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Holis-Tick : Identification et maitrise des maladies vectorielles transmises par les tiques en Corse

Vincent Cicculli

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**UNIVERSITA DI CORSICA-PASQUALE PAOLI
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UR 7310 BIOSCOPE MEDITERRANEE**

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BIOLOGIE**

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Le 16 décembre 2022

**HOLIS-Tick : Identification et maîtrise des maladies vectorielles transmises
par les tiques en Corse.**

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Abréviations

A : *Anaplasma*

ADN : Acide désoxyribonucléique

AHFV: Alkhumra Hemorrhagic Fever virus

ARN : Acide ribonucléique

B : *Borrelia*

BRBV : Bourbon virus

CCHFV : Crimean-Congo hemorrhagic fever virus

CTFV : Colorado Tick Fever virus

D : *Dermacentor*

ECDC : European Centre for Disease Prevention and Control

FDCCS : Fédération de chasse de Corse du sud

H : *Hyalomma*

HRTV : Heartland virus

I : *Ixodes*

ICTV : Comité International sur la Taxonomie des Virus (International Committee on Taxonomy of Viruses)

INSEE : Institut national de la statistique et des études économiques

INSERM : Institut national de la santé et de la recherche médicale

JMTV: Jingmen Tick virus

JMTVs: Jingmen Tick virus group

MLE : maximum likelihood estimation

OHFV : Omsk Haemorrhagic fever virus

OMS : Organisation Mondiale de la Santé

PCR : Réaction de Polymérisation en Chaîne ou Polymerase Chain Reaction

POWV : Powassan virus

PPAV : Virus de la Peste Porcine Africaine

R : *Rhipicephalus*

Ri. : *Rickettsia*

RdRp : RNA-dependant RNA polymerase

SARS-CoV 2: Severe Acute Respiratoy Syndrome Coronavirus 2

SFTSV: Severe Fever with Thrombocytopenia Syndrome virus

TBEV : Tick borne encephalitis virus

UR : Unité de recherche

UVE : Unité des Virus Emergents

Liste des publications scientifiques

- 1) Cicculi V, Maitre A, Ayhan N, Mondoloni S, Paoli JC, Vial L, de Lamballerie XN, Charrel R, Falchi A. Lack of Evidence for Crimean-Congo Hemorrhagic Fever Virus in Ticks Collected from Animals, Corsica, France. *Emerg Infect Dis.* 2022 May;28(5):1035-1038. doi: 10.3201/eid2805.211996. PMID: 35447051; PMCID: PMC9045455.

Rang: Q1

IF: 7

Nombre de citations: 0

- 2) Cicculi V, Capai L, Quilichini Y, Masse S, Fernández-Alvarez A, Minodier L, Bompard P, Charrel R, Falchi A. Molecular investigation of tick-borne pathogens in ixodid ticks infesting domestic animals (cattle and sheep) and small rodents (black rats) of Corsica, France. *Ticks Tick Borne Dis.* 2019 Apr;10(3):606-613. doi: 10.1016/j.ttbdis.2019.02.007. Epub 2019 Feb 13. PMID: 30777731.

Rang: Q1

IF: 3.75

Nombre de citations: 20

- 3) Cicculi V, Oscar M, Casabianca F, Villechenaud N, Charrel R, de Lamballerie X, Falchi A. Molecular Detection of Spotted-Fever Group Rickettsiae in Ticks Collected from Domestic and Wild Animals in Corsica, France. *Pathogens.* 2019 Sep 4;8(3):138. doi: 10.3390/pathogens8030138. PMID: 31487899; PMCID: PMC6789605.

Rang: Q1

IF: 4.4

Nombre de citations: 17

- 4) Cicculi V, De Carreaux D, Ayhan N, Casabianca F, de Lamballerie X, Charrel R, Falchi A. Molecular screening of Anaplasmataceae in ticks collected from cattle in Corsica, France. *Exp Appl Acarol.* 2020 Aug;81(4):561-574. doi: 10.1007/s10493-020-00527-w. Epub 2020 Jul 29. PMID: 32728778.

Rang: Q1

IF: 2.3

Nombre de citations: 6

- 5) Cicculi V, de Lamballerie X, Charrel R, Falchi A. First molecular detection of *Rickettsia africae* in a tropical bont tick, *Amblyomma variegatum*, collected in Corsica, France. *Exp Appl Acarol.* 2019 Feb;77(2):207-214. doi: 10.1007/s10493-018-00336-2. Epub 2019 Jan 17. PMID: 30656468.

Rang: Q1

IF: 2.3

Nombre de citations: 22

- 6) Cicculi V, Masse S, Capai L, de Lamballerie X, Charrel R, Falchi A. First detection of *Ehrlichia minasensis* in *Hyalomma marginatum* ticks collected from cattle in Corsica, France. *Vet Med Sci.* 2019 May;5(2):243-248. doi: 10.1002/vms3.140. Epub 2019 Jan 21. PMID: 30663864; PMCID: PMC6498527.

Rand: Q1

IF: 2.2

Nombre de citations: 22

- 7) Cicculi V, Ayhan N, Luciani L, Pezzi L, Maitre A, Decarreaux D, de Lamballerie X, Paoli JC, Vial L, Charrel R, Falchi A. Molecular detection of parapoxvirus in Ixodidae ticks collected from cattle in Corsica, France. *Vet Med Sci.* 2022 Mar;8(2):907-916. doi: 10.1002/vms3.700. Epub 2022 Jan 28. PMID: 35092189; PMCID: PMC8959304.

Rand : Q1

IF : 2.2

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Chapitre 1 :
Synthèse bibliographique

Introduction

Les dernières années témoignent du fardeau en santé publique de l'émergence des maladies vectorielles à tiques. Ces arthropodes parasites hématophages stricts sont aujourd'hui, les premiers vecteurs de maladies chez les humains dans l'hémisphère nord, et les premiers chez les animaux dans le monde avant les moustiques [1]. Les tiques revêtent une importance primordiale en santé publique et vétérinaire car elles sont des vecteurs d'agents pathogènes à la fois pour les humains et pour les animaux. Ces ectoparasites peuvent à la fois transmettre et être réservoir d'un panel très large de micro-organismes (bactéries, parasites et virus) [2].

Parmi les agents pathogènes responsables de maladies infectieuses émergentes, 75% sont d'origine zoonotiques [3], une proportion significative est vectorisée par les tiques. La prévalence de ces agents pathogènes augmente à l'échelle mondiale en raison des changements anthropogéniques (réchauffement climatique, artificialisation des milieux, urbanisation, morcellement des forêts, disparition des espèces prédatrices) qui mettent l'homme en contact plus étroit avec les tiques et augmentent leur aire de répartition [4, 5]. Les animaux restent un réservoir de microorganismes à partir desquels des agents pathogènes inconnus continuent à émerger. On estime que seulement environ un cinquième à un dixième des espèces pathogènes ont été répertoriées [3]. Considérées en priorité par leur impact en santé animale, ce n'est que récemment que les tiques et leurs maladies associées revêtent une importance d'intérêt médical. De nombreux foyers épidémiques de maladies à tique tendent à émerger à travers le globe, avec la découverte de nombreux nouveaux virus pathogènes. Parmi eux, nous pouvons citer le virus Bourbon et le virus Heartland aux Etats-Unis, le syndrome de fièvre sévère avec thrombocytopenie en Chine et de façon plus ubiquiste le virus Jingmen tick [6-8]. Des études approfondies sont nécessaires pour la compréhension de l'épidémiologie des maladies transmises par les tiques, afin d'éclairer, leur impact, leur transmission et leur pathogénicité et la mise en place des mesures diagnostiques. En effet, la capacité de diagnostic d'une épidémie est fortement liée à la capacité d'endiguement de celle-ci. La détection des pathogènes permet notamment une surveillance entomologique pour anticiper et se préparer avant l'urgence sanitaire.

Partie I : Généralités sur les tiques

Les premières traces écrites sur les tiques ont été référencées pour la première fois en Egypte selon les écrits par Von Oefele (1911), où elles sont nommées ricinus pour leur ressemblance avec les grains de ricin (*Ricinus communis*). Le rôle des tiques en tant que vecteurs de maladies est démontré beaucoup plus tardivement grâce aux travaux de T Smith et FL Kilborn de 1889 à 1893, qui en plus de prouver leur rôle infectieux, constituent la première démonstration complète de la transmission d'un organisme pathogène par un arthropode [9].

1. Taxonomie des tiques

Les tiques de par leur morphologie, avec la présence d'appendices articulés et d'un exosquelette appartiennent à l'embranchement des arthropodes au sein du règne animal [9].

Les tiques sont classées en trois familles monophylétiques : les Nuttalliellidae, les Argasidae appelées « tiques molles » et enfin les « tiques dures » de la famille des Ixodidae, qui seront le sujet de cette thèse.

Ce groupe se compose de plusieurs genres : *Amblyomma*, *Anomalohimalaya*, *Bothriocroton*, *Cosmiomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes Margaropus*, *Nosomma*, *Rhipicentor* et *Rhipicephalus*, dont au moins six (*Amblyomma*, *Ixodes*, *Hyalomma*, *Rhipicephalus*, *Haemaphysalis* et *Dermacentor*) ont déjà été identifiées en Corse [10-13].

2. Biologie des tiques

Les tiques sont des arthropodes, arachnides, acariens parasites hématophages stricts des vertébrés, où elles alternent leur phase parasitaire de quelques jours, à quelques semaines, avec des phases libres de plusieurs mois.

2.1 Cycle de vie

Les tiques dures possèdent trois stades de développement. Après un repas sanguin et un accouplement, la tique femelle tombe sur le sol et pond ses œufs dans la litière. Les larves qui éclosent se mettent à la recherche d'un hôte. Une fois gorgées, les larves muent en nymphes qui muent, après repas sanguin, en individus adultes.

Il existe chez les tiques dures trois sortes de cycles biologiques [9] :

- Monophasique où tous les stades se nourrissent et se métamorphosent sur le même hôte
- Diphasique composé de deux stades parasitaires distincts où la larve et la nymphe se nourrissent sur le même hôte puis tombent pour réaliser leur métamorphose en adulte afin de se nourrir sur un nouvel hôte
- Tri phasique qui comporte trois phases parasitaires : où chaque stade effectue son repas sur un hôte différent; c'est la situation la plus fréquente.

2.2 L'hôte

Les préférences trophiques des tiques sont complexes, car elles dépendent de plusieurs facteurs selon les relations étroites avec un hôte spécifique, selon l'écologie et selon les biotopes. Elles sont référencées par les travaux de Morel en 1969. Lorsqu'une espèce ne parasite qu'une seule espèce hôte, voire une famille d'hôtes, on appelle cela la spécificité parasitaire. On la retrouve dans des micro-habitats restreints où le nombre d'hôtes est limité. Les tiques ont évolué en relation étroite avec leur hôte. Il arrive pour certaines espèces de tiques que leur propre évolution spécialisée pour un hôte spécifique ne leur permette plus de se nourrir différemment.

Il existe aussi une sélectivité parasitaire où certains stades vont préférer certains hôtes. Les larves et les nymphes vont favoriser les hôtes de petite taille avec une peau plus fine (oiseaux ou rongeurs), alors que les stades adultes vont préférer les grands mammifères.

Certaines tiques n'ont pas de spécificité parasitaire et se nourrissent sur un nombre d'hôte très large. La même espèce peut donc se retrouver dans un grand nombre d'habitats, tels que les forêts, les prairies et les ourlets forestier. On peut citer comme exemple la tique *Ixodes ricinus* (*I ricinus*).

3. Morphologie des tiques

3.1 Anatomie externe

Les tiques dures du genre *Ixodidae* ont un corps divisé en deux parties le capitulum et l'idiosome

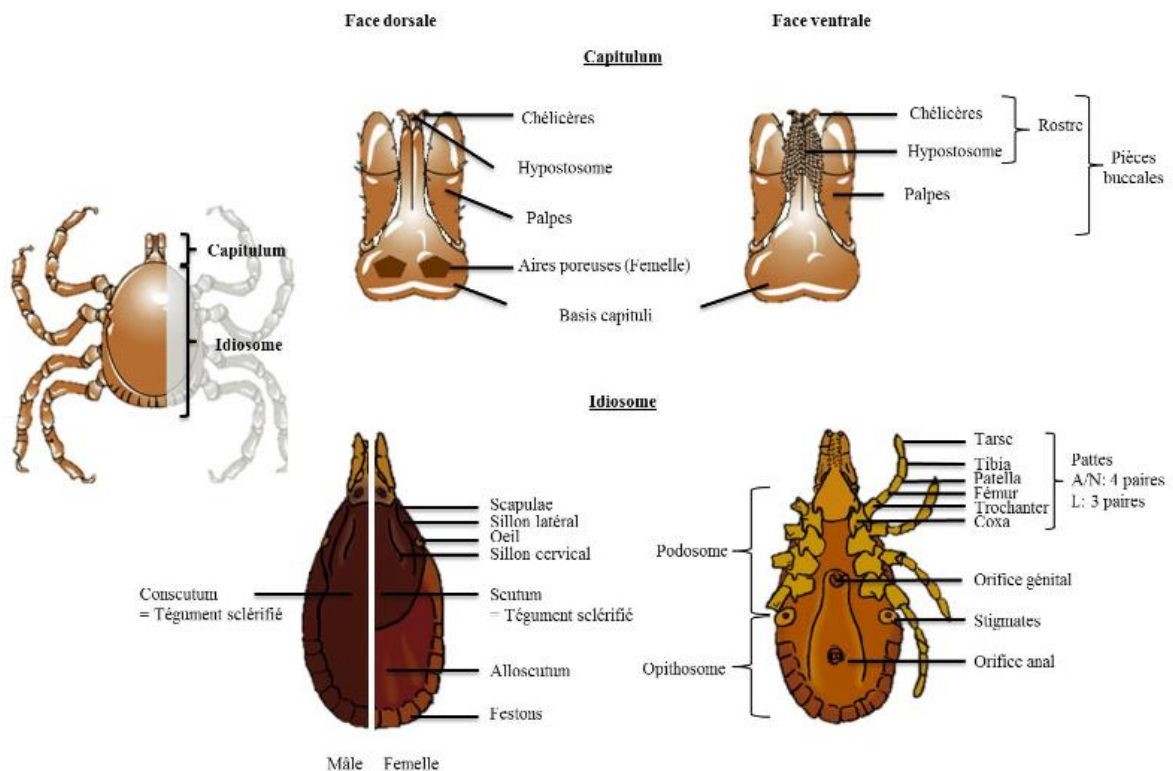


Figure 1 : Morphologie externe des tiques dures (McCoy et Boulanger 2015; Pérez-Eid 2007)

3.1.1 Le capitulum

Le capitulum permet à la tique de se nourrir de sang, il est composé de la partie basse « *basis capituli* » et des parties buccales constituées des palpes (rôle sensoriel) et du rostre qui pénètre dans la peau. Les chélicères permettent au parasite de couper dans la chair. Elles sont couplées à un appendice harpon appelé hypostome muni de rangées de dents dirigées vers l'arrière. Une salive digestive et anesthésiante semblable à du venin d'araignée permet de dissoudre les tissus et facilite le repas sanguin. Une fois installée, la tique parfait son ancrage par la sécrétion d'une substance appelée ciment. Les femelles possèdent des aires poreuses situés sur leur *basis*

capituli, ces organes ont un rôle lors de la ponte car ils secrètent des substances lipidiques anti-oxydantes dont la femelle enrobe les œufs pour les protéger de la déshydratation [9].

En systématique, le capitulum est souvent utilisé, car il échappe à la déformation dû au gorgement et sa forme varie selon les genres.

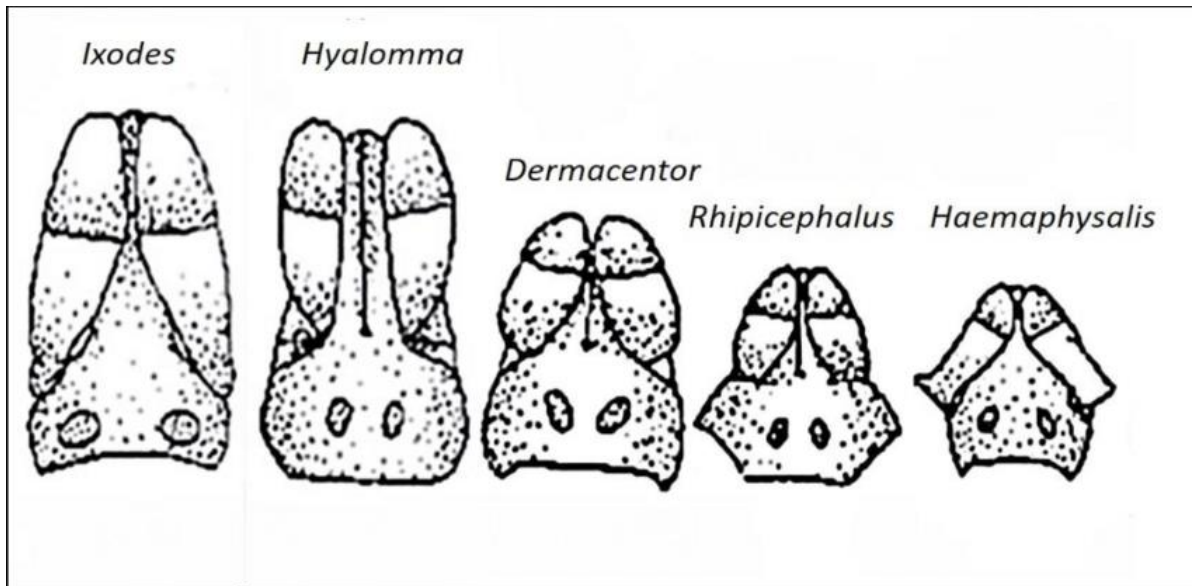


Figure 2 : Capitulum des cinq genres de tiques dures présentes en zone méditerranéenne (Rhodain et Pérez, 1985)

3.1.2 L'idiosome

L'idiosome est la partie antérieure de la tique, il est composé d'un tégument qui comporte des parties rigides chitinisées et sclérifiées et des parties souples. La composition du tégument varie selon les stades et le sexe de la tique. Chez les stades immatures et chez la femelle seule, la partie antérieure appelée scutum est sclérifiée, la partie postérieure l'alloscutum est plus souple et lui permet de s'étendre lors du gorgement. Pour les mâles, le tégument sclérifié qui recouvre la face dorsale entièrement est appelé conscutum [14].

3.1.3 Les organes sensoriels

La tique possède un arsenal sensoriel qui lui permet de chasser soit à l'affut (*Ixodes*) ou en chasse active (*Hyalomma*). Ces organes sensoriels varient selon l'espèce ou le genre. On retrouve des yeux, des poils tactiles, des pores, des sensilles. Il existe à l'extrémité des premières paires de pattes, l'organe de Haler qui permet au parasite de suivre la trace d'un hôte

à 15 mètres de distance et de capter les phéromones émises par leur congénère, ainsi que l'humidité ambiante [15].

3.2 Anatomie interne

3.2.1 Le système digestif

Les tiques alternent des phases d'émission de salive avec des phases d'aspiration du sang. On retrouve les glandes salivaires au fond de la cavité buccale, en forme de grappe (les acini). Elles permettent la fabrication d'une salive digestive qui aide le rostre à pénétrer dans la peau, puis facilitent le prélèvement de sang grâce à des molécules anesthésiantes, anticoagulantes, anti-immunitaires et anti-inflammatoires [16].

Les glandes comportent 4 types d'acini selon la fonction de la salive, à savoir la formation de ciment, la régulation de l'eau ou la formation de complexe moléculaire pour lyser les tissus.

L'estomac des tiques est très développé, il est composé d'une cavité centrale ramifiée en diverticules où la digestion du sang, des tissus et des fragments cellulaires est réalisée. C'est là que l'assimilation des produits de la digestion se fait; ceci s'explique par le fait que leur intestin est très court et ne sert qu'à l'évacuation des selles.

3.2.2 Le système respiratoire et circulaire

Chez les larves, le tégument très fin permet une respiration transcutanée à travers la cuticule. Les nymphes et les adultes disposent, comme la plupart des arachnides, d'un système trachéolaire constitué de multiples canaux qui permettent les échanges gazeux grâce aux différentes contractions/décontractions du thorax. Le système circulatoire est aidé par un cœur primaire qui permet de propulser l'hémolymphe dans les organes. L'hémolymphe a plusieurs fonctions, notamment le transport des métabolites, des hormones, le maintien de la pression osmotique, et le système immunitaire. Il est riche en plasma et en hémocytes, cellules de l'immunité qui synthétisent des protéines antimicrobiennes [9].

3.2.3 Reproduction

Lorsque la femelle trouve un hôte, elle commence à se nourrir et ne pourra finir son repas qu'en cas d'accouplement. En effet, des substances inhibitrices sont inactivées lors de la rencontre avec un mâle. Ce mécanisme intervient car le gorgement engendre la maturation du système reproductif et donc du mécanisme de ponte, sans fécondation les œufs seront stériles [17]. Les mâles restent plusieurs semaines sur l'hôte en attendant les femelles, ils alternent des phases de repas avec des phases de recherche de femelles et d'accouplement ou ne se nourrissent pas pour

certaines espèces. Les femelles ne restent que le temps du repas et tombent au sol une fois gorgées pour pondre. Elles enrobent leurs œufs dans des substances anti-oxydantes et cireuses riches en lipides synthétisées dans les aires poreuses pour éviter leur déshydratation,.

4. Risque médical et vétérinaire

Les tiques sont des parasites des vertébrés. C'est lors de ce parasitisme hématophage qu'elles peuvent acquérir certains pathogènes et les disséminer. En participant à la maintenance de ces pathogènes dans les milieux par différents moyens de transmission intra-espèce (passage trans-ovarien, accouplement, passage trans-stadial) mais aussi par la transmission à leur hôte, cela les qualifie de vecteur et de réservoir de pathogènes. Les infestations de tiques peuvent avoir des conséquences néfastes sur la productivité des élevages et des denrées alimentaires qu'ils produisent. Les tiques sont responsables d'épidémies émergentes et réémergentes en santé animal et humaine [18]. Il est donc nécessaire de connaître leurs cycles biologiques pour comprendre l'épidémiologie des infections qu'elles transmettent, afin de mettre en place des moyens de lutte et de gestion des risques efficaces contre celles-ci.

4.1 Risques directs

Il existe un impact direct dû à l'infestation des tiques sur l'animal. En cas d'infestation massive, la spoliation sanguine peut être un problème majeur, notamment lorsque le nombre de tiques sur un hôte est trop important ou bien lorsque des espèces plus grosses sont présentes. Il existe aussi des risques de paralysie, toxicose, dyshidrose tropicale, réaction allergique pour certaines espèces. De plus, les morsures de tiques sont une entrée aux différents pathogènes infectieux de la peau et provoquent des plaies cutanées qui engendrent, dans certain des cas, des surinfections [18]. Les pertes économiques dues au cout de la lutte chimique s'estime à des centaines de millions de dollars par an et par pays [18].

4.2 Risques indirects

Les tiques sont les premières vectrices de maladies chez les animaux et les premières chez l'Homme dans les pays développés [1]. Elles représentent la plus grande diversité des pathogènes transmis (virus, bactérie, protiste, parasite)[14]. Elles agissent à la fois comme vecteurs mais aussi comme réservoir de certains pathogènes car la capacité de transmission trans-générationnelle, c'est-à-dire d'un parent à sa descendance, permet la survie des

pathogènes dans le milieu. La circulation des germes transmis par les tiques est accrue par leur comportement et leur biologie, car à toutes les étapes de leur vie, les tiques se nourrissent de sang. A chacun des stades elles changent, pour la plupart, d'hôtes à chaque repas et pour des individus (hôte) d'espèces variées, ce qui décuple l'effet disséminateur. Leurs hôtes peuvent les transporter sur des distances considérables, particulièrement grâce aux oiseaux migrateurs [12]. Leur longévité assure une persistance sur une zone et dans le temps, des pathogènes.

5. Les tiques et les changements globaux

On s'attend à ce que la région méditerranéenne subisse de profonds changements climatiques et anthropiques dus à l'activité humaine. La proximité de la zone méditerranéenne et particulièrement de la Corse avec le continent africain, en fait, une aire particulièrement exposée à l'importation d'espèces de tiques d'Afrique et des pathogènes qu'elles véhiculent [19]. Il existe de nombreux facteurs responsables de l'émergence des tiques et des maladies vectorisées par les tiques. Les changements climatiques favorisent l'apparition de nouvelles espèces de tiques dans des nouveaux territoires, avec par exemple, l'apparition de la tique *Hyalomma marginatum* (*H. marginatum*) dans le sud de la France continentale ou encore la tique *I. ricinus* doublant sa zone de répartition dans le nord de la Suède ou du Canada [20, 21]. Chaque espèce de tique est vectrice de pathogènes qui lui sont plus ou moins spécifiques; l'apparition de nouvelles espèces engendre un risque d'introduction de nouveaux pathogènes. Le principal effet du changement climatique sur les tiques et leurs agents pathogènes est l'augmentation des températures [22]. Elle induit une amélioration des conditions de survie et de reproduction des tiques et un développement plus rapide, ce qui conduit à une accélération du cycle de vie et à une augmentation de l'abondance. De plus, une augmentation des températures permet l'accroissement de leur activité de quête par l'augmentation de nombres de jours d'activité saisonnière [22].

Parmi les facteurs responsables de cette émergence, on retrouve aussi la perte de biodiversité et des prédateurs par l'urbanisation incontrôlée, la chasse, le morcellement des forêts et la pollution, comme précurseurs de l'émergence des maladies vectorielles à tiques. L'analyse de la structure paysagère des parcelles de forêt réalisée à l'aide d'images satellites, a révélé un lien positif entre la fragmentation et la densité des tiques/prévalence de pathogènes chez ces dernières. L'augmentation de la fragmentation du paysage par la diminution de la taille des parcelles a vu augmenter la prévalence d'infestation par les tiques, tandis que l'augmentation de la distance entre les parcelles a vu augmenter la densité des tiques. L'effet du paysage sur la

prévalence de l'infection par les tiques est médié par la composition des petits mammifères et des oiseaux, dont beaucoup sont des réservoirs compétents [23]. En particulier, il a été démontré que les rongeurs ont une relation positive avec la fragmentation des habitats dans lequel la taille des parcelles de forêt a été réduite. Ces événements favorisent la prolifération des espèces réservoir et augmentent ainsi la circulation de maladies vectorisées dans leur environnement [24, 25]. De plus, des études ont indiqué que la lutte contre la fragmentation réduirait le risque de maladie de Lyme en influençant le risque entomologique [26]. On peut citer aussi la disparition des prédateurs principalement du renard roux (*Vulpes vulpes*). Ce prédateur est spécialiste des rongeurs; il régule leurs populations et abaisse le nombre de tiques présentes l'environnement [27]. La raréfaction de ce prédateur a des effets en cascade sur l'augmentation de l'incidence des maladies transmises par les tiques. La protection des espèces prédatrices telles que le renard roux, la martre, le putois ou le blaireau permet de diminuer la prévalence des maladies transmises par les tiques [5, 27].

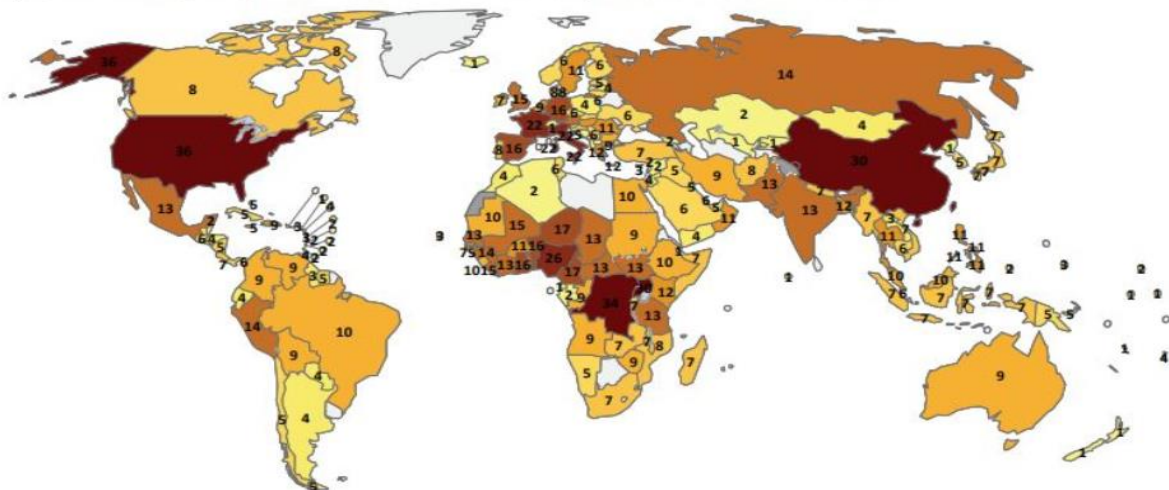
Partie II : les Pathogènes d'intérêts transmis par les tiques

1. Emergence et réémergence des maladies infectieuses

Les maladies infectieuses évoluent aussi en suivant le changement de leurs environnements. L'émergence et la réémergence des maladies infectieuses en est la preuve avec l'apparition de nouvelles menaces infectieuses dans notre environnement. Dans les années 70, l'arsenal dont disposait la médecine permettant de traiter plus efficacement la plupart des infections bactériennes par l'antibiothérapie et les campagnes de vaccination de masse firent éclore l'idée d'un contrôle absolu et de l'éradication des maladies infectieuses. En 1978, le docteur William H. Stewart aurait déclaré « *Le moment est venu de fermer le livre des maladies infectieuses et de déclarer gagnée la guerre contre les pestilences* ». Trois ans plus tard et pendant 40 ans suivant, le virus de l'immunodéficience humaine causa la mort de 32 millions de personnes et constitue une des épidémies les plus meurtrières. La capacité d'évolution de la nature se montre sans limite, s'adaptant à de nouvelles espèces, aux antibiotiques, aux antiviraux et aux vaccins. L'histoire nous apprend qu'une maladie ne disparaît que rarement, préférant laisser la place à une nouvelle. (Figure 3)

Burden of epidemics: illustrations

Epidemic events* globally, 2011 – 2017** : A total of 1,307 epidemic events, in 172 countries



* Analysis excluded Poliomyelitis. The following epidemic and pandemic diseases were analysed: Avian Influenza A(H5N1), A(H7N9), A(H7N6), A(H10N8), A(H3N2), A(H5N6), A(H9N2), Chikungunya, Cholera, Crimean-Congo haemorrhagic fever, Ebola virus disease, Lassa fever, Marburg virus disease, Meningitis, MERS-CoV, Monkeypox, Nodding syndrome, Nipah virus infection, Plague, Rift Valley fever, Shigellosis, Typhoid fever, Viral haemorrhagic fever, West Nile fever, Yellow fever, Zika virus disease. If a disease caused more than 1 epidemic event in a country, it was only counted once for the year it occurred in that country. Includes cases imported or locally transmitted.

** WHO/IHM data as of 12 January 2018 (note: 2017 data is not complete)

Source: data reported to WHO and in media

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2018. All rights reserved

Figure 3 : Poids des épidémies au niveau mondial, selon les alertes épidémiques de l'Organisation Mondiale de la Santé.

Une définition épidémiologique de l'émergence est proposé en 2003 par Toma et Thiry: *maladie dont l'incidence réelle augmente de manière significative dans une population donnée, dans une région donnée ou pendant une période donnée, par rapport à la situation épidémiologique habituelle de cette maladie* [28]. L'augmentation de cette incidence responsable d'une émergence d'un pathogène, est un phénomène qui peut avoir différentes origines. Parmi elles, la mise en place d'un nouvel outil diagnostique peut faire "artificiellement" penser à une augmentation d'incidence bien que la maladie soit déjà bien installée. Dans le cas d'une émergence vraie, l'incidence de la maladie augmente après son apparition dans une nouvelle population. On peut citer comme exemple, l'apparition d'un nouveau pathogène (SARS-CoV-2), l'introduction d'un pathogène dans de nouvelles zone géographique (virus West Nile aux Etats-Unis) ou une augmentation des cas, caractérisé par une forme de résistance aux médicaments (tuberculose)[29].

2. Maladies zoonotiques et arbovirus

La plupart des maladies infectieuses sont des zoonoses, c'est à dire des maladies qui peuvent se transmettent des animaux vertébrés à l'homme et vice-versa. Elles causent des millions de cas humains chaque année restant ainsi une barrière dans le développement social et économique de nombreux pays en voie de développement [30]. Parmi elles, les arbovirus (arthropode-borne virus), sont virus transmis par des arthropodes vecteurs. La transmission directe entre humains est soit inexistante soit très limitée. Parmi les vecteurs arthropodes d'arbovirus, on retrouve les moustiques, les tiques, les phlébotomes ou encore les culicoïdes.

1,307 epidemic events happened between 2011 and 2017

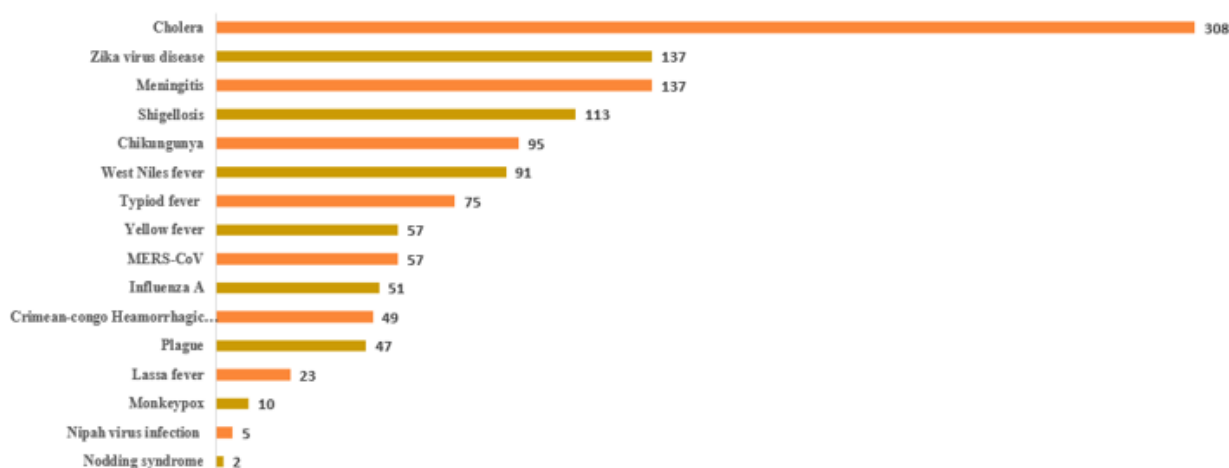


Figure 4 : Evènements épidémiques apparus entre 2011 et 2017 selon l'organisation mondiale de la santé.

L'écologie des vecteurs limite et détermine le cycle des arbovirus; c'est le cas des préférences trophiques des vecteurs vis-à-vis de certains hôtes vertébrés. Pour beaucoup d'arbovirus, l'homme est un hôte accidentel, une impasse épidémiologique, qui ne participe pas au cycle naturel du virus. Dans le cas de la dengue, le moustique *Aedes aegypti* a une préférence trophique pour l'homme qui joue le double rôle de réservoir et de victime [31]. Dans certains cas, le vecteur arthropode a un rôle de réservoir en participant au maintien du virus dans un milieu, grâce notamment aux transmissions transovarienne ou vénérienne [32].

Les arbovirus sont des virus à génome ARN (à l'exception de African Swine Fever virus); leur taux de mutation est plus important que celui des virus à génome ADN en raison d'un manque de propriété de relecture de leur ARN polymérase ARN dépendante. On peut citer l'adaptation de la souche E1 A226V du virus Chikungunya qui rend les moustiques plus infectieux et qui s'est adaptée à un autre vecteur que celui d'origine [33]. D'autres phénomènes participent à la variabilité génétique de ces virus, comme la recombinaison génétique ou les réassortiments génétiques. Qu'il s'agisse de virus ou de bactéries, leur multiplication est dépendante de leurs interactions avec les espèces qu'ils infectent. Plus ces interactions sont nombreuses notamment lors d'une épidémie, plus la multiplication des agents pathogènes est importante et plus l'évolution de ces agents pathogènes sera favorisée.

Plusieurs facteurs peuvent conduire à l'émergence et la réémergence des maladies infectieuses zoonotiques, les trois principaux sont :

- Les changements climatiques sont un exemple de la manière dont nous avons altéré l'interface entre l'homme et les réservoirs sauvages. Parmi les nombreux effets en cause,

on peut citer l'expansion de l'aire de répartition des vecteurs qui augmente le risque d'épidémie d'arbovirus. Comme c'est le cas d'*Aedes albopictus* et *A. aegypti* qui ont profité d'une augmentation des températures et de la pluviométrie pour se développer au-delà des frontières [34]. Les épidémies d'arbovirus, telles que Chikungunya en Amérique et en Europe, Zika en Amérique du Sud et de fièvre jaune au Brésil en sont la preuve [35-37]. D'autres changements environnementaux dus aux activités anthropiques favorisent l'apparition de ces arbovirus comme la déforestation, l'agriculture intensive et l'urbanisation. L'homme envahit les espaces naturels, altérant les étroites relations entre l'environnement, les vecteurs et les hôtes [38]. En conséquence, les contacts entre arthropodes et vertébrés augmentent rapidement, parallèlement au risque d'épidémie. Les modifications de l'habitat naturel des vecteurs et des réservoirs sont les conditions nécessaires pour que les cycles de transmission sauvage deviennent des cycles de transmission urbaine, caractérisés par une rapide augmentation de la transmission humain-moustique-humain [39].

