Molecular investigation and phylogeny of *Anaplasmataceae* species infecting domestic animals and ticks in Corsica, France

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**Abstract**

**Backgrounds:** Corsica is a French island situated in the Mediterranean Sea. The island provides suitable natural conditions to study disease ecology, especially tick-borne diseases and emerging diseases in animals and ticks. The family *Anaplasmataceae* is a member of the order *Rickettsiales*; it includes the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia* and *Wolbachia*. Anaplasmosis and ehrlichiosis traditionally refer to diseases caused by obligate intracellular bacteria of the genera *Anaplasma* and *Ehrlichia*. The aim of this study was to identify and estimate the prevalence of *Anaplasmataceae* species infecting domestic animals and ticks in Corsica.

**Methods:** In this study, 458 blood samples from sheep, cattle, horses, goats, dogs, and 123 ticks removed from cattle, were collected in Corsica. Quantitative real-time PCR screening and genetic characterisation of *Anaplasmataceae* bacteria were based on the 23S rRNA, *rpoB* and *groEl* genes.

**Results:** Two tick species were collected in the present study: *Rhipicephalus bursa* (118) and *Hyalomma marginatum marginatum* (5). Molecular investigation showed that 32.1% (147/458) of blood samples were positive for *Anaplasmataceae* infection. *Anaplasma ovis* was identified in 42.3% (93/220) of sheep. *Anaplasma marginale* was amplified from 100% (12/12) of cattle and two *R. bursa* (2/123). Several potentially new species were also identified: *Anaplasma cf. ovis*, “*Candidatus Anaplasma corsicanum*”, “*Candidatus Anaplasma mediterraneum*” were amplified from 17.3% (38/220) of sheep, and *Anaplasma* sp. marginale-like was amplified from 80% (4/5) of goats. Finally, one *R. bursa* tick was found to harbour the DNA of *E. canis*. All samples from horses and dogs were negative for *Anaplasmataceae* infection.

**Conclusions:** To our knowledge, this study is the first epidemiological survey on *Anaplasmataceae* species infecting animals and ticks in Corsica and contributes toward the identification of current *Anaplasmataceae* species circulating in Corsica.

**Keywords:** Corsica Island, Animals, Ticks, *Anaplasma ovis*, *Anaplasma marginale*, *Anaplasma* sp., *Ehrlichia canis*
Background
Bacteria from the genera *Anaplasma* and *Ehrlichia* are obligate intracellular bacteria transmitted by arthropods, mainly ticks, from one vertebrate host to another. Transmission usually occurs transstadially [1, 2], although transovarial transmission has been reported [3]. In the vertebrate host, the bacteria infect hematopoietic cells [4, 5]. *Anaplasma* and *Ehrlichia* can cause a persistent infection in vertebrate hosts, which allows these hosts to be reservoirs [1, 5]. Probable cases of *Anaplasmataceae* infection in domestic animals were known as early as the beginning of the twentieth century. However, wide interest in studying these bacteria arose when discovering species pathogenic for humans [1]. *Anaplasma phagocytophilum* was known to cause disease in domestic ruminants in Europe and the USA decades before its identification in humans [6]. The first European case of human anaplasmosis was reported in 1995 in Slovenia; after that, human cases have been reported in many countries of Europe [7–11]. Bovine anaplasmosis due to *A. marginale* results in the development of mild to severe anaemia and occurs in tropical and subtropical regions, including South and Central America, the United States, southern Europe, Africa, Asia and Australia [12]. In India, mortality due to bovine anaplasmosis is estimated at between 5 and 40% but may reach up to 70% during a severe outbreak [13]. The economic loss due to infections caused by *Babesia* and *Anaplasma* infections in India was estimated to be $57 million [14]. In Europe, *A. marginale* has spread up to the northern latitudes of Switzerland, Austria and Hungary [15]. *Anaplasmataceae*, a less pathogenic organism but closely related to *A. marginale*, was reported in cattle in Sicily, Italy [16], and from roe deer in Spain [17]. *Anaplasma ovis* is an intraerythrocytic pathogen of sheep, goats and wild ruminants [18]. It is thought to cause only mild clinical symptoms, thus being of minor economic importance [19]. Ovine anaplasmosis appears to be widespread and found in different regions of the world. The extent of the infection and the loss of livestock productivity remain poorly understood [19]. The historical record of this bacterium in Europe was established in Russia in 1929 and 1930 by Yakimoff et al. [19], and in France by Cuille et al. in 1935 and 1936 [19]. In 2007, *A. ovis* human infection was reported in a 27-year-old woman in Cyprus [20].

