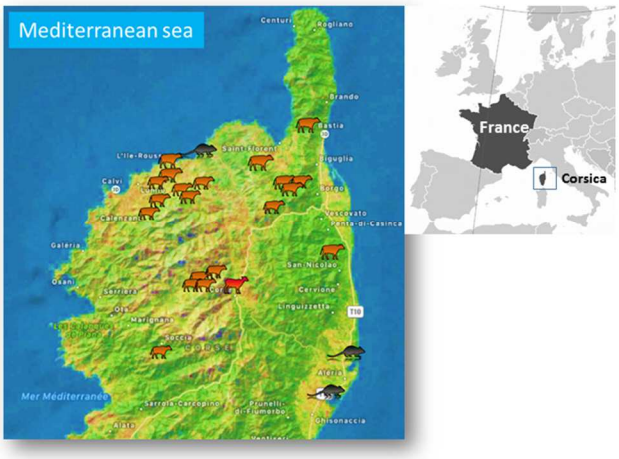


Figure 1: Map of Corsica showing the study area where the animals lived and the GPS coordinates.

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).

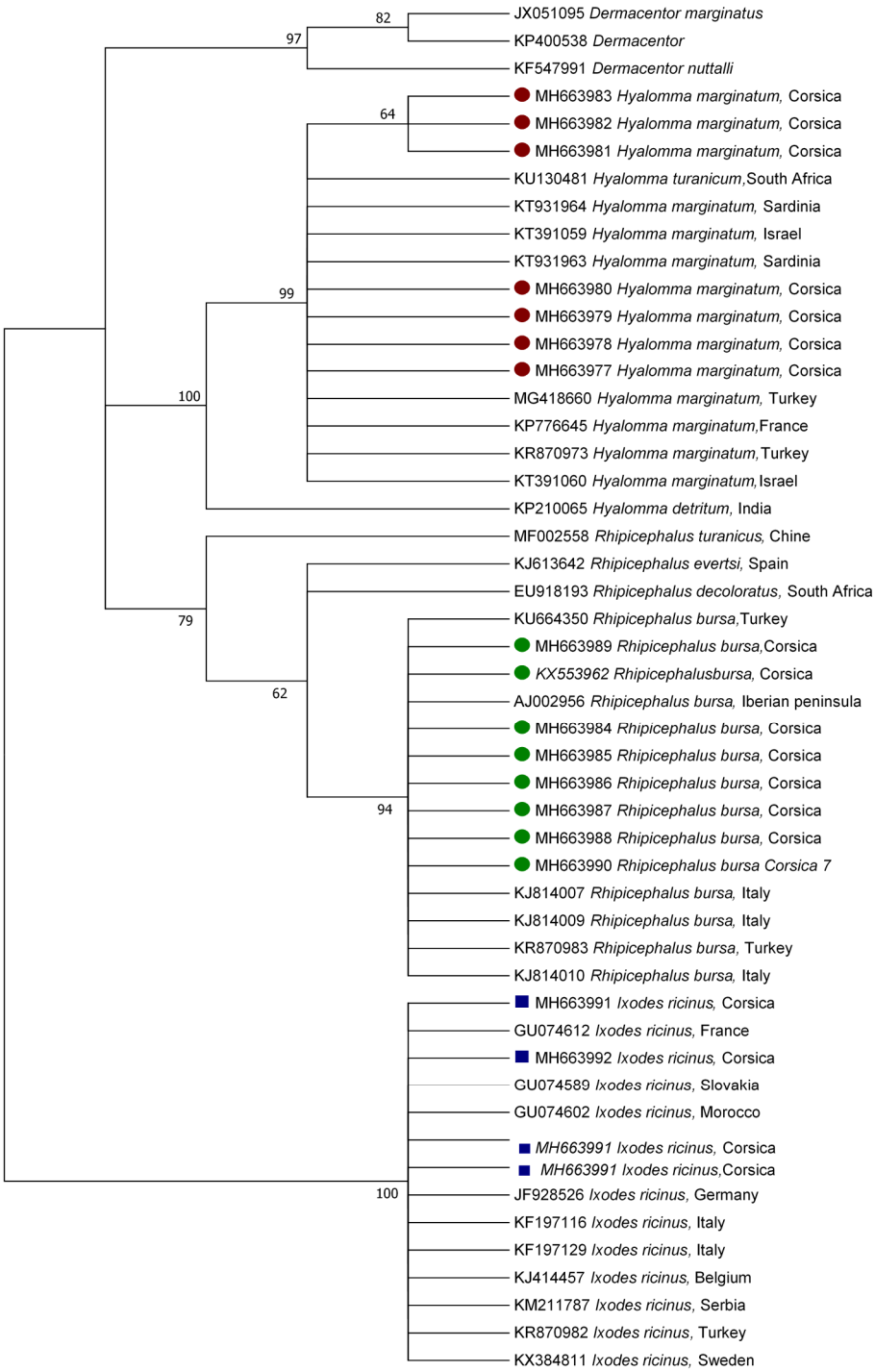


Figure 2. Phylogenetic tree showing the position of *Rhipicephalus bursa (green circles)*, *Hyalomma marginatum (red circles)* and *Ixodes ricinus (blue squares)* compared to other tick species based on 16S rDNA sequences. The evolutionary history was inferred by using the Maximum Likelihood method.