- Un facteur important de l'émergence des maladies est la modification des interactions entre homme et vecteurs, notamment dans le cas d'une forte croissance démographique ou d'une urbanisation incontrôlée. Ces endroits offrent un milieu de vie favorable aux vecteurs par la présence d'hôtes humains et de conteneurs artificiels d'eau pour les stades immatures. Le moustique trouvant donc toutes les conditions pour un établissement permanent. Une forte urbanisation présente un impact dans les populations de tique car le morcellement des forêts favorise la survie des espèces réservoirs par la disparition des espèces prédatrices [24, 25, 40].
- Enfin, la mondialisation favorise la dissémination des maladies infectieuses par l'augmentation des voyages et le commerce international. Les voyages permettent la rapide dissémination des pathogènes et parfois des vecteurs et des animaux réservoirs. Il existe aussi le risque pour les voyageurs/touristes de ramener dans leur pays d'origine un virus étranger, les voyageurs agissent donc comme transporteurs. Ce phénomène a été notamment référencé avec le virus MERS-CoV. En effet, en 2015 un voyageur infecté au moyen orient a importé virus en Corée initiant une épidémie de près de 200 cas (38 décès) [41]. De même le virus Zika a été introduit au Brésil lors de la coupe du monde de 2014 [42].

Ces facteurs sont couplés à des facteurs sociaux, économiques, environnementaux et écologiques. Ils sont tous impliqués dans l'apparition et l'émergence des maladies infectieuses.

3. Les virus transmis par les tiques

Les virus transmis par les tiques sont des arbovirus transmis lors du repas sanguin de la tique sur son hôte vertébré. Il existe plus de 170 virus transmis par les tiques. Ceux-ci ont été détectés dans plusieurs familles de tiques Argasidae et Ixodidea qui comprennent les genres, *Amblyomma*, *Argas*, *Ixodes*, *Haemaphysalis*, *Hyalomma*, *Dermacentor*, *Rhipicephalus*, *Boophilus* et *Ornithodoros*. A l'exception du virus de l'encéphalite à tique et de la fièvre hémorragique Crimée-Congo, ils sont moins étudiés que les virus transmis par d'autres arthropodes: sur PubMed NCBI, les mots clés "virus+mosquito" retrouvent près de 19000 références alors que la combinaison "virus+tick" rapporte 7650 références; en remplaçant virus par bacteria, la tendance reste la même: 16628 contre 7649 références. Pourtant, récemment deux pathogènes majeurs ont été découverts, Dabie bandavirus et le virus Bourbon [43, 44].

3.1 Le virus de la fièvre hémorragique Crimée-Congo



Figure 5 : Répartition géographique du virus de la Fièvre Hémorragique Crimée-Congo

Le virus :

Le virus de la fièvre Hémorragique Crimée-Congo (CCHFV) appartient à la famille des *Bunyaviridae* et fait partie du genre *Orthonairovirus*. Son génome est constitué de trois segments S, M et L qui codent respectivement pour, la nucléocapside, le précurseur de la glycoprotéine et l'ARN polymérase ARN dépendante. Ce virus a été découvert pour la première fois dans la péninsule de Crimée causant une épidémie de fièvre hémorragique sévère. Il fut nommé Fièvre Hémorragique Crimée-Congo suite à son isolement en 1958 au Congo.

Géographie :

Son aire de répartition dépend fortement de celle de ses tiques vectrices principalement du genre *Hyalomma*. Le cycle enzootique du virus implique un grand nombre d'espèces de tiques et d'hôtes mammifères pour la plupart asymptomatiques, seuls les simiens présentent une forme hémorragique. Les lagomorphes semblent jouer un rôle important dans le cycle naturel du virus [45].

Epidémiologie :

Les tiques sont elles-mêmes vectrices et réservoir du virus car les transmissions transovariennes, transstadiales et vénériennes sont décrites [46]. La Fièvre hémorragique

Crimée-Congo est responsable de nombreux cas humains chaque année principalement en Turquie avec plus de mille cas par an [47] et en Russie avec 1654 cas entre 1999 et 2013 [48]. En Europe entre 2013 et 2021, selon le centre Européen de prévention et de contrôle des maladies (ECDC), 48 cas ont été référencés dont 8 morts, principalement en Bulgarie avec plus de 70% des cas européens. Les trois autres pays sont l'Espagne avec 10 cas, la Grèce avec 1 seul cas et le Royaume-Uni avec un cas importé de Bulgarie. De plus entre 2013 et 2017, l'Organisation Mondiale de la Santé a enregistré 49 évènements épidémiques de CCHFV sur 1307 évènements épidémiques causés par les 20 maladies responsables d'épidémies les plus fréquentes, classent le virus 11^{ème} en nombre d'évènements épidémiques sur la même période. La transmission se fait principalement par morsure de tique, mais d'autres formes de transmissions ont déjà été décrites, notamment nosocomiales après contact avec les fluides corporels ou de tissus biologiques infectés. Dans ce cadre, les ouvriers d'abattoirs et les éleveurs sont à fort risque d'exposition au virus. En Turquie, une étude de séroprévalence montre que les personnes rapportant des morsures de tiques étaient plus susceptibles d'être porteuses d'anticorps spécifiques [49]. Enfin, on retrouve une saisonnalité des cas en lien avec l'activité des tiques vectrices: en Turquie, 70 % des cas ont lieu en Juin et Juillet [50]. Sur les 48 cas rapportés en Europe depuis 2013, 47 cas ont été liés à des morsures de tiques et un cas est une transmission nosocomiale.

Symptômes :

L'identification des foyers est aisée car ils se manifestent par des poussées épidémiques estivales. Après une incubation de 24h à 9 jours, les signes cliniques décrits sont peu spécifiques à type de fièvre, de rash cutané, de myalgies, de céphalées, de nausées, et de douleurs digestives. Les signes hémorragiques apparaissent après 3 à 5 jours. Le taux de létalité de 5-80% selon les pays est lié au niveau sanitaire du pays et aux possibilités de prise en charge médicale et réanimatoire [51].

3.2 Le virus de l'encéphalite à tiques

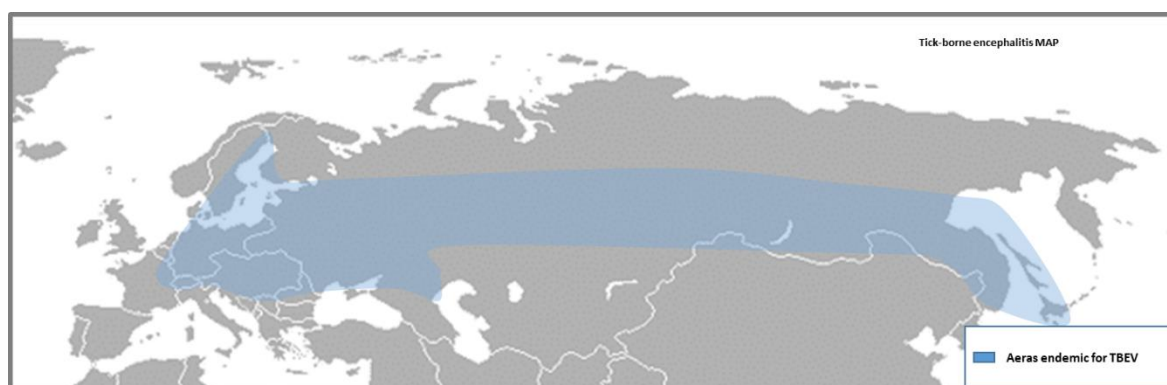


Figure 6 : Répartition géographique du virus de l'encéphalite à tique

Le virus :

Le virus de l'encéphalite à tiques (TBEV) appartient à la famille des *Flaviviridae* et au genre *Flavivirus*, Le génome du virus fait environ 11kb et est constitué d'ARN simple brin à polarité positive qui code pour une polyprotéine par la suite clivée en 10 protéines virales [52]. La maladie est décrite pour la première fois en Autriche en 1931 et le virus est isolé pour la première fois en Russie en 1937 [53].

Géographie :

L'encéphalite à tiques représente l'une des plus importantes maladies transmises par les tiques en Europe et en Asie avec plusieurs milliers de cas humains chaque année [54]. Il existe trois génotypes viraux: la souche Européenne, transmise par *I. ricinus*, couvre la majeure partie de l'Europe occidentale, la souche Sibérienne et la souche Far-Eastern, toutes deux transmises par *I. persulcatus* sont présentes en Sibérie et dans les régions de l'Est, respectivement [53]. Cependant, des cas ont déjà été rapportés en dehors de ces zones de manière sporadique comme en Sicile [55].

Epidémiologie :

Les rongeurs et les populations de tiques sont impliqués dans le cycle du virus. Les réservoirs sont des micromammifères de types campagnols et mulots [56]. Les tiques sont considérées comme vectrices, mais aussi comme réservoirs, car des transmission par co-feeding et par voie trans-ovarienne ont déjà été décrites [57]. Les cas apparaissent de manière saisonnière et correspondent à l'activité saisonnière des tiques au printemps. Si la morsure de tiques reste la principale cause d'infection à TBEV, d'autres modes de transmission sont possibles notamment la consommation de produits laitiers non pasteurisés. On peut citer le récent foyer de cas d'encéphalites à tiques lié à la consommation de fromage de chèvre au lait cru dans l'Ain en

2020 en France. Plus de 40 cas ont été référencés dont deux ont été admis en réanimation, ce foyer épidémique est le premier identifié en France lié à la consommation de produits laitiers non pasteurisés [58].

Symptômes :

Les symptômes apparaissent entre une et quatre semaines après l'infection et s'accompagnent d'un syndrome grippal. Dans la moitié des cas symptomatiques des signes méningés surviennent, tels que des troubles de l'équilibre, tremblements et des troubles de la coordination, et dans moins de 10% des cas, des signes de myélite. Enfin, une forme chronique peut intervenir dans 10% à 20% et se manifeste par des séquelles nerveuses importantes et des troubles neuropsychiatriques [53].

3.3 Le virus de la Peste Porcine Africaine

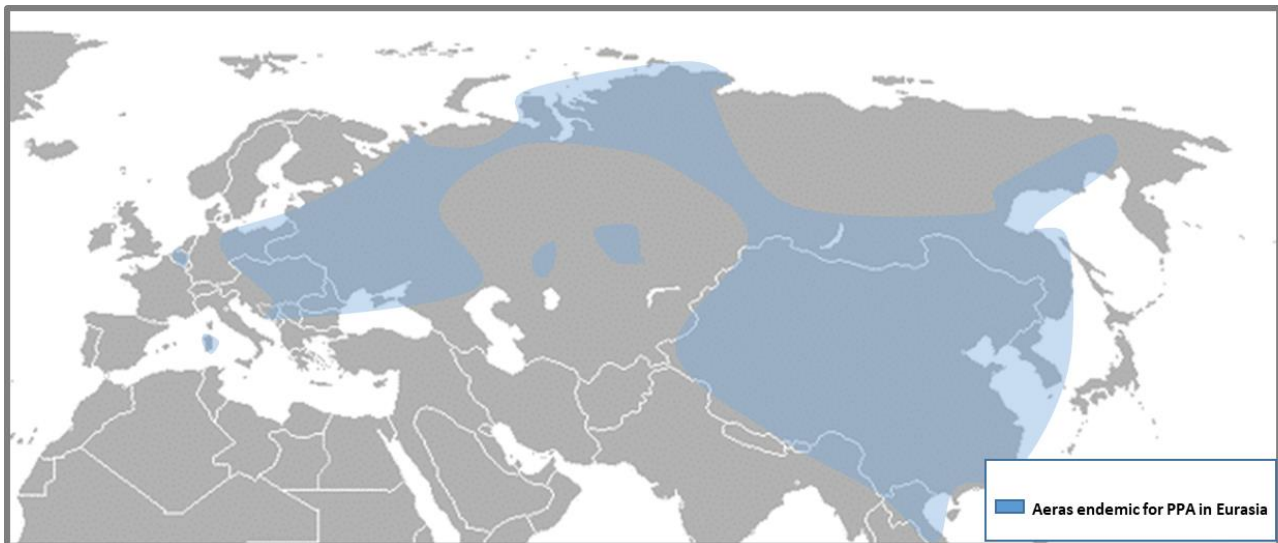


Figure 7 : Répartition géographique du virus de la Peste Porcine Africaine

Le virus :

Le virus de la Peste Porcine Africaine (ASFV, African swine fever virus) appartient à la famille des *Asfarviridae* et au genre *Asfivirus*. Le virus possède un ADN double brin enveloppé qui varie entre 170 et 193kbp. Il est le seul représentant décrit de sa famille et infecte exclusivement les suidés.

Géographie :

La peste Porcine Africaine est enzootique en Afrique de l'Est et Australe. Dans les années 1957 à 1960, elle s'est propagée au Portugal puis s'est étendue en Europe. L'éradication de ce fléau s'est achevée en 1990 en Europe, à l'exception de la Sardaigne où la maladie reste endémique aujourd'hui [59]. Une nouvelle introduction en Géorgie en 2007 annonce une nouvelle période de diffusion de la maladie en Europe de l'Est jusqu'en Belgique. La Chine principal producteur de viande porcine est touché en 2018 et la dissémination se propage vers tout le bassin Asiatique, de la Mongolie aux Philippines et jusqu'en Corée du Nord [59]. Récemment le Département Américain de l'Agriculture a signalé l'apparition de la maladie dans les Caraïbes, en République Dominicaine; l'origine de cette introduction reste inexpliquée. Cependant, une analyse du risque d'introduction de la PPA dans les pays de l'OIRSA (Organismo Internacional Régional de Sanidad Agropecuaria) par l'introduction de manière légale ou illégale de produits d'origine porcine qualifiait le risque d'introduction de la PPA d'élevé en provenance des pays Européen affectés [60]. En effet, le virus est très résistant, dans l'environnement et dans les

carcasses et les déjections des animaux infectés [61]. Le virus reste infectieux 3 mois dans les selles et 4 mois dans les muscles [62].

Epidémiologie et symptômes :

Le virus se transmet par contact direct entre animaux, par consommation et contact avec des aliments ou milieux souillés et par morsure de tiques molles de type *Ornithodoros* jouant un rôle de vecteur mais aussi de réservoir [63]. L'infection des porc domestiques provoque une fièvre hémorragique avec un taux de létalité avoisinant les 100%. Les suidés sauvages africains sont asymptomatiques. L'impact social et économique de la maladie est très important dû à la perte d'activités et les coûts de contrôle de la maladie affectant les cheptels [59].

3.4 Les Jingmen tick virus

Les virus :

Le virus Jingmen tick représente en réalité un groupe de nouveaux virus appartenant à une nouvelle famille virale. Ce sont des virus segmentés dont certaines protéines présentent de fortes similitudes avec les protéines non structurales des *Flavivirus*. Leur ARN est enveloppé et est formé de 4 segments, simple brin à polarité positive d'environ 11 kbp. Le Jingmen Tick virus (JMTV) a été découvert pour la première fois en Chine en 2014, presque simultanément au Brésil dans des tiques et sur des bovins virémiques [64]. Différentes études tendent à prouver son caractère d'arbovirus comme une transmission trans-stadiale et trans-ovarienne [65-67], une infection expérimentale du virus dans les tiques et une migration du virus dans les glandes salivaires [68]. En 2019, le virus Alongshan très proche génétiquement et structurellement du JMTV original est détecté chez plusieurs patients en Chine et en Mongolie. Il est associé à des symptômes cliniques communs avec d'autres arbovirus et le virus a pu être isolé à partir de sérum de patients infectés après morsure de tique [69]. La même année des symptômes cliniques sont associés avec le JMTV en Chine chez l'Homme [68].

Géographie :

Les JMTVs sont présent de manière ubiquiste car leur présence est avérée en Chine, Uganda, Kenya, Brésil, Turquie, Kosovo, Japon et au Cambodge [70]. Le virus Alongshan a été détecté en Chine, Finlande et en France [70].

Epidémiologie et symptômes :

Après la première découverte du JMTV, d'autres virus génétiquement proches ont été identifiés chez des arthropodes, des reptiles, des mammifères, et des humains en Asie, en Amérique, en Afrique et en Europe. Diverses d'espèces de tiques ont été trouvées positives: *Amblyomma variegatum*, *I ricinus*, *Rhipicephalus microplus*, *Haemaphysalis longicornis*, *Rhipicephalus bursa* (*R bursa*) et *H marginatum*. Cependant, l'implication des tiques dans le cycle naturel toujours pas claire. Dans une étude récente, des tiques *Amblyomma javanense* ont été infectées expérimentalement: le virus se réplique, migre à travers les intestins jusqu'aux glandes salivaires, suggérant que les tiques pourraient jouer un rôle dans la transmission [70]. De nombreuses espèces de tiques ont été détectées positives dans les différentes régions où le virus circule, ce qui rend difficile la compréhension du cycle de répllication. Certaines espèces de tiques ne semblent être positives que grâce à un phénomène de co-feeding, sans réellement

jouer un rôle dans le cycle [70]. Les symptômes provoqués par les virus de la famille des JMTVs sont encore peu connus, on retrouve cependant des maux de tête, fièvre, des comas, une augmentation des aspartates aminotransférases relatives à des lésions hépatiques dans 25% des cas et musculaire dans 10% des cas [69].

3.5 Les virus transmis par les tiques en Amérique du Nord

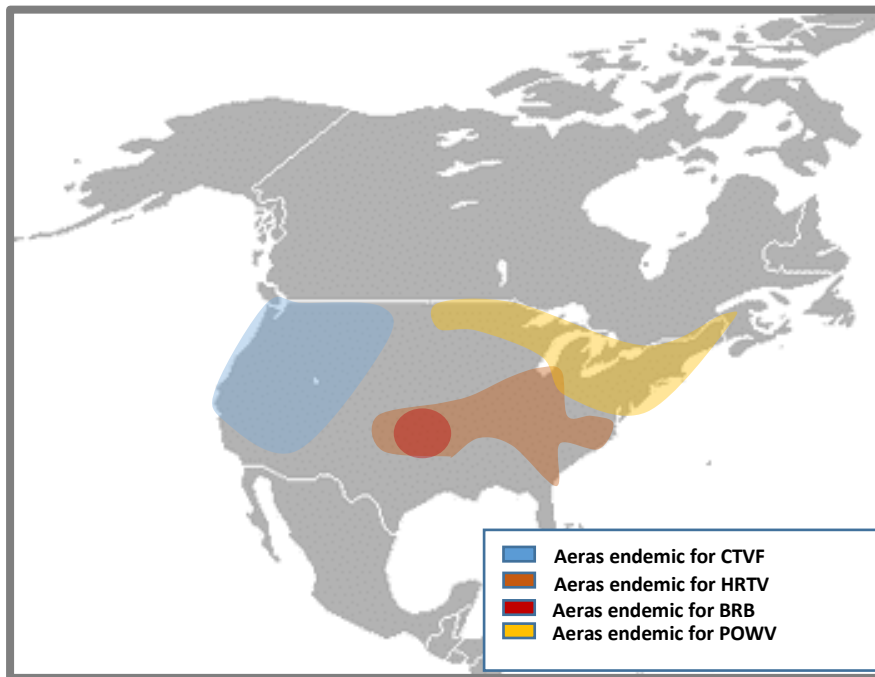


Figure 8 : Répartition géographique des virus transmis par les tiques en Amérique du Nord

Le Colorado Tick Fever Virus (CTFV) est un virus appartenant au genre des *Coltivirus* et à la famille des *Reoviridae*. Le virus est transmis par *Dermacentor andersoni* dans l'Ouest des Etats-Unis et au Canada[71]. Aux Etats-Unis, selon le CDC, 59 cas humains ont été décrits entre 2010 et 2019. Les hôtes réservoirs sont certains micromammifères et les cervidés [72]. Les principaux symptômes sont une fièvre biphasique, des frissons, des céphalées, une photophobie, des myalgies et, dans certains cas, une éruption pétéchiale. La gravité de la maladie conduit à l'hospitalisation d'environ 20 % des patients atteints, tandis que de rares cas de fièvre hémorragique et de décès chez les enfants ont également été rapportés [73]. Dans 5-10% des cas pédiatriques, CTFV provoque une méningite aseptique, une méningo-encéphalite ou une encéphalite [73]. Toutefois, les cas semblent diminuer depuis les 20 dernières années [74].

Le virus Heartland (HRTV) est un *Phlebovirus* isolé pour la première fois chez deux agriculteurs du nord-ouest du Missouri hospitalisés pour fièvre, leucopénie et thrombocytopénie en 2009 [75]. Le génome de ce virus est constitué de trois segments d'ARN simple brin à sens négatif qui codent pour les protéines de la nucléocapside (segment S), les protéines de l'enveloppe (segment M) et la polymérase (segment L). Les premiers cas index avaient des antécédents d'exposition aux tiques *Amblyomma americanum*. De 2012 à 2017, un 17 cas ont été identifiés aux Etats-Unis [76]. Les études décrivent une circulation continue du virus chez *Amblyomma americanum* [77, 78]; 1 % des donneurs de sang possèdent des anticorps spécifiques.

Le virus Bourbon (BRBV) est a été isolé pour la première fois chez un patient fébrile après morsure de tique dans le comté de Bourbon, au Kansas (États-Unis); le patient est décédé [79]. D'autres cas ont été signalés dans le Midwest et le Sud des États-Unis [79]. BRBV appartient à la famille *Orthomyxoviridae*, et au genre *Thogotovirus*, qui est distribué dans le monde entier et comprend les virus Araguari, Aransas Bay, Dhori, Jos, Thogoto et Upolu.

Le virus Powassan (POWV) a été isolé pour la première fois à partir du cerveau d'un jeune garçon décédé d'une encéphalite dans la ville de Powassan au Canada [80]. Il s'agit du seul membre du genre *Flavivirus* transmis par les tiques en Amérique du Nord. La majorité des cas symptomatiques se présente sous forme d'un prodrome fébrile suivi de mal de gorge, somnolence, maux de tête et désorientation. Les formes sévères évoluent vers une encéphalite ou méningo-encéphalite avec une issue fatale dans 10% des cas et des séquelles neurologiques graves et durables dans 50 % des cas [81, 82]. En 1997, un virus génétiquement proche, deer tick virus (DTV) a été isolé de la tique *I. scapularis*. Récemment, l'incidence des cas humains rapportés est en hausse soit à cause d'un renforcement de la surveillance et du dépistage soit liée à une augmentation réelle du nombre de cas.

3.6 Les autres virus transmis par les tiques

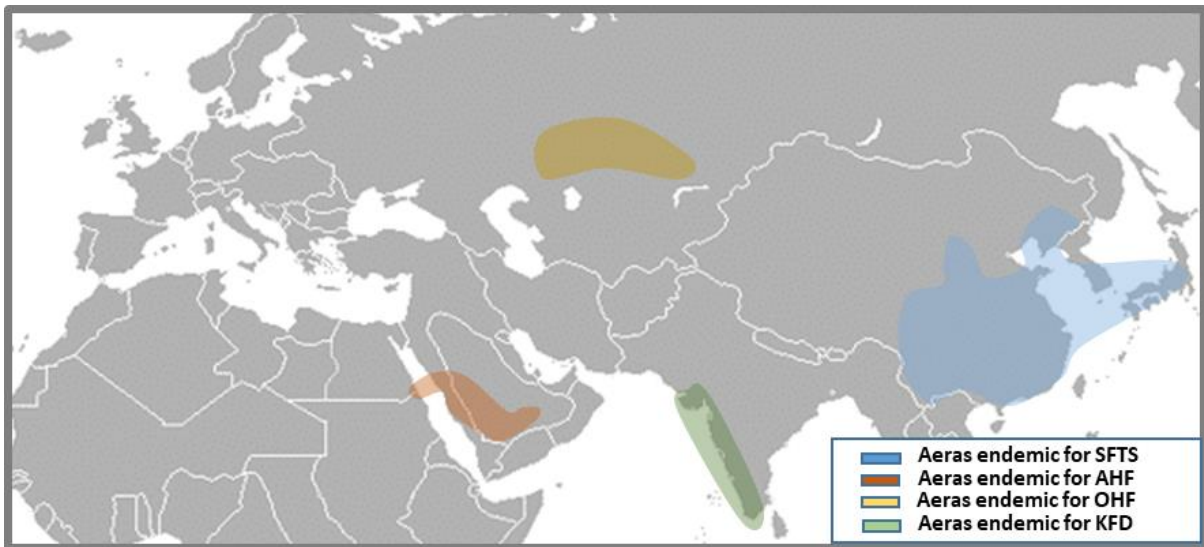


Figure 9 : Répartition géographique des virus transmis par les tiques du vieux monde

Le virus Severe Fever with Thrombocytopenia Syndrome (SFTSV) est un *Phlebovirus* découvert pour la première fois en Chine en 2009 [83]. De 2010 à 2016, plus de 5000 cas ont été signalés dans au moins 23 provinces de Chine continentale avec un taux de létalité de 5,3% [84, 85]. Des cas ont également été signalés en Corée du Sud, au Japon et au Vietnam. L'ICTV l'a récemment renommé Dabie Bandavirus. La transmission interhumaine a également été signalée.

Le virus Alkhumra Hemorrhagic Fever (AHFV) est un *Flavivirus* identifié pour la première fois en Arabie Saoudite en 1995. Entre 2001 et 2007, 28 cas ont été référencés dans le même pays, suivi d'un foyer épidémique dans la région de 70 cas en 2008[86]. Des cas sporadiques ont été décrits, dont des cas importés en Italie chez des touristes au retour d'Egypte, suggérant que la distribution de AHFV s'étend au-delà des frontières de l'Arabie Saoudite. Un syndrome grippal fébrile aigu, une hépatite, des manifestations hémorragiques et, plus rarement, une encéphalite sont les principales caractéristiques cliniques. Le rôle des tiques dans la transmission du virus est très probable reste encore à prouver [86].

Le virus Omsk hemorrhagic fever (OHFV) est un membre de la famille des *Flaviviridae*. Il a été découvert entre 1945 et 1947 à Omsk, en Russie, chez des patients atteints de fièvre hémorragique. Il est distribué exclusivement dans les quatre régions de Sibirie occidentale (Kurgan, Tyumen, Omsk et Novosibirsk) en Russie [87]. La maladie se présente comme une fièvre modérée pouvant provoquer des atteintes du système nerveux centrale dans de rares cas. Les manifestations hémorragiques sont généralement représentées par des hémorragies

capillaires qui surviennent 7-12 jours après les premiers signes. La maladie est bénigne et présente un faible taux de létalité de 1 % [88].

Le virus Kyasanur Forest Disease (KFDV) est un *Flavivirus* proche de Alkhumra Hemorrhagic Fever Virus. Il a été découvert pour la première fois en 1957 de manière épizootique chez les singes dans la forêt de Kyasanur dans la région du Karnataka en Inde. Le virus fut identifié plus tard dans plusieurs états voisins [89]. Entre 2003 et 2012, plus de 3200 cas ont été répertoriés dans ces régions, avec un taux de létalité de 4% [90]. Le virus est transmis par les tiques du genre *Haemaphysalis*, mais de nombreux autres genres de tiques se sont montrés capables de transmettre le virus [90].

4. Les bactéries transmises par les tiques

Les tiques sont les vecteurs qui transmettent le plus large panel de pathogènes. Au niveau des bactéries deux genres *Borrelia* spp. et *Rickettsia* spp. et une famille Anaplasmataceae sont responsables de la majorité des cas de maladie humaine ou animale d'origine bactérienne transmises par les tiques.

4.1 La borréliose de Lyme

La bactérie :

Il existe un nombre important de bactéries du genre *Borrellia* responsables de la maladie de Lyme, elles sont toutes regroupées dans le groupe *Borrelia burgdorferi* sensu lato avec plus de 20 espèces identifiées. Certaines d'entre elles ne provoquent cependant aucun symptôme. La borréliose de Lyme est une maladie à transmission vectorielle, vectorisée principalement par les tiques du genre *Ixodes*. La maladie de Lyme est observée en Europe depuis la fin du 19ème siècle.

Géographie :

Cette maladie, répandue dans tout l'hémisphère nord, continue de se développer à l'échelle mondiale en raison de multiples facteurs environnementaux et à l'incursion accrue de l'Homme dans les habitats qui abritent les tiques [91]. Elle est couramment signalée en Amérique du Nord et en Europe, mais on la retrouve également dans certaines régions d'Asie.

Epidémiologie :

Le US CDC estime que ~300 000 nouveaux cas de la maladie surviennent chaque année aux États-Unis [92]. En Europe et en Asie, l'incidence signalée à l'échelle nationale va de faible au Royaume-Uni, en Turquie et au Japon, à élevé (>80 cas pour 100 000 individus) aux Pays-Bas, en Belgique, en Autriche, en Slovénie, en Lituanie et en Estonie [93, 94]. Les rongeurs et les oiseaux constituent les principaux réservoirs de la bactérie

Symptômes :

Chez l'Homme la manifestation clinique la plus fréquente est l'érythème migrant, une inflammation cutanée circulaire correspondant à la dispersion de la bactérie dans l'organisme de l'hôte. Elle évolue en stade secondaire en une infection multiple polymorphe qui peut se manifester par des symptômes neurologiques, arthrites inflammatoires, syndromes cardiaques, oculaires et dermatologiques, un stade tertiaire plus focalisé à des organes peut apparaître [95].

4.2 Les Rickettsies

La bactérie :

Les *Rickettsia* (*Ri.*) sont des bactéries gram négatives parasites intracellulaires obligatoires. Il existe plusieurs espèces de *Rickettsia* dont la plupart sont des organismes symbiotiques des tiques, mais certaines d'entre elles sont pathogènes et provoquent des symptômes chez les humains et les animaux. Les *Rickettsia* sont divisées en deux groupes, le groupe des typhus (TG : typhus groupe) responsables d'épidémies mortelles tout au long de l'histoire et le groupe des fièvres pourprées (SFG : Spotted Fever group) responsables de fièvres estivales [96].

Géographie :

En Europe, les rickettsioses sont principalement causées par les *Rickettsiae* SFG transmises par les tiques. Dans la région méditerranéenne, des espèces de *Rickettsiae* ont été détectées chez plusieurs espèces de tiques, mais on considère généralement que les rickettsioses humaines sont causées par *Ri. conorii*, l'agent responsable de la fièvre boutonneuse méditerranéenne. *Ri. aeschlimanii*, *Ri. massiliae* et *Ri. monacensis* peuvent également provoquer une présentation clinique similaire [97, 98]. D'autres espèces qui ont été associées à des maladies humaines et détectées en Europe comprennent *Ri. slovaca* et *Ri. raoultii*, toutes deux responsables de tick-borne lymphadenopathy et *Dermacentor*-borne necrosis lymphadenopathy (TIBOLA/DEBONEL) [99].

Epidémiologie :

Certaines espèces ne sont transmissibles que par une seule espèce de tique, alors que d'autres peuvent l'être par différentes espèces selon les régions. Les réservoirs des bactéries dépendent de l'espèce mais l'on retrouve les ongulés, les canidés et les rongeurs comme principaux réservoirs [100]. Il existe 21 espèces de SFG et 20 d'entre elles sont vectorisées par les tiques et 19 sont des zoonoses que l'on retrouve sur tous les continents [11, 96]

Symptômes :

La plupart des rickettsioses ont les mêmes signes cliniques: une fièvre brutale suivie par des myalgies et des céphalées. Le diagnostic peut être orienté par la présence d'une escarre nécrosée au point de morsure de la tique. Les jours suivants, un exanthème d'abord maculeux puis papuleux de couleur violacé se développe sur tout le corps, symptôme qui lui donne son nom vernaculaire de fièvre pourprée (Spotted fever en anglais). La guérison spontanée est habituelle mais une forme sévère peut se développer dans 6% des cas. Le taux de létalité de ces maladies est de 2%, principalement pour les personnes dont le diagnostic est tardif ou les immunodéprimés [14].

4.3 Les Anaplasmataceae

La Famille :

La famille des Anaplasmataceae, membre des *Rickettsia*, appartient à la sous-classe alpha des Proteobacteria organisée en quatre genres : *Anaplasma*, *Ehrlichia*, *Neorickettsia* et *Wolbachia* [101].

Les Bactéries :

Anaplasma et *Ehrlichia* peuvent provoquer une infection persistante chez les hôtes vertébrés, dont certains deviennent un réservoir de la bactérie [102]. Les hôtes réservoirs comprennent de nombreux animaux sauvages et domestiqués. Les bactéries sont transmises principalement par des tiques dures de la famille des *Ixodidae*.

Les six espèces reconnues appartenant au genre *Anaplasma* (*A*) sont : *A phagocytophilum*, *A marginale*, *A centrale*, *A platys*, *A bovis* et *A ovis*. Ces espèces sont responsables d'anaplasmose chez un large éventail de vertébrés sauvages et domestiques dans le monde. Les anaplasmoses restent une cause importante de pertes économiques dans les élevages, et la reconnaissance d'*Anaplasma* comme un genre d'importance pour la santé publique a contribué à l'intérêt croissant pour ces bactéries notamment de leur biologie moléculaire et leur pathogénicité.

Ehrlichia (*E*) comprend les six espèces suivantes, toutes transmises par les tiques: *E. chaffeensis*, *E. canis*, *E. ewingii*, *E. muris*, *E. ruminantium* et *E. minasensis* [103]. L'ehrlichiose est une maladie causée par les bactéries du genre *Ehrlichia* spp. Ces bactéries sont responsables d'un nombre important de maladies à transmission vectorielle et sont responsables de diverses infections émergentes chez les animaux et les humains.

Symptômes :

Chez l'homme l'infection s'accompagne d'un syndrome fébrile peu grave; cependant des formes sévères avec des atteintes multi viscérales sont décrites [104]. En santé animale, les anaplasmoses et ehrlichioses sont responsables de nombreux avortements [105].

5. Le rôle du diagnostic lors d'une épidémie

La détection et l'endiguement d'une épidémie sont fortement reliés à la capacité de diagnostic. Comme démontré par les récentes épidémies et pandémies qui ont éclaté à travers le globe ces 15 dernières années, le diagnostic est un outil clé pour investiguer et surveiller les pathogènes. Ces deux facteurs sont un aspect essentiel dans la stratégie de préparation en réponse à une épidémie. Dans de nombreux pays du monde, les faibles capacités de diagnostic ont toujours été une barrière dans la surveillance mais surtout pour la détection précoce des pathogènes et des épidémies associées. Dans le cas de l'épidémie d'Ebola de 2013-2016 en Afrique de l'Ouest, il y a eu un délai de 3 mois entre le cas index et l'identification de l'agent causal; les analyses effectuées après l'épidémie suggèrent qu'en diagnostiquant 60% des patients dans un délai d'un jour au lieu de 5 jours, on aurait pu réduire le taux d'attaque de 80% à près de 0% [106]. En conséquence, durant les premières phases d'une épidémie, le pathogène peut circuler pendant une période importante, les cas ne sont pas alors investigués ni même rapportés. Les chaînes de transmission augmentent de manière exponentielle, la maladie se propage et devient difficile à contrôler. De nombreux exemples démontrent que les faibles capacités de diagnostic ont augmenté les délais d'identification et de mesure de contrôle lors d'épidémies [107]. Ces faibles capacités de diagnostic concernent la plupart des pathogènes faisant partie du programme de surveillance de l'Organisation Mondiale de la Santé: les virus Ebola, de la fièvre de Lassa, Zika [107-109]. Il a été estimé que si les anciennes épidémies d'Ebola avaient été identifiées deux semaines plus tôt, cela aurait permis de diminuer de moitié le nombre total de cas [110]. Cet exemple démontre que l'identification précoce d'une épidémie est la clé de sa maîtrise efficace.

6. Plateforme de diagnostic moléculaire à l'Unité des Virus Emergents (UVE).

Une large plateforme de diagnostic est présente à l'Unité des Virus Emergents à Marseille depuis 2009. Elle est un élément crucial pour le fonctionnement des activités de recherches du Laboratoire, ainsi que les nombreuses collaborations internationales. Depuis sa création, la plateforme ne cesse d'évoluer pour atteindre en 2020, un catalogue de 400 tests moléculaires pour la détection de 250 pathogènes d'origines virale et bactérienne. La capacité de production de tests de la plateforme avoisine les 50000 tests par semaine.

Elle dispose de plusieurs avantages qui en font un outil indispensable en cas d'épidémie.

1. La plateforme propose un large panel de tests moléculaire, alimenté en permanence par l'acquisition de nouveaux systèmes de détection publiés dans la littérature ou la création et la validation de nouveaux systèmes adaptés à des situations spécifiques.
2. Le format lyophilisé des systèmes permet un envoi et un stockage des systèmes à température ambiante pendant plusieurs mois et assure une reproductibilité des résultats, à faible coût.

Des témoins positifs artificiels quantifiés sont disponibles et créés à partir de plasmides; ils comportent une séquence exogène unique qui permet de reconnaître les contaminations croisés au laboratoire.