The management of vector-borne diseases requires increased communication between physicians and veterinarians, particularly when physicians are dealing with patients with unexplained febrile illnesses in an endemic area were pathogen like *Anaplasmataceae* largely interconnected in an epidemiological network involving animals, vectors and humans [21]. Corsica is a French island in the Mediterranean Sea close to the south-east French coast, Sardinia, and the west Italian coast. Highly endemic flora and fauna and endemic pathologies are characteristic in Corsica [22]. Recently, we reported the emergence of Toscana virus in dogs in this region [23] and West Nile virus in domestic animals [24]. Our main objective was to continue the epidemiologic investigation of neglected infectious diseases in animals. To date, the occurrence of *Anaplasmataceae* bacteria in Corsica in domestic animals has never been reported. The aim of this study was to screen for the presence and the prevalence of *Anaplasmataceae* species infecting and currently circulating in domestic animals and their ticks in this region.

Methods
Sampling
From 2014 to 2015, EDTA blood samples were obtained from domestic animals on different farms from 14 different areas situated on the east coast of Corsica, France (Fig. 1). Sheep and goats were sampled on the Aléria plain. Cattle and ticks were sampled from one farm in Centu Mezzini, Balagne (42°34′58.242″N, 8°58′38.015″E), whereas dogs and horses were sampled in different localities along the east coast of Corsica island, including Cap Corse (42°56′44″N, 9°26′28″E), Furiani (42°39′32″N, 9°24′54″E), Biguglia (42°37′41″N, 9°25′14″E), Lucciana (42°32′48″N, 9°25′5″E), Vescovato (42°29′41″N, 9°26′26″E), Castellare (42°28′7″N, 9°28′27″E), Tallone (42°13′55″N, 9°24′53″E), Ghisonaccia (42°′13′3″N, 9°24′20″E), Solenzara (41°55′36″N, 9°24′19″E), Lecci (41°40′48″N, 9°19′5″E), Borgo (42°33′17″N, 9°25′41″E), and Ventiseri-Solenzara (41°55′36″N, 9°24′19″E) (Table 1). Sheep blood samples (230) were collected from three farms. In two farms, the sheep appeared healthy; however, the farmers declared that their sheep experienced many health problems during the winter of 2014, including respiratory disorders and a drop in milk production. At the third sheep farm, the farmer declared that the sheep at his farm were currently unhealthy, with a variety of symptoms, including recurrent fever, abortion, and some sheep died. A cattle herd in Balagne consisted of 16 cows. The cows in this herd had pronounced anaemia with icterus, and some of them died in 2015. Goats (n = 5) were all sampled on one farm; they had anaemia and a drop in milk production. In addition, blood samples were collected from horses at a different ranch. Dogs sampled in the present study included hunting dogs, sheep dogs, military working dogs and some pet dogs. Animals were examined with the assistance of their owners. Blood samples were collected by a veterinarian. After transport to the laboratory in Marseille, all samples were stored at -80 °C.

Tick collection and identification
From the cattle farm, ticks were collected manually from adult cattle and stored in 70% ethanol until identification. Morphological identification was performed with a
binocular microscope. Ticks were classified by family, genus and species using available taxonomic keys and morphometric tables [25, 26]. In addition, to confirm the morphological identification, three morphologically identified specimens of each species and all ticks that were not identified, or identified only to the genus level (engorged females and damaged ticks) were subjected to molecular identification using primers targeting the mitochondrial 12S rRNA gene, as previously described [27].

**DNA extraction**

DNA extraction was performed on the BioRobot EZ1 (Qiagen, Courtaboeuf, France) using a commercial EZ1 DNA Tissue Kit (Qiagen) according to the manufacturer’s instructions. DNA was extracted from 200 μl of blood from all animal samples. Ticks were recovered from ethanol, rinsed with distilled water and dried on sterile filter paper in a laminar-flow hood. Each tick was cut in half lengthways (the blades were discarded after each tick was cut). DNA was individually extracted from one half, and the remaining halves of the ticks were frozen at -80 °C for subsequent studies, as previously described [28].