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

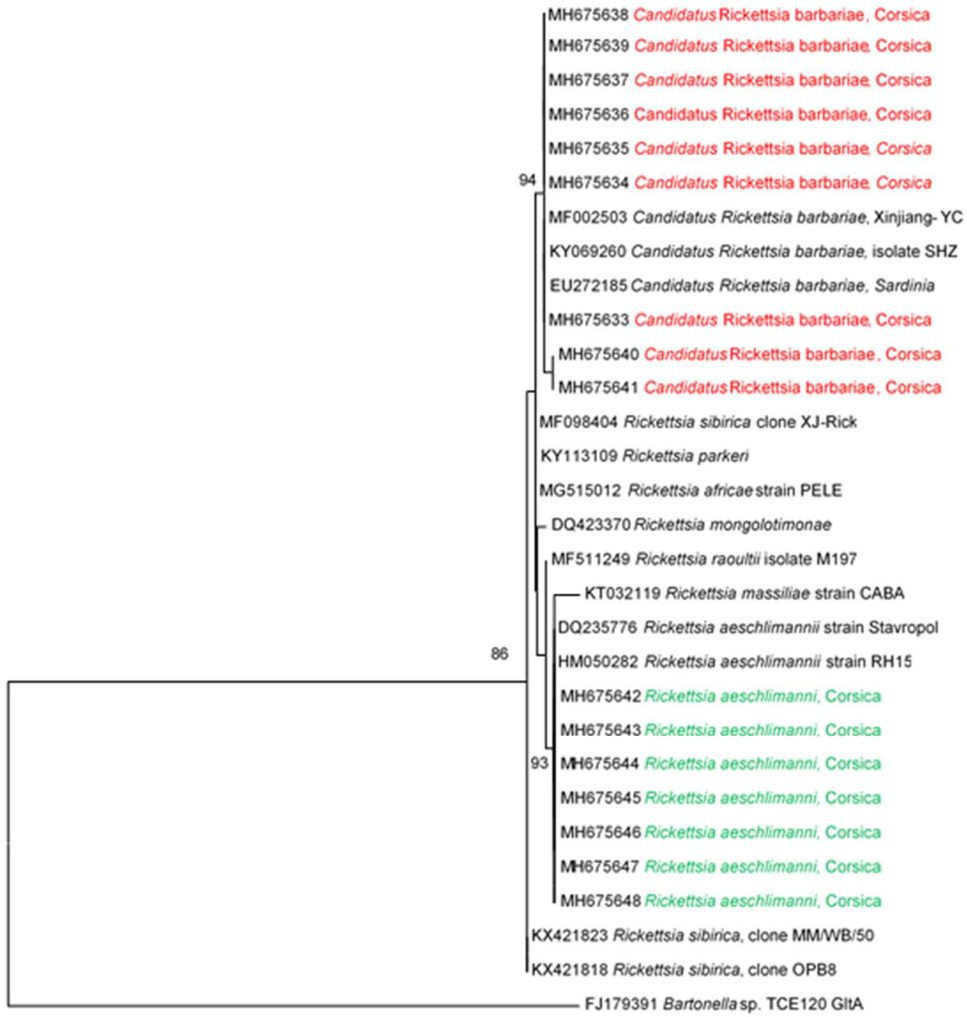


Figure 3. Phylogenetic analysis of *Rickettsia* spp. identified in Corsica. The evolutionary history was inferred by using the Maximum Likelihood method based on Rickettsia *gltA* sequences. The analysis involved 30 nucleotide sequences. All positions containing gaps and missing data were eliminated.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