3. Un bactériophage est utilisé en tant que contrôle externe et permet de valider l'extraction, la reverse transcription et l'amplification. Il permet d'éviter les faux négatifs causés par des inhibiteurs de PCR ou des erreurs de procédure.

Ces tests sont actuellement utilisés, (i) dans les programmes de recherche de l'UVE, (ii) pour les activités de diagnostic hospitalier notamment à l'Institut Hospitalier Universitaire Méditerranée Infection, (iii) dans le cadre de projets internationaux sur le diagnostic et la surveillance de pathogènes et en soutien aux activités de l'OMS, du ECDC, du GOARN.

De nombreux systèmes de diagnostic produits par la plateforme de l'UVE ont pu être distribués à travers le monde en réponse aux épidémies. Notamment, lors d'épisodes d'émergence ou de réémergence: pour les détection des virus Zika et Chikungunya lors de leur globalisation entre 2013 et 2015, pour le virus Ebola en 2014 en Afrique de l'Ouest et plus récemment pour le SARS-CoV-2 dans plus de 38 pays différents.

Plusieurs systèmes lyophilisés de la plateforme de l'UVE ont été utilisé lors de cette thèse dans le cadre de la détection des pathogènes viraux, particulièrement TBEV et CCHFV. Les

nouveaux systèmes de diagnostic créés lors de ce travail rejoindront le catalogue proposé par la plateforme.

Chapitre 2 :
Contexte et objectifs

1. Présentation des laboratoires

La thèse Holis-Tick s'est déroulée en codirection dans deux laboratoires :

L'UR7310 est localisée à l'Université de Corse. La thématique de recherche principale est l'étude de la dynamique de maladies infectieuses en milieu insulaire. Le laboratoire effectue des recherches épidémiologiques et virologiques en santé humaine et animale et est spécialisé dans la surveillance des maladies respiratoires communautaires et des maladies transmises par les tiques, et plus largement des zoonoses. L'UR7310 est une antenne du réseau *Sentinelles*, et possède une collaboration importante avec les médecins généralistes en France métropolitaine dans le cadre de nombreux projets de recherche en lien avec la médecine générale. Le laboratoire UR7310 met en lien de nombreux acteurs de terrain comme, les médecins, les chercheurs, les vétérinaires et les naturalistes pour effectuer une surveillance complète entre vecteurs, animaux sauvages et domestiques et populations humaines.

L'Unité des Virus Emergents est localisée à l'Université d'Aix-Marseille. Elle travaille depuis plus de 15 ans sur les maladies virales émergentes. Bien que le laboratoire soit spécialisé dans les arbovirus, d'autres thématiques de recherches sur les virus émergents sont entreprises, comme les virus respiratoires communautaires lors d'épisodes d'émergences ou les virus à risque d'émergence tels les Poxvirus. Plusieurs plateformes techniques sont opérationnelles dans les domaines du diagnostic, du développement d'approches vaccinales, des thérapies antivirales, de l'évolution virale et de la séro-épidémiologie

2. Aire d'étude

La Corse et son statut insulaire, représente un environnement de choix pour les études épidémiologiques et la surveillance des facteurs d'émergences. La population de l'île est composée de 337 000 habitants [111], et possède une superficie de 8679 kilomètres carrés. Sa densité de population reste faible avec 39 habitants/km², principalement située autour des deux principales agglomérations sur le littoral, Bastia et Ajaccio. L'intérieur des terres est peu habité et délaissé. La Corse est appelée l'île montagne, car elle possède de nombreux pics montagneux importants, avec plus de 100 sommets supérieurs à 2000 mètres de haut et un point culminant de 2706 mètres. Situé au Sud de la méditerranée, la Corse possède un climat chaud, et son insularité lui permet d'avoir un climat plus clément que les terres continentales à la même latitude. Le relief, le type de végétation et le climat varient énormément entre les microrégions. Deux climats sont présents sur l'île, un climat méditerranéen et un climat montagnard. On

retrouve donc un environnement propice à l'apparition et l'adaptation de nouvelles espèces de vecteurs, ainsi qu'une diversité de ceux-ci.

en Corse, l'élevage repose sur des modèles traditionnels. La chambre d'agriculture Corse recense 110000 ovins, 63000 porcins, 55000 bovins et 45000 caprins [112]. Les pratiques d'élevage traditionnel des ruminants reposent sur la pratique du parcours en semi-liberté voire en liberté totale. Les parcours libres représentent un type d'élevage particulier à la Corse, induisant de nombreux contacts avec la faune sauvage, les autres animaux domestiques et les populations humaines [113]. De plus, les pratiques culturelles en Corse sont caractéristiques du terroir et naissent de l'héritage rural et insulaire. Ces pratiques comme la chasse à plumes ou au gros gibier, suivies du travail de la viande, comme le dépeçage et l'éviscération, augmentent les contacts entre l'homme, les animaux et l'environnement. Près de 5% de la population corse possède un permis de chasse, contre 2 % au niveau continental [114]. Sur l'île, de nombreuses pratiques d'élevage de loisir notamment, porcins sont présentes. Si cette pratique facilite les échanges de pathogènes, elle est surtout responsable d'abattage à domicile rendant difficile le suivi sanitaire des animaux. Il est important de noter la réintroduction de deux espèces emblématiques et endémiques de l'île: le mouflon de Corse et le cerf élaphe corse qui eux aussi partagent leur territoire avec les animaux domestiques.

Ces élevages traditionnels, couplés aux fortes activités anthropiques (tourisme, chasse, élevage) mettent en relation les animaux sauvages, domestiques et les hommes. **La Corse possède toutes les caractéristiques d'une région sentinelle vis-à-vis des maladies émergentes. Des conditions favorables à une diversité de vecteurs et de leurs pathogènes, la présence de nombreux échanges entre les animaux sauvages et domestiques et une interface importante entre population humaine et faune animale dûe aux activités anthropiques.**

Plusieurs exemples sont les témoins de l'importance de la Corse en tant qu'espace sentinelle. On peut citer le virus Toscana responsable d'une forte séroprévalence (22,5%) en population humaine ou encore, le virus de l'Hépatite E qui montre une séroprévalence (56,1%) importante en population générale [115, 116]. On peut citer aussi, sur l'île, en 2014, 106 cas de touristes infectés par un hybride de *Schistosoma haematobium* et *Schistosoma bovis*. De plus, depuis 2015, 10 autres cas autochtones ont été rapportés liés à des baignades traduisant la persistance du parasite sur l'île [117]. Pour finir, du à la présence de la tique vectrice de CCHFV, une enquête sérologique a été réalisée sur 4000 sérums d'ovins, caprins et bovins. Neuf % des animaux présentaient des anticorps spécifiques, dont 13% des bovins. Toutes les tiques testées pour la présence de l'ARN de CCHFV se sont avérées négatives.

3. Objectifs de la thèse

Cette thèse et ses objectifs ont évolué au fil du temps en fonction de: i) **la mise en place** au niveau de l'UR7310 d'une thématique sur les maladies émergentes transmises par les tiques; en 2018, au début donc de ce projet de thèse il existait très peu de données sur la diversité des tiques et de leurs pathogènes bactériens et viraux en Corse ; ii) **l'adaptation à l'évolution de la situation épidémiologique** notamment celle de CCHFV. En effet, les données de séroprévalence publiées chez les ruminants montrant la trace sérologique d'une éventuelle circulation d'un virus CCHF ou CCHF-like en Corse associées à la présence de son vecteur principal *H marginatum* (deuxième espèce de tiques la plus prévalente en Corse) ont amené à la mise en place d'une surveillance de CCHF sur l'île. Cette surveillance démarrée en 2019 et a été portée par l'UR7310 en collaboration avec l'UMR ASTRE (CIRAD de Montpellier), l'UVE et l'équipe de l'INRAe Laboratoire de Recherche sur le Développement de l'Élevage de Corse. Cette surveillance a été intégrée à ma thèse et enrichie avec la recherche d'autres familles de virus à risque d'émergence en Corse.

La thèse a donc suivi deux objectifs principaux sur deux périodes chronologiquement distinctes :

Période 2018-2019 : Le principal objectif (objectif 1) de cette première partie était d'identifier les principales espèces de tiques présentes en Corse et d'en détecter les principaux pathogènes. Nous avons décidé de nous concentrer sur les pathogènes bactériens car ils sont bien décrits, et bénéficient de techniques de détection bien établies.

Elle a consisté à:

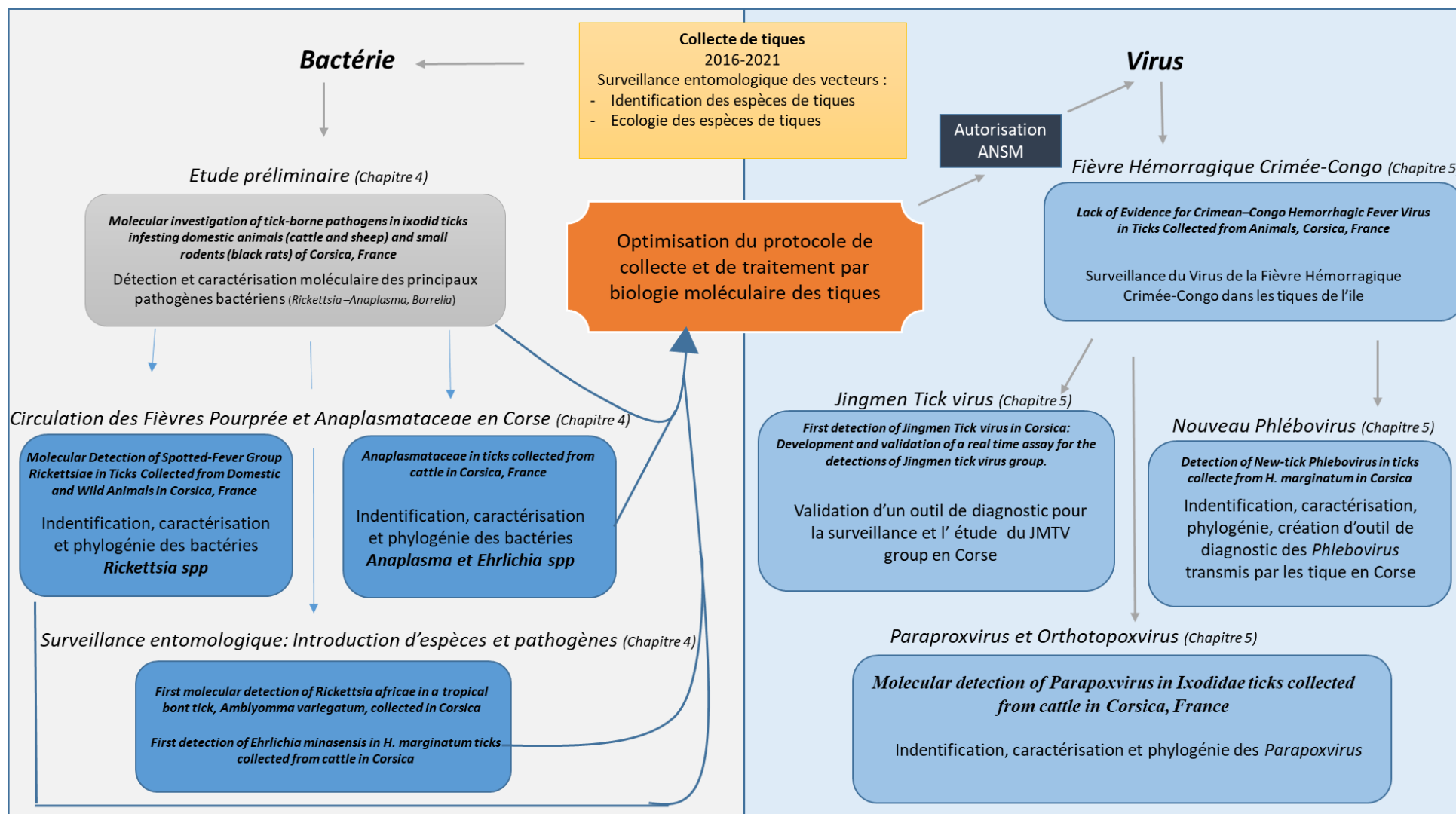
- Optimiser la collecte des tiques à l'abattoir avec une base de données pour un suivi statistiquement représentatif des vertébrés hôtes et des espèces de tiques.
- Perfectionner les protocoles de traitement des tiques (broyage, extraction) en amont des techniques de détection moléculaire.

Période 2020-2022 : L'objectif de cette seconde partie de thèse (objectif 2), était d'une part d'identifier des virus à risque d'émergence en Corse, ainsi que de développer des outils de détection moléculaire des pathogènes d'origine virale, applicables dans un contexte de diagnostic rapide.

Le choix de privilégier les virus répond à plusieurs raisons: (i) l'UVE travaille sur les arboviroses et offre une expertise, des techniques et des moyens performants pour l'étude des virus; (ii) les virus transmis par les tiques à l'exception de TBEV et CCHFV sont peu étudiés, en particulier en Corse, et ce même si de nombreux virus transmis par les tiques continuent d'émerger, de re-émerger à travers le globe [118]. Ces deux principales raisons ont fait des pathogènes viraux une priorité par rapport aux pathogènes bactériens pour la poursuite de la thèse (Figure 10). Le développement de systèmes de diagnostic basés sur la technique de PCR en temps réel a été abordé.

Le schéma suivant montre les différentes étapes de la thèse par ordre chronologique et qui seront présentées dans les chapitres résultats (4 & 5).

Figure 10 : Schéma chronologique représentant les étapes de la thèse



Chapitre 3 :
Méthodologie

1. Collecte des tiques

Collecte à l'abattoir : La collecte de tique a été réalisée principalement à l'abattoir de Ponte-Leccia (centre de Corse) uniquement sur bovins. La fermeture du second abattoir à Cuttoli en 2018 a facilité notre travail de collecte en transférant l'abatage de la plupart des bovins du Sud de la Corse à l'abattoir de Ponte-Leccia (Figure 12). Toutes les deux semaines, les tiques présentes sur les peaux de bovin ont été collectées et tracées par le numéro de boucle auriculaire de l'animal; ce numéro, permet de retrouver l'origine des animaux et donc indirectement des tiques, grâce à la base de données nationale d'identification animale (*BDNI*). La collecte des tiques avait lieu toute les deux semaines sur l'année entière (2018-2022).



Figure 12 : Localisation de l'abattoir de Ponte-Leccia et exemple de tiques collectées (*Dermacentor marginatus*).

Collecte sur les chevaux : Les tiques ont été collectées sur les chevaux tous les jours lors de randonnée équestre en été (Juin-Aout des années 2019-2020-2021). Les dates et les lieux de collecte ont été enregistrés par le technicien chargé de la collecte. La collecte a été réalisée par l'INRAe LRDE en collaboration avec l'UMR ASTRE. La Figure 13 montre la localisation des 25 centres équestres ayant participé à la collecte.

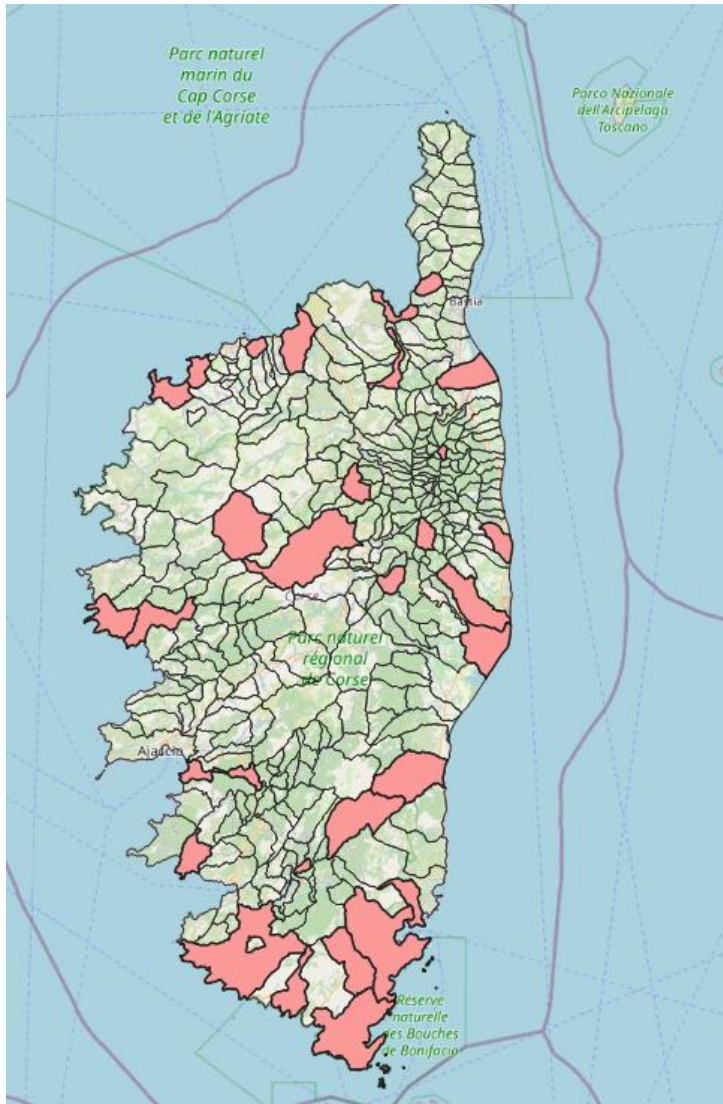


Figure 13 : Localisation des communes pour la collecte des tiques sur chevaux (rose).

Collecte sur les sangliers : les tiques ont été collectées lors de la période de chasse d’Août à Janvier en 2018-2019-2020 (Figure 14). Les dates et les lieux de collecte ont été enregistrés par les chasseurs.

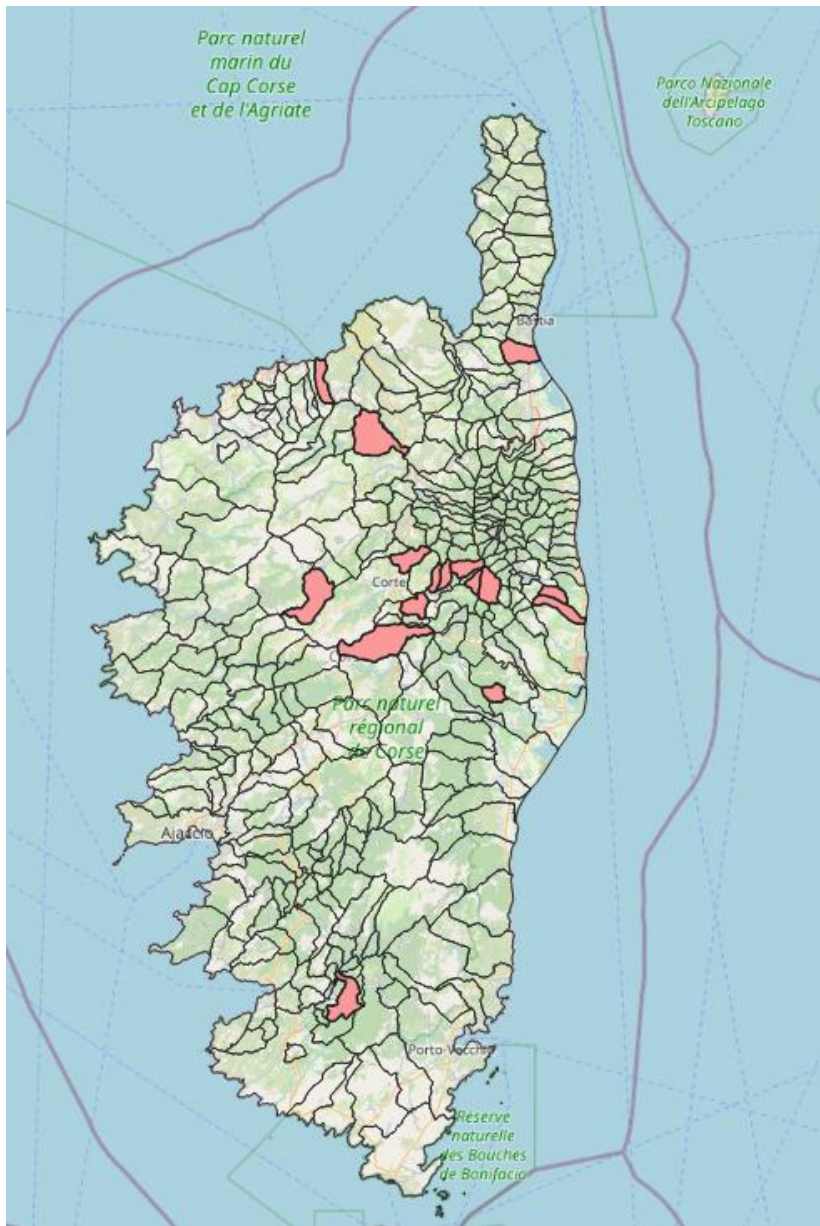


Figure 14 : Localisation des communes de collecte des tiques sur sanglier (rose).

Les tiques ont été collectées sporadiquement sur brebis, cerfs, animaux de compagnie et sur rongeurs respectivement lors de la traite, captures par les techniciens du Parc Naturel Régional de Corse, suivi de vétérinaire et suivi sanitaire.

2. Traitement des tiques

Identification des tiques : Les tiques ont été identifiées vivantes à l'aide des clés morphologiques sous loupe binoculaire x10 et x20 ou sous microscope x 100 [119]. Lors des premières études, pour me former à l'identification des tiques, une identification des vecteurs par PCR et séquençage du gène mitochondrial 16s a été menée parallèlement à cette identification morphologique (Tableau 1).

Formation des pools : en raison du grand nombre de tiques, nous avons décidé de pooler les tiques pour la détection des pathogènes. Les pools étaient constitués de 1 à 10 tiques, selon ces trois critères : l'espèce de l'hôte vertébré, le stade de la tique, le genre de la tique.

Broyage et extraction des tiques : Les tiques individuelles ou les pools de tiques ont été broyées dans du Milieu Minimum Essentiel de Eagle à l'aide du TissueLyser II (Qiagen, Hilden, Allemagne) à 5500 rpm pendant 3 minutes avec deux billes de tungstène. L'extraction des acides nucléiques (ADN et ARN) a été réalisée à l'aide de la plateforme automatique d'extraction QIAcube HT (Qiagen) et du kit QIAamp Cador Pathogen Mini. Afin de vérifier la qualité de l'extraction, des contrôles ont été ajoutés à chaque plaque d'extraction : 1 témoin négatif et un control interne d'extraction (2 bactériophages ADN et ARN, MS2 et T4).

Les acides nucléiques étaient élués avec 100 µL d'eau RNase free, et conservés à -80°C pour future utilisation.

Tableau 1 : Nombre de tiques collectées et testées par hôte, espèces et année.

	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupens</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total
Rongeurs 2016 ^{1 4}	0	0	0	0	0	0	13	0	13
Bovins 2016 ^{1 4}	495	91	0	0	0	0	0	0	586
Bovins 2017 ^{2 3 4}	237	80	0	0	0	0	4	0	321
Bovins 2018 ^{2 3 4 5}	360	136	0	0	0	0	6	0	502
Bovins 2019 ^{4 5 6 7}	460	305	152	4	6	78	59	0	1064
Bovins 2020 ^{4 6 7}	1 850	731	130	10	68	52	16	2	2859
Chevaux 2019 ^{4 5 6 7}	586	707	0	0	0	0	0	0	1293
Chevaux 2020 ^{4 6 7}	51	319	0	0	27	0	0	0	397
Sangliers 2018 ^{2 4}	8	1	0	0	0	0	13	293	315
Sangliers 2019 ^{4 5 6 7}	1	0	0	0	0	0	0	439	440
Sangliers 2020 ^{4 6 7}	0	12	0	0	0	0	0	174	186
Brebis 2016 ^{1 4}	61	0	0	0	0	0	0	0	61
Brebis 2019-2020 ^{4 6 7}	38	0	0	0	0	0	0	0	38
Mouflons et Cerfs 2019_2020 ⁴	17	4	0	0	0	0	1	0	22
Animaux de compagnie 2018 ^{2 4}	0	0	0	0	24	0	0	1	25
Total	4164	2386	282	14	125	130	112	909	8122

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

2: Molecular Detection of Spotted-Fever Group Rickettsiae in Ticks Collected from Domestic and Wild Animals in Corsica, France

3: Molecular screening of Anaplasmataceae in ticks collected from cattle in Corsica, France

4: Lack of Evidence for Crimean–Congo Haemorrhagic Fever Virus in Ticks Collected from Animals, Corsica

5: Molecular detection of Parapoxvirus in Ixodidae ticks collected from cattle in Corsica, France

6: First detection of Jingmen Tick virus in Corsica: Development and validation of a real time assay for the detections of Jingmen tick virus group to face a potential emergence

7: Detection of New tick *Phlebotomus* of M deficient segment group in Corsica, France

3. Détection des pathogènes bactériens

Dans un premier temps les tiques ont été analysées pour les *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp. et *Borrelia burgdorferi* car ils font partie des principaux genres et espèces de pathogènes bactériens transmis par les tiques [4]. Ces pathogènes bactériens ont été recherchés par genre ou famille grâce à des systèmes de PCR et qPCR décrits dans la littérature (Tableau 2).

Tableau 2 : Liste de genres et familles bactériennes recherchées au niveau de tiques collectées.

Bactérie	Méthode	Gène	Détection	Référence
<i>Rickettsia</i> spp.	qPCR	<i>gltA</i>	Genre	[10]
<i>Anaplasmataceae</i>	qPCR	<i>23S RNA</i>	Famille	[10]
<i>Borrelia burgdorferi sl</i>	qPCR	<i>23S RNA</i>	Espèces	[10]
<i>Rickettsia</i> spp.	PCR	<i>gltA</i>	Genre	[10]
<i>Anaplasma</i> spp.	PCR	<i>rpoB</i>	Genre	[10]
<i>Ehrlichia</i> spp.	PCR	<i>groEL</i>	Genre	[120]

Les Anaplasmataceae ont été détectées à l'aide d'amorces spécifiques du genre *Anaplasma* ciblant un fragment de 525 pb du gène de la sous-unité bêta de l'ARN polymérase (*rpoB*) et des amorces spécifiques du genre *Ehrlichia* ciblant un fragment de 590 pb du gène de la protéine *groEL* [121, 122]. Les *Rickettsia* spp. ont été recherchées à l'aide d'amorces amplifiant le fragment de 850 pb du gène *gltA* codant pour la citrate synthase [122]. *Borrelia Burgdorferi sl* a été détectée par un RT-qPCR ciblant le gène *23S RNA* [123]. Les échantillons positifs ont été séquencés (méthode Sanger) afin d'identifier les espèces.

4. Détection des pathogènes viraux et création d'outils de détection

Le génome viral a été recherché par RT-qPCR et RT-PCR conventionnelle à l'aide de systèmes publiés ou de systèmes "maison" (Tableau 3).

Les genres de virus testés en PCR conventionnelle Pan-genre étaient les *Flavivirus*, les *Phlebovirus*, les *Nairovirus*, les *Orbivirus* et les *Ortobunyavirus* [4]. La récente détection de

Parapoxvirus dans des tiques collectées au Burkina-Faso nous a incité à ajouter les genres *Orthopoxvirus* et *Parapoxvirus* dans les tiques collectées en Corse [124].

D'autres virus d'intérêt ont été recherchés, CCHFV, RVFV, TBEV, le virus Dugbe et ASFV (Tableau 3). Certains des systèmes de RT-qPCR utilisés ont été créés lors de cette thèse pour plusieurs raisons :

- Le Jingmen tick virus et JMTVs, est un groupe de virus ubiquitaires dont i) la présence en Corse était possible mais non documentée ; ii) pour lesquels on ne disposait d'aucun système de détection par RT-qPCR ;
- Le Thogoto virus, est un virus i) pathogène pour l'Homme transmis par les tiques en Méditerranée ; ii) et ne disposait pas de système de détection par RT-qPCR ;
- Le New Corsica tick Phlebovirus, un nouveau phlébovirus découvert lors de cette thèse grâce au système de détection *Pan-Phlebovirus*.

Tableau 3 : Liste de genres et espèces virales recherchés dans les tiques.

Virus	Méthode	Détection	Référence
<i>Pan-Flavivirus</i>	PCR	Genre	[125]
<i>Pan-Nairovirus</i>	PCR	Genre	[126]
<i>Pan-Phlebovirus</i>	PCR	Genre	[127]
<i>Pan-Orbivirus</i>	PCR	Genre	[128]
<i>Pan-Orthobunyavirus</i>	PCR	Genre	[129]
<i>Pan-Orthopoxvirus</i>	PCR	Genre	[130]
<i>Pan-Parapoxvirus</i>	PCR	Genre	[131]
<i>Crimean-Congo Hemorrhagic Fever virus</i>	RT-Qpcr	Espèces	[132]
<i>Rift Valley Fever Virus</i>	RT-Qpcr	Espèces	[133]
<i>Tick-Born Encephalitis Virus</i>	RT-QPCR Duplex	Espèces	In house
<i>Dugbe virus</i>	RT-Qpcr	Espèces	[134]
<i>Jingmen tick virus segment 1</i>	RT-Qpcr	Espèces	In house
<i>Jingmen tick virus segment 2</i>	RT-Qpcr	Espèces	In house
<i>African swine fever virus</i>	RT-Qpcr	Espèces	[135]
<i>Thogoto virus</i>	RT-Qpcr	Espèces	In house
<i>New Corsica tick Phlebovirus</i>	RT-Qpcr	Espèces	In house

Prévalence de pathogènes dans les pools

La prévalence des pathogènes présents dans les pools de tiques a été calculée à l'aide de la méthode Maximum Likelihood Estimation (MLE) avec un Intervalle de Confiance à 95 % et exprimée en tant que MLE de la prévalence pour 100 tiques. Les calculs étaient basés sur le nombre de pools, la taille des pools (nombre d'individus par pool) et le nombre de pools positifs [136].

Analyse phylogénétique

Les séquences nucléotidiques de gènes ou de génome complet de chaque bactérie et virus ont été alignées avec des séquences présents sur Genbank en utilisant MAFFT version 7. Les analyses phylogénétiques ont été réalisées en utilisant la méthode d'estimation du maximum de vraisemblance implémentée dans Mega X, l'outil « trouver le meilleur Model » présent sur le logiciel Mega a été utilisé pour le choix du Model propre à chaque article.

Chapitre 4 :
Identification par biologie moléculaire des principales familles
bactériennes vectorisées par les tiques en Corse

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

Contexte

Bien que l'élevage (ovins, caprins, porcins et bovins) soit une activité économique importante en Corse, les connaissances sur la faune des tiques et les micro-organismes qu'elles transportent restent rares. Cette étude avait pour but de réaliser la caractérisation moléculaire par le séquençage de gènes d'intérêt des Anaplasmataceae, *Rickettsia* spp. et *Borrelia burgdorferi* sensu lato (sl) présents dans les espèces de tiques collectées en Corse.

Méthode

Des tiques de bovins (*Bos taurus*), de moutons (*Ovis aries*) et de rongeurs (*Rattus rattus*) ont été collectées de mai à septembre 2016 (Tableau 1) (Figure 12). Les tiques ont été identifiées grâce à une clé morphologique et un séquençage du gène *16s*. Un panel d'amorces ciblant les gènes d'intérêt pour chaque genre bactérien (*Anaplasma* spp., *Erlhichia* spp., *Rickettsia* spp. et *Borrelia burgdorferi* sensu lato (sl) a été utilisé (Tableau 2).

Résultats

Au total, 660 tiques ont été collectées sur 111 animaux au cours de l'étude (Figure 12). L'espèce de tique la plus abondante chez les bovins était *R. bursa* (n = 495 ; 84,5%), suivie par *H. marginatum* (n = 91 ; 15,5%). *R. bursa* et *I. ricinus* étaient les seules espèces de tiques collectées chez les moutons et les rongeurs, respectivement. Globalement, *Rickettsia* était le groupe d'agents pathogènes le plus commun (n = 48 ; 24%). L'analyse des séquences des gènes partiels *gltA* et *ompA* a révélé la présence de *Ri. aeschlimannii* et de *Candidatus Ri. barbariae*. L'ADN des pathogènes de la famille d'Anaplasmataceae a été détecté dans huit (6 %) des 127 pools de tiques de bovins et dans une tiques (2 %) des 61 spécimens de *R. bursa* prélevés sur des moutons. L'analyse de la séquence du gène *rpoB* a révélé la présence d'une espèce d'*Anaplasma*, *A. marginale*. L'ADN de *Borrelia burgdorferi* sl a été détecté dans un pool de *H. marginatum* collecté sur des bovins et dans deux (15%) des 13 pools de *I. ricinus* collectés sur neuf rats noirs (*Rattus rattus*) (Figure 15).

Conclusion

Cette première étude avait pour but d'identifier les principales espèces de tiques en Corse, et la diversité des principaux pathogènes bactériens. Il s'agissait d'une étude préliminaire, qui pose

les bases des prochaines investigations sur les différentes espèces de tiques et de quatre genres bactériens qu'elles transmettent. À notre connaissance, il s'agit du premier rapport sur l'occurrence et la caractérisation moléculaire de Candidatus *Ri. barbariae*, un membre émergent du groupe des *Rickettsia*, en Corse. La détection de l'ADN de *Borrelia burgdorferi* sl, , confirme la présence de cet agent en Corse.



Original article

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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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.

1. Introduction

Ticks, ubiquitous ectoparasitic arthropods that belong to the sub-class 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

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

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

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

2. Materials and methods

2.1. Tick collection and morphological identification

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

2.2. DNA extraction

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

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

2.3. Molecular analysis of ticks and bacteria

Morphological identification of ticks was confirmed by polymerase chain reaction (PCR) amplification and sequencing mitochondrial 16S rDNA (Table 1) (Black and Piesman, 1994). At least two specimens from each species were randomly selected and subjected to molecular identification. The PCR assays for Anaplasmataceae (Dahmani et al., 2017), *Rickettsia* spp. (Labruna et al., 2004), *B. burgdorferi* sl (Courtney et al., 2004) and *B. miyamotoi* (Diaz et al., 2012) were performed using the primers and probes listed in Table 1. Reactions were performed on a 96-well Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System using QuantiFast Pathogen + Internal Control Kits (Qiagen). Internal and negative controls were included in each run. Each run was repeated three times. Samples that were positive for Anaplasmataceae were tested by conventional PCR using *Anaplasma* genus-specific primers targeting the 525-bp fragment of the RNA polymerase subunit beta (*rpoB*) gene (Dahmani et al., 2017) (Table 1). Positive samples for *Rickettsia* spp. were analyzed using primers that amplified the 850-bp fragment of the *gltA* gene encoding citrate synthase (Table 1) (Mediannikov et al., 2004; Roux et al., 1997) and primers to amplify the 532-bp fragment of the 190-kDa outer membrane protein (*ompA*) gene (Regnery et al., 1991). The reactions were carried out using Applied Biosystems GeneAmp PCR System 9700 (Courtabouef, France). Negative and positive controls were included. The PCR products were visualized in 2% agarose gels in Tris-Acetate-EDTA (TAE Buffer) and were visualized under ultraviolet light after staining with ethidium bromide. A 100-bp DNA ladder was used as a standard marker.

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

2.4. Sequencing and phylogenetic analysis

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

3. Results

3.1. Tick identification

In total, 660 ticks were collected during the study (Table 2). Forty-two cattle were inspected in the Ponte-Leccia slaughterhouse from June to July 2016 and 586 ticks were collected. Among the 42 infested cows, 57% were infested with less than 10 ticks, 28% with 10–30 ticks, and 15% with more of 30 ticks. Two tick species were identified morphologically on cattle (Table 2). The most abundant species was *R. bursa* (n = 495; 84.5%), followed by *H. marginatum* (n = 91; 15.5%). Among the 586 ticks collected from cattle, 541 (94.1%) were adults (38.6% females and 55.5% males) and 34 (5.9%) were nymphs. Sex and life stage were not determined for 11 ticks. Sixty-one ticks were collected from 60 sheep in one farm on May 2016 (Corte, Haute-Corse) (Table 2). All these were *R. bursa*; 51 (83.6%) were adults (33% males and 51% females). Thirteen *I. ricinus* ticks (all adult females) were collected from nine rodents (black rats) captured in Lozari, Urbino, and Terrenzana (Haute-Corse) in September 2016. Among them, six were infested by one tick, two by two ticks, and one by three ticks. The sequences of

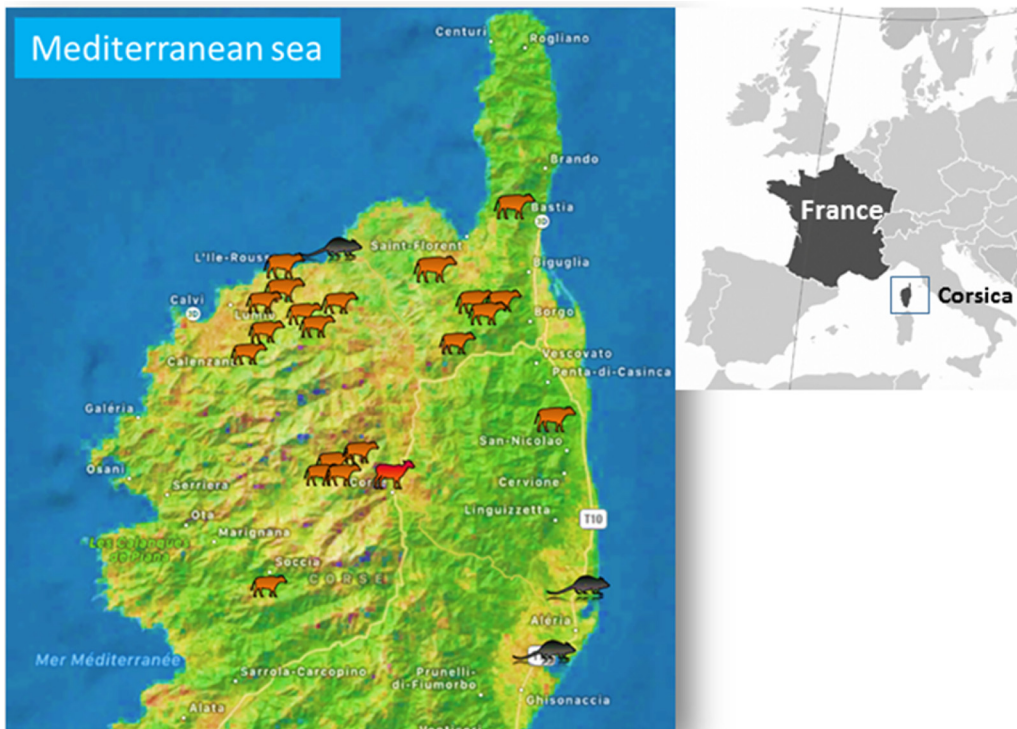


Fig. 1. Map of Corsica showing the study area where the animals lived and the GPS coordinates.