**PCR amplification**

For the molecular identification of the species of selected ticks, the DNA samples were subjected to standard PCR to amplify a 360-base-pair (bp) fragment of the mitochondrial 12S rRNA gene (Table 2). To investigate the presence of *Anaplasmataceae* in Corsican ticks and domestic animals, DNA from ticks and blood were initially screened by a qPCR targeting the 23S rRNA gene. This qPCR has been reported to amplify most bacteria belonging to the family *Anaplasmataceae* [29]. Then, all positive samples were subjected to conventional PCR using the primers that amplify a 485 bp fragment of the 23S rRNA gene, as previously described [29].

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**Table 1** Origin of animal and tick samples collected and investigated in this study

| Species | Number | Origin   | Tick infestation | No. of ticks |
|---------|--------|----------|-----------------|--------------|
| 2014    |        |          |                 |              |
| Sheep   | 201    | Aléria Plain | not found       | –            |
| Horse   | 98     | East coast | not found       | –            |
| Dog     | 73     | East coast | not found       | –            |
| 2015    |        |          |                 |              |
| Sheep   | 19     | Aléria Plain | not found       | –            |
| Cattle  | 12     | Balagne  | *R. bursa*       | 118          |
|         |        |          | *Hy. m. marginatum* | 5            |
| Goat    | 5      | Aléria Plain | not found       | –            |
| Dog     | 50     | not found |                 | –            |
| Totals  | 458    |          |                 | 123          |
In order to mine deeper into the identification of *Anaplasmataceae* species in domestic animals or ticks, positive samples were tested by PCR using *Anaplasma* genus-specific primers targeting the 525 bp fragment of the RNA polymerase subunit beta (*rpoB*) gene, and *Ehrlichia* genus-specific primers targeting the 590 bp fragment of the heat shock protein (*groEL*) gene [28] (Table 2).

**Table 2 Primers and probes used in this study**

| Targeted microorganisms | Targeted gene | Primers and probe<sup>a</sup> | Sequences 5’-3’ | Annealing temperature (°C) | References |
|-------------------------|---------------|-------------------------------|-----------------|-----------------------------|------------|
| *Anaplasmataceae*       | 23S rRNA      | TrAna-F, TrAna-R, TrAna-S<sup>5</sup> | TGACACGGCTACCTTGGCAT, GTAACAGGGTTCGCGTTCCTCA, FAM-CTTGCTTCTGGCTCTAATCC-TAMRA | 60          | [28, 29] |
|                         |               |                               |                 |                             |            |
|                         |               |                               |                 |                             |            |
| *Anaplasmataceae*       | 23S rRNA      | Anana23S-121F, Anana23S-753r | TAAAGCTCGAGGAGTGTGC, TGCAGAAGGTACGCTGTCAC | 55          | [28, 29] |
|                         |               |                               |                 |                             |            |
| *Anaplasma* spp.        | *rpoB*        | Ana-rpoB<sup>F</sup>, Ana-rpoB<sup>R</sup> | GCTGGCTCTAGGCTYCTTACGGCA, AATCRCAGCAYAGGCCCCCTRATGCGA | 55          | [27]     |
|                         |               |                               |                 |                             |            |
| *Ehrlichia* spp.        | *groEL*       | Ehr-groEL-F, Ehr-groEL-R | GTGAAAAARCTGATGATGATGCA, ACACGRTCTTACGGCYTACCA | 50          | [27]     |
| *Ticks*                 | 12S rRNA      | T1B, T2A                        | AAACCTAGGATTAGGATACCC, AATGAGAGCGACGGGCGATGT | 51          | [26]     |

<sup>a</sup>Probe

**Sequencing and phylogenetic analyses**

Sequencing analyses were performed on the Applied Biosystems 3130xl Genetic Analyzer (Thermo Fisher Scientific, France) using the DNA sequencing BigDye Terminator Kit (Perkin-Elmer) according to the manufacturer’s instructions. The obtained sequences were assembled using ChromasPro 1.7 software (Technelysium Pty Ltd., Tewantin, Australia) and the sequences of primers were removed. Sequences obtained in this study were aligned with other ticks or *Anaplasmataceae* species sequences available on GenBank using CLUSTALW implemented on BioEdit v3 [31]. The sequence of 12S rDNA from ticks and the sequences of bacterial 23S rDNA, *rpoB*, and *groEL* genes were first aligned individually, gaps and missing data were eliminated, and then, for the sequences of *Anaplasmataceae* species, the alignment of the 23S rDNA with *rpoB* genes and 23S rDNA with *groEL* gene sequences were concatenated for phylogenetic tree construction for the *Anaplasma* and *Ehrlichia* species, respectively. Phylogenetic and molecular evolutionary analysis were inferred using the maximum likelihood method implemented on MEGA7 [32], with the complete deletion option, based on the Hasegawa-Kishino-Yano (HYK) model for nucleotide sequences. A discrete gamma distribution was used to model evolutionary rate differences among sites. Initial trees for the heuristic search were obtained automatically by applying the neighbor-joining and BIONJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. Statistical support for internal branches of the trees was evaluated by bootstrapping with 1000 iterations.