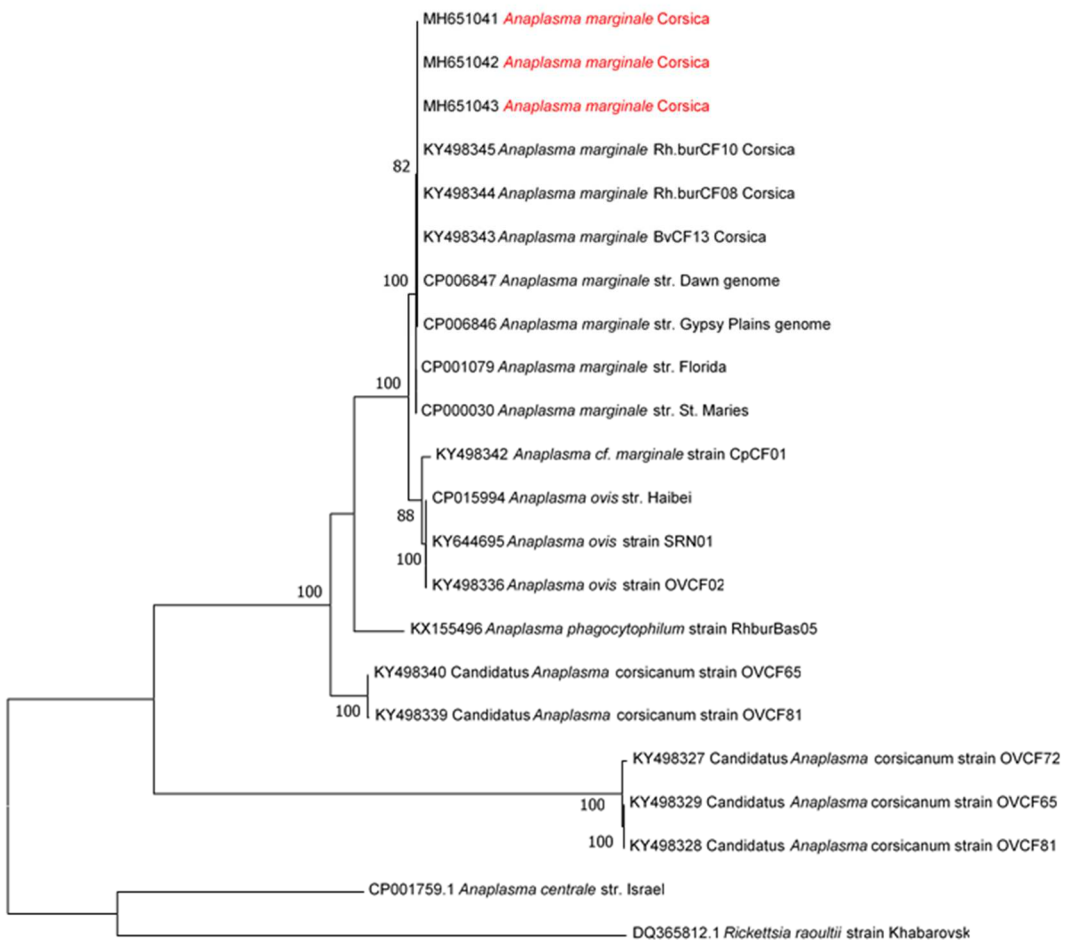


Figure 4. Phylogenetic analysis of Anaplasmatacea identified in Corsica. The evolutionary history was inferred by using the Maximum Likelihood method based on Anaplasmatacea *rpob* gene sequences. The analysis involved 30 nucleotide sequences. There were a total of 331 positions in the final dataset.