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' 28" N, 9° 00' 56" E), Urbinu (42° 02' 53" N, 9° 28' 22" E), and Terrenzana (42° 9' 28" N, 9° 32' 58" E).

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

Species	Target	Name	Sequence	Annealing temperature (°C)	References	
		qPCR				
<i>B. burgdorferi</i> sl	23S rRNA	<i>Bb23Sf</i> <i>Bb23Sfr</i> <i>Bb23Sp</i>	CGAGTCTTAAAGGGCGATTAGT GCTTCAGCTGGCCATAAATAG AGATGTGGTAGACCCGAAGCCGAGTG	60	Courtney et al. (2004)	
<i>B. miyamotoi</i>	<i>glpQ</i>	<i>Bmi-F</i> <i>Bmi-R</i> <i>Bmi-P</i>	CACGACCCAGAAATTGACACA GTGTGAAGTCAGTGGCGTAAT TCGTCCGTTTTCTAGCTCGATTGGG	60	Diaz et al. (2012)	
<i>Rickettsia</i> spp.	<i>gltA</i>	<i>Rssp-F</i> <i>Rssp-R</i> <i>Rssp-P</i>	GAGAGAAAATATATCCAAATGTTGAT AGGGTCTTCGTGCATTTCTT CATTGTGCCATCCAGCCTACGGT	60	Labruna et al. (2004)	
Anaplasmataceae	23S rRNA	<i>Tt-Ana-F</i> <i>Tt-Ana-R</i> <i>Tt-Ana-P</i>	TGACACGCTACCTTTTGCAT GTAACAGGTTCCGGTCTCCA GGATTAGACCCGAAACCAAG	60	Dahmani et al. (2017)	
		Conventional PCR (sequencing)				
<i>Rickettsia</i> spp.	<i>gltA</i>	<i>CS2D</i> <i>CSEndR</i> <i>409D</i> <i>1258R</i>	ATGACCAATGAAAATAATAAT CTTATACTCTATGTACA CCTATGGCTATTATGCTTGC ATTGCAAAAAGTACAGTGAACA	54 54	Roux et al. (1997), Mediannikov et al. (2004)	
	<i>ompA</i>	<i>Rr190.70p</i> <i>Rr190.602n</i>	ATGGCGAATATTTTCCAAAA AGTGCAAGCATTCCGCCCT	48	Regnery et al. (1991)	
<i>Anaplasma</i> spp.	<i>rpoB</i>	<i>Ana-rpoBF</i> <i>Ana-rpoBR</i>	GCTGTCTCCTAGGCTYCTTACGCCGA AATCRAGCCAVGACCCCTRTAWGG	55	Dahmani et al. (2017)	
Ticks	16S rDNA	16S+1 16S-1	CTGCTCAATGATTTTTTAAATTGCTGTGG CCGGTCTGAACTCAGATCAAGT	48 and 54	Black and Piesman (1994)	

mitochondrial 16S rDNA fragments of 16 ticks selected in this study after blast analysis were confirmed to be *R. bursa* (n = 7), *H. marginatum* (n = 7), and *I. ricinus* (n = 2) (GenBank accession numbers MH663984-90 for *R. bursa*, MH663977-83 for *H. marginatum* and MH663991-92 for *I. ricinus*). The seven 16S rDNA sequences of *H. marginatum* had 98–100% identity with each other and 99–100% identity with *H. marginatum* from Sardinia (KT931964), Israel (KT391060), Turkey (KR870973), and Algeria (KP776645) (Fig. 2).

The seven (MH663984-90) 16S rDNA sequences of *R. bursa* were

identical to each other and showed 100% identity with *R. bursa* from the Iberian Peninsula (AJ002956), Italy (KJ814007-KJ814010), and Turkey (KR870983.1-KU664350.1) (Fig. 2). The two (MH663991-92) 16S rDNA sequences of *I. ricinus* were identical to each other and showed 100% identity with *I. ricinus* from Germany (JF928526), Italy (KF197116), Sweden (KX384811), Belgium (KJ414457), and Turkey (KR870982). They had 98% identity with 16S rDNA sequences from Morocco (GU074602) and France (GU074612) (Fig. 2). Seventy-four individual ticks (61 collected from sheep and 13 from rodents) and 127

Table 2
Total tick species collected from hosts.

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 ^a	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)

^a Sex and life were not determined for 11 ticks.

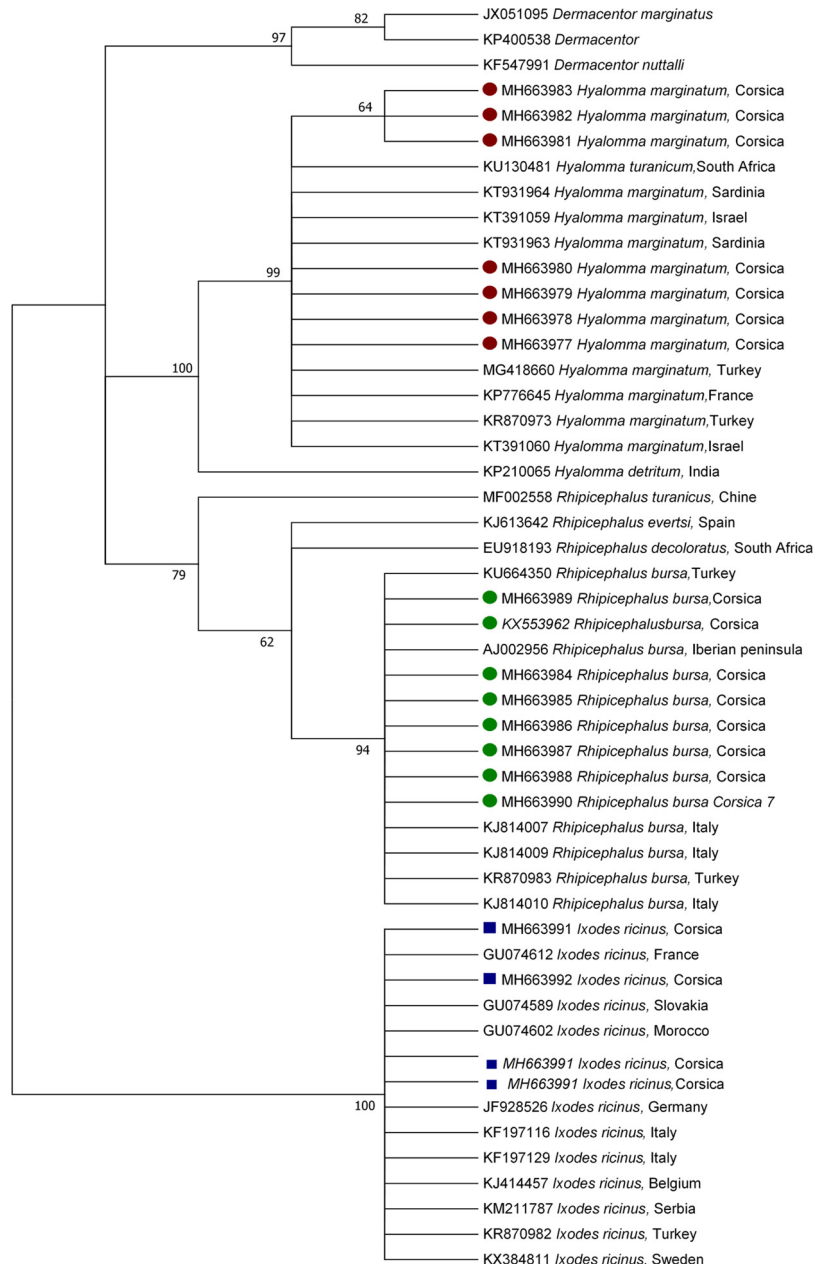


Fig. 2. Phylogenetic tree showing the position of *Rhipicephalus bursa* (green circles), *Hyalomma marginatum* (red circles) and *Ixodes ricinus* (blue squares) compared to other tick species based on 16S rDNA sequences. The evolutionary history was inferred by using the Maximum Likelihood method. The analysis involved 50 nucleotide sequences. There were a total of 291 positions in the final dataset. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Infection rate of bacteria in ticks collected from cattle.

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 (%)		
			<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)

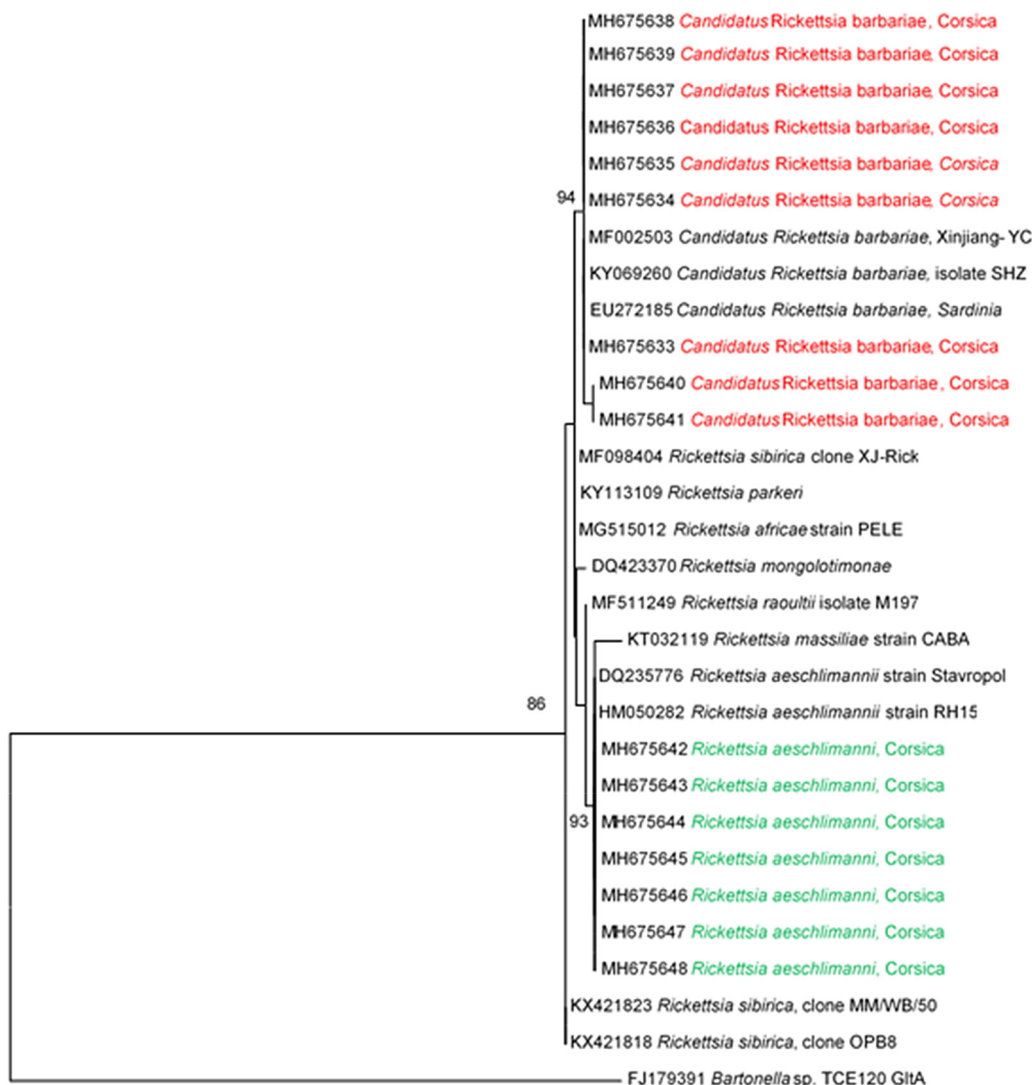


Fig. 3. Phylogenetic analysis of *Rickettsia* spp. identified in Corsica. The evolutionary history was inferred by using the Maximum Likelihood method based on *Rickettsia gltA* sequences. The analysis involved 30 nucleotide sequences. All positions containing gaps and missing data were eliminated. There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

pools prepared with cattle ticks (100 pools of *R. bursa* and 27 pools of *H. marginatum* in cattle) were analyzed (Tables 2 and 3).

3.2. Detection of *Rickettsia* spp

In ticks collected from cattle, *Rickettsia* spp. DNA was detected in 37 pools (29%), with the highest detection rate in *H. marginatum* (59%) compared with *R. bursa* (21%) ($p = 0.00$; Table 3). Similar *Rickettsia* spp. detection rates were observed in *R. bursa* collected from cattle and

sheep (21% and 18%, respectively; $p = 0.68$). The 13 *I. ricinus* specimens collected from rodents were all negative for *Rickettsia* spp. Nine pools of ticks (three *R. bursa* and six *H. marginatum*) and seven individual ticks (*R. bursa*) collected from cattle and sheep, respectively, were screened for the presence of the *gltA* gene (GenBank accession numbers MH675633-MH675648). Sequence analysis of the *gltA* sequences revealed the presence of two *Rickettsia* species: *Ri. aeschlimanii* and *Candidatus Ri. barbariae*. The seven *gltA* sequences derived from five pools of *H. marginatum* and two pools of *R. bursa* collected from

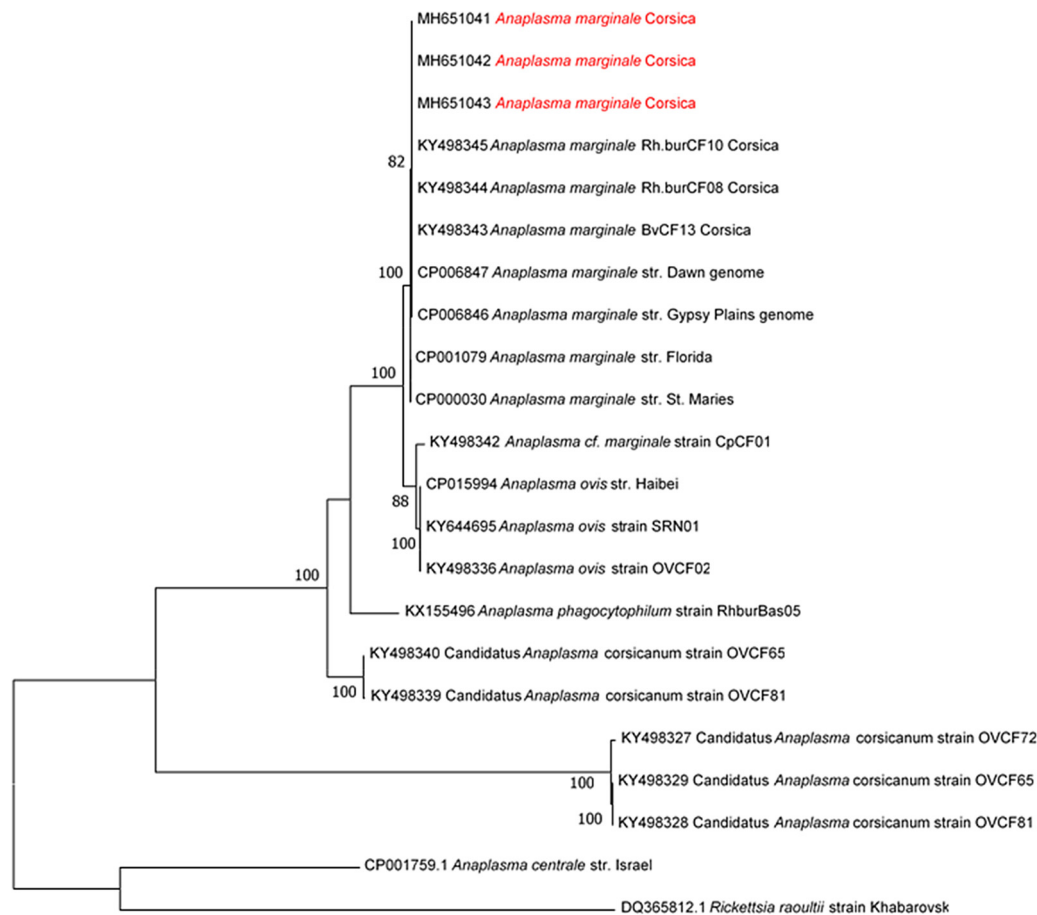


Fig. 4. Phylogenetic analysis of Anaplasmataceae identified in Corsica. The evolutionary history was inferred by using the Maximum Likelihood method based on Anaplasmataceae *rpoB* gene sequences. The analysis involved 30 nucleotide sequences. There were a total of 331 positions in the final dataset.

cattle were 100% identical to each other and clustered together with *Ri. aeschlimannii* sequences (KU961540, DQ235776, and HM50282) (Fig. 3). Nine *gltA* sequences derived from eight *R. bursa* specimens collected from sheep and one pool of *H. marginatum* ticks collected from cattle showed 99–100% identity with each other and 99% identity with *Candidatus Ri. barbariae* collected in Sardinia (EU272185) (Fig. 3). The analyses of the *ompA* gene (GenBank accession numbers MH797764–MH79777) confirmed these results (99% of identity with *Candidatus Ri. barbariae* with KY233248–49 from Lebanon).

3.3. 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). Anaplasmataceae DNA was detected in one (2%) of the 61 *R. bursa* ticks collected from sheep and analyzed individually. Anaplasmataceae 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) (Fig. 4).

3.4. 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.

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

Rhipicephalus bursa was the most abundant tick species collected from cattle (> 80%) and the only tick species collected from small ruminants. This was in line with previous findings from Corsica (Dahmani et al., 2017; Grech-Angelini et al., 2016). In the Mediterranean basin, this species is considered as the major ectoparasite of sheep (Yeruham et al., 2000). The majority of *R. bursa* specimens collected in this study were adults, as they were collected during the summer period (Ferrolho et al., 2016). Majority of *R. bursa* ticks analyzed were positive for *Rickettsia* spp., with a similar detection rate between cattle and sheep. Sequence analysis revealed the detection of *Ri. aeschlimannii* and *Candidatus Ri. barbariae* in *R. bursa*. *Rickettsia* spp., such as *Ri. aeschlimannii* and *Ri. massiliae*, had previously been detected in *R. bursa* in several Mediterranean countries in a wide range of animal hosts (Parola et al., 2013); however, to the best of our knowledge, this is the first detection report of *Candidatus Ri. barbariae* in *R. bursa* ticks collected

from domestic animals in Corsica. These species were described previously in several tick species in Portugal (*R. bursa*), Cyprus (*R. turanicus*), Sardinia (*R. turanicus*), and China in the flea (*Vermipysylla alakurt*) (Zhao et al., 2016). Here, 3% of *R. bursa* tick cattle pools were positive for Anaplasmataceae DNA. We found a detection rate of Anaplasmataceae DNA of 2% among the 61 *R. bursa* collected from sheep, which is similar to the detection rate reported in other Mediterranean regions (Satta et al., 2011; Torina et al., 2010). We also detected the DNA of *A. marginale*. This is consistent with a previous study reporting that the DNA of *A. marginale* was detected in two engorged female ticks removed from cattle (2%) and from all (12/12) cattle blood samples in Corsica (Dahmani et al., 2017).

Hyalomma marginatum was the second most abundant tick species collected from cattle (15.5%), as described previously (Grech-Angelini et al., 2016). *Hyalomma marginatum*, which is the vector of CCHFV, is present in southeastern continental France (Vial et al., 2016), but also in Mediterranean islands such as Sicily (Italy) and Minorca (Spain) (Castella et al., 2001). More than 50% of *H. marginatum* pools were positive for DNA of *Ri. aeschlimannii* and *Candidatus Ri. barbariae*. Such a high infection rate is in agreement with *Ri. aeschlimannii* infection rate of *Hyalomma* (> 70%) previously reported in Corsica (Matsumoto et al., 2004) and Croatia (64%) (Punda-Polic et al., 2002). Lower rates of *Rickettsia* spp. were observed in Sicily (4%) (Torina et al., 2010) and Turkey (6.5%) (Keskin et al., 2016). These differences in results could be related to differences in environmental factors, animal species, the numbers of ticks collected and the analysis methods. In our study, the detection of *Candidatus Ri. barbariae* in a pool of *H. marginatum*, not a recognized vector for this *Rickettsia* species (Parola et al., 2013), could also be explained by the presence of this bacterium in the blood meal of the ticks.

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

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.

Funding

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The inspected cattle were slaughtered for human consumption. Living sheep were examined with the assistance of their owner. Trapping of black rats was organized near to livestock facilities or fence of dwellings and provided according to Regulation No. 20 (order of 29 January 2007 and of 29 June 2011, L. 427-8 of the Environment Code) of the French Ministry of Ecology, Energy and Sustainable Development.

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2. Molecular Detection of Spotted-Fever Group *Rickettsiae* in Ticks Collected from Domestic and Wild Animals in Corsica, France;

Contexte

Suite aux résultats de l'étude préliminaire sur les pathogènes bactériens présents dans les tiques en Corse, ces bactéries du groupe des fièvres pourprées ont fait l'objet d'une étude plus approfondie afin de mieux les caractériser d'un point de vue moléculaire et identifier les espèces circulantes en Corse.

Méthode

Les tiques ont été collectées sur des bovins (*Bos taurus*), des moutons (*Ovis aries*), des sangliers (*Sus scrofa*) et des animaux de compagnie en juillet-août 2017 et en juillet 2018-janvier 2019 (Tableau 1) (Figure 12). La présence de l'ADN de *Rickettsia* spp. a été évaluée par qPCR ciblant le gène *gltA* et par le séquençage partiel des gènes *gltA* et *ompA* pour l'identification des espèces et l'analyse phylogénétique (Tableau 2). Les taux de prévalence des pathogènes ont été calculés selon le MLE avec un IC à 95%.

Résultats



Au total, 1117 tiques appartenant à quatre genres différents (*Rhipicephalus*, *Hyalomma*, *Ixodes* et *Dermacentor*) ont été collectées. Dans l'ensemble, l'ADN de *Rickettsia* spp. a été détecté dans 208 des 349 pools de tiques (MLE = 25,6 %, IC 95 % : 22,6-28,8 %)(Figure 15). L'analyse moléculaire a révélé cinq espèces de Rickettsies différentes du groupe des fièvres pourprées. A savoir, *Ri. Barbariae* dans *R. bursa* et *Ri. aeschlimanii* dans *H. marginatum*. *Ri. slovaca* a été détectée dans *Dermacentor marginatus* collectée sur des sangliers. Cette étude a démontré la présence de *Ri. monacensis* dans une tique *I. ricinus* collectée sur un chien domestique (Figure 15).

Conclusion

Dans l'ensemble, les résultats ont révélé une large circulation des Rickettsies du groupe des fièvres pourprées soulignant la nécessité d'une surveillance du risque d'infection pour les personnes vivant et/ou travaillant à proximité d'animaux infectés ou infestés.

Article

Molecular Detection of Spotted-Fever Group *Rickettsiae* in Ticks Collected from Domestic and Wild Animals in Corsica, France

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Abstract: To obtain a better understanding of the current magnitude of tick-borne rickettsioses in Corsica, we used molecular methods to characterize the occurrence of *Rickettsia* spp. in ixodid ticks collected from domestic and wild animals. The presence of *Rickettsia* spp. was evaluated using real-time polymerase chain reaction targeting the *gltA* gene and by sequencing of *gltA* and *ompA* partial genes for species identification and phylogenetic analysis. Infection rates were calculated as the maximum-likelihood estimation (MLE) with 95% confidence intervals (CI). In total, 1117 ticks belonging to four genera (*Rhipicephalus*, *Hyalomma*, *Ixodes*, and *Dermacentor*) were collected from cattle, sheep, wild boars, and companion animals during July–August 2017 and July 2018–January 2019. Overall, *Rickettsia* DNA was detected in 208 of 349 pools of ticks (MLE = 25.6%, 95% CI: 22.6–28.8%). The molecular analysis revealed five different rickettsial species of the spotted-fever group (SFG). We highlighted the exclusive detection of *Candidatus* *Ri. barbariae* in *R. bursa* and of *Ri. aeschlimanii* in *H. marginatum*. *Rickettsia slovacica* was detected in *D. marginatus* collected from wild boars. This study provides the first evidence of the presence of *Ri. monacensis* in *I. ricinus* ticks isolated from a dog in Corsica. In conclusion, our data revealed wide dispersal of SFG *Rickettsiae* and their arthropod hosts in Corsica, highlighting the need for surveillance of the risk of infection for people living and/or working close to infected or infested animals.

Keywords: ticks; Corsica; *Rickettsia*; wild animals; domestic animals; host

1. Introduction

Rickettsiae are obligate intracellular Gram-negative bacteria that are transmitted to vertebrate hosts by arthropod vectors such as ticks, fleas, lice, and mites. The genus *Rickettsia* (family Rickettsiaceae; order Rickettsiales) comprises 31 species that cause diseases in vertebrate hosts, including humans and domestic and wild animals [1]. Ticks can transmit these bacteria to humans and animals by feeding. Members of the genus *Rickettsia* can be classified into four phylogenetic groups: (1) the spotted-fever group (SFG), (2) the typhus group (TG), which includes the agents of epidemic typhus and murine typhus, (3) the ancestral group (AG), consisting of *Ri. bellii* and *Ri. canadensis*, and (4) the transitional group, the *Ri. akari* group (TRG) [2]. The SFG and AG *Rickettsiae* are mainly associated with ticks, while TG and TRG *Rickettsiae* are associated with other arthropods such as lice, fleas, and mites [3].

Rickettsia spp. and their associated human clinical diseases vary depending on geographical location [4]. In Europe, rickettsioses are caused mainly by tick-borne SFG *Rickettsiae* [4]. In the

Mediterranean area, Rickettsiae species have been detected in several tick species, but human rickettsioses are usually considered to be caused by *Ri. conorii*, the causative agent of Mediterranean spotted fever (MSF) [1,5]. *Rickettsia aeschlimanii*, *Ri. massiliae*, and *Ri. monacensis* can also cause a similar clinical presentation. Other species that have been associated with human disease and detected in Europe include *Ri. slovaca* and *Ri. raoultii*, both causes of tick-borne lymphadenopathy and *Dermacentor*-borne necrosis lymphadenopathy (TIBOLA/DEBONEL) [6,7].

Information about the circulation and distribution of Rickettsia-infected tick species in Corsica, a French Mediterranean island, is scarce and fragmented. *Rickettsia aeschlimanii* and *Candidatus Ri. barbariae* were identified in *Hyalomma marginatum* and *Rhipicephalus bursa* collected from domestic animals in 2016 [8]. *Rickettsia slovaca* has been detected in ticks collected from vegetation. *Rickettsia massiliae* was reported from an *R. turanicus* collected from a dog in Corsica in 2005, and *Ri. africae* was detected in one *Amblyomma variegatum* collected from bovine in August 2018.

To obtain a better understanding of the current magnitude of tick-borne rickettsioses in Corsica, we aimed to use molecular methods to describe the presence of tick-borne Rickettsiae in tick species collected from domestic and wild animals. From a public health perspective, a better understanding of local tick species and of *Rickettsia* species carried by them is essential to improve local surveillance systems.

2. Results

2.1. Sampling and Identification of Ticks

In total, 1117 ticks were collected from 1014 animals (381 cattle, 500 sheep, 119 wild boars, 13 dogs, and 1 cat) (Table 1). Of these, 1075 (96% of 1117) were adult ticks and 674 (60% of 1117) were male ticks (Table 1). The sequences of mitochondrial 16S rDNA fragments of 12 ticks selected in this study, were confirmed, after BLAST analysis, to be *D. marginatus* ($n = 5$), *R. bursa* ($n = 1$), *H. marginatum* ($n = 1$), *I. ricinus* ($n = 1$), and *R. sanguineus s.l.* ($n = 4$).

Overall, 834 ticks were collected from 381 cattle skins, from which, three tick species were morphologically identified. The most abundant species was *R. bursa* ($n = 608$; 73.0% of ticks collected in cattle) followed by *H. marginatum* ($n = 216$; 25.8%), and *I. ricinus* ($n = 10$; 1.2%) (Table 1).

Ten ticks were collected from 500 sheep in one farm (Biguglia, Haute-Corse) in June 2017. All these were *R. bursa* (Table 1).

Two-hundred forty-five ticks were collected from 119 wild boars from August 2018 to January 2019. Four tick species were identified in wild boars. The most abundant species was *D. marginatus* ($n = 223$; 91.0%), followed by *I. ricinus* ($n = 13$; 5.3%), *R. bursa* ($n = 8$; 3.3%), and *H. marginatum* ($n = 1$; 0.4%) (Table 1).

Twenty-seven ticks were collected from 13 dogs from July to August 2017. Two tick species were identified in dogs: *Rhipicephalus sanguineus s.l.* ($n = 24$; 88.8%) and *I. ricinus* ($n = 3$; 11.2%). One *D. marginatus* was collected from one cat.

Table 1. Total tick species collected from hosts sampled.

Host (n of Hosts Inspected; Infestation Rate (%))	Tick Species	n Ticks (%)	Male n (%)	Female n (%)	Nymph n (%)
Cattle (n = 381; 37.7%)	<i>R. bursa</i>	608 (73.0)	350 (57.6)	225 (37.0)	33 (5.4)
	<i>H. marginatum</i>	216 (25.8)	163 (75.4)	53 (24.6)	0 (0.0)
	<i>I. ricinus</i>	10 (1.2)	3 (3.0)	7 (7.0)	0 (0.0)
	Total	834	516 (62.0)	285 (34.0)	33 (4.0)
Sheep (n = 500; 2.0%)	<i>R. bursa</i>	10 (100)	10 (100.0)	0	0
	Total	10	10		
Wild boars (n = 119; 38.6%)	<i>D. marginatus</i>	223 (91.0)	144 (64.5)	79 (35.5)	0 (0.0)
	<i>I. ricinus</i>	13 (5.3)	1 (8.0)	12 (92.0)	0 (0.0)
	<i>R. bursa</i>	8 (3.3)	1 (12.5)	7 (87.5)	0 (0.0)
	<i>H. marginatum</i>	1 (0.4)	0 (0.0)	1 (100)	0 (0.0)
	Total	245	146 (60.0)	99 (40.0)	0 (0.0)

Table 1. Cont.

Host (n of Hosts Inspected; Infestation Rate (%))	Tick Species	n Ticks (%)	Male n (%)	Female n (%)	Nymph n (%)
Dogs (n = 13 * §)	<i>R. sanguineus</i> s.l.	24 (89.0)	2 (8.3)	14 (58.3)	8(33.3)
	<i>I. ricinus</i>	3 (11.0)	0	3 (100)	0
	Total	27	2 (7.4)	17 (63.0)	8 (29.6)
Cat (n = 1 * §)	<i>D. marginatus</i>	1 (100)	0	0	1(100)
Total (n = 1014)	All species	1117	674 (60.4)	401 (35.8)	42 (3.8)

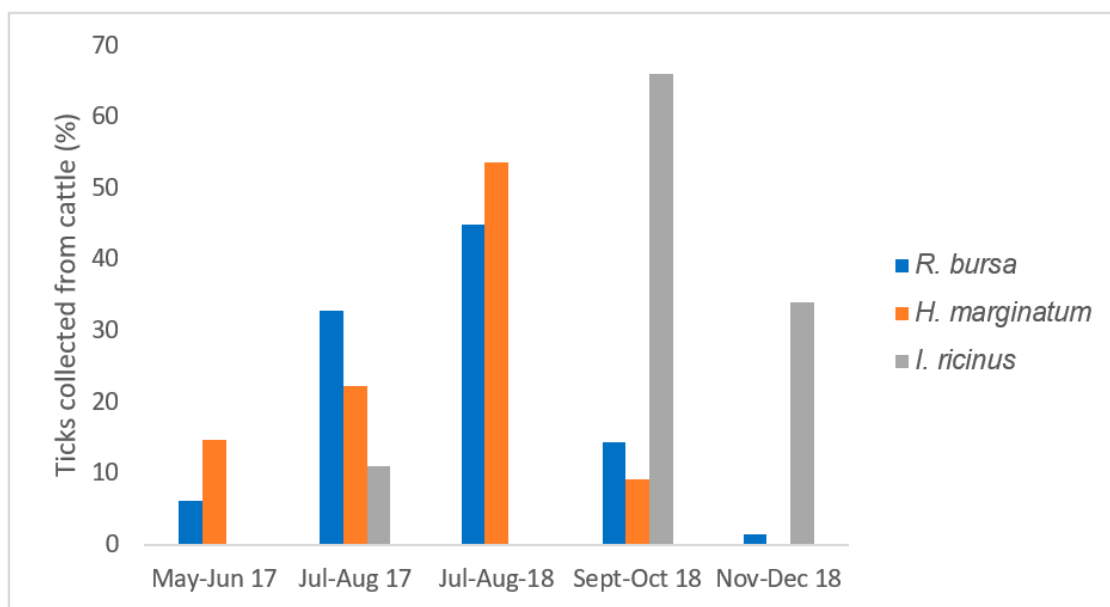
* Tick collected by veterinarian. § infestation rate not estimable.

2.2. Infestation Rate in Cattle, Sheep, and Wild Boars

The infestation rate in cattle was 19.2% (27/140) in 2017 (May–August 2017) and 36.0% (87/241) in 2018 (July–December 2018). Overall, the July–August 2018 infestation rate (65.1%; 54/83) was significantly higher than the July–August 2017 infestation rate (46.5%; 40/86) ($p = 0.02$), with a peak in July 2018 (70.7%; 29/41) (Figure 1a). The observed proportion of *H. marginatus* was significantly higher in July–August 2018 (53.7%; 116/216) than in July–August 2017 (22.2%; 48/216) ($p < 0.0001$) (Figure 1). Similarly, the observed proportion of *R. bursa* was significantly higher in July–August 2018 (45.0%; 208/608) than July–August 2017 (33.0%; 273/608) ($p < 0.0001$) (Figure 1a). *Ixodes ricinus* showed its peak activity in September–October 2018 (60.0%; 6/10).

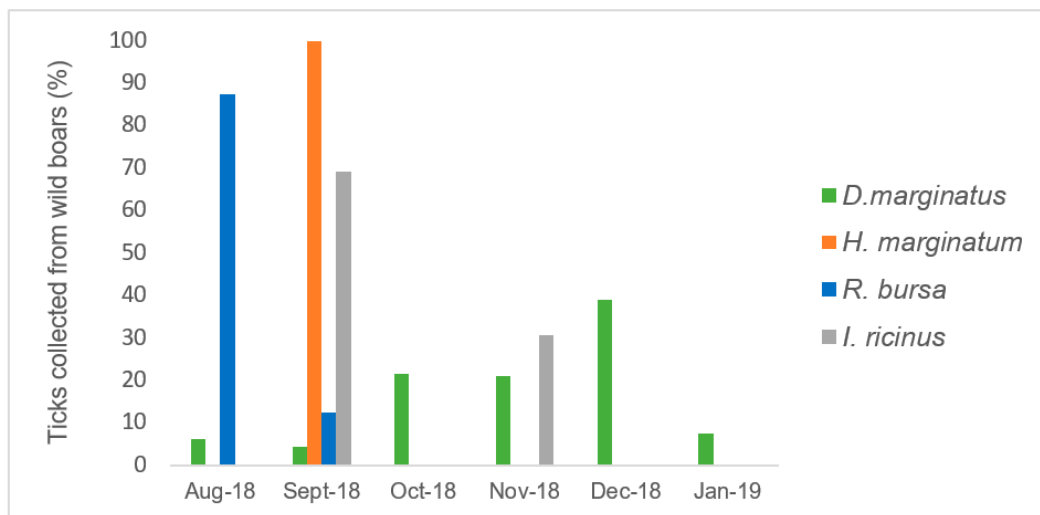
The infestation rate of sheep was 2% and it was not drastically different from the 4% observed in cattle during the same period (May 2017) ($p = 0.68$).