**Results**

**Tick identification and *Anaplasmataceae* screening**

In total, 123 ticks were collected. Eighty-five removed ticks were identified as *Rhipicephalus bursa*, and 3 as *Hyalomma marginatum*. Thirty-five damaged ticks, including 32 engorged ticks, were only morphologically identified to the genus level as follows: 29 ticks *Rhipicephalus* sp., 2 *Hyalomma* sp., and 4 ticks were not identified. Two or three specimens from each tick identified at
a species level were selected randomly, and all 35 damaged/engorged ticks were subjected to molecular identification. After 12S rDNA amplification and blast analysis, the six morphologically identified specimens were confirmed to be R. bursa and Hy. marginatum. From the 35 damaged ticks, 32 ticks were identified as R. bursa, and 3 were identified as Hy. m. marginatum. All 12S rDNA sequences of the R. bursa were identical to each other and showed 100% identity with R. bursa from Italy (KU51295, KC243833, AM410572), and 99% identity with R. bursa ticks reported from Spain (KC243834) (Fig. 1). All five sequences of Hy. m. marginatum were also identical to each other and showed 100% identity with Hy. m. marginatum from Italy (KC817304), Israel (KT391046), Morocco (AF150034) and Yemen (HE819151) (Fig. 2). Overall, the ticks collected in this study were as follows: 118 (95.9%) were identified as R. bursa; 75 were female, including 30 engorged females, and 43 were male. Five (4.1%) were identified as Hy. m. marginatum; two were engorged females, and three were male.

Anaplasmataceae DNA was detected in three R. bursa of the 123 ticks examined (2.4%). After the 23S rRNA gene sequencing of the Anaplasmataceae DNA present in the three ticks, A. marginale was identified in two ticks. The two sequences of A. marginale were identical to each other and showed 100% homology with the A. marginale strain Dawn (CP006847) and Gypsy Plains (CP006846) reported from Australia, and 99% with the A. marginale strain Florida (CP001079) and St. Maries (CP000030) reported from the USA (Fig. 3). Finally, based on the 23S rRNA analysis, E. canis was identified from the third positive tick. These sequences presented 99% homology with the E. canis strain Jack (CP000107) reported from the USA (Fig. 4).

**Anaplasmataceae species screening from animal blood**

The results are summarised in the (Table 3). Of the total of 458 blood samples analysed (Table 1), 32.1% (147) were positive for the initial 23S rRNA qPCR screening. The prevalence of Anaplasmataceae infections was as follows: sheep 59.5% (131/220), cattle 100% (12/12) and goats 80% (4/5), whereas all blood samples from horses and dogs were negative. Identification of bacterial species was achieved by amplification followed by sequencing of the portion of the 23S rRNA gene. Seventy-one percent (93/131) of Anaplasmataceae-positive sheep samples were infected by A. ovis. The 23S sequences obtained were identical to each other and showed 100% identity with A. ovis strain KMND Niayes-14 reported in sheep from Senegal [33]. All the other 38 qPCR-positive sheep (29%) were found to be infected by several as yet uncharacterised and potentially new species of Anaplasma. In 13/131 (9.9%) of infected sheep, the obtained sequences were identical to each other and showed only 96% identity with the A. ovis strain KMND Niayes-14. Due to the absence of additional data on this Anaplasma and genetic relatedness to A. ovis, we refer to this genotype here as Anaplasma cf. ovis. There were 3/131 (2.3%) sheep infected by another genotype of Anaplasma. These three sequences had 96–98% identity to each other and showed 91–94% identity with the A. phagocytophilum strain Norway Variant 2, reported from sheep in Norway (CP015376). We are provisionally calling this incompletely characterised bacterium “Candidatus Anaplasma corsicanum”. Finally, a third genotype was found infecting 22/131 (16.8%) sheep, sampled only in 2015. All sequences of this genotype were identical to each other and showed 95% identity with the A. centrale strain Israel (CP001759) reported from Israel (Fig. 3). We are provisionally calling this bacterium “Candidatus Anaplasma mediterraneum”.

All 12 cattle tested were positive in qPCR and conventional PCR (100%) for Anaplasmataceae bacteria. Sequencing analyses showed that all cattle were infected by A. marginale. The sequences were identical to each other, and also to the sequences of A. marginale identified in the R. bursa ticks removed from the same animals.

Finally, 4 of 5 goats were found to be infected by a potentially new species of Anaplasma similar to A. marginale. All sequences were identical to each other and showed 99% homology with the A. marginale strain Dawn (CP006847), A. centrale strain Israel (CP001759) and 99% with A. ovis strain KMND Niayas-14 (KM021411) (Fig. 3).

Additional characterisation of detected Anaplasmataceae bacteria was performed by amplification/sequencing of a portion of the rpoB gene (for Anaplasma-positive samples) or groEL gene (for Ehrlichia-positive samples). RpoB sequences from A. ovis-positive samples were also identical to each other and showed 100% identity with A. ovis strain KMND Niayes-14. rpoB sequences from two A. marginale-positive R. bursa ticks and the four other sequences obtained from cattle blood samples were identical to each other and showed 100% identity with A. marginale strain Dawn (CP006847) and Gypsy Plains (CP006846) and 99% with A. marginale strain Florida (CP001079) and St. Maries (CP000030). For the E. canis identified in one R. bursa tick, the DNA sample was amplified using groEL Ehrlichia genus-specific primers and sequenced. The sequence showed 99% homology with the E. canis strain Jack (CP000107) (Fig. 4).

Analysis of rpoB sequences of all three novel genotypes of Anaplasma produced results similar to the 23S gene analysis. RpoB sequences from A. cf. ovis showed 98% identity with A. ovis strain KMND Niayes-14. The three rpoB sequences from “Ca. Anaplasma corsicanum” had 99% identity to each other, and only 80% with A. phagocytophilum strains Norway Variant 2 (CP015376), Dog2 (CP006618), JM (CP006617) and HZ (CP000235).
RpoB sequences of “Ca. Anaplasma mediterraneum” presented 84% identity with A. centrale strain Israel (CP001759). Finally, Anaplasma cf. marginale from four goats had rpoB sequences that shared 98% identity with A. ovis strain KMND Niayes-14 (KX155494) and strain RhburBas11 (KX155495), 93% with A. marginale strain Florida (CP001079), St. Maries (CP000030), 89% strain Dawn (CP006847) and Gypsy Plains (CP006846), and 87% with A. centrale strain Israel (CP001759) (Fig. 3).

Phylogenetic analyses of the potentially new species

The phylogenetic tree inferred from the Anaplasmataceae concatenated 23S rRNA, and the rpoB genes provide evidence that “Ca. Anaplasma corsicanum”, Anaplasma cf.
"Ca. Anaplasma mediterraneum" from sheep and Anaplasma cf. marginale from goats could potentially be new species. “Ca. Anaplasma corsicanum” clustered separately from the recognised species A. phagocytophilum, A. platys, A. ovis, A. marginale and A. centrale (Fig. 2).

The sequence of Anaplasma cf. ovis from sheep and the sequence of Anaplasma cf. marginale from goats clustered together with the sequence of A. ovis strain KMND Niayes-14 from Senegal and A. ovis from sheep identified in this study with high bootstrap values and separately
from the cluster of A. marginale species. Finally, the sequence of “Ca. Anaplasma mediterraneum” obtained from sheep form well-defined branches with high bootstrap values (93–95%) (Fig. 3).

All sequences obtained in the present study were submitted to GenBank under the following accession numbers: (i) for the 23S rRNA gene: Anaplasma ovis OVCF02 (KY498325), Anaplasma cf. ovis OVCF115 (KY498326), “Ca. Anaplasma corsesicanum” OVCF72 (KY498327), “Ca. Anaplasma corsesicanum” OVCF81 (KY498328), “Ca. Anaplasma corsesicanum” OVCF65 (KY498329), “Ca. Anaplasma mediterraneum” OVCF0215 (KY498330), Anaplasma cf. marginale CpCF01 (KY498331), Anaplasma marginale BvCF13 (KY498332), A. marginale Rh.burCF08 (KY498334), A. marginale Rh.burCF10 (KY498335), Ehrlichia canis Rh.burCF07 (KY498333); (ii) for the rpoB gene: Anaplasma ovis OVCF02 (KY498325), Anaplasma cf. ovis OVCF115 (KY498336), “Ca. Anaplasma corsesicanum” OVCF72 (KY498338), “Ca. Anaplasma corsesicanum” OVCF81 (KY498339), “Ca. Anaplasma corsesicanum” OVCF65 (KY498340), “Ca. Anaplasma mediterraneum” OVCF0215 (KY498341), Anaplasma cf. marginale CpCF01 (KY498342), Anaplasma marginale BvCF13 (KY498343), A. marginale Rh.burCF08 (KY498344), A. marginale Rh.burCF10 (KY498345); (iii) For the groEl gene: Ehrlichia canis Rh.burCF07 (KY498324). For tick