The infestation rate observed in wild boars was 38.6% (46/119). *Dermacentor marginatus* was mainly collected from wild boars throughout the surveillance period (August 2018–January 2019), with a peak of activity in December 2018. September 2018 was characterized by the co-circulation of four species of ticks in wild boars (Figure 1b).



(a)

Figure 1. Cont.



(b)

Figure 1. (a) Seasonal distribution of ticks collected from cattle (May–August 2017 and July–December 2018); (b) Seasonal distribution of ticks collected from wild boars (August 2018–January 2019).

2.3. Detection of Rickettsial DNA

Overall, *Rickettsia* DNA was detected in 208 of 349 pools of ticks collected with an infection rate (maximum-likelihood estimation (MLE)) of 25.6% (95% CI: 22.6–28.8%). In ticks collected from cattle, *Rickettsia* spp. DNA was detected in 157 of 255 pools, with the highest infection rate for *H. marginatum* (50.5%, 95% CI: 37.0–64.4%) compared with *R. bursa* (16.6%, 95% CI: 13.3–20.4%) ($p < 0.0001$) and *I. ricinus* (2.7%, 95% CI: 0.1–11.6%) ($p < 0.0001$) (Table 2).

In sheep, the 10 ticks were analyzed in two pools. The detection rate of *Rickettsia* spp. DNA for *R. bursa* was 100%.

In ticks collected from wild boars, *Rickettsia* spp. DNA was detected in 46 of 78 pools with an infection rate of 24.4% (95% CI: 18.5–31.1%). The highest infection rate was observed for *D. marginatus* (23.0%, 95% CI: 16.9–30.0%) (Table 3).

The *Rickettsia* spp. DNA infection rate observed in ticks collected from cattle during July–August months of 2018 (34.7%, 95% CI 28.4–41.6%) was similar to that of 2017 during the same period (26.3%, 95% CI: 22.7–30.1%) ($p = 0.219$).

Rickettsia spp. DNA was detected in 2 (15.3%) of 13 tick pools collected from dogs. The infection rate was 5.0% (95% CI: 0.8–14.9%). The *D. marginatus* collected from one cat was also positive for *Rickettsia* spp. DNA.

Table 2. Distribution of detected *Rickettsia* spp. in the tick species collected from cattle in 2017 (May–August 2017) and 2018 (July–December 2018) surveillance periods.

Number of Individual Ticks or Ticks per Pool (<i>n</i>)	Number of Pools with <i>n</i> Ticks				Positive <i>Rickettsia</i> spp. <i>n</i> s(%)			
	2017 (July–August)	<i>H. marginatum</i>	<i>R. bursa</i>	<i>I. ricinus</i>	Total	<i>H. marginatum</i>	<i>R. bursa</i>	<i>I. ricinus</i>
1	15	19	1	35	11 (73)	6 (32)	0 (0)	17 (49)
2	13	9	0	22	11 (85)	1 (11)	0 (0)	12 (55)
3	6	6	0	12	4 (67)	1 (17)	0 (0)	5 (42)
4	1	7	0	8	1 (100)	3 (43)	0 (0)	4 (50)
5	1	8	0	9	1 (100)	5 (63)	0 (0)	6 (67)
6	2	19	0	21	1 (50)	8 (42)	0 (0)	9 (43)
Total pools	38	68	1	107	29 (76)	24 (35)	0 (0)	53 (50)
MLE (95% CI)					50.5% (37.0–64.4)	12.2% (8.1–17.4)	0	20.7% (16.0–26.1)
2018 (July–December)	<i>H. marginatum</i>	<i>R. bursa</i>	<i>I. ricinus</i>	Total	<i>H. marginatum</i>	<i>R. bursa</i>	<i>I. ricinus</i>	Total
1	25	12	1	38	24 (96)	5 (42)	0 (0)	29 (76)
2	9	7	1	17	5 (55)	5 (71)	0 (0)	10 (62)
3	14	8	1	23	13 (93)	4 (50)	1 (100)	18 (78)
4	5	15	0	20	4 (80)	7 (46)	0 (0)	11 (55)
5	5	14	0	19	5 (100)	10 (66)	0 (0)	15 (79)
6	1	30	0	31	1 (100)	20 (64)	0 (0)	21 (68)
Total pools	59	86	3	148	52 (88)	51 (58)	1 (33)	104 (70)
MLE (95% CI)					65.3% (52.8–77.3)	20.1% (15.3–25.6)	2.8% (0.1–11.9)	30.7% (25.6–36.2)
2017 and 2018	<i>H. marginatum</i>	<i>R. bursa</i>	<i>I. ricinus</i>	Total	<i>H. marginatum</i>	<i>R. bursa</i>	<i>I. ricinus</i>	Total
1	40	31	2	73	35 (87)	11 (35)	0 (0)	46 (63)
2	22	16	1	39	16 (73)	6 (37)	0 (0)	22 (56)
3	20	14	1	35	17 (85)	5 (36)	1 (100)	23 (66)
4	6	22	0	28	5 (83)	10 (45)	0 (0)	15 (53)
5	6	22	0	28	6 (100)	15 (68)	0 (0)	21 (75)
6	3	49	0	52	2 (67)	28 (56)	0 (0)	30 (58)
Total pools	97	154	4	255	81 (83)	75 (48)	1 (25)	157 (61)
MLE (95% CI)					58% (49.4–67.9)	16.6% (13.3–20.4)	2.7% (0.1–11.6)	26.3% (22.7–30.1)

Table 3. Distribution of detected *Rickettsia* spp. in the tick species collected from wild boars (August 2018–January 2019).

Number of Individual Ticks or Ticks per Pool (<i>n</i>)	Number of Pools with <i>n</i> Ticks					Positive <i>Rickettsia</i> spp. <i>n</i> (%)				
	<i>D.</i> <i>marginatus</i>	<i>I.</i> <i>ricinus</i>	<i>R.</i> <i>bursa</i>	<i>H.</i> <i>marginatum</i>	Total	<i>D.</i> <i>marginatus</i>	<i>I. ricinus</i>	<i>R. bursa</i>	<i>H.</i> <i>marginatum</i>	Total
1	15	1	3	1	20	8 (53)	1 (100)	2 (67)	1 (100)	12 (60)
2	10	2	1	0	13	7 (70)	1 (50)	1(100)	0 (0)	9 (62)
3	11	0	1	0	12	7 (64)	0 (0)	1(100)	0 (0)	8 (67)
4	11	2	0	0	13	8 (73)	0 (0)	0 (0)	0 (0)	8 (62)
5	9	0	0	0	9	3 (34)	0 (0)	0 (0)	0 (0)	3 (33)
6	11	0	0	0	11	6 (56)	0 (0)	0 (0)	0 (0)	6 (55)
Total pools	67	5	5	1	78	39 (58)	2 (40)	4 (80)	1 (100)	46 (59)
MLE (95% CI)						23.0% (16.9–30.0)	4.9% (0.0–14.4)	10.9% (3.5–23.7)	2.4% (0.0–10.2)	24.4% (18.5–31.1)

2.4. *Rickettsia* Species Identification Based on Sequence Analysis

The molecular characterization of the Rickettsiae in ticks was based on *gltA* ($n = 91$ of 208 positive pools; 43.8%). A subset of positive samples ($n = 33$) was also characterized by sequencing *ompA* to confirm *Rickettsia* spp. (Table 4). The molecular analysis of *gltA* and *ompA* sequences revealed five different rickettsial species.

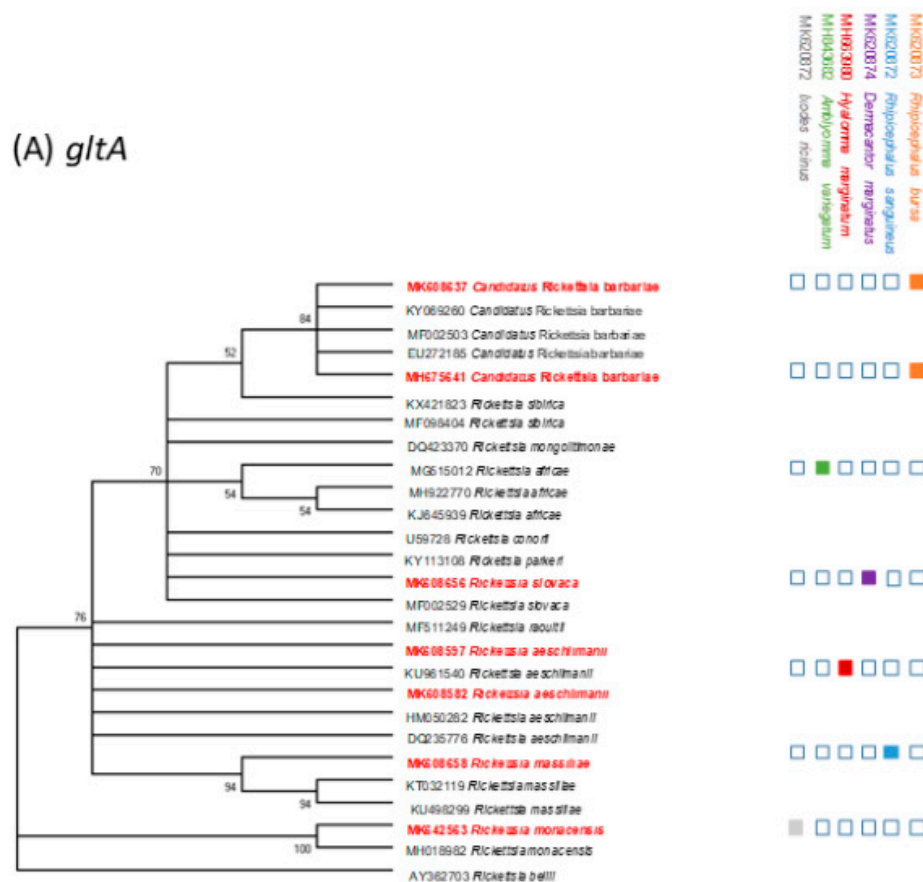
All *Rickettsia* detected in *H. marginatum* tick pools ($n = 56$) collected from cattle and wild boars were exclusively *Ri. aeschlimanii* (Table 4). *Candidatus Ri. barbariae* was the only bacterium detected in *R. bursa* tick pools collected from sheep ($n = 2$) and from cattle in 2017 ($n = 5$). The DNA of *Ri. aeschlimanii* and *Candidatus Ri. barbariae* were respectively detected in five and eleven *R. bursa* pools collected from cattle in 2018. The DNA of *Ri. slovaca* was detected in six *D. marginatus* tick pools, in two *I. ricinus* tick pools and in four *R. bursa* tick pools, all collected from wild boars. It is to be noted that *Ri. slovaca* DNA was also detected in one *D. marginatus* tick collected from a cat. *Rickettsia massiliae* and *Ri. monacensis* DNA have been detected in one pool of *R. sanguineus s.l.* ticks and in one pool of *I. ricinus* ticks collected from dogs.

Table 4. The spotted-fever group Rickettsiae identified in tick species collected from different hosts.

Host	Tick Species	No. of Pools Positive for Rickettsiae	No of <i>gltA</i> Sequences	No of <i>ompA</i> Sequences	Identified <i>Rickettsia</i> Species
2017					
Cattle	<i>H. marginatum</i>	29	24	5	<i>Ri.aeschlimanii</i>
	<i>R. bursa</i>	24	5	2	<i>Candidatus Ri. barbariae</i>
Sheep	<i>R. bursa</i>	2	2	1	<i>Candidatus Ri. barbariae</i>
Dogs	<i>R.sanguineuss.l.</i>	1	1	1	<i>Ri. massiliae</i>
	<i>I. ricinus</i>	1	1	1	<i>Ri. monacensis</i>
Cat	<i>D. marginatus</i>	1	1	0	<i>Ri. slovaca</i>
Total pools 2017		58	34	10	
2018					
Cattle	<i>H. marginatum</i>	52	31	13	<i>Ri.aeschlimanii</i>
	<i>R. bursa</i>	51	14	5	<i>Ri. aeschlimanii; Candidatus Ri. barbariae</i>
	<i>I. ricinus</i>	1	1	0	<i>Ri.aeschlimanii</i>
Wild boars	<i>D. marginatus</i>	39	6	3	<i>Ri. slovaca</i>
	<i>I. ricinus</i>	2	2	0	<i>Ri. slovaca</i>
	<i>R. bursa</i>	4	2	2	<i>Ri. slovaca</i>
	<i>H. marginatum</i>	1	1	0	<i>Ri.aeschlimanii</i>
Total pools 2018		150	57	23	
Total pools 2017–2018		208	91	33	<i>Ri. aeschlimanii; Candidatus Ri. barbariae; Ri. massiliae; Ri. monacensis; Ri. slovaca</i>

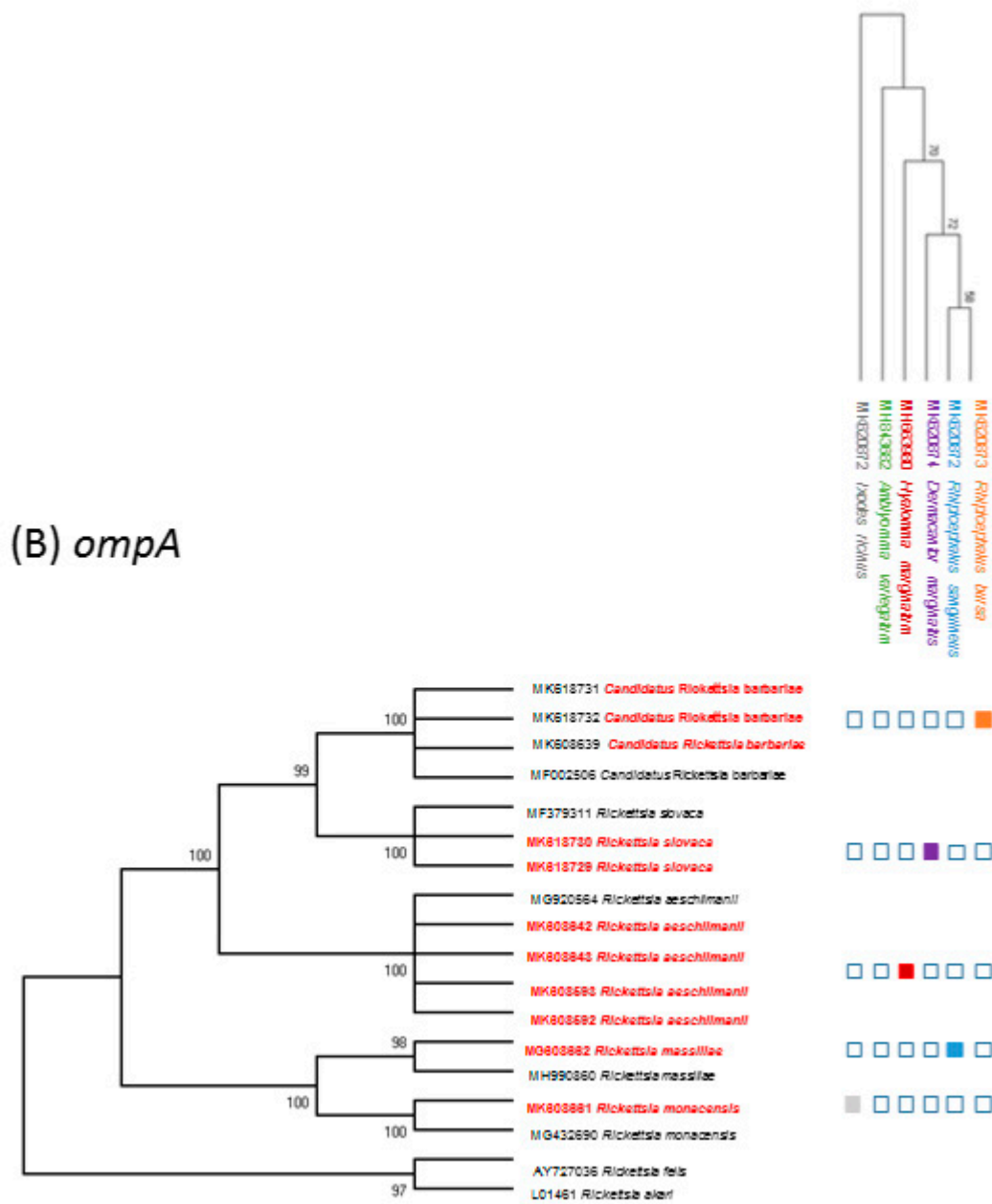
2.5. Phylogenetic Analysis

The results of phylogenetic trees based on *gltA* and *ompA* sequences are illustrated in Figure 2A,B). Cluster I corresponded to *Ri. aeschlimanii*: the sequences were 100% identical to each other and to the *Ri. aeschlimanii* sequences deposited in GenBank (KU961540 (Russia) [*gltA*], MG920564 (Turkey) [*ompA*]). Cluster II represented the *Candidatus Ri. barbariae* group. Our sequences were 99–100% identical to each other and to the *Candidatus Ri. barbariae* detected in Sardinia (EU272185 [*gltA*] and MF002506 [*ompA*]). Cluster III represented *Ri. slovacae*; sequences were 100% identical to each other and showed 99.9% identity with that in GenBank (MF002529 (China) [*gltA*] and MF379311 (Turkey) [*ompA*]). Cluster four corresponded to the *Ri. monacensis* sequences group (100% identical to *Ri. monacensis* MH18982 (Poland) [*gltA*] and MG32690 (Italy) [*ompA*]). Cluster five included *Ri. massiliae* sequences and displayed 99.8% identity with *Ri. massiliae* (MH990860 (Pakistan) [*gltA*] and MH990860 (Pakistan) [*ompA*]).



(A)

Figure 2. Cont.



(B)

Figure 2. (A) A phylogenetic tree of spotted fever-group Rickettsiae based on the *gltA* gene sequences. The analysis was performed using a maximum-likelihood method with the Kimura 2-parameter model. This analysis involved 27 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 744 positions in the final dataset. The sequences detected in this study are indicated in red. The simplified tick phylogeny consisting of four species is indicated on the top right. The colored boxes indicate the presence of Rickettsia DNA in each tick species. (B) A phylogenetic tree of spotted-fever group *Rickettsiae* based on the *ompA* gene sequences. The analysis was performed using a maximum-likelihood method with the Kimura 2-parameter model. This analysis involved 18 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 493 positions in the final dataset. The sequences detected in this study are indicated in red. The simplified tick phylogeny consisting of four species is indicated on the top right. The colored boxes indicate the presence of Rickettsia DNA in each tick species.

3. Discussion

To our knowledge, this is the first reported evidence of the circulation of five bacteria from the genus of *Rickettsia* in four genera of ticks (*Rhipicephalus*, *Hyalomma*, *Dermacentor*, and *Ixodes*), collected from wild and domestic animals mainly reared in Northern Corsica.

In the present study, five tick species were collected, namely, *R. bursa*, *H. marginatum*, *I. ricinus*, *D. marginatus*, and *R. sanguineus s.l.* *Rhipicephalus* ticks were the most abundant species collected in this study. The diversity and seasonal pattern of ticks observed in this study, though collected not over 12 months and on selected animals kept under various conditions, is consistent with that reported previously for Corsica [8,9] and other Mediterranean countries [10–12]. In this study, overall, the low infestation rate observed in sheep with respect to cattle could be due to the short collection period and to a low density of tick populations usually reported in pastures where livestock had grazed with respect to undisturbed fallow lands [13].

In the present study, *R. bursa* was the tick species most frequently collected in cattle and sheep, with a peak of activity observed in the summer. This typical Mediterranean species is well established in the whole of Corsica [8,9]. In this study overall, almost 50% of *R. bursa* tick pools, mainly collected from cattle and sheep, were positive for *Rickettsia* spp. Interestingly, *Candidatus* *Ri. barbariae* DNA was exclusively detected in tick pools of *R. bursa* removed from sheep (100% of sequenced tick pools) and mostly in cattle (76%). Our detection of *Candidatus* *Ri. barbariae* in *R. bursa* collected from domestic animals reinforces its presence in Corsica ([8], pp. 606–613) and highlights the possible role played by *R. bursa* ticks in the natural maintenance of this bacterium. To understand better the role of *R. bursa* in the maintaining of *Candidatus* *Ri. barbariae*, ticks should be collected from nature. *Candidatus* *Ri. barbariae* is a presumed new species of the genus *Rickettsia* genetically characterized for the first time in *R. turanicus* from Sardinia [14], a Mediterranean island separated from Corsica by 10 km of sea. The presence of this bacterium has also been reported in several *Rhipicephalus* spp. in Portugal, Italy, France, Algeria, Cyprus, Israel, Cameroon, Lebanon [12,15–19], and in *Hyalomma* ticks in the West Bank [20]. The pathogenic role of *Candidatus* *R. barbariae* remains unknown for humans and animals, although it has been identified in an *R. bursa* tick removed from a woman in Greece [21].

Hyalomma marginatum was the second main tick species collected from cattle in the northern region of Corsica. This tick species was almost exclusively collected from cattle (99%). One *H. marginatum* was collected from a wild boar. *Rickettsia* spp. DNA was detected in more than 80% of *H. marginatum* pools collected from cattle sampling in Northern Corsica. *Hyalomma marginatum* ticks were exclusively infected with the human pathogen *Ri. aeschlimanii*, regardless of the host species (cattle or wild boars). In Corsica, cattle are reared outside all year round and acaricide treatments are rarely performed, increasing the opportunity of a wide circulation of this pathogen in Corsica. Such a high infection rate is consistent with the previously reported rate of infection of *Hyalomma* by *Ri. aeschlimanii* (>50% and >70%) in Corsica [8,22], in Croatia (64%) [23], and in Germany (50%) [24]. *Rickettsia aeschlimanii* may be spread through migratory birds from Africa, and it was detected in *Hyalomma* ticks collected from barn swallows (*Hirundo rustica*) in Corsica [25]. The first human case of infection was described in a patient who developed symptoms after returning from Morocco [26].

Even if wild boars and cattle share the same environment in Corsica [9], dominant tick species collected in the present study from these animals differed. In the present study, we confirmed that *D. marginatus* was the main tick species collected from wild boars, confirming the host preference previously reported. This tick species was not observed in cattle investigated in this study.

In the present study, most specimens of *D. marginatus* were collected during winter because the adults are active within a temperature range of 4–16 °C [27]. More than half of the pools of *D. marginatus* collected were positive for *Rickettsia* spp. Sequence analyses revealed for the first time the presence of *Ri. slovacica* DNA in *D. marginatus* ticks collected from wild boars in Corsica. This bacterium was previously detected in *D. marginatus* collected by flagging in Corsica and in other regions of the northern Mediterranean area. *Rickettsia slovacica* was identified in *D. marginatum* ticks collected on swine and wild boars in Sardinia [28]. Overall, these results suggested that the role of

D. marginatus is important for the maintenance of *Ri. slovaca*. In Corsica, recreational hunting of wild boars could provide an ideal environment for the transmission of *Ri. slovaca* between wild boars, humans, and companion animals (e.g., hunting dogs). At present, the *D. marginatus* tick is recognized as the main vector and reservoir for *Ri. slovaca* in Mediterranean areas, including southern Europe and North Africa [1]. This human pathogen has been detected in almost half of *D. marginatus* ticks collected from rodents in the northern Apennines (Italy) and by flagging in Kazakhstan [29,30]. *Rickettsia slovaca* is associated with a syndrome characterized by neck lymphadenopathy following tick bites [6]. Human infection with *Ri. slovaca* has been described in several European countries, including France [31]. We also reported for the first time the detection of *Ri. slovaca* DNA in one *D. marginatus* collected from a cat.

In this study, *R. sanguineus s.l.* ticks were exclusively collected from dogs. We reported the detection of *Ri. massiliae* DNA in one pool of *R. sanguineus s.l.* collected from a dog. *Rhipicephalus sanguineus s.l.* is the likely reservoir of *Ri. massiliae*, with transovarian passage rates up to 100%. *Rickettsia massiliae* was reported from a specimen of *R. turanicus* collected from a dog in Corsica [32] and has been putatively linked to mild to moderately severe illnesses in dogs in California [33]. *Rickettsia massiliae* is recognized as a pathogenic Rickettsia causing spotted fever in humans [1].

In the present study, *I. ricinus*, mainly collected during autumn, was sporadic and represented almost 2% of all ticks sampled. The DNA of *Ri. slovaca* was detected in pools of *I. ricinus* ticks collected from wild boars. We report for the first time the detection in Corsica of *Ri. monacensis* DNA in one pool of *I. ricinus* ticks collected from one dog. *Rickettsia monacensis*, an emerging human pathogen of the SFG Rickettsiae family, has been previously detected in *I. ricinus* collected from dogs in Spain [34] and in dog blood samples from the Maio Islands [35].

This study has some limitations. Finding the DNA of human and veterinary pathogens in feeding ticks is only a marker of the community of tick-transmitted pathogens circulating in the target territory [36]. Ticks were mostly analyzed in pooled samples and not individually. Working with pools can lead to underestimation of the infection rate, which must be interpreted with caution.

The ticks were mostly collected in a municipal slaughterhouse in Northern Corsica. We have no data about the southern part of the island. Moreover, ticks from cattle, wild boars, and dogs were collected from several locations while the sheep were only from one farm.

4. Methods

4.1. Study Area and Ticks

Ticks were manually collected at several sites in Corsica (Figure 3) as follows: (i) in May 2017 from sheep ($n = 500$) bred in one farm located at the edge of the largest lagoon in Corsica, the Biguglia Nature Reserve; (ii) in July–August 2017 and July–December 2018 from cattle ($n = 140$ and $n = 241$, respectively) in the Ponte-Leccia slaughterhouse, and (iii) from wild boars ($n = 119$) during the 2018 hunting season (August 2018–January 2019). In July–August 2017, ticks were sampled from 13 dogs and one cat that presented at three veterinary clinics for a variety of reasons and not specifically for symptoms related to tick infestation.

Ticks were collected from sheep during milking mainly around the perineum and udder. The farmer declared he had never treated sheep against ectoparasites. The breeding system was extensive; thus, the sheep live in open pastures.

During each visit to the slaughterhouse, the whole skin of slaughtered animals was inspected, and ticks were collected manually. The national cattle identification system, which uses ear tags, allowed the origin of the animals to be tracked and the farm owners to be identified. One to 20 ticks were collected from each infested animal.

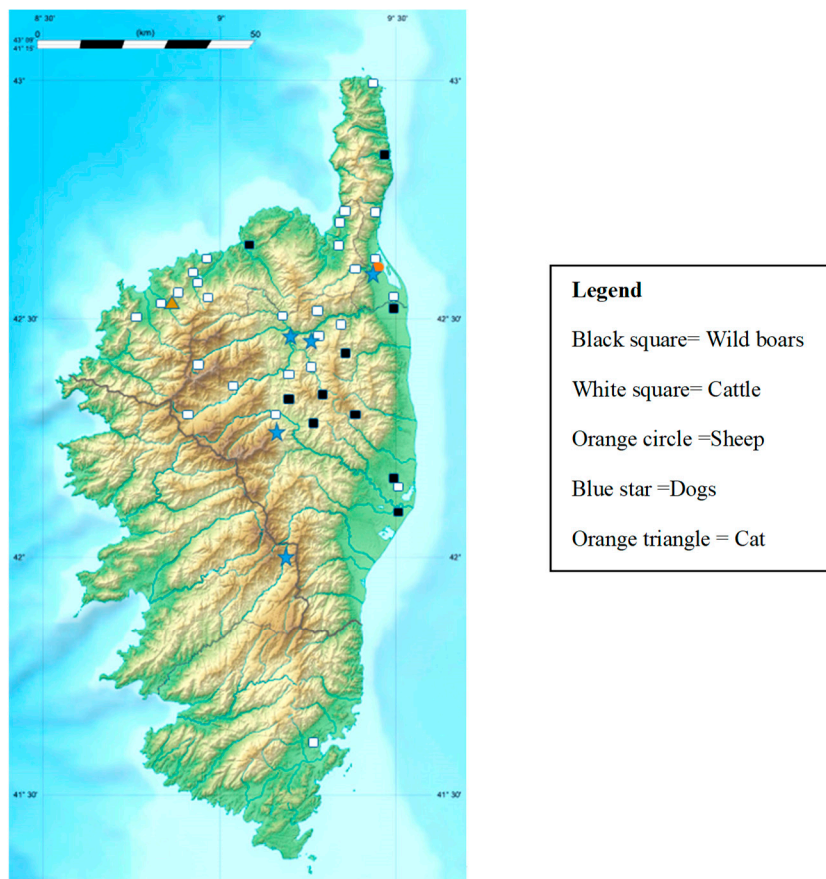


Figure 3. Map of Corsica, France, indicating the tick collection sites and the animal species. **GPS coordinates:** **Cattle:** Oletta (42°38'00" N, 9°21'22" E), Filicetu (42°32'40" N, 8°56'09" E), Corti (42°18'23" N, 9°09'05" E), Nessa (42°33'04" N, 8°56'57" E), Portivechju (41°35'30" N, 9°16'49" E), Omessa (42°22'16" N, 9°12'39" E), Lama (42°34'39" N, 9°10'22" E), Monticellu (42°37'05" N, 8°57'16" E), Lucciana (42°32'48" N, 9°25'05" E), Calinzana (42°30'31" N, 8°51'21" E), Lentu (42°31'22" N, 9°16'57" E), San Iurenzu (42°23'06" N, 9°17'28" E), San Martinu di Iota (42°43'26" N, 9°27'21" E), Casamaccioli (42°19'06" N, 9°00'07" E), Patrimoniu (42°41'54" N, 9°21'44" E), Olmeta-di-tuda (42°36'44" N, 9°21'16" E), Farinole (42°43'58" N, 9°21'58" E), Penta-Acquatella (42°27'55" N, 9°21'49" E), Zilia (42°31'52" N, 8°54'06" E), Castellu di rustinu (42°27'52" N, 9°18'56" E), Pietralba (42°32'51" N, 9°11'11" E), Santa-Reparata-di-Balagna (42°36'16" N, 8°55'45" E), Cateri (42°34'21" N, 8°53'33" E), Ruglianu (42°57'25" N, 9°25'08" E), Filicetu (42°32'40" N, 8°56'09" E), Ascu (42°27'16" N, 9°01'59" E), Corscia (42°21'20" N, 9°02'36" E), Vilone Ornetu (42°24'06" N, 9°28'18" E), Furiani (42°39'32" N, 9°24'54" E), Monte grossu (42°30'06" N, 8°55'22" E), Tallone (42°13'55" N, 9°24'53" E). **Wild boars:** Chiatra (42°17'34" N, 9°28'34" E), Tralonca (42°20'39" N, 9°12'26" E), Quercitellu (42°25'37" N, 9°21'02" E), Bustanicu (42°19'24" N, 9°18'03" E), Favalellu (42°17'43" N, 9°16'20" E), Venzolasca (42°29'06" N, 9°27'26" E), Sermanu (42°18'54" N, 9°16'06" E), Palasca (42°35'24" N, 9°02'36" E), Pianellu (42°17'26" N, 9°21'39" E), Cagnanu (42°52'34" N, 9°25'50" E), Santa-Lucia-di-Mercuriu (42°19'37" N, 9°13'18" E), Aleria (42°06'53" N, 9°30'48" E), Mazzola (42°18'05" N, 9°18'40" E). **Dogs:** Biguglia (42°37'41" N, 9°25'14" E), Casanova (42°15'19" N, 9°10'30" E), Castellu di Rustinu (42°27'52" N, 9°18'56" E), Ponte-Leccia (42°26'10" N, 9°18'01" E), Palneca (41°58'14" N, 9°10'26" E). **Cat:** Muru (42°32'47" N, 8°54'54" E). **Sheep:** Biguglia (42°37'41" N, 9°25'14" E).

4.2. Morphologic Identification of Ticks at the Species Level

Ticks were identified at species level using a pictorial guide [37]. Specific attention was brought to *Ixodes* ticks to identify *I. inopinatus* [38].

4.3. DNA Extraction

Ticks were washed once in 70% ethanol for 5 min and then twice in distilled water for 1 min each time. Following species identification, ticks were analyzed individually or using monospecific pools consisting of two to six ticks according to developmental stage (nymphs, not engorged females, and male adults) and host. Individual or pools of ticks were crushed in phosphate-buffered saline using TissueLyser II (Qiagen, Hilden, Germany) at 5500 rpm for 20 s. DNA extraction was performed using QIAcube HT (Qiagen) and a QIAamp cadour Pathogen Mini Kit according to the manufacturer's instructions. DNA was eluted in 150 µL of buffer and stored at −20 °C. For each polymerase chain reaction (PCR) reaction, the template DNA was at a final amount of <200 ng.

4.4. Molecular Identification of Ticks at the Species Level

Morphological identification of ticks was confirmed using PCR amplification and sequencing of mitochondrial 16S rDNA [39] (Table 5).

Table 5. Primers and probes used in this study.

Species	Target	Name	Sequence	Annealing Temperature (°C)	References
rPCR					
<i>Rickettsia</i> spp.	<i>gltA</i>	<i>Rsp-F</i> <i>Rsp-R</i> <i>Rsp-P</i>	GAGAGAAAATTATATCCAAATGTTGAT AGGGTCTTCGTGCATTCTT CATTGTGCCATCCAGCCTACGGT	60	
Conventional PCR (Sequencing)					
<i>Rickettsia</i> spp.	<i>gltA</i>	<i>CS2D</i>	ATGACCAATGAAAATAATAAT	54	[40,41]
		<i>CSEndR</i>	CTTATACTCTCTATGTACA		
		<i>409D</i>	CCTATGGCTATTATGCTTGC	54	
	<i>ompA</i>	<i>1258R</i>	ATTGCAAAAAGTACAGTGAACA	54	
<i>Rr190.70p</i> <i>Rr190.602n</i>		ATGGCGAATATTTCTCCAAAA AGTGCAGCATTTCGCTCCCCT	48		
Ticks	16S rDNA	<i>16S + 1</i> <i>16S - 1</i>	CTGCTCAATGATTTTTTAAATTGCTGTGG CCGGTCTGAACTCAGATCAAGT	48 and 54	[39]

4.5. PCR Detection of *Rickettsia*

The rPCR assays for *Rickettsia* spp. were performed using the primers and probes previously described for the amplification of the citrate synthase gene (*gltA*) (Table 5) [42]. Reactions were performed on a 96-well Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System using QuantiFast Pathogen + Internal Control Kits (Qiagen). Internal and negative controls were included in each run.

Positive samples detected using rPCR were then analyzed using conventional PCR using primers that amplified the 850-bp fragment of the *gltA* gene common to the *Rickettsia* genus [40,41] and primers to amplify the 532-bp fragment of the 190-kDa outer membrane protein (*ompA*) gene specific to the SFG Rickettsiae [43] (Table 5). The reactions were carried out using an Applied Biosystems GeneAmp PCR System 9700 (Applied Biosystems, Courtaboeuf, France). The PCR products were separated on 2% agarose gels in tris-acetate-ethylenediaminetetraacetic acid (TAE buffer) and were visualized under ultraviolet light after staining with ethidium bromide. A 100-bp DNA ladder was used as a standard marker.

Strong positive samples were purified and directly sequenced using an Applied Biosystems model 3730XL (Fisher Scientific S. A. S., Illkirch-Graffenstaden, France). The newly generated sequences were aligned using Mega X [44]. All sequences were assembled and compared with similar sequences retrieved from the GenBank nucleotide database using BLASTn [45]. Phylogenetic analyses were inferred using the maximum-likelihood method implemented in the Mega X program [44].

4.6. Statistical Analysis

Infection rates for pooled ticks were calculated using the MLE method with 95% CI for unequal pool sizes and expressed as the MLE of infection rate per 100 ticks. MLE calculations are based on the number of pools, pool sizes (number of individuals per pool), and the number of positive pools [46].

The rates of infection or of tick infestation were compared using Fisher's exact test ($p < 0.05$). The analysis was performed using the R statistical platform (version 3.1.2) [47].

5. Conclusions

In conclusion, our results contribute to a better knowledge about SFG Rickettsiae in Corsica and provide a useful contribution to understanding their epidemiology. This study reports the first evidence of the circulation of five species of the *Rickettsia* genus in Corsican ticks representing four genera, namely, *Rhipicephalus*, *Hyalomma*, *Dermacentor*, and *Ixodes*, that were collected from wild and domestic animals. We highlighted the exclusive detection of *Candidatus Ri. barbariae* in *R. bursa* collected from sheep and the wide circulation of the human pathogen *Ri. aeschlimanii* in *H. marginatum* collected from cattle. Moreover, the high percentage of *D. marginatus* with positive detection to *Ri. slovaca* in wild animals indicates a wide distribution of this human pathogen in Corsica. This study reports evidence for the first time that *Ri. monacensis*, a human pathogen, could circulate in ticks collected from dogs in Corsica. Implications for human health of the circulation of these two pathogens merits further investigation and should be taken into account by physicians seeing patients with unexplained febrile illness.

6. Declarations

6.1. Ethics Approval and Consent to Participate

The inspected cattle were slaughtered for human consumption. Living sheep were examined with the assistance of their owner. Companion animals were examined by veterinarians with the agreement of the owners. The wild boars collected were legally hunted during the hunting season.