Table 3 Overall results and Anaplasmataceae species reported in the present study

| Species                          | Sheep (%) | Cattle | Goats (%) | Equine (%) | Dogs (%) | R. bursa (%) | Hy. m. marginatum |
|----------------------------------|-----------|--------|-----------|------------|----------|--------------|-------------------|
| A. ovis                          | 93/220 (71%) | 0      | 0         | 0          | 0        | 0            | 0                 |
| A. marginale                     | 0         | 0      | 0         | 0          | 0        | 2/118 (1.7%) | 0                 |
| Anaplasma cf. marginale          | 0         | 12/12 (100%) | 4/5 (80%) | 0          | 0        | 0            | 0                 |
| Anaplasma cf. ovis               | 13/220 (9.9%) | 0      | 0         | 0          | 0        | 0            | 0                 |
| “Candidatus Anaplasma corsesicanum” | 3/220 (2.3%) | 0    | 0         | 0          | 0        | 0            | 0                 |
| “Candidatus Anaplasma mediterraneum” | 22/220 (16.8%) | 0    | 0         | 0          | 0        | 0            | 0                 |
| E. canis                         | 0         | 0      | 0         | 0          | 1/118 (0.8%) | 0          | 0                 |
| Totals                           | 131/220 (59.5%) | 12/12 (100%) | 4/5 (80%) | 0          | 0        | 3/118 (2.5%) | 0                 |

Data presented as No. of infected/No. of examined (Prevalence %)
species, the 12S rRNA sequences were submitted under the following accession number: Hy. m. marginatum (KYS95783) and R. bursa (KYS95784).

**Discussion**

Livestock farming in Corsica is an important economic activity involving approximately 150,000 sheep, 48,000 goats, 40,000 pigs and 70,000 cattle [22]. The significance of anaplasmosis in animals in Corsica is not yet known. *Anaplasma* infection may likely be neglected because of its unknown economic importance in small ruminants. To our knowledge, the present study is the first report of the incidence of *Anaplasmataceae* species in ticks and animals in Corsica. Furthermore, the presence and molecular traits of six species belonging to the genus *Anaplasma* from ruminants and ticks infesting cattle, and one *Ehrlichia*, are shown. The typical Mediterranean environment of Corsica with hot summers, along with the geographical location, favours the spread of seasonal tick infestations. Two tick species were collected and confirmed by the morphological and molecular investigation as *R. bursa* and *Hy. m. marginatum* (Fig. 1). Neither the tick fauna of Corsica nor the transmitted pathogens have been fully investigated. Here, ticks were only collected from cattle; infestation of other animals, including sheep, goats, horses and dogs, was not observed. A previous study demonstrated the presence of three species of the genus *Hyalomma* in Corsica: *Hy. marginatum*, *Hy. aegyptium* and *Hy. rufipes* [22]. While *Hy. marginatum* is found on many hosts, *Hy. aegyptium* was identified once in Corsica on a *Testudo hermanni* tortoise, while *Hy. rufipes* has been collected from migrating birds [22]. Recently, *Hy. scupense* was also identified and collected from Corsican cattle by Grech-Angelini et al. [22]. *Rhipicephalus bursa* was the most common tick infesting cattle in our study. This two-host species occurs in the entire Mediterranean, Adriatic and Aegean basins, including their islands, and North Africa [25, 34]. *Rhipicephalus bursa* prefers grassy slopes and low to medium altitude mountain slopes, as well as certain modified steppe and semi-desert environments [35]. However, this tick species is recorded in cold regions, including the Atlantic region of Europe, the French Basque country, Spanish Basque country, and north-west Portugal [28, 35]. Corsica is a typical Mediterranean ecosystem, which favours the spread of these ticks. *Rhipicephalus bursa* mature and adults infest many hosts, including cattle, sheep, goats and other domestic animals, whereas wild ungulates are the original host [36]. This species is a recognised vector of many pathogens, including *Babesia ovis*, *Theileria* spp., *A. marginale* and *A. ovis* [36]. DNA of *Coxiella burnetii* and *A. phagocytophilum* have also been amplified from these ticks [28, 35]. Here, the DNA of *A. marginale* was amplified from two engorged female ticks removed from cattle infected by *A. marginale*. Previous studies have reported *A. marginale* from *R. bursa* removed from cattle in Portugal [34], and from Iberian red deer and European wild boar in Spain [37]. It is likely that the presence of *A. marginale* DNA in these two ticks was due to the presence of this pathogen in the blood meal. However, the percentage of *R. bursa*-engorged females in our study was 42.9% (30/70 female); only two engorged ticks were found to harbour *A. marginale*.