6.2. Availability of Data and Materials

The GenBank accession numbers obtained in this study were MK608560-MK608588; MK608594-MK608637; MK608656-MK608660; MK624992-MK624999; MK652441-MK652442 for *gltA* gene ($n = 91$) and MK608589-MK608593; MK608638-MK608655; MK618727-MK618732; MK609661; MK600862; MK608661-MK608662 and MK618727-MK618732 for *ompA* gene ($n = 33$). For tick identification (16S rDNA) the GenBank accession numbers obtained were: MK620874–MK620878 (*Dermacentor marginatus*), MK620873 (*R. bursa*), MH663977 for (*H. marginatum*) MK620872 (*I. ricinus*) and MK761069-MK761072 (*R. sanguineus s.l.*). All other relevant data are included in the article.

Author Contributions: V.C. and A.F. conceived and designed the study and critically revised the manuscript. V.C., N.V., and O.M. performed the experiments and analyzed the data. R.C., F.C., and X.d.L. contributed to writing the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare that they have no competing interests.

Abbreviations

DEBONEL	<i>Dermacantor</i> -borne necrosis lymphadenopathy
<i>gltA</i>	citrate synthase
MLE	maximum likelihood estimation
MSF	Mediterranean spotted fever
<i>ompA</i>	outer membrane proteins A
PCR	polymerase chain reaction
SFG	spotted fever group
TIBOLA	tick-borne lymphadenopathy

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3. First detection of *Ehrlichia minasensis* in *Hyalomma marginatum* ticks collected from cattle in Corsica, France

Contexte

Nous rapportons ici la première détection de l'ADN de la bactérie *E. minasensis* en Corse. Cette bactérie n'avait été identifiée auparavant qu'au Canada, en Ethiopie et au Brésil. Depuis sa découverte en Corse et la publication de l'article relatant sa présence, la bactérie a été retrouvée dans de nombreux autres pays, notamment Israël, Pakistan, Colombie et Afrique du Sud, faisant de cet article le plus cité du journal en 2019 [137, 138].

Méthode

Les tiques ont été collectées en Haute Corse sur des bovins en juin et juillet 2016 à l'abattoir de Ponte-Leccia (Figure 12) (Tableau 1). La présence de la bactérie du genre *Erlichia* a été déterminée par séquençage partiel du gène *16S* et *groEL* (Tableau 2).

Résultats

Au total, 91 tiques de l'espèce *H. marginatum* ont été collectées et regroupées en 27 pools, dont un (3,7%) contenait de l'ADN de *E. minasensis* (Figure 15). Ce pool était constitué de six tiques collectées sur un bovin élevé dans le nord-ouest de la Corse. Les séquences des gènes *16S* et *groEL* ont montré 100% (303/303 bp) et 99,8% (555/556) d'identité nucléotidique avec *E. minasensis*. Les analyses phylogénétiques ont démontré la plus grande proximité avec le génotype UFMG-EV *E. minasensis*.

Conclusion

A notre connaissance, ces résultats représentent la première détection de la bactérie *E. minasensis* en Europe. Ces résultats montrent la grande diversité de pathogènes bactériens retrouvés en Corse et confirme son rôle d'espace sentinelle.

Original Article

First detection of *Ehrlichia minasensis* in *Hyalomma marginatum* ticks collected from cattle in Corsica, France

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Abstract

Ehrlichiosis are severe, feverish tick-borne illnesses caused by specific species within the genus *Ehrlichia* (Anaplasmataceae family). Recent data suggest that ruminants in Corsica area reservoir for several Anaplasmataceae species. The purpose of our study was to determine whether *Ehrlichia* species could be detected in ticks collected in Corsican ruminants by using molecular methods. Ticks were collected in northern Corsica: (i) in May 2016 from sheep bred in one farm located in a 5000-inhabitants village and (ii) from cattle in June and July 2016 in a slaughterhouse. There sheep and cattle whole skin was inspected and ticks were collected manually. A total of 647 ticks was collected in northern Corsica during this study: 556 (86%) belonged to the *Rhipicephalus bursa* species and 91 (14%) to *Hyalomma marginatum*. The 91 *H. marginatum* ticks were organized in 27 pools, of which one (3.7%) was found positive for the presence of *E. minasensis*; this pool consisted of six ticks collected from a cow bred and raised northwestern Corsica. Ehrlichial DNA was not detected in *R. bursa* ticks. The 16S rRNA and *groEL* gene sequences of *Ehrlichia* detected in the *H. marginatum* pool showed 100% (303/303 bp) and 99.8% (555/556) of nucleotide identity with *E. minasensis*, respectively. Phylogenetic analyses demonstrated the highest closeness with *E. minasensis* UFMG-EV genotype than to any other *E. canis* strains. To our knowledge, this is the first report of *E. minasensis* outside of Brazil, Ethiopia and Canada. This identification of *E. minasensis* in *H. marginatum* merits to be further investigated and pleads for translational studies addressing the potential impact of vector-borne diseases of human and veterinary impact through large-scale research and surveillance programmes in Corsica.

Keywords: *Ehrlichia minasensis*, *Hyalomma marginatum*, Corsica, France.

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Introduction

Ehrlichiosis are severe, feverish tick-borne illnesses caused by specific species within the genus *Ehrlichia* (Anaplasmataceae family) (Nicholson *et al.* 2010). The genus *Ehrlichia* consists of *E. chaffeensis*, *E. canis*, *E. ewingii*, *E. muris* and *E. ruminantium*, all of which are capable of causing infections in both humans and domestic animals (Rar & Golovljova 2011; Vieira *et al.* 2011). *Ehrlichia minasensis* is a recently described species most closely related with although clearly distinct from *E. canis*. *Ehrlichia*

minasensis (i) was discovered in naturally infected dairy cattle and mule deer in Canada (genotype BOV2010) (Gajadhar *et al.* 2010), (ii) was detected in the haemolymph of Brazilian *Rhipicephalus microplus* ticks (*Ehrlichia* sp. UFMG-EV) (Cabezas-Cruz *et al.* 2016), (iii) isolated in Brazil (strain UFMT-BV), where it proved to be pathogenic for cattle (Aguar *et al.* 2014) and (iv) isolated (strain UFMG-EV) from a partially engorged *R. microplus* female tick (Cabezas-Cruz *et al.* 2016). *Ehrlichia minasensis* was established as a new species in 2016. *Ehrlichia minasensis* can be grown in *Ixodes*

scapularis cell lines (ID8) and dog macrophages (DH82) (Cabezas-Cruz *et al.* 2016). Corsica is a French Mediterranean island characterized by a warm-summer Mediterranean climate with a high variability of microclimates because of peculiar geographical situation (Grech-Angelini *et al.* 2016). Corsican livestock farming (sheep, goats, pigs, and cattle) is mainly of the extensive type; thus frequent interactions between livestock, wildlife and human populations favour the circulation of ticks and tick-borne microorganisms (Grech-Angelini *et al.* 2016).

Recent data suggest that ruminants in Corsica area reservoir for several Anaplasmataceae species (Dahmani *et al.* 2017b). *Ehrlichia canis* was detected once in Corsica in a non-engorged *R. bursa* tick collected from a cow (Dahmani *et al.* 2017b). The purpose of our study was to determine whether other *Ehrlichia* species could be detected in ticks collected in Corsican ruminants by using molecular methods.

Material and methods

Ticks were collected in northern Corsica: (i) in May 2016 from sheep bred in one farm located in Corte, a 5000-inhabitants village and (ii) from cattle in June and July 2016 in the Ponte-Leccia slaughterhouse. There sheep and cattle whole skin was inspected and ticks were collected manually. They were identified at the species level based on taxonomic keys and morphometric tables using a binocular microscope (Estrada-Pena *et al.* 2004). Morphologic identification was confirmed by mitochondrial 16S rDNA sequence analysis (Table 1) (Black & Piesman 1994). Ticks were washed once in 70% ethanol for 5 min and twice in distilled water for 5 min. Ticks collected from sheep were analysed individually. Pools consisting of 2–6 ticks collected from cattle (same species, same animal) were analysed. Ticks were crushed using the TissueLyser II (Qiagen, Hilden, Germany) in a phosphate-buffered saline solution at 2800 g for 20 s. DNA extraction was performed on a QIAcube HT (Qiagen) using QIAamp Cador Pathogen Mini kit according to the manufacturer's instructions. DNA was eluted in 150 μ l of buffer and stored at -20°C . Samples were analysed by using a qPCR for detection of *Ehrlichia*

spp. specific including a positive (*Ehrlichia_spp.*) and a negative control (distilled water) (genesig[®] Standard Kit). *Ehrlichia* DNA was also identified by conventional PCR using (i) tick-borne Anaplasmataceae specific primers targeting a 345-bp region of the 16S rRNA (Parola *et al.* 2001) and (ii) genus-specific primers targeting a 590-bp region of the heat shock protein (*groEL*) gene (Dahmani *et al.* 2017a). PCR conditions and primer sequences are described in Table 1. The reactions were carried out using a GeneAmp PCR Systems 9700 Applied Biosystems (Courtaboeuf, France). The PCR products were UV light-visualized in 2% agarose gel in Tris-Acetate-EDTA (TAE Buffer) after staining with ethidium bromide. Sequences obtained in this study were deposited in the GenBank using the National Center for Biotechnology Information (NCBI) BankIt 3.0 submission tool (<https://www.ncbi.nlm.nih.gov/WebSub/>) (accession number for *H. marginatum* MH663977-83 and for *R. bursa* MH663984-90). Sequences of *Ehrlichia* 16S rRNA and *groEL* genes correspond to acc. nos MH657222 and MH675614 respectively. All sequences were assembled and compared with selected homologous sequenced retrieved from the GenBank nucleotide database using BLASTn (Altschul *et al.* 1997). Each model was inferred using the Maximum Likelihood method implemented in Mega X (Kumar *et al.* 2018). The bootstrap consensus tree was conducted with 1000 replicates (Felsenstein 1985).

The pathogens detected in pools were expressed as the percentage and minimum infection rate based on the assumption that each PCR-positive pool contained at least one positive tick (Sosa-Gutierrez *et al.* 2016).

Results and discussion

A total of 647 ticks was collected during this study; 586 ticks were taken from 42 cows, and the remaining 61 ticks were removed from 60 sheep. In cattle, *R. bursa* was the most abundant species ($n = 495$; 84.5%), followed by *Hyalomma marginatum* ($n = 91$; 15.5%). Ticks collected from sheep all belonged to the *R. bursa* species. The 586 ticks collected in cattle were organized as 127 pools consisting of 100 pools

Table 1. Primers and probes used in this study

Species	Target	Name	Sequence	Cycles	References
Conventional PCR* <i>Ehrlichia</i> ssp.	<i>groEL</i>	<i>Ehr-groel-F</i>	GTTGAAAARACTGATGGTATGCA	95°C 5 min, 40 × [95°C 60 s, 50°C 60 s, 72°C 60 s], 72°C 7 min	(Dahmani <i>et al.</i> 2017a)
		<i>Ehr-groel-R</i>	ACACGRTCTTTACGYTCYTTAAC		
Ticks	16S rRNA**	<i>Ehr-16S-D</i>	GGTACCYACAGAAGAAGTCC	95°C 5 min, 40 × [95°C 60 s, 55°C 60 s, 72°C 60 s], 72°C 7 min	(Parola <i>et al.</i> 2001)
		<i>Ehr-16S-R</i>	TAGCACTCATCGTTTACAGC		
		<i>16S+I</i>	CTGCTCAATGATTTTTTAAATGTGTGG		
	16S rDNA	<i>16S-I</i>	CCGGTCTGAACTCAGATCAAGT	95°C 5 min, 10 × [92°C 60 s, 48°C 60 s, 72°C 90 s], 32 × [92°C 60 s, 54°C 35 s, 72°C 90 s], 72°C 7 min	(Black & Plesman 1994)

*For each PCR reaction, the template DNA had a final concentration <200 ng. **Primers designed to amplify a fragment of the 16S rRNA gene from bacteria within the family of Anaplasmataceae.

of *R. bursa* and 27 pools of *H. marginatum*. Ehrlichial DNA was detected neither in the 61 *R. bursa* ticks from sheep, not in the 100 pools of *R. bursa* collected from cattle.

Ehrlichial DNA (16S rRNA and *groEL*) was detected in one of the 27 pools (3.7%) of *H. marginatum* ticks collected from cattle. This pool consisted of six ticks collected from an animal raised in a county of northwestern Corsica. The 16S rRNA sequence of *Ehrlichia* showed 100% identity (303/303 bp) with *E. minasensis* strain (JX629805) and 99% of identity with *E. canis* TWN (GU810149.1). The 556-bp *groEL* gene sequence showed 99.8% identity (555/556 bp) with the homologous sequence of *E. minasensis* strain UFMG-EV (JX629806) and 97% of identity (521/554 bp) with the *E. canis* strain previously described in Corsica (KY498324). These values were closed to those obtained by comparing the 16S rRNA and *groEL* gene sequences of *Ehrlichia* sp. UFMG-EV to *E. canis* TWN (98.3% and 97.2% respectively) (Cabezas-Cruz *et al.* 2016). Phylogenetic analyses proved that our Corsican strain was most closely related with *E. minasensis* UFMG-EV than to *E. canis* strains (Fig. 1) (Cabezas-Cruz *et al.* 2016).

To the best of our knowledge, this is the first description of *E. minasensis* in Corsica after recent identification in the Americas (Cabezas-Cruz *et al.* 2016). In agreement with previous phylogenetic analyses, we observed that *E. minasensis* is a sister taxa of *E. canis*. This is also the first description of *E. minasensis* in *H. marginatum* tick. So far, it has been reported in haemolymph of *R. microplus* ticks (Cabezas-Cruz *et al.* 2016), and in blood of an apparently healthy cattle in Ethiopia, where *R. microplus* is not described (Hailemariam *et al.* 2017). Interestingly, *H. marginatum* is present in Ethiopia (ECDC.EURO-PA.EU, 2018).

The role of *H. marginatum* in the transmission of *E. minasensis* remains unknown. The presence of a bacterium in an engorged tick could be due to the presence in the blood meal. The presence of *H. marginatum* in Corsica, endemic in southern Europe, was previously reported (Matsumoto *et al.* 2004; Grech-Angelini *et al.* 2016). At the outset of this

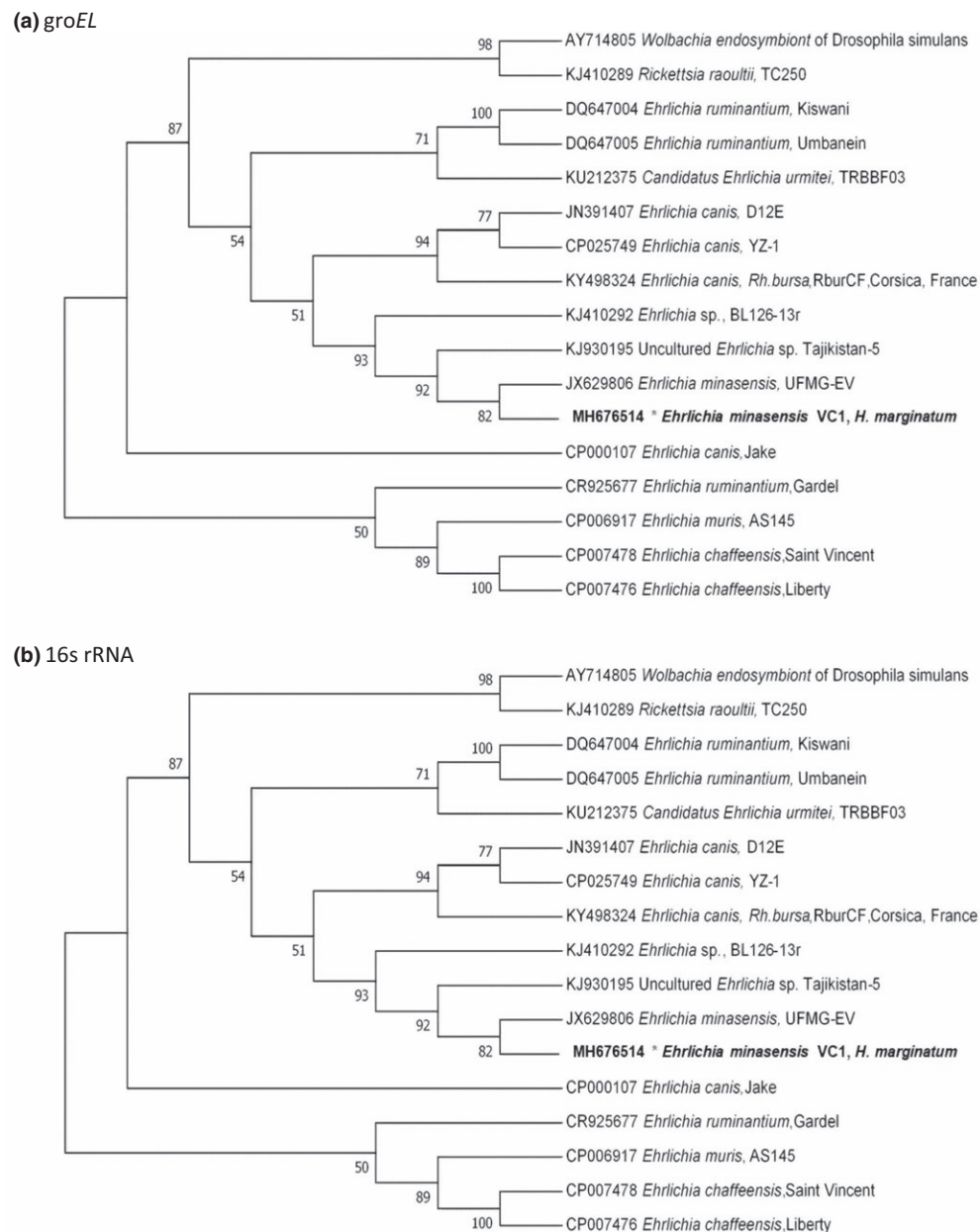


Fig. 1. Phylogenetic position of *Ehrlichia minasensis* detected in the *Hyalomma marginatum* pool collected on cattle in Corsica using *groEL* (a) and 16S rRNA (b). All sequences were assembled and compared with homologous sequenced retrieved from the GenBank nucleotide database using BLASTn (Altschul et al. 1997). The Hasegawa–Kishino–Yano and the Tamura 3-parameter models were identified as the best-fit models under the Akaike Information Criterion, for 16S rRNA and *groEL* sequences respectively. Each model was inferred using the Maximum Likelihood method implemented in Mega X (Kumar et al. 2018). The bootstrap consensus tree was conducted with 1000 replicates (Felsenstein 1985).

study, *H. marginatum* ticks collected in Corsica from ruminants had never been detected positive through PCR for microorganisms belonging to

Anaplasmataceae (Dahmani et al. 2017b). Identification of *E. minasensis* in *H. marginatum* merits to be further investigated and pleads for translational

studies addressing the potential impact of vector-borne diseases of human and veterinary impact through large-scale research and surveillance programmes in Corsica.

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Source of funding

This work was supported by the Corsican Territorial Collectivity and the University of Corsica.

Conflicts of interest

The authors of the work have no conflict of interests to disclose.

Ethical statement

No ethical approval was required, as this study does not involve clinical trials or experimental procedures. The cattle inspected were slaughtered for human consumption. The slaughterhouse staff gave permission to collect ticks from the whole skins of animals. This study did not involve endangered or protected species.

Contributions

VC and AF conceived the study, analysed data and draft the manuscript. SM microbiological diagnosis of bacteria. CL and VC collected ticks. XdL and RC draft the manuscript.

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Molecular screening of Anaplasmatataceae in ticks collected from cattle in Corsica, France

Contexte

Cette étude visait à fournir un aperçu détaillé et une mise à jour épidémiologique concernant la détection d'ADN d'Anaplasmatataceae dans les tiques *Ixodidae* collectées sur des animaux domestiques en Corse et à déterminer la diversité de ces bactéries sur le territoire. Ceux-ci, afin d'obtenir une meilleure compréhension des espèces de bactéries que chaque espèce de tiques peut transmettre et l'intérêt en santé humaine et animal de celle-ci.

Méthode

Les tiques ont été collectées sur des bovins (*Bos taurus*) pendant la période juillet-août 2017 et juillet 2018-janvier 2019 (Figure 12) (Tableau 1). L'ADN des Anaplasmatataceae a été détecté en utilisant une qPCR ciblant le gène 23S. Le séquençage partiel du gène *rpoB* et *groEL* a permis d'identifier les espèces et de réaliser des analyses phylogénétiques (Tableau 1). Les taux de prévalence des pathogènes ont été calculés selon le MLE avec un IC à 95%.

Résultats

Au total, 597 *R. bursa*, 216 *H. marginatum* et sept *I. ricinus* ont été collectées. Dans l'ensemble, l'ADN de la famille des Anaplasmatataceae a été détecté dans 15 des 255 pools de tiques (MLE = 1,7 % ; IC 95 % 0,9-2,7 %) (Figure 15). L'analyse moléculaire a révélé deux espèces au sein du genre *Anaplasma* : *A. marginale* et *A. phagocytophilum*. Plusieurs bactéries du genre *Ehrlichia* ont été détectées. A savoir, la bactérie *E. minasensis* dans des pools de tiques *H. marginatum* et *R. bursa* collectés sur des bovins et pour la première fois la bactérie Candidatus *E. urmitei* détectée dans des tiques *R. bursa* collectées en Corse, ainsi qu'une nouvelle espèce potentielle, Candidatus *E. corsicanum*.


Conclusion

Dans la présente étude, une vue d'ensemble des bactéries Anaplasmatataceae détectées dans les tiques gorgées sur des bovins en Corse a été fourni. À notre connaissance, il s'agit de la première étude à détecter Candidatus *E. urmitei* dans des tiques *R. bursa* corses et une nouvelle espèce potentielle, Candidatus *E. corsicanum*, également détectée dans des tiques *R. bursa*. La détection de nouvelles espèces a de nombreuses limites, car toutes les espèces précédemment décrites n'ont pas été caractérisées d'un point de vue moléculaire ; ainsi, la détection d'une

nouvelle espèce pourrait être la redécouverte d'une espèce incomplètement caractérisée. Des études supplémentaires sont nécessaires pour clarifier si ces nouveaux variants génétiques représentent une nouvelle espèce.



Molecular screening of Anaplasmatataceae in ticks collected from cattle in Corsica, France

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Abstract

Bacteria belonging to the family Anaplasmatataceae cause infections in humans and domestic animals. The consequences of infection can be significant economic losses for farmers. To better understand the epidemiology of tick-borne Anaplasmatataceae in Corsica, we used molecular methods to detect and characterize Anaplasmatataceae in ixodid ticks collected from cattle. Anaplasmatataceae were detected by using a real-time polymerase chain reaction (PCR) targeting the 23S rRNA gene. Partial sequencing of *rpoB* and *groEL* allowed identifying species and conducting phylogenetic analyses. Infection rates were calculated using maximum likelihood estimation (MLE) with 95% confidence intervals (CIs). In total, 597 *Rhipicephalus bursa*, 216 *Hyalomma marginatum*, and seven *Ixodes ricinus* were collected from cattle during July–August 2017 and July–December 2018. Overall, Anaplasmatataceae DNA was detected in 15 of 255 tick pools (MLE = 1.7%; 95% CI 0.9–2.7%). The molecular analysis revealed two species within the genus *Anaplasma*: *A. marginale* and *A. phagocytophilum*. We also detected bacteria within the genus *Ehrlichia*: we confirmed the detection of *E. minasensis* DNA in *H. marginatum* and *R. bursa* tick pools collected from cattle in Corsica and detected, for the first time to our knowledge, *Candidatus E. urmitei* in Corsican *R. bursa* ticks and a potential new species, *Candidatus E. corsicanum*. Further studies are needed to ascertain the pathogenesis and zoonotic potential of the strains and their importance for animals and public health.

Keywords Anaplasmatataceae · Cattle · Ticks · Corsica · *Ehrlichia* · *Anaplasma*

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Introduction

The Anaplasmataceae family, a member of the Rickettsiales, belongs to the alpha subclass of the Proteobacteria organized into the following four genera: *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia* (Dumler et al. 2001). *Anaplasma* and *Ehrlichia* can cause a persistent infection in their vertebrate host, which becomes a reservoir of the infection (Rar and Golovljova 2011). The reservoir hosts include numerous wild and domesticated animals (Dumler et al. 2001). These bacteria are transmitted mainly by hard ticks of the Ixodidae family.

The six recognized species belonging to the *Anaplasma* genus are: *A. phagocytophilum*, *A. marginale*, *A. centrale*, *A. platys*, *A. bovis*, and *A. ovis*. These species are responsible for anaplasmosis in a large range of wild and domesticated vertebrates worldwide (Battilani et al. 2017). Anaplasmosis remains an important cause of economic losses in livestock farming, and the recognition of *Anaplasma* as a genus of public health significance has contributed to rising interest about these bacteria, their molecular biology, and their pathobiology (Woldehiwet 2010; Battilani et al. 2017). Bovine anaplasmosis is caused by *A. phagocytophilum*, *A. centrale*, *A. marginale*, and *A. bovis* (Kocan et al. 2010).

Ehrlichiosis, which is caused by *Ehrlichia* spp., is an important vector-borne disease responsible for various emerging infections in animals and humans (Adelabu et al. 2019). The genus *Ehrlichia* consists of the following six recognized tick-transmitted species: *E. chaffeensis*, *E. canis*, *E. ewingii*, *E. muris*, *E. ruminantium*, and *E. minasensis* (Cabezas-Cruz et al. 2016). *Ehrlichia minasensis* is a newly described pathogen that causes bovine ehrlichiosis in Brazil (Moura de Aguiar et al. 2019).

Corsica is an island of France located in the northwestern part of the Mediterranean Sea. The average number of cattle kept in Corsica between 2013 and 2017 was approximately 65,000, highlighting the importance of meat production there (Corse 2018). Despite this, data about Anaplasmataceae prevalence in cattle blood and their detection in ticks collected from cattle are fragmentary. A cross-sectional study in 2014–2015 found that cattle in Corsica seem to be a reservoir of *Anaplasma* spp., with a prevalence of 100% in cattle blood (Dahmani et al. 2017a, b). *Anaplasma marginale* DNA was detected in *Rhipicephalus bursa*, *Hyalomma marginatum*, and *Rh. (B.) annulatus* tick pools collected from cattle in Corsica between 2014 and 2016 (Cicculi et al. 2019a; Grech-Angelini et al. 2019). *Anaplasma phagocytophilum* DNA was also detected in *Ixodes ricinus* collected mainly from cattle, but its sequence was not obtained (Grech-Angelini et al. 2019). *Ehrlichia canis* DNA was detected in *R. bursa* and *E. minasensis* DNA in *H. marginatum* pooled ticks collected from cattle (Dahmani et al. 2017a, b; Cicculi et al. 2019b). To our knowledge, this is the first report of *E. minasensis* in Europe, confirming the wide geographical distribution of this bacterium (Cabezas-Cruz et al. 2019).

The present study aims to provide a detailed overview and an epidemiological update regarding the detection of Anaplasmataceae DNA in Ixodid ticks feeding on cattle in 2017–2018, and to determine the genetic diversity of these bacteria.

Materials and methods

Study area and ticks

Ticks were collected in July–August 2017 and July–December 2018 from cattle ($n=140$ and 241 , respectively) in the Ponte-Leccia slaughterhouse. As previously described (Cicculli et al. 2019a), during each visit, the whole skin of slaughtered animals was inspected, and ticks were collected manually. The national cattle identification system, which uses ear tags, allowed the origin of animals to be tracked and the farm owners to be identified. One to 20 ticks were collected from each infested animal. Ticks were identified microscopically to the species level using a pictorial guide (Estrada-Pena et al. 2004).

DNA extraction

Ticks were washed once in 70% ethanol for 5 min and then twice in distilled water for 1 min each time. Ticks were analyzed individually or using monospecific pools consisting of 2–6 ticks, according to developmental stage and sex, collected from the same animal. Individual or pools of ticks were crushed in phosphate-buffered saline using Tissue-Lyser II (Qiagen, Hilden, Germany) at 5500 rpm for 20 s. DNA extraction was performed using QIAcube HT (Qiagen) and a QIAamp cador Pathogen Mini kit according to the manufacturer's instructions. DNA was eluted in 150 μ l of buffer and stored at -80 °C. For each polymerase chain reaction (PCR) reaction, the template DNA was at a final concentration <200 ng.

Molecular identification of ticks at the species level

Morphological identification of ticks was confirmed by PCR amplification and sequencing of mitochondrial 16S rDNA (Black and Piesman 1994) (Table 1). The distribution of tick species has previously been published in a study of Spotted Fever Group bacteria (Cicculli et al. 2019c).

PCR detection of Anaplasmataceae

rPCR assays for Anaplasmataceae were performed using specific primers and probes targeting the 23S rRNA gene (Table 1). Reactions were performed on a 96-well Applied Biosystems QuantStudio 3 Real-Time PCR System using QuantiFast Pathogen and Internal Control Kits (Qiagen). Internal and negative controls were included in each run. Each run was repeated 3 \times . All Anaplasmataceae-positive samples were tested by conventional PCR using *Anaplasma* genus-specific primers targeting a 525-bp fragment of the gene for RNA polymerase subunit beta (*rpoB*) and *Ehrlichia* genus-specific primers targeting a 590-bp fragment of the gene for heat shock protein (*groEL*). For better molecular characterization, Anaplasmataceae genus-specific primer targeting a 485-bp fragment of the 23S rRNA gene and the 345-bp region of the 16S rRNA gene (Parola et al. 2001; Dahmani et al. 2017a, b) (Table 1) was used for uncharacterized *Ehrlichia*. Samples were also confirmed by using rPCR to detect *Ehrlichia* spp. including a positive (*Ehrlichia* spp.) and a negative control (distilled water) (genesig Standard Kit). The reactions were conducted using an Applied

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

PCR type	Species	Target	Name	Sequence	Annealing temperature (°C)	References
rPCR	Anaplasmataceae	23S rRNA	<i>Tt-Ana-F</i>	TGACAGCGTACCTTTTGCAT	60	Dahmani et al. (2017a, b)
			<i>Tt-Ana-R</i>	GTAAACAGGTTCCGGTCTCTCCA		
			<i>Tt-Ana-P</i>	GGATTAGACCCCGAAACCAAG		
Conventional PCR (sequencing)	<i>Anaplasma</i> spp.	<i>rpoB</i>	<i>Ana-rpoBF</i>	GCTGTTCTAGGCTYCTTACGCCGA	55	Dahmani et al. (2017a, b)
			<i>Ana-rpoBR</i>	AATCRAGCCAVGAGCCCCCTRTAWGG		
	<i>Ehrlichia</i> ssp.	<i>groEL</i>	<i>Ehr-groel-F</i>	GTTGAAAARACTGATGGTATGCA	50	Parola et al. (2001)
			<i>Ehr-groel-R</i>	ACAGRTCTTTACGYTCYTTAAC		
			<i>Ehr-16S-D</i>	GGTACCYACAGAAAGAGTCC		
Anaplasmataceae	23S rRNA	<i>Ehr-16S-R</i>	TAGCACTCATCGTTTACAGC	55	Parola et al. (2001)	
		<i>Ana23S-212f</i>	ATAAGCTGCGGGGAGTTGTC			
Ticks	16S rDNA	<i>Ana23S-753r</i>	TGCAAAAGGTACGCTGTCCAC	54	Black and Piesman (1994)	
		<i>16S + I</i>	CTGCTCAATGATTTTTTAAATTGCTGTGG			
			<i>16S-1</i>	CCGGTCTGAACCTCAGATCAAGT		

Biosystems 9700 GeneAmp PCR system (Courtaboeuf, France). The PCR products were visualized in 1% agarose gels in Tris–acetate-EDTA (TAE Buffer) and visualized under ultraviolet light after staining with ethidium bromide. A 100-bp DNA ladder was used as a standard marker.

Strongly positive samples were purified and sequenced directly using an Applied Biosystems 3730XL system (Fisher Scientific, Illkirch-Graffenstaden, France). The newly generated sequences were aligned using Mega X software (Kumar et al. 2018). All sequences were assembled and compared with similar sequences retrieved from the GenBank nucleotide database using BLASTn (Altschul et al. 1997). Phylogenetic analyses were inferred using the maximum likelihood estimation (MLE) method implemented in Mega X (Kumar et al. 2018).

Statistical analysis

Infection rates for pooled ticks were calculated using the maximum likelihood estimation (MLE) method with 95% confidence intervals (CIs) for unequal pool sizes and expressed as the MLE of infection rate per 100 ticks. MLE calculations are based on the number of pools, pool sizes (number of individuals per pool), and the number of positive pools (Biggerstaff 2005). The MLE is defined as the infection rate most likely observed given the test results.

Results

Sampling and identification of ticks

In total, 820 ticks belonging to three species were collected from 381 cows from May to August 2017 and July to December 2018. The study area included 39 municipalities (Fig. 1). *Rhipicephalus bursa* was the main tick species ($n=597$; 73%), followed by *H. marginatum* ($n=216$; 26%) and *I. ricinus* ($n=7$; 1%). Overall, 255 pools of ticks were obtained (Table 2).

Detection of Anaplasmataceae DNA

The 23S rRNA gene-based rPCR screening detected Anaplasmataceae DNA in 15 of the 255 tick pools, i.e., an infection rate (MLE) of 1.7% (95% CI 0.9–2.7%) (Table 2). Among these, Anaplasmataceae DNA was detected in 13 of the 154 *R. bursa* tick pools and in two of the 97 *H. marginatum* tick pools. The overall infection rate was 2.3% (95% CI 1.2–3.7%) for *R. bursa* and 0.9% (95% CI: 0.2–2.8%) for *H. marginatum* (Table 2).

Anaplasmataceae species identification based on sequence analysis

The *rpoB* sequencing identified two species of *Anaplasma* in 11 of the 15 tick pools analyzed. *Anaplasma marginale* DNA was detected in 10 tick pools and *A. phagocytophilum* DNA in one tick pool (Table 3).