*Ehrlichia canis* was amplified from one non-engorged *R. bursa* female. In Europe, *E. canis* is associated with the presence of the brown dog tick *R. sanguineus* [38]. However, in the Mediterranean area, *E. canis* has also been reported from *R. bursa* collected from goats in Sardinia, Italy [39] and *Cediopsylla inaequalis* collected from red foxes in Sicily, Italy [40]. In other European countries, there are reports of *E. canis* from *D. marginatus* collected from dogs, *Ixodes canisuga* collected from red foxes, and *I. ricinus* collected from vegetation in Hungary [41, 42]. Domestic animals are now recognised as the primary hosts of *R. bursa* [36]; however, the role of *R. bursa* and the other arthropod species in the transmission of *E. canis* remains unknown.

None of the five *Hy. m. marginatum* ticks were positive for *Anaplasmataceae* infection. However, in Spain, *Hy. m. marginatum* has been identified as a potential biological vector for *A. marginale* [43]. These ticks are also the vectors of *Babesia caballi*, causing babesiosis in horses and *Theileria annulata* infection under laboratory conditions [26]. Other studies are needed to clarify and list the pathogens associated with these ticks in Corsica.

The prevalence of *Anaplasma* spp. in our study was surprisingly high in ruminants. Based on the 23S rRNA gene molecular investigations, the individual prevalence observed was 59.5% in sheep, 100% in cattle, and 80% in goats. However, none of the canine or equine blood samples was positive. Genetic characterisation using 23S rRNA and the rpoB genes identified *A. ovis*, *A. marginale*, and several potentially new species, all belonging to the genus *Anaplasma*. These data confirm the relevance of ruminants as important hosts and reservoirs of different *Anaplasma* species in the Mediterranean ecosystem. The prevalence of *Anaplasma* spp. in ruminants examined by us was lower than the prevalence data reported from Sardinia [44]. The prevalence of *A. marginale* infection in cattle was higher than that observed in cattle in Sicily [45]; however, in that study, the number of samples analysed was greater than in our study. In sheep, the prevalence reported in our study was lower than that reported in Sicily [46].

Sheep, goats, and cattle sampled in this study manifested poor health. In sheep, most clinical manifestations observed were relapsing fever, drop in milk production
and mortality. Molecular and phylogenetic analysis of sequences amplified from sheep blood samples were identified A. ovis, and three potentially new species, “Ca. Anaplasma corsicanum”, “Ca. Anaplasma mediterraneum”, and Anaplasma cf. ovis. In the Mediterranean area, A. ovis is reported to be endemic to Sicily [47, 48]. This pathogen has also been reported from Greece and Cyprus [21, 49]. In Europe, A. ovis has also been reported from Portugal, Hungary [19] and Slovakia [47]. Anaplasma ovis infection in the mouflon and the European roe deer has been reported from Cyprus and southern Spain, respectively [50, 51]. The main vector of A. ovis in Europe is R. bursa [28]. However, A. ovis DNA was amplified from I. ricinus removed from cattle in Hungary [15], Haemaphysalis sulcata removed from moufflons in Cyprus [50], and the sheep ked (Melophagus ovinus) and deer ked (Lipoptena cervi) in Hungary [48]. In addition, in provinces of Palermo and Ragusa (Italy), A. ovis was amplified from foxes, and a flea, Xenopsylla cheopis, removed from these foxes [40]. The role of these arthropods and insects in the transmission of A. ovis remains unclear. Anaplasmosis in sheep is usually subclinical. This bacterium can lead to severe infection with severe illness in sheep; severe illness can occur in some extreme conditions, such as the association with more than one parasitic disease or other stress factors [49, 52].

All cattle sampled in this study were infected with A. marginale. In this farm, the farmer reported mortality in his livestock. Anaemia and icterus were most observed in other cattle (Table 1). Bovine anaplasmosis due to A. marginale causes mild to severe anaemia, icterus, fever, weight loss, abortion and lethargy [53]. In Europe, A. marginale is mainly present in the Mediterranean region, alpine, and eastern areas [16]. In the Mediterranean region, the DNA of this bacterium has been amplified from D. reticulatus, D. marginatus, R. turanicus, Haemaphysalis punctata, Hy. m. marginatum and R. bursa [34, 37, 43]. Interestingly, A. marginale has been amplified from Xenopsylla cheopis removed from red foxes in Italy [40]. Outside of the Mediterranean region, A. marginale has been amplified from I. ricinus and Tabanus bovis in Hungary [15, 43]. The role of I. ricinus, Tabanus bovis and Xenopsylla cheopis in the transmission of A. marginale remains unclear.

The potentially new species “Ca. Anaplasma corsicanum” and “Ca. Anaplasma mediterraneum” have genetic features which are different from other species of the genus Anaplasma (Fig. 3). Phylogenetic analysis based on the concatenated 23S rRNA and rpoB genes showed that “Ca. Anaplasma corsicanum” is related to A. phagocytophilum, but clustered separately from recognised species. “Ca. Anaplasma mediterraneum” is related to A. centrale and forms a distinct subcluster. Two other identified genotypes, Anaplasma cf. ovis and Anaplasma cf. marginale, grouped with the sequences of A. ovis (Fig. 3). Interestingly, despite this grouping, Anaplasma cf. marginale is closer to A. marginale than to A. ovis, based on the 23S rRNA comparison. The rpoB encodes the RNA polymerase subunit beta and gives a better statistical score for differentiating between the closest species of Anaplasma spp., with more sequence variations [28]. The observed prevalence of the potentially new Anaplasma species in sheep was low (17.3%, 38/220) compared to the prevalence of A. ovis; however, 80% (4/5) of the goats sampled in this study were infected by Anaplasma cf. marginale. The importance of this amplified Anaplasma species remains to be understood.

Mortalities in animals were reported by the farmers in the sheep and cattle herds. Unfortunately, we did not have access to body tissue of fluid from the dead animals to perform a post-mortem diagnosis. The different reported symptoms and the results found in the present study with the high prevalence of A. marginale in cattle and A. ovis and the others amplified Anaplasma spp. in sheep and goats suggest that the mortalities can be linked to these Anaplasma species. However, other tick- or vector-borne diseases can also lead to mortalities like Piroplasmosis [54, 55]. In addition, co-infection by two or more pathogens can lead to increase the pathogenicity and clinical manifestations in animals and resultant varying outcomes on host health and survival [56]. The involvement or not involvement of the Anaplasmataceae species amplified in the present study should be considered with caution do to the possible implication of other pathogens.

In our study, we did not find A. phagocytophilum in animal or tick samples. Anaplasma platys and E. canis were also not found in dogs, although E. canis was found in Rh. bursa collected from a cow.

**Conclusion**

The present study demonstrates that ruminants in Corsica are a reservoir for multiple Anaplasma species, whereas R. bursa seems to be a vector of A. marginale in cattle. The prevalence of Anaplasma spp. infection was high. The use of quantitative real-time PCR complemented with sequencing and genetic characterisation using two genes, rpoB and groEL, revealed an interesting diversity of Anaplasma spp. infection in small ruminants and R. bursa, including potentially new species and E. canis in one R. bursa tick. Nevertheless, characterisation studies are needed to ascertain the pathogenesis and/or the zoonotic potential of the strains and their significance for animals and public health.

**Abbreviations**

GroEL gene: Heat shock protein gene; qPCR: Quantitative real-time polymerase chain reaction; RpoB gene: RNA polymerase subunit beta gene
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Availability of data and materials

The data supporting the conclusions of this article are included within the article.

Authors’ contributions

MD, DT, BD, FF, OM and DR designed the study. MD, BD, FF, OM designed and MD carried out the data analysis. MD and OM drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All animals sampled in this study were examined with the assistance of their owners. Blood samples were collected by a veterinarian.

Consent for publication

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