As shown in Table 3 and Fig. 1, *A. marginale* DNA was detected in seven *R. bursa* tick pools collected from cattle in four municipalities (July–August 2017) and in three *R.*

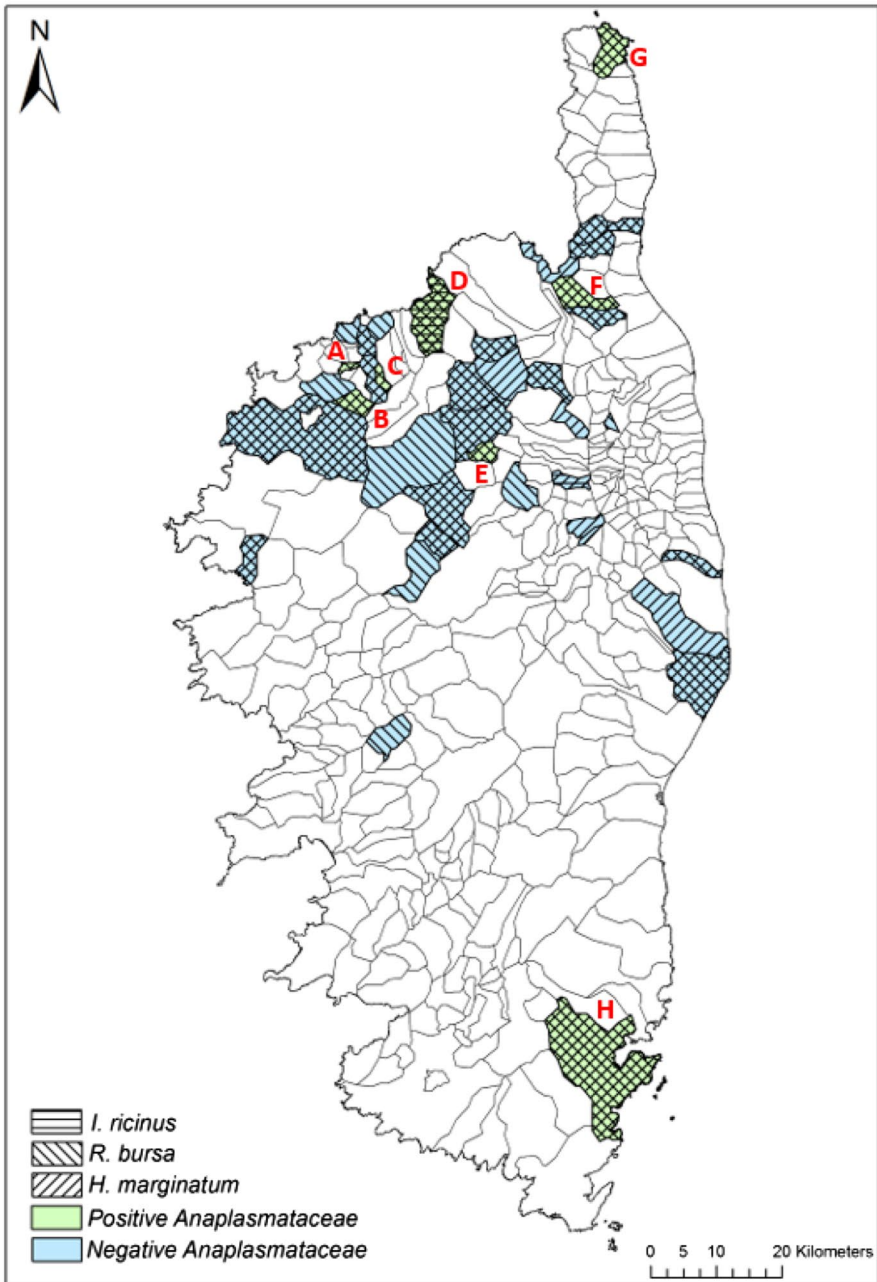


Fig. 1 Map of Corsica, France, indicating the tick collection sites and positive areas. Municipalities positive for Anaplasmatidae: **a** Cateri (42° 34' 21" N, 8° 53' 33" E), **b** Zilia (42° 31' 52" N, 8° 54' 06" E), **c** Nessa (42° 33' 04" N, 8° 56' 57" E), **d** Palasca (42° 35' 24" N, 9° 02' 36" E), **e** Popolasca (42° 26' 24" N, 9° 07' 59" E), **f** Oletta (42° 38' 00" N, 9° 21' 22" E), **g** Rogliano (42° 57' 25" N, 9° 25' 08" E), and **h** Porto-Vecchio (41° 35' 30" N, 9° 16' 49" E)

Table 2 Distribution of Anaplasmatidae DNA in ticks collected from cattle in the 2017 (May–August 2017) and 2018 (July–December 2018) surveillance periods

Year	Number of ticks per pool (n)	Number of pools with n ticks			Positive Anaplasmatidae spp. n (%)			Total
		<i>H. marginatum</i>	<i>R. bursa</i>	<i>I. ricinus</i>	<i>H. marginatum</i>	<i>R. bursa</i>	<i>I. ricinus</i>	
2017	1	15	19	1	0 (0)	0 (0)	0 (0)	0 (0)
	2	13	9	0	1 (8)	1 (11)	0 (0)	2 (9)
	3	6	6	0	0 (0)	2 (33)	0 (0)	2 (17)
	4	1	7	0	0 (0)	3 (43)	0 (0)	3 (38)
	5	1	8	0	0 (0)	0 (0)	0 (0)	0 (0)
	6	2	19	0	0 (0)	1 (5)	0 (0)	1 (5)
Total pools		38	68	1	1 (3)	7 (10)	0 (0)	8 (7)
MLE (95% CI)					0.95 (0.3–2.2)	3.1 (1.3–5.8)	0	2% (0.9–3.7)
2018	1	25	12	1	0 (0)	0 (0)	0 (0)	0 (0)
	2	9	7	1	1 (11)	1 (14)	0 (0)	2 (12)
	3	14	8	1	0 (0)	0 (0)	0 (0)	0 (0)
	4	5	15	0	0 (0)	0 (0)	0 (0)	0 (0)
	5	5	14	0	0 (0)	3 (21)	0 (0)	3 (16)
	6	1	30	0	0 (0)	2 (7)	0 (0)	2 (6)
Total pools		59	86	3	1 (2)	6 (7)	0 (0)	7 (5)
MLE (95% CI)					0.74 (0.04–3.2)	1.7 (0.7–3.5)	0	1.2% (0.5–2.3)
Overall	1	40	31	2	0 (0)	0 (0)	0 (0)	0 (0)
	2	22	16	1	2 (9)	2 (13)	0 (0)	4 (10)
	3	20	14	1	0 (0)	2 (14)	0 (0)	2 (6)
	4	6	22	0	0 (0)	3 (14)	0 (0)	3 (11)
	5	6	22	0	0 (0)	3 (14)	0 (0)	3 (10)
	6	3	49	0	0 (0)	3 (6)	0 (0)	3 (6)
Total pools		97	154	4	2 (2)	13 (8)	0 (0)	15 (6)
MLE (95% CI)					0.93 (0.16–2.8)	2.3 (1.2–3.7)	0	1.7% (0.9–2.7)

The infection rate for pooled ticks was estimated by the maximum likelihood estimation (MLE) with 95% confidence intervals (CIs)

Table 3 Number of positive pools (DNA detection) per tick species and pathogen species

Year	Number of tick/pools	Tick species	<i>Anaplasma (rpoB)</i>	<i>Ehrlichia (groEL)</i>	GenBank acc. no	Municipality	GPS coordinates	Cow	Breed	Period	
2017	2	<i>H. marginatum</i>	<i>A. phagocytophilum</i>		MT075488	Palasca	42° 35' 24" N, 9° 02' 36" E	1985	1	23/08/2017	
	4	<i>R. bursa</i>	<i>A. marginale</i>		MT018472	Palasca	42° 35' 24" N, 9° 02' 36" E	6366	1	23/08/2017	
	3	<i>R. bursa</i>	<i>A. marginale</i>		MT018473	Palasca	42° 35' 24" N, 9° 02' 36" E	6366	1	23/08/2017	
	2	<i>R. bursa</i>	<i>A. marginale</i>		MT018474	Popolasca	42° 26' 24" N, 9° 07' 59" E	192	2	19/07/2017	
	4	<i>R. bursa</i>	<i>A. marginale</i>		MT018475	Popolasca	42° 26' 24" N, 9° 07' 59" E	194	2	19/07/2017	
	3	<i>R. bursa</i>	<i>A. marginale</i>		MT018476	Cateri	42° 34' 21" N, 8° 53' 33" E	6221	3	08/08/2017	
	4	<i>R. bursa</i>	<i>A. marginale</i>		MT018477	Cateri	42° 34' 21" N, 8° 53' 33" E	6214	3	08/08/2017	
	6	<i>R. bursa</i>	<i>A. marginale</i>		MT018478	Oletta	42° 38' 00" N, 9° 21' 22" E	2584	4	08/08/2017	
	2018	2	<i>H. marginatum</i>		<i>E. minasensis</i>	MT035968	Nessa	42° 33' 04" N, 8° 56' 57" E	1823	5	21/08/2018
		6	<i>R. bursa</i>		<i>E. minasensis</i>	MT035969	Nessa	42° 33' 04" N, 8° 56' 57" E	9372	5	25/07/2018
5		<i>R. bursa</i>	<i>A. marginale</i>		MT018479	Porto-Vecchio	41° 35' 30" N, 9° 16' 49" E	8884	6	25/07/2018	
5		<i>R. bursa</i>		<i>E. urmitiei</i>	MT035970	Rogliano	42° 57' 25" N, 9° 25' 08" E	1813	7	25/07/2018	
5		<i>R. bursa</i>		<i>Candidatus E. corsicanum</i>	MT075489	Zilia	42° 31' 52" N, 8° 54' 06" E	6959	8	08/08/2018	
6	<i>R. bursa</i>	<i>A. marginale</i>		MT018480	Zilia	42° 31' 52" N, 8° 54' 06" E	401	8	10/07/2018		
2	<i>R. bursa</i>	<i>A. marginale</i>		MT018481	Zilia	42° 31' 52" N, 8° 54' 06" E	6924	8	21/08/2018		

bursa tick pools collected from cattle in two municipalities (July–August 2018). The infection rate for *A. marginale* was 3.1% (95% CI 1.3–5.9%) in July–August 2017 and 0.8% (95% CI 0.2–2.2%) in July–August 2018. *Anaplasma marginale* DNA was detected in ticks collected from cattle belonging to six breeds. The 10 sequences of *rpoB* of *A. marginale* had 99–100% identity, and showed 99–100% identity with *A. marginale* strains previously reported in *R. bursa* collected from cattle in Corsica (Table 3, Fig. 2).

Anaplasma phagocytophilum DNA was detected in one pool of two *H. marginatum* ticks collected in August 2017 (Table 3). The infection rate for *A. phagocytophilum* DNA was 1.1% (95% CI 0.0–5.4%). *Anaplasma phagocytophilum* sequences of *rpoB* showed 100% (513/513) identity with *A. phagocytophilum* str. Norway 2 (GenBank acc. no. CP015376) identified in sheep (Fig. 2). Breed 1 localized in the municipality of Palasca (northwestern Corsica; Fig. 1) was characterized by the codetection of *A. phagocytophilum* and *A. marginale* DNA during August 2017 (Table 3).

The *groEL* sequencing identified two species of *Ehrlichia* (one *Candidatus Ehrlichia urmitei* and two *E. minasensis*) and of one *Ehrlichia* spp. (Table 3). After *groEL* amplicon analysis, *E. minasensis* DNA was found in two tick pools: one *R. bursa* pool (comprising six ticks) and one *H. marginatum* pool (comprising two ticks) (Table 3). These two tick pools were collected from cows of the same breed in a county in northwestern Corsica in July and August 2018, respectively. Overall, the infection rate for *E. minasensis* was 0.3% (95% CI 0.0–1.0%), ranging from 0.3% (95% CI 0.0–1.2%) for *R. bursa* to 0.7% (95% CI 0.0–3.2%) for *H. marginatum*. The two sequences of *E. minasensis* showed 100% identity with *E. minasensis* (509/509) previously detected in one *H. marginatum* tick pool collected in 2016 from a cow raised in a county in northwestern Corsica (GenBank acc. no. MH675614) (Fig. 3).

The *groEL* DNA of *Candidatus E. urmitei* was detected in a pool of five *R. bursa* ticks collected in July 2018 from a cow of breed 7 localized in a municipality of the northernmost part of Corsica (Table 3, Fig. 1). The infection rate of *Candidatus E. urmitei* was

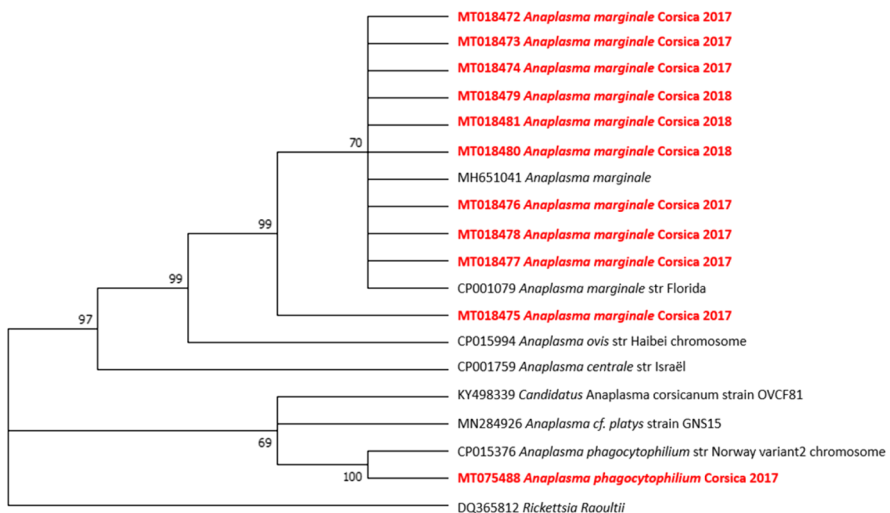


Fig. 2 Phylogenetic analysis of Anaplasmataceae identified in Corsica, inferred using maximum likelihood estimation (MLE) based on *Anaplasma rpoB* sequences. The analysis involved 19 nucleotide sequences. There was a total of 360 positions in the final data set

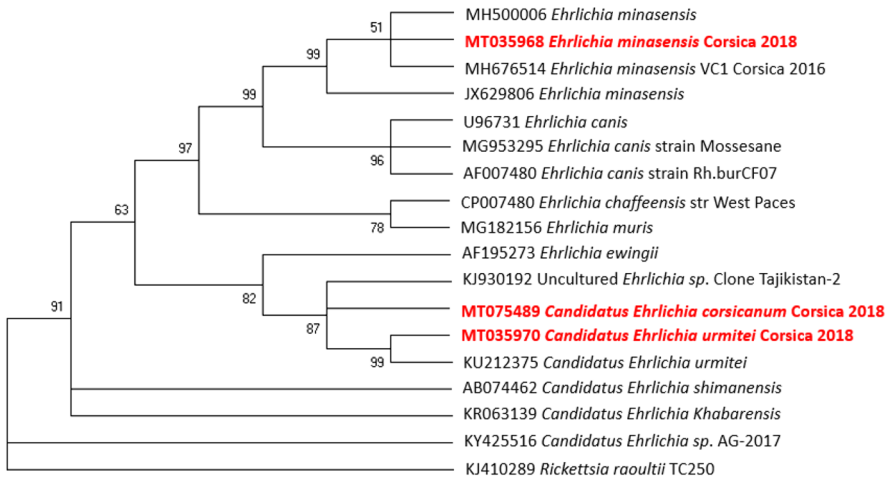


Fig. 3 Phylogenetic analysis of Anaplasmataceae identified in Corsica, inferred using maximum likelihood estimation (MLE) based on *Ehrlichia groEL* sequences. The analysis involved 19 nucleotide sequences. There was a total of 628 positions in the final data set

0.3% (95% CI: 0.0–1.2%). The *groEL* amplicon showed 100% identity (564/564) with *Candidatus* *E. urmitei* DNA identified in *R. bursa* ticks collected in French Basque country from sheep (GenBank acc. no. KU212375) (Fig. 3).

Analysis of the *groEL* amplicon detected DNA of *Ehrlichia* spp. (strain KcattleRB72) in a pool of *R. bursa* (comprising five ticks) collected in August 2018 in breed 8 (Table 3, Fig. 1) revealed: (i) 94.7% (538/568) identity with uncultured *Ehrlichia* spp. (GenBank acc. no. KJ930192.1) detected in Tajikistan from *H. anatolicum* and (ii) 95.2% (530/568) with *Candidatus* *E. urmitei* (GenBank acc. no. KU212375.1) detected in French Basque Country from *R. bursa*. Breed 8 was characterized by the codetection of this *Ehrlichia* spp. and *A. marginale* DNA during August 2017 (Table 3). The infection rate of this *Ehrlichia* spp. (strain KcattleRB72) was 0.3% (95% CI 0.0–1.2%).

Additional characterization of this bacterium, provisionally named ‘*Candidatus* *E. corsicanum*’, was performed by the analysis of the 16S and 23S rRNA gene sequences. The 16S rRNA gene showed 99.7% (288/289) identity with *Ehrlichia* spp. detected in *H. anatolicum* collected from cattle in Pakistan (GenBank acc. no. MH250197) and 99.3% (288/290) identity with *E. canis* (GenBank acc. no. MN227484) detected in Crete. The 23S rRNA gene showed 97.4% identity (417/428) with the *Ehrlichia muris* complete genome (GenBank acc. no. CP006917) and 96.7% (414/428) with *Candidatus* *E. urmitei* detected in *R. bursa* collected from sheep in the French Basque Country (GenBank acc. no. KM021422).

Phylogenetic analysis based on partial *groEL* sequences showed that *Candidatus* *E. corsicanum* was on a separate branch situated between uncultured *Ehrlichia* spp. clone Tajikistan (GenBank acc. no. KJ930192) and a separate *Candidatus* *E. urmitei* (GenBank acc. no. KU212375) cluster (Fig. 3). The bacterium closest to *Candidatus* *E. corsicanum* was *Candidatus* *E. urmitei*, and the closest recognized species was *E. ewingii*. The genetic distances between the *Candidatus* *E. corsicanum* (MT075489) and *Candidatus* *E. urmitei* detected in French Basque Country (KU212375) and the *Candidatus* *E. urmitei* (MT035970) detected in Corsica were estimated at 0.052 and 0.051, respectively.

Discussion

In the present study, we provided an overview of potential Anaplasmataceae bacteria detected in feeding ticks collected from cattle in Corsica. We identified two species of *Anaplasma*: *A. marginale* and *A. phagocytophilum*. Interestingly, we confirmed the presence of *E. minasensis* in *H. marginatum* and *R. bursa* tick pools collected from cattle in Corsica (Cicculli et al. 2019b). To our knowledge, this is the first study to detect *Candidatus E. urmitei* in Corsican *R. bursa* ticks and a potential new species, *Candidatus E. corsicanum*, also detected in *R. bursa* ticks. As previously explained (Diarra et al. 2017), the detection of new species has many limitations, as not all previously described species have been characterized molecularly; thus, the detection of a new species could be the rediscovery of an incompletely characterized species. Further studies are needed to clarify whether these new genetic variants represent a new species.

Bovine anaplasmosis can lead to substantial economic losses to cattle industries (Kocan et al. 2003). The DNA of *A. marginale* was detected in *R. bursa*, which is its natural vector, confirming that *A. marginale* was widespread in Corsica (Dahmani et al. 2017a, b; Cicculli et al. 2019a; Grech-Angelini et al. 2019). Among 39 municipalities investigated, we detected *A. marginale* DNA in six breeds from six municipalities. Among these six, three had two groups of positive pool ticks collected from two distinct cows (Table 3). Moreover, we detected *A. marginale* DNA in two tick pools collected from the same cow (no. 6366; breed 1). In Corsica, *R. bursa* seems to be a proven vector of *A. marginale*, the causative agent of erythrocytic bovine anaplasmosis (Aubry and Geale 2011).

Anaplasma phagocytophilum is a zoonotic agent mostly transmitted to humans through the bites of ticks of the *Ixodes* genus that is responsible for human granulocytic anaplasmosis, which occurs in the USA, Europe, and Northern Africa, and is increasingly found in some Asian countries (Heyman et al. 2010; Zhang et al. 2011; Elhamiani Khatat et al. 2016). This bacterium also causes tick-borne fever (or pasture fever) in ruminants and equine anaplasmosis. In the European sub-Mediterranean area, *A. phagocytophilum* has been reported in cattle from Sicily (Italy) and Spain (De La Fuente et al. 2005; Torina et al. 2008), and in ruminants from Sicily (Torina et al. 2010), Sardinia (Zobba et al. 2014), Turkey (Aktas and Ozubek 2015), and French Basque Country (Dahmani et al. 2017a, b). Although the only vector indicated for *A. phagocytophilum* in Europe are *Ixodes* ticks, we detected *A. phagocytophilum* DNA in the *H. marginatum* tick pool collected from a breed positive for *A. marginale* in August 2018. The detection of *A. phagocytophilum* DNA in *H. marginatum* collected from a whitethroat migratory bird was previously reported in Sardinia (Chisu et al. 2018). In Corsica, *A. phagocytophilum* DNA was previously reported once in *I. ricinus* in cattle and once in bovine blood, but no sequence has been published (Grech-Angelini et al. 2019). In the present study, the *A. phagocytophilum* strain detected in *H. marginatum* ticks was shown to be very close to *A. phagocytophilum* pathogenic strains isolated in Europe (Norway). As *Ixodes* ticks represent only a very small percentage of those occurring in Corsica, further research is essential to investigate the possible role of *Hyalomma* ticks in the circulation of *A. phagocytophilum*.

Interestingly, we confirmed the presence of *E. minasensis* in *H. marginatum* and *R. bursa* tick pools collected from cattle in Corsica. These two tick pools were collected from cattle of the same breed in July and August 2018, which shows the circulation of these bacteria in the breed through ticks. *Ehrlichia minasensis* is a newly described member of genus *Ehrlichia* most closely related to *E. canis*, found for the first time in cattle and mule deer in Canada in 2010 (Gajadhar et al. 2010). Since its discovery, *E. minasensis* has been

identified in Brazil (Cruz et al. 2012), Ethiopia (Hailemariam et al. 2017), South Africa (Iweriebor et al. 2017), Pakistan (Rehman et al. 2019), Malaysia (Koh et al. 2018), China (Cicculi et al. 2019b), Israel (Thomson et al. 2018), and Corsica (Cicculi et al. 2019b), and in many tick species of the genera *Rhipicephalus*, *Amblyomma*, *Hyalomma*, and *Haemaphysalis* (Cruz et al. 2012; Iweriebor et al. 2017; Cicculi et al. 2019b; Rehman et al. 2019). The wide distribution of this bacterium can be explained by the large number of tick species competent for its transmission. If no human cases have been reported, this bacterium causes blood infections with anemia, leukopenia, and thrombocytopenia in cattle, and can cause economic loss to grazers (Moura de Aguiar et al. 2019).

To our knowledge, this study reports for the first time *Candidatus E. urmitei* in Corsica *R. bursa* ticks. This potentially new bacterium has been identified for the first time in *R. bursa* ticks collected from sheep in French Basque Country, in *A. variegatum* and *R. microplus* collected from cattle in Cote d'Ivoire, and in *R. microplus* in Mali (Ehounoud et al. 2016; Dahmani et al. 2017a, b; Diarra et al. 2017). This bacterium and its pathogenicity for animals or humans remains unknown. Further studies are necessary to increase knowledge about the distribution and pathogenicity of this bacterium. The DNA of Anaplasmataceae was not found in *I. ricinus* ticks, which could be explained by the low number of ticks of this species tested.

This study has some limitations. As the ticks were collected in a municipal slaughterhouse in Northern Corsica, we have no data about the southern part of the island. As we collected feeding ticks, the identification of the pathogen genome is only a marker of the community of tick-transmitted pathogens circulating in the target territory. Working with pools leads to underestimation of the number of ticks positive for DNA pathogens. Moreover, tick collection had not been conducted for the full year; therefore, we are unable to provide data on the seasonality of the different Anaplasmataceae species.

Conclusion

To our knowledge, no human cases of anaplasmosis or ehrlichiosis have been reported in Corsica. However, these diseases are still little known by clinicians, and in most cases, laboratory diagnostic test results are lacking. It is important to continue to study the epidemiological data of such emerging pathogens, which may be the source of disease complications in both animals and humans. The present study appears to confirm that *R. bursa* is a vector of *A. marginale* in cattle. Further studies are needed to ascertain the pathogenesis and zoonotic potential of the strains and their importance for animals and public health.

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Data availability The GenBank accession Nos. obtained in this study were MT075488 and MT018472-81 for *rpoB* (n=11) and MT035968-70 and MT075489 for *groEL* (n=4), MT065823 and MT012311-13 for the 23S rRNA gene (n=4), and MT065824 (n=1) for the 16S rRNA gene. All other relevant data are included in the article.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval and consent to participate The cattle inspected were slaughtered for human consumption.

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4. First molecular detection of *Rickettsia africae* in a tropical bont tick, *Amblyomma variegatum*, collected in Corsica

Contexte

Nous rapportons ici la première détection de l'espèce *Amblyomma variegatum*, une espèce de tique d'importance médicale et vétérinaire, et la première preuve de détection de l'ADN de l'agent pathogène, *Ri. africae*, elle aussi nouvelle en Corse. En août 2018, une tique à la morphologie inhabituelle a été prélevée sur la partie ventrale de la peau d'une vache dans un abattoir situé dans le village de Ponte-Leccia (Haute-Corse).

Méthode

L'espèce de la tique a été identifiée à l'aide d'un microscope binoculaire en utilisant des clés morphologiques [119]. L'espèce a été confirmée en analysant la séquence partielle du gène *16S* et la séquence du gène *ITS-2* de la tique. Des amorces ciblant la région du gène *16S* et la région du gène de la protéine *groEL* (Tableau 2) ont été utilisés pour la détection des Anaplasmataceae. L'ADN de *Rickettsia* a été détectée par PCR avec des amorces amplifiant la région du gène *gltA* codant pour la citrate synthase (Tableau 2).

Résultats

Les séquences *16S* et *ITS-2* ont confirmé l'identification morphologique de *Amblyomma variegatum*. L'analyse de la séquence *16S* du spécimen de tique a montré des identités de 99% avec *Amblyomma variegatum* du Sénégal, de 98% avec *Amblyomma variegatum* du Nigeria et de São Tomé, de 97% avec *Amblyomma variegatum* du Nigeria. L'analyse de la séquence *ITS-2* de la tique *Amblyomma variegatum* identifiée en Corse était également identique à 99% avec *Amblyomma variegatum* identifiée au Burkina Faso, au Mali, au St. Kitts et aux îles Vierges américaines. Cette tique mâle s'est révélée positive à la présence du genre *Rickettsia* mais négative à la présence de la famille des Anaplasmataceae. L'analyse de la séquence *gltA* obtenue a révélé 99 à 100% d'identité avec *Ri. africae* détectée à partir d'une tique *Amblyomma variegatum* collectée en Ethiopie.

Conclusion

A ce jour, il y a peu ou pas de risque d'introduction et d'établissement d'une population viable de l'espèce *Amblyomma variegatum* en Corse notamment par les oiseaux migrateurs. Les adultes isolés devraient rencontrer le sexe opposé pour se reproduire, et même dans ce cas, il

est peu probable que l'espèce survive aux conditions hivernales. Cependant, si elle y parvenait (réchauffement climatique), elle pourrait entraîner des pertes économiques majeures pour la production de bétail surtout en raison de son association avec une dermatophilose sévère.



First molecular detection of *Rickettsia africae* in a tropical bont tick, *Amblyomma variegatum*, collected in Corsica, France

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Abstract

Here we report the first detection of *Amblyomma variegatum*, a tick species of medical and veterinary importance, and the first molecular evidence of a pathogen, *Rickettsia africae*, both new to Corsica (France). In August 2018, an ixodid tick with an unusual morphology was removed from the ventral part of a cow's whole skin in a slaughterhouse located in the village of Ponte-Leccia (Haute-Corse). The tick was morphologically identified as an adult male of *A. variegatum*. This result was confirmed by 16S rDNA sequence analysis with a close relative being a sequence from Senegal showing 99% nucleotide identity. We tested the tick for *Ehrlichia* and *Rickettsia*. The tick was positive to *Rickettsia* and the corresponding sequence matched with *R. africae*. There is little or no risk of the introduction and establishment of a viable population of *A. variegatum* in Corsica by migrating birds. However, if it did, it could produce major economic losses for livestock production. Further studies and sustained surveillance are indicated, not only focusing on this species of tick and this rickettsia, but also on other microorganisms of veterinary and medical importance that might be transmitted in Corsica and other Mediterranean islands.

Keywords *Amblyomma variegatum* · *Rickettsia africae* · Cattle · Corsica · Mediteranean basin

Introduction

Amblyomma variegatum (Fabricius) (Acari: Ixodidae) is among the most important and widely distributed ticks of tropical livestock (Estrada-Pena et al. 2007). Adults can cause considerable direct losses in livestock by reducing growth and milk production and causing mortality by transmitting *Ehrlichia ruminantium*, the rickettsial agent that causes heart-water in ruminants (Allsopp 2015; Daubney 1930). *Amblyomma variegatum* adults are

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also associated with dermatophilosis (Walker and Lloyd 1993), and this tick is the natural vector of human pathogens including several species of *Rickettsia* and viruses (Jongejan and Uilenberg 2004). *Amblyomma variegatum* carries and transmits *Rickettsia africae*, the agent of African tick bite fever in humans, in Africa and the Caribbean region (Kelly et al. 1996, 2010).

Corsica is a French Mediterranean island characterized by a warm-summer Mediterranean climate with 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 mainly of the extensive type thus important interactions between livestock, wildlife and human populations favour the circulation of tick-borne diseases. This first systematic survey of tick fauna on Corsican livestock reported a dominance of typical Mediterranean species (*Rhipicephalus bursa* and *Hyalomma marginatum*) (Grech-Angelini et al. 2016). The existence of established populations of tick species usually found in dry (*Hyalomma scupense*), or even in cool areas (*Ixodes ricinus*) has also been reported.

In order to extend knowledge about tick species infesting hosts (livestock) and their potential to cause zoonotic diseases of veterinary and medical importance it was decided: (i) to perform a systematic tick-survey from slaughtered animals, and (ii) to test for zoonotic pathogens commonly described depending upon the species. Here we report morphological and genetic identification of an exotic tick species for Corsica Island (France). We tested the tick for *Ehrlichia* and *Rickettsia* spp. and found molecular evidence of the presence of *R. africae*.

Materials and methods

The exotic tick was collected in the Corsican cattle slaughterhouse of Ponte-Leccia (Haute-Corse) during a tick survey carried out in July–August 2018. The national cattle identification system, which uses ear tags, allowed us to trace the origin of animals and to identify farming owners.

The species was identified (i) with a binocular microscope using morphological keys (Walker et al. 2003), and (ii) by analyzing the sequence of one mitochondrial 460 bp fragment of the 16S rDNA (Black and Piesman 1994) and the sequence of one 1200 bp nuclear fragment of the ribosomal internal transcribed spacer-2 (ITS-2) (Grech-Angelini et al. 2016) (Table 1).

DNA extraction was performed on a QIAcube HT (Qiagen) using a QIAamp cadore Pathogen Mini kit according to the manufacturer's instructions. *Ehrlichia* DNA was detected using PCR performed with (i) tick-borne *Anaplasmataceae* specific primers targeting a 345-bp region of the 16S rRNA and (ii) with the genus-specific primers amplifying a 590-bp region of the heat shock protein (*groEL*) gene (Dahmani et al. 2017) (Table 1). *Rickettsia* DNA was detected using PCR with primers amplifying a 850-bp region of the *gltA* gene encoding citrate synthase (Mediannikov et al. 2004) (Table 1). A negative (sterile water) and a positive control (*E. minasensis*¹ and *R. aeschlimannii*) were included in every PCR run. PCR products of the expected size were purified and directly sequenced using an Applied Biosystem 3730XL. Sequences resulting from this study were deposited

¹ A new *Ehrlichia* species recently found in the tick *Rhipicephalus microplus* in Brazil (Cabezas-Cruz et al. 2016).

Table 1 Sequences of primers and conditions of PCR

Species	Target	Name	Sequence	Cycles	References
Conventional PCR					
Ticks	<i>16S rDNA</i>	<i>16S+1</i>	CTGCTCAATGATTTTTAAATTGCTGTGG	†	Black and Plesman (1994)
		<i>16S-1</i>	CCGGTCTGAACCTCAGATCAAGT		
	<i>ITS2</i>	<i>H1</i>	TGGCTTCGTCTGTCTGAGGGTC	‡	Grech-Angelini et al. (2016)
		<i>H2</i>	TGCTTAAATTCAGCGGGTTGTC		
<i>Rickettsia</i> spp.	<i>gltA</i>	<i>CS2D</i>	ATGACCAATGAAAATAATAAT	□	Mediannikov et al. (2004)
		<i>CSEndR</i>	CTTATACTCTCTATGTACA		
	<i>409D</i>	CCTATGGCTATTATGCTTGC	□		
	<i>1258R</i>	ATTGCAAAAAGTACAGTGAACA			
	<i>groEL</i>	<i>Ehr- groel- F</i>	GTTGAAAARACTGATGGTATGCA	¶	Dahmani et al. (2017)
<i>16S rRNA*</i>	<i>Ehr- groel- R</i>	ACACGRTCTTTACGGYTCYTTAAC			
	<i>Ehr-16S-D</i>	GGTACCYACAGAAGAAGTCC	□	Parola et al. (2001)	
	<i>Ehr-16S-R</i>	TAGCACTCATCGTTTACAGC			

*Primers designed to amplify a fragment of the 16S rRNA gene from bacteria within the family of Anaplasmataceae

†95 °C 5 min, 10 × [92 °C 60 s, 48 °C 60 s, 72 °C 90 s], 32 × [92 °C 60 s, 54 °C 35 s, 72 °C 90 s], 72 °C 7 min

‡95 °C 5 min, 35 × [95 °C 40 s, 52 °C 40 s, 72 °C 60 s], 72 °C 5 min

□95 °C 5 min, 35 × [95 °C 60 s, 55 °C 60 s, 72 °C 60 s], 72 °C 10 min

¶95 °C 5 min, 40 × [95 °C 60 s, 50 °C 60 s, 72 °C 60 s], 72 °C 7 min

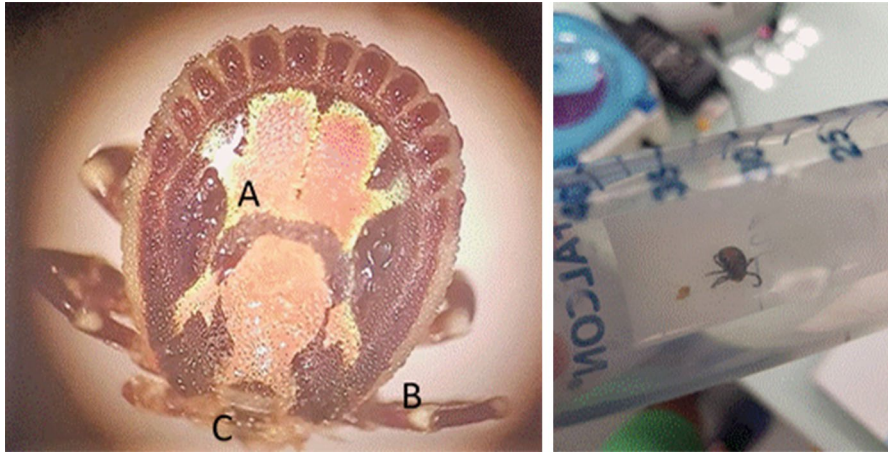


Fig. 1 Adult male tropical bont tick, *Amblyomma variegatum*, dorsal view. A, Medium sized punctations on the bright, yellow-gold scutum; B, banded legs; C, rostrum had been broken during removal. (Color figure online)

in GenBank accession no. for the corresponding sequences: MH843682 for 16S rDNA, MH910967 for ITS2 and MH922770 for *gltA* of *Rickettsia*. The phylogenetic analysis was performed with Maximum Likelihood method implemented on Mega X. Best fitting substitution models were determined with the Aikake Information Criterion using the ML model test implemented in Mega X (<https://www.megasoftware.net/>). The bootstrap consensus tree was conducted with 1000 replicates (Felsenstein 1985).

Ethics statement

No ethical approval was required, as this study does not involve clinical trials or experimental procedures. The cattle inspected were slaughtered for human consumption. The slaughterhouse staff gave permission to collect ticks from the whole skins of animals. This study did not involve endangered or protected species.

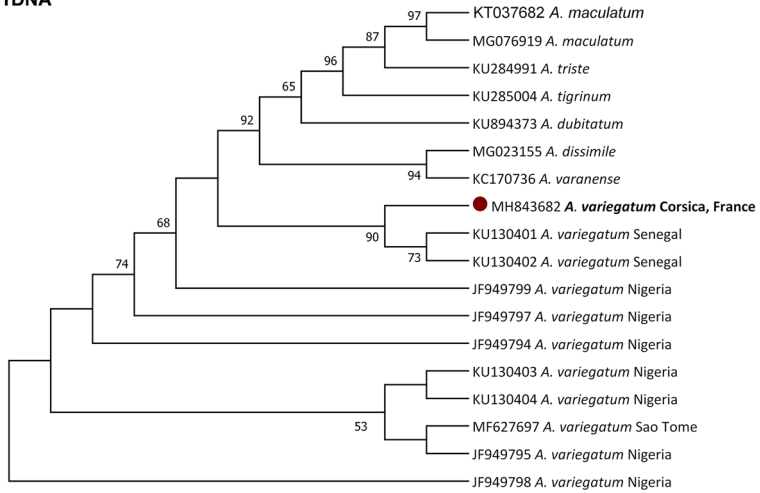
Results

Three hundred and fifty-six ticks were collected from July to August 2018 in the Ponte-Leccia slaughterhouse. Among the 59 inspected cows 43 (73%) were infested. Two main tick species were morphologically identified: *R. bursa* (n=255; 72%) and *H. marginatum* (n=100; 28%).

One of the 356 ticks was morphologically identified as a male adult of *A. variegatum* (Fig. 1). The tick was manually removed from the skin of a cow (*Bos taurus*) on the ventral bodysurface on the 7th of August 2018. The cow had been raised in a county of northeastern Corsica.

The 16S rDNA and ITS-2 sequences confirmed the morphological identification of *A. variegatum*. The 16S rDNA sequence analysis of the tick specimen showed identities (i) of 99% with *A. variegatum* from Senegal (KU130401), (ii) of 98% with *A. variegatum* from

a) 16s rDNA



b) ITS-2

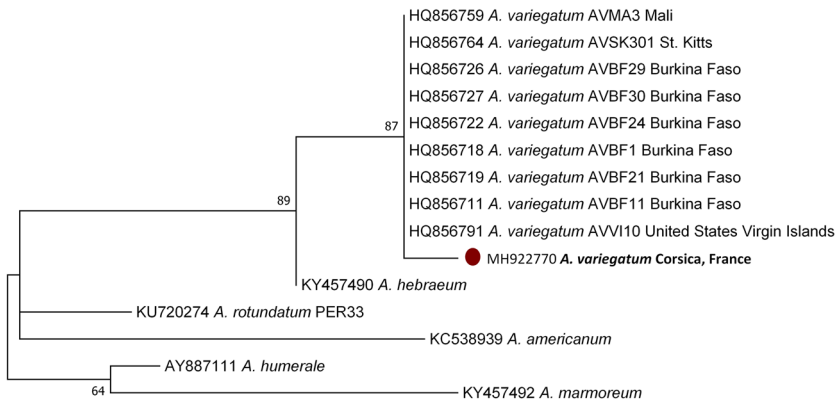


Fig. 2 Molecular phylogenetic analysis by Maximum Likelihood method of *Amblyomma variegatum* detected in Corsica by using 16S rDNA and ITS-2 genes. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. **a** The analysis involved 18 nucleotide sequences. There were a total of 277 positions in the final dataset. **b** The analysis involved 15 nucleotide sequences. There were a total of 90 positions in the final dataset

Nigeria (KU130403 and JF949794) and from São Tome (MF627697), (iii) of 97% with *A. variegatum* from Nigeria (JF949795, JF949797–99) (Fig. 2a). The ITS-2 sequence analysis of *A. variegatum* from Corsica was also 99% identical with *A. variegatum* from Burkina Faso (HQ856711, HQ856718–19, HQ856722, HQ856726–27), Mali (HQ856759), St. Kitts (HQ856764) and United States Virgin Islands (HQ856791) (Fig. 2b). This male tick was found positive for the presence of *Rickettsia* DNA but it was negative for the presence of *Erlischia* DNA. Sequence analysis of the *gltA* sequence obtained revealed 99 to

100% of identity with *Rickettsia africae* amplified from *A. variegatum* collected in Ethiopia (U59733) and in Madagascar (KJ645937-39), respectively.

Discussion

To the best of our knowledge, this report describes for the first time: (ii) the detection of *A. variegatum* in Corsica Island (France) and (ii) its positivity to *R. africae*, the most widespread spotted fever agent in sub-Saharan Africa, where it causes African tick-bite fever (Kelly et al. 1996, 2010). This tick species is widely distributed in the Caribbean Basin, in sub-Saharan Africa and in Atlantic and Indian Ocean islands. It tolerates a large climatic and environmental range from dry savannahs to more humid sylvatic regions. Sporadic occurrences of *A. variegatum* in the Mediterranean basin (Greece, Cyprus, Italy and Sicily) have been linked to the introduction by migratory birds, in particular the cattle egret, *Bubulcus ibis*, via transportation of larvae and nymphs (Deem 1998; Estrada-Pena et al. 2007; Pascucci et al. 2007). Interestingly, the cattle egret is present in Corsica (Thibault and Bonaccorsi 1999). The identification of the adult stage on a cow suggests the introduction of a nymph on a migrating bird from sub-Saharan Africa during spring migration and its moult to the adult stage during the warm summer of Corsica. There are examples of isolated reports of adults of other tropical tick genera, even in northern Europe, linked to the introduction of immatures by migratory birds from sub-Saharan Africa.

There is little or no risk of the introduction and establishment of a viable population of *A. variegatum* in Corsica by migrating birds. Isolated adults would have to meet the opposite sex in order to reproduce, and even then the species is unlikely to survive winter conditions. However, if it did (global warming!), it could produce major economic losses for livestock production through reduced growth, and perhaps most importantly by its association with severe dermatophilosis (Daubney 1930; Walker and Lloyd 1993); if infected (which is more unlikely), also by transmitting heartwater (Daubney 1930). Both are major diseases of ruminants. It could also have an impact in the medical field as one of the vectors of *R. africae*. In addition, this tick species can also transmit various viruses belonging to the *Orthomyxoviridae*, *Reoviridae*, and *Bunyaviridae* families, including the Crimean-Congo hemorrhagic fever virus (Jones et al. 1989; Kobayashi et al. 2017; Konstantinov 1990; Papa et al. 2017).

In Corsica, arthropod-borne pathogens were already reported such as Toscana virus in dogs (Dahmani et al. 2016) and West Nile virus in domestic animals (Maquart et al. 2017). Further studies and sustained surveillance are indicated, not only focusing on this species of tick and pathogen but also on other microorganisms of veterinary and medical importance that might be transmitted in Corsica and other Mediterranean islands.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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Chapitre 5 :
Identification des virus transmis par les tiques en Corse,
création d'outil de détection

1. La Fièvre Hémorragique Crimée-Congo: Lack of Evidence for Crimean–Congo Haemorrhagic Fever Virus in Ticks Collected from Animals, Corsica

Contexte

Les dernières années ont vu émerger le CCHFV en Europe, notamment en Espagne, où le premier cas humain est décrit en 2013 (rétrospectivement) puis suivi de 9 cas jusqu'en 2022. En Corse, la présence de CCHFV est possible car son vecteur, la tique *H. marginatum*, a été retrouvée. Face aux risques liés à la circulation du virus en Corse, nous avons mené une surveillance entomologique pendant 4 ans en testant toutes les tiques collectées pour la présence de l'ARN de CCHFV. Une étude sérologique récente menée sur les bovins de Corse rapporte une séroprévalence de 9%. Dans ce contexte, nous avons obtenu une autorisation de l'ANSM qui a validé le protocole de recherche moléculaire réalisé pour CCHFV qui est un virus qui relève de la législation française sur les microorganismes et toxines (MOT).

Méthode

Les tiques collectées (Tableau 1) (Figure 2) ont été organisées en pools et testées pour la présence de l'ARN du CCHFV en utilisant une RT-qPCR et à la présence de l'ARN de *Nairovirus* en utilisant une RT-PCR pan-genre (Tableau 3). Sur la base d'une prévalence attendue du CCHF (P) de $\approx 0,2\%$ et d'une taille de pool (k) de 2 tiques, un total de 7676 tiques devait être testées pour une estimation de la prévalence avec un IC de 95% et une précision (d) fixée à $+0,001$ car la prévalence de la maladie a été estimée à $<0,1$ (10%).

Résultats

Parmi les 8051 tiques collectées, 7156 tiques avaient été prélevées sur 3674 animaux domestiques et 895 tiques prélevées sur 188 animaux sauvages. Elles étaient constituées de 4 177 tiques *R. bursa* (51,8%), 2386 *H. marginatum* (29,6%), 839 *D. marginatus* (10,4%) et 282 *H. scupense* (3,5%). Aucune des tiques n'a été retrouvée positive au virus CCHF ou tout autre *Nairovirus*. En outre, nous avons détecté de l'ARN viral correspondant à de nouveaux virus appartenant au genre *Phlebovirus* dans 40 échantillons (5 %) et au genre *Flavivirus* dans 7 échantillons (0,9 %) (Figure 15).

Conclusion

Les résultats de cette surveillance, ainsi que l'absence de cas humain documenté, permettent de déclarer que la Corse n'est pas un hot-spot pour CCHFV et que la menace en population humaine reste pour l'instant très limitée.

Lack of Evidence for Crimean–Congo Hemorrhagic Fever Virus in Ticks Collected from Animals, Corsica, France

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In Corsica, France, 9.1% of livestock serum samples collected during 2014–2016 were found to have antibodies against Crimean–Congo hemorrhagic fever virus (CCHFV), an emerging tickborne zoonotic disease. We tested 8,051 ticks for CCHFV RNA and *Nairovirus* RNA. The results indicate that Corsica is not a hotspot for CCHFV.

Crimean–Congo hemorrhagic fever (CCHF) is a tickborne zoonotic disease that is characterized by hemorrhagic fever and can progress from mild, nonspecific signs to a severe and fatal hemorrhagic disease. The CCHF virus (CCHFV) is an enveloped, segmented, negative-sense, single-stranded RNA member of the family *Nairoviridae*, genus *Orthonairovirus*. CCHFV has been detected in >35 species of ticks worldwide, among which ticks belonging to the genus *Hyalomma* are the primary vectors in humans and wild and domestic animals (1). Humans are infected through tick bites and direct contact with infected blood and body fluids during occupational exposure (e.g., farming, slaughtering, and medical and nursing care).

CCHF is endemic in Africa, Asia, and the Balkan region (2). In Western Europe, autochthonous human cases were reported only in Spain, where CCHFV

was identified in *H. lusitanicum* ticks (3). In Corsica, a French Mediterranean island, 9.1% of livestock (i.e., cattle, goats, sheep) serum samples contained CCHFV-specific IgG during 2014–2016 (4). Entomologic surveys revealed that the *H. marginatum* tick, a vector of CCHFV, was present in Corsica (5).

The Study

To assess whether CCHFV circulates in Corsica, we collected 8,051 ticks from wild and domestic animals in selected sites on the island during 2016–2020 (Table, Figure). These 8,051 ticks included 7,156 ticks taken from 3,674 domestic animals and 895 ticks taken from 188 wild animals. They consisted of 4,177 *Rhipicephalus bursa* (51.8%), 2,386 *H. marginatum* (29.6%), 839 *Dermacentor marginatus* (10.4%) and 282 *H. scupense* (3.5%) ticks. We identified ticks at the species level by using a pictorial guide and confirmed morphologic identification by using sequencing of mitochondrial 16S rDNA (5). We then pooled up to 10 ticks per pool on the basis of developmental stage (nymphs, non-engorged females, and male adults) and host (Table). Pools, containing an average of 2.5 ticks (range 1–10 ticks) were crushed in phosphate-buffered saline with TissueLyser II (QIAGEN, <https://www.qiagen.com>) at 5,500 rpm for 3 min. We spiked each pool before extraction with a predefined amount of MS2 bacteriophage to monitor the subsequent steps (nucleic acid extraction, reverse transcription, and PCR amplification) and to detect the presence of inhibitors and enzymatic reactions as described (6). We performed DNA extraction by using QIAcube HT and a QIAamp cador Pathogen Mini Kit (QIAGEN), according to the manufacturer's instructions. We eluted DNA in 150 µL of buffer and stored at –20°C. We tested each pool for the presence of CCHFV RNA by using a real-time, reverse transcription PCR (7) and

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the presence of *Nairovirus* RNA by using a pangeneric reverse transcription PCR (8).

We detected neither CCHFV RNA nor *Nairovirus* RNA in the 8,051 ticks. The absence of CCHFV or *Nairovirus* RNA was not attributable to technical problems or presence of inhibitors, which were ruled out by MS2 bacteriophage monitoring. Moreover, we detected viral RNA corresponding to new tickborne *Phleboviruses* in 40 samples (5%) and *Flavivirus* in 7 samples (0.9%); these samples remain under investigation, and results will be reported after detailed characterization.

Conclusions

We considered whether CCHFV RNA was not detected because of a low minimum infection rate (MIR) that a larger number of ticks would have been required. We calculated the theoretical power that could be achieved by using the number of ticks obtained in our study. On the basis of an expected CCHFV prevalence (P) of $\approx 0.2\%$ and a pool size (k) of 2 ticks, a total of 7,676 ticks have to be tested for a prevalence estimation with a 95% CI and a precision (d) set at ± 0.001

because the disease prevalence is <0.1 (10%) (9). Thus, with a sample of 8,051 ticks, we were able to detect a prevalence of $\geq 0.2\%$.

The rate of CCHFV-infected ticks in countries in Europe with enzootic foci ranges from 0.50% to 3.70% among *Hyalomma* spp. ticks (2.8% [44/1,579 *H. lusitanicum* ticks] in Spain, 3.7% [6/161 *H. marginatum* ticks] in Bulgaria, and 0.5% [1/199 *H. marginatum* ticks] in Kosovo) and from 1.5% to 6.2% among *Rhipicephalus* spp. (1.5% [2/123 *R. sanguineus* ticks] in Bulgaria and 6.2% (8/130 *R. bursa* ticks] in Kosovo) (10). Other studies conducted outside of Europe have largely reported MIR values $>0.2\%$ among ticks: 0.71% in South Africa (1.6% [15/914] *H. truncatum* and 0.2% [2/1,149] *H. rufipes*) (11); 2.6% in Mauritania (39/1,517 *Hyalomma* spp.) (12); 3.8% (20/525 *Hyalomma* spp.) in Pakistan (13); and 51.5% (103/200 *H. marginatum*) in Turkey (14). These studies were conducted during the past 5 years using methods comparable to those of our study. The number of *Hyalomma* ($n = 2,682$) and *Rhipicephalus* ($n = 4,177$) ticks that we tested are much higher than reported in these previous studies. Therefore, our study would

Table. Ticks collected, by host, number of ticks, and number of tick pools, in a study of Crimean–Congo hemorrhagic fever virus in ticks from wild and domestic animals, Corsica, France, 2016–2020

Host and tick species	No. ticks	No. tick pools
Cattle, $n = 1,211$		
<i>Rhipicephalus bursa</i>	3,413	818
<i>Hyalomma marginatum</i>	1,343	475
<i>H. scupense</i>	282	96
<i>Boophilus annulatus</i>	130	47
<i>Ixodes ricinus</i>	85	33
<i>H. punctata</i>	14	10
<i>R. sanguineus</i>	96	32
<i>Dermacentor marginatus</i>	2	2
Total	5,365	1,513
Horses, $n = 201$		
<i>H. marginatum</i>	1,026	247
<i>R. bursa</i>	637	135
<i>R. sanguineus</i>	27	10
Total	1,690	392
Wild boar, $n = 182$		
<i>D. marginatus</i>	837	222
<i>H. marginatum</i>	13	7
<i>R. bursa</i>	9	6
<i>I. ricinus</i>	13	5
<i>R. sanguineus</i>	1	1
Total	873	241
Sheep, $n = 773$		
<i>R. bursa</i>	101	93
Total	101	93
Deer, $n = 4$		
<i>R. bursa</i>	9	4
<i>H. marginatum</i>	4	1
Total	13	5
Mouflon sheep, $n = 2$		
<i>R. bursa</i>	8	5
<i>I. ricinus</i>	1	1
Total	9	6
Overall	8,051	2,250

have been able to recognize CCHFV presence for a prevalence $\geq 0.2\%$, which is 10 times lower than the lowest overall prevalence value reported to date in countries where CCHFV is present: 2.1% (95% CI 1.3%–2.9%) according to a recent meta-analysis (10). Furthermore, another study addressing the presence of CCHFV RNA in *Hyalomma* spp. ticks (362 *H. marginatum* and 135 *H. scupense*) and *Rhipicephalus* ticks (n = 518) collected in 2014 from domestic and wild animals in Corsica also provided only negative results (15). In all countries where CCHF cases are described, the observed MIR of ticks is ≥ 2.5 times higher than the detection limit in our study (0.2%). Another argument that strongly supports the contention that the lack of detection of CCHFV or *Nairovirus* RNA was not caused by technical problems is based on the consideration that the protocol used in this study enables the detection of a wide variety of different CCHFV strains, a fact that confirms the accuracy of the results (7,8).

Recent studies determine whether CCHFV is present in Corsica and to what extent it is a threat for human populations, provide contrasting data. On one hand, tick species that are able to transmit CCHFV are present and widely distributed, and a serologic study based on ELISA screening and neutralization test for confirmation supports the presence of CCHFV or an antigenically related agent. On the other hand, the absence of detection of CCHFV RNA (or an antigenically related agent) in a large number of ticks, together with the absence of a CCHF case, supports the absence of CCHFV in Corsica to date.

In any case, the absence of a documented case of CCHF together with the lack of detection of CCHFV RNA in tick species that are recognized as a competent vector enables us to declare that Corsica is not a hotspot for CCHFV and that the threat to the human population is very limited. However, this discrepant set of data pleads for a One Health approach for dealing with the CCHF question in Corsica and the potential exposure of island population. To do so, the roadmap established by the World Health Organization's R&D blueprint (<https://www.who.int/teams/blueprint/about>) should be followed. Because the accuracy of CCHFV serologic assays has been questioned, several tests must be combined as advocated. Then, serologic studies in animals and humans must be synchronized with virus detection in ticks and systematic screening of patients with uncharacterized febrile illness during the tick season. A need exists for a large-scale One Health prospective program for surveillance of ticks, vertebrates, and humans in Corsica.

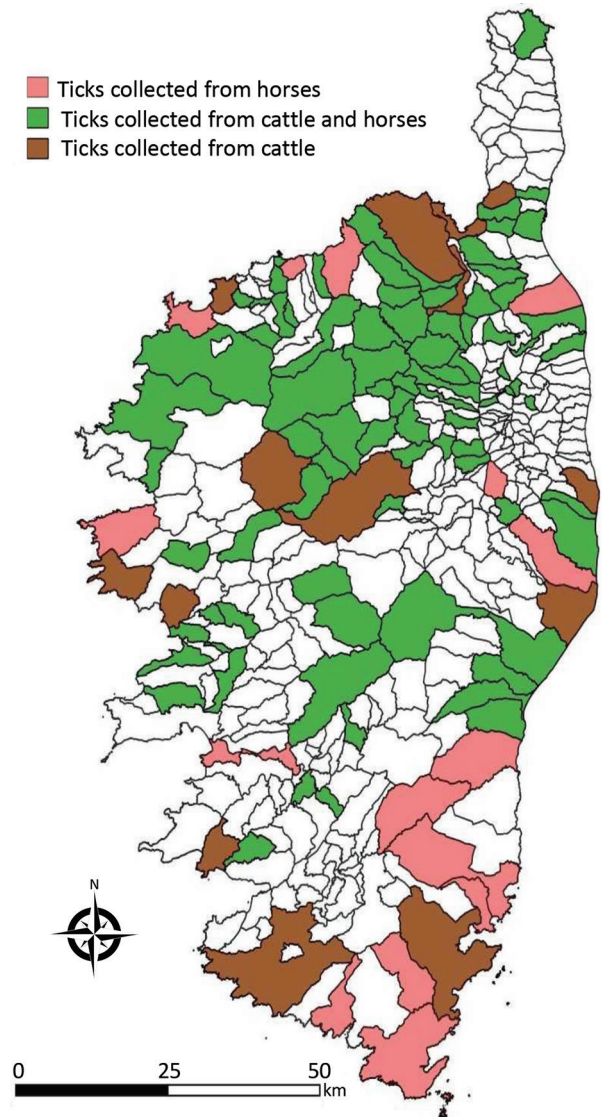


Figure. Locations of tick collection sites (for cattle and horses) for a study of Crimean–Congo Hemorrhagic fever virus in ticks from wild and domestic animals, Corsica, France, 2016–2020.

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2. Parapoxvirus et Orthopoxvirus : Molecular detection of Parapoxvirus in Ixodidae ticks collected from cattle in Corsica, France

Contexte

Les virus appartenant aux genres *Orthopoxvirus* et *Parapoxvirus* sont des virus à ADN double de la famille des *Poxviridae*. Les *Poxvirus* ont une importance majeure sur le plan vétérinaire et humain et infectent divers vertébrés et invertébrés, y compris l'homme. Les virus du genre *Parapoxvirus* sont : *Orf virus*, *Bovine Papular Stomatitis virus*, *Pseudocowpox virus*, et *Parapoxvirus* of Red Deer in New Zealand. Parmi les *Orthopoxvirus* on retrouve : le virus Monkeypox responsable de nombreux cas à travers le monde en 2022, le virus Cowpox, la variole et le virus de la vaccine qui sont associés à des épidémies en Afrique, en Europe, en Amérique du Sud et en Asie. Seule le virus de la variole présente une spécificité pour l'homme: la variole, véritable fléau de l'humanité au cours des siècles, reste à ce jour la seule maladie entièrement éradiquée grâce à la vaccination de masse. Cependant depuis l'arrêt de la vaccination en 1980 l'immunité globale de la population est en train de diminuer. Cela s'associe à l'émergence et l'épidémie actuelle de Monkeypox particulièrement inquiétante. Parmi les *Poxvirus*, les *Orthopoxvirus* ne sont pas le seul genre à causer des infections humaines. Cependant, depuis l'éradication de la variole le 8 mai 1980, la fréquence des travaux portant sur les *Poxvirus* baisse drastiquement. C'est ce qui a poussé l'UVE à s'intéresser à cette famille (thèse « Les *Poxvirus* et leur implication en santé humaine » soutenue en décembre 2021 par Léa Luciani). Une partie des travaux de cette thèse ont montré, la présence de deux *Parapoxvirus*, le *Pseudocowpoxvirus* et *Bovine Papular Stomatitis virus* dans des tiques prélevées sur des zébus dans l'est du Burkina Faso. Bien que l'interaction naturelle entre les tiques et le *Parapoxvirus* détecté dans cette étude soit inconnue, ces résultats montrent que les tiques peuvent être un bon indicateur de la propagation de ces pathogènes. Nous avons donc décidé de mener une étude corolaire, dans le but de confirmer et d'élargir les résultats pour tenter de comprendre l'implication des tiques dans la circulation de ces virus.

Méthode

Les tiques ont été collectées sur des brebis, des bovins, des sangliers et des chevaux de Mai 2018 à Janvier 2019 (Figure 12) (Tableau 1). Pour identifier et caractériser les poxvirus, des couples d'amorces Pan-*Orthopoxvirus* et Pan-*Parapoxvirus* capables de détecter et d'amplifier tous les virus du genre ont été utilisés (Tableau 2). L'utilisation de ces amorces a été encouragée par la diversité génétique et l'ancienneté de cette famille rendent la découverte de nouvelles

espèces virales encore inconnues, fréquente même chez les insectes. La prévalence a été calculée en utilisant le MLE.


Résultats

Un total de 3555 tiques a été collecté sur 1549 animaux différents (687 bovins, 538 chevaux, 106 moutons et 218 sangliers). L'ADN *Parapoxvirus* a été détecté dans 6,6 % (36/544) des tiques collectées sur 23 vaches provenant de 20 fermes. Les 3011 autres tiques collectées sur des chevaux, des sangliers et des moutons étaient négatives. Le MLE des tiques collectées sur les bovins était de 2,45% (IC95% : 1,32%–4,07%) en 2018 et de 2,32% (IC95%: 1,52%-3,36%) en 2019.

Conclusion

Les résultats ont montré que seules les tiques collectées sur bovin étaient positives à l'ADN de *Parapoxvirus*, alors que les mêmes espèces de tiques collectées sur des hôtes différents (Equins, Ovins, Porcins) étaient négatives et ce, de manière significative. Ces données suggèrent que l'ADN du virus présent dans les tiques proviendrait du sang des bovins infectieux et non d'une réplication du virus dans la tique. En effet, le *Pseudocowpoxvirus* affecte uniquement les bovins (et rarement l'Homme) et ne crée aucune immunité chez son hôte. Une faible partie des animaux d'un troupeau se retrouvent alors virémiques à faible bruit, tout comme les tiques qui se nourrissent sur ces animaux.

Molecular detection of parapoxvirus in Ixodidae ticks collected from cattle in Corsica, France

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Abstract

Background: Several viruses belonging to the family Poxviridae can cause infections in humans and animals. In Corsica, livestock farming (sheep, goats, pigs, and cattle) is mainly mixed, leading to important interactions between livestock, wildlife, and human populations. This could facilitate the circulation of zoonotic diseases, and makes Corsica a good example for studies of tick-borne diseases.

Objectives: To gain understanding on the circulation of poxviruses in Corsica, we investigated their presence in tick species collected from cattle, sheep, horses, and wild boar, and characterized them through molecular techniques.

Methods: Ticks were tested using specific primers targeting conserved regions of sequences corresponding to two genera: parapoxvirus and orthopoxvirus.

Results: A total of 3555 ticks were collected from 1549 different animals (687 cattle, 538 horses, 106 sheep, and 218 wild boars). They were tested for the presence of parapoxvirus DNA on one hand and orthopoxvirus DNA on the other hand using Pangeneric real-time TaqMan assays. Orthopoxvirus DNA was detected in none of the 3555 ticks. Parapoxvirus DNA was detected in 6.6% (36/544) of ticks collected from 23 cows from 20 farms. The remaining 3011 ticks collected from horses, wild boars, and sheep were negative. The infection rate in cow ticks was 8.0% (12/148) in 2018 and 6.0% (24/396) in 2019 ($p = 0.57$). Parapoxvirus DNA was detected in 8.5% (5/59) of *Hyalomma scupense* pools, 8.2% (15/183) of *Hyalomma marginatum* pools, and 6.7% (16/240) of *Rhipicephalus bursa* pools ($p = 0.73$). We successfully amplified and sequenced 19.4% (7/36) of the positive samples which all corresponded to pseudocowpox virus.

Conclusions: Obviously, further studies are needed to investigate the zoonotic potential of pseudocowpox virus and its importance for animals and public health.

KEYWORDS

cattle, epidemiology, ticks, zoonoses

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1 | INTRODUCTION

Viruses belonging to the orthopoxvirus and parapoxvirus genera are large, enveloped, linear double-stranded DNA viruses in the family Poxviridae (McFadden, 2005). Poxviruses are of major veterinary and human importance and infect various vertebrates and invertebrates, including humans. The genus *Parapoxvirus* contains five virus species: orf virus, bovine papular stomatitis virus, pseudocowpox virus, and parapoxvirus of red deer in New Zealand (Buttner & Rziha, 2002). There are three known zoonotic orthopoxvirus species: monkeypox virus, cowpox virus, and vaccinia virus which are associated with outbreaks in Africa, Europe, South America, and Asia (Singh et al., 2007). Humans are susceptible to monkeypox virus, cowpox virus, vaccinia virus, bovine papular stomatitis virus, orf virus, and pseudocowpox virus. Although the complete host range of these viruses is unclear, domestic animals such as sheep, goats, cats, dogs, and dairy cows can be infected with orthopoxvirus and/or parapoxvirus (Cicculi et al., 2020). Infected humans play an important role in the spread of orthopoxvirus and parapoxvirus among domestic animals, especially during milking and other livestock-related occupational activities (Cicculi et al., 2020; McFadden, 2005). Clinically, the exanthematous lesions caused by zoonotic orthopoxvirus and parapoxvirus species are very similar, especially in humans and cows, and can be diagnosed in areas of orthopoxvirus/parapoxvirus cocirculation (Inoshima et al., 2000).

Recently, the presence of two parapoxvirus (pseudocowpox virus and bovine papular stomatitis virus) was reported in ticks collected from zebu cattle in Eastern Burkina Faso (Ouedraogo et al., 2020). Although the natural interaction between ticks and the detected parapoxvirus in that study is unknown, this finding shows that ticks may be a good indicator of the spread of these pathogens.

In Corsica, a French Mediterranean island, ticks of the genus *Ixodes*, *Hyalomma*, *Dermacentor*, *Haemaphysalis*, and *Rhipicephalus* have been identified and can act as vectors for a variety of emerging diseases (Cicculi, Capai, et al., 2019; Cicculi, de Lamballerie, et al., 2019; Cicculi et al., 2020; Cicculi, Masse, et al., 2019; Cicculi, Oscar, et al., 2019; Grech-Angelini et al., 2020). Since mixed livestock farming (sheep, goats, pigs, and cattle) is extensive in Corsica, high interactions between livestock, wildlife, and human populations can facilitate the circulation of zoonotic diseases in the island. To our knowledge, there has been no investigation of the presence of poxviruses in domestic and wild animals in Corsica. Thus, the aim of this study was to provide new information about the potential circulation of parapoxvirus and orthopoxvirus by investigating their presence in tick species collected from cattle, sheep, horses, and wild boars in Corsica.

2 | MATERIALS AND METHODS

2.1 | Study area and collection of ticks

Ticks were collected (i) in May and June, 2019 from one sheep-breeding farm located in the centre of Corsica (42.298899N, 9.153161E); (ii) between July and December, 2018 and January

and December, 2019 from cattle in the Ponte-Leccia slaughterhouse, which is the main active slaughterhouse in Corsica; (iii) from August to December, 2018 and 2019 (hunting season) from wild boars in the northeast of Corsica; and (iv) between March and August, 2019 from horses on farms after they had been used for horseback riding in the natural environment across Corsica (Figure 1).

For each animal, all ticks were collected and kept alive until identification and storage. Living ticks were identified at species level under a stereomicroscope using an identification key, and immediately stored at -80°C (Estrada-Pena et al., 2014).

2.2 | DNA extraction and polymerase chain reaction detection

Ticks were washed once in 70% ethanol for 5 min and then twice in distilled water for 5 min. Ticks were analyzed as pools consisting of 1–6 ticks of the same species, same stage, and collected from the same animal (Table 2). Individual ticks or pools of ticks were crushed in minimal essential medium containing antibiotics and fungicide, using the TissueLaser II (Qiagen, Hilden, Germany) at 30 cycles/s of 3 min. DNA extraction was performed on a QIAcube HT (Qiagen) using a QIAamp Cador Pathogen Minikit according to the manufacturer's instructions. DNA was eluted in 100 μl of buffer and stored at -80°C . Extraction was monitored by systematic spiking of each sample with MS2 bacteriophage and subsequent quantitative polymerase chain reaction (qPCR) to assess PCR-inhibitory factors. Individual ticks or tick pools were tested using a set of qPCR assays for the detection of parapoxvirus (Kulesh et al., 2004; Nitsche et al., 2006) (Table 1).

Reactions were performed on a 96-well Applied Biosystems QuantStudio 3 Real-Time PCR System using QuantiFast Pathogen. Internal and negative controls were included in each run. Samples with $\text{Ct} \geq 32$ were considered as negative. Positive samples detected using qPCR were then analyzed by two different PCR protocols to obtain DNA fragments for sequencing (Table 1). The two PCR protocols target a 992 bp B2L gene fragment (open reading frame ORF 011) and a 1170 bp region within ORF 032. The ORF 011 (B2L) locus is a well-known and commonly used target gene for sequence analysis and comparison of parapoxvirus DNA. Moreover, ORF 032 is highly heterogeneous and provides an excellent basis for the assessment of the relationship between and within parapoxvirus species (Friederichs et al., 2014). Positive samples were purified and sequenced using an Applied Biosystems model 3730XL (Fisher Scientific, Illkirch-Graffenstaden, France). The newly generated sequences were aligned using X (ClustalW, Muscle, Mafft) via Mega X (Kumar et al., 2018).

2.3 | Sequence alignment and phylogenetic analysis

For comparative analysis, additional partial B2L gene and ORF 032 sequences of other parapoxvirus were retrieved from GenBank and

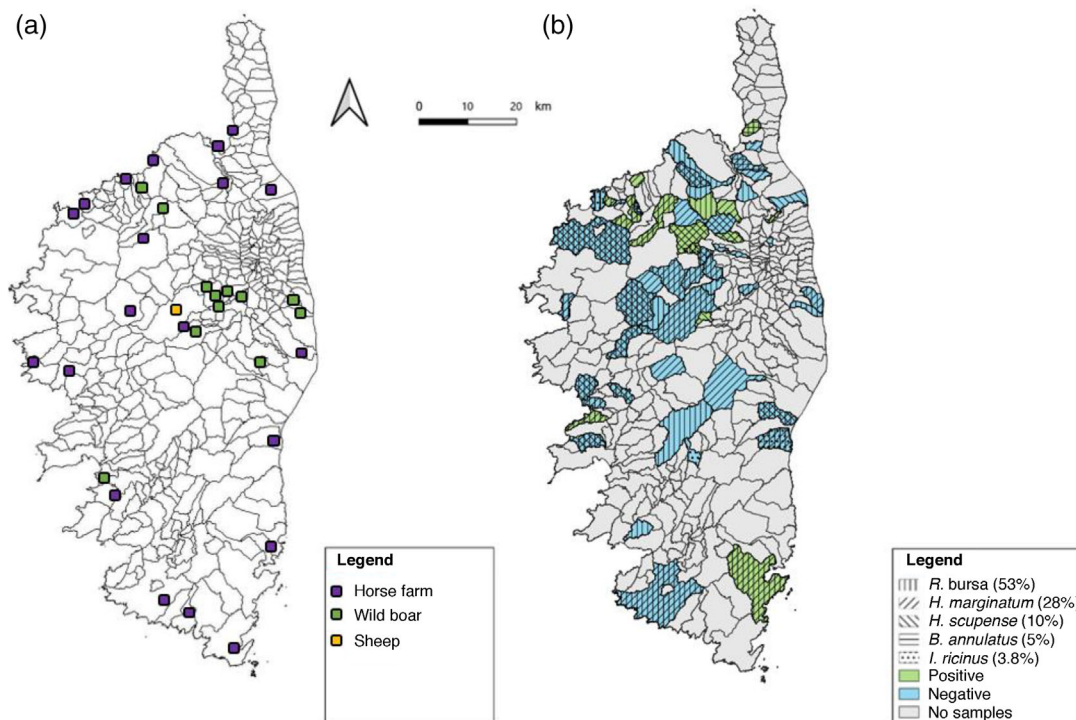


FIGURE 1 (a) Map of Corsica, France, indicating the tick collection sites and the animal species and farm and (b) tick species and positive pools of ticks collected from cattle in the study area, Corsica. *R. sanguineus* ($n = 6$) and *H. punctata* ($n = 4$) were not included

TABLE 1 Primers and probes used for the detection and amplification of parapoxvirus and orthopoxvirus

Genus or species	Primer and probe	5' → 3' Sequence	Gene	Reference
<i>Pan-Parapox virus</i>	Forward	TCGATGCGGTGCAGCAC	B2L	(Nitsche et al., 2006)
	Reverse	GCGGCGTATTCTTCTCGGAC		
	Probe	TGCGGTAGAAGCC		
<i>Pan-Orthopox virus</i>	Forward	GAA CAT TTT TGG CAG AGA GAG CC	HA (J7R)	(Kulesh et al., 2004)
	Reverse	CAA CTC TTA GCC GAA GCG TAT GAG		
	Probe	CAG GCT ACC AGT TCA A		
<i>Pan-Parapox virus</i>	Forward	GTG CGC GAA GGT GTC Kuleshov CA	ORF 011 (B2L)	(Friederichs et al., 2014)
	Reverse	ATGTGGCCGTTCTCTCCATC		
<i>Pan-Parapox virus</i>	Forward	CGAGCTTTAAATAGTGGAACACAGC	ORF 032	(Friederichs et al., 2014)
	Reverse	GCACCATCATCTGTACTTCCTC		

screened to remove short and duplicate sequences (Altschul et al., 1997). The final data set for phylogenetic analyses comprised 15 sequences for B2L, including three pseudocowpox sequences from this study, one pseudocowpox virus from cattle, one from reindeer, two from humans, four orf viruses, and four bovine popular stomatitis viruses. The final data set for phylogenetic analyses of ORF 032 comprised 24 sequences including seven pseudocowpox virus sequences from this study, one pseudocowpox virus from cattle, one from reindeer, three from humans, eight orf virus, and four bovine popular stomatitis viruses. Phylogenetic analyses were inferred using the maximum

likelihood estimation method implemented in Mega X (Kumar et al., 2018). The bootstrap consensus tree was conducted with 1000 replicates.

2.4 | Statistical analysis

The pathogens detected in pools were expressed as the percentage and minimum infection rate (maximum likelihood estimation (MLE)) method with 95% confidence intervals (CIs) based on the assumption

that each PCR-positive pool contained at least one positive tick (Sosa-Gutierrez et al., 2016). Infection rate of DNA viruses was compared by using Fisher exact test ($p < 0.05$). The analysis was conducted using the R statistical platform (version 3.1.2) (Team, 2015).

3 | RESULTS

3.1 | Tick collection and morphological identification

In total, 3555 ticks were collected from 1549 different animals (687 cattle, 538 horses, 106 sheep, and 218 wild boars) (Table 2). Of these, 3490 (98%) were adult ticks and 1529 (43%) were female ticks. Overall, 1566 ticks were collected from 687 cattle from 83 different cattle-breeding farms (Table 2). The most abundant species was *Rhipicephalus bursa* ($n = 820$; 52% of ticks collected in cattle), followed by *Hyalomma marginatum* ($n = 441$; 28%), *Hyalomma scupense* ($n = 152$; 10%), *Boophilus annulatus* ($n = 78$; 5%), *Ixodes ricinus* ($n = 59$; 4%), *Rhipicephalus sanguineus* s.l ($n = 6$; 0.4%), and *Haemaphysalis punctata* ($n = 4$; 0.3%) (Figure 1b). In total, 685 ticks were collected from 218 wild boars. The most abundant species was *Dermacantor marginatus* ($n = 662$; 96.6% of ticks collected in wild boars), followed by *I. ricinus* ($n = 13$; 2%), *R. bursa* ($n = 9$; 1.3%), and *H. marginatum* ($n = 1$; 0.1%). A total of 1285 ticks were collected from 538 horses from 21 farms. The most abundant species was *H. marginatum* ($n = 707$; 55% of ticks collected in horses), followed by *R. bursa* ($n = 578$; 45%). Thirty ticks were collected from 106 sheep. The only collected species was *R. bursa* ($n = 30$; 100%).

3.2 | Detection of pathogens

Overall parapoxvirus DNA was detected in 6.6% (36/544) of tick pools collected from 23 cows from 20 farms (Table 3 and Figure 1) with an infection rate (MLE) of 2.36% (95% CI: 1.68%–3.21%). The parapoxvirus DNA detection was 8% (12/148) in 2018 and 6.0% (24/396) in 2019 ($p = 0.57$) with an MLE of 2.45% (95% CI: 1.32%–4.07%) and of 2.32% (95% CI: 1.52%–3.36%), respectively (Table 2).

The parapoxvirus DNA infection rate detected in *H. marginatum*, *H. scupense*, and *R. bursa* was not significantly different between these three tick species ($p = 0.73$) (Table 2). The 2018 infection rate of *R. bursa* (7%; 6/86) (MLE = 1.71% (95% CI: 0.68%–3.43%)) was similar to that observed in 2019 (6.5%; 10/154) (MLE = 2.25% (95% CI: 1.13%–3.92%)) ($p = 1$). Similar infection rates were also observed for *H. marginatum* in 2018 (10.1%, 6/59) (MLE = 4.53% (95% CI: 1.83%–8.97%)) and 2019 (7.6%, 9/124) (MLE = 3.04% (95% CI: 1.47%–5.42%)) ($p = 0.57$). *H. scupense* was collected only in 2019 (Table 2). Parapoxvirus DNA was not detected in tick pools collected from horses, wild boars, or sheep. Orthopoxvirus DNA was not identified in any of the 3555 ticks collected.

3.3 | Phylogenetic analysis

We successfully sequenced 19.4% (7/36) of the positive tick pools. The seven sequences were obtained from ticks collected from five cows belonging to seven farms (Table 3). Three B2L sequences were obtained from two *H. marginatum* pools and from one *R. bursa* pool. The phylogenetic tree based on B2L gene sequences indicated that the three samples showed 99% and 100% nucleotide and amino acid identity, respectively. The three sequences showed 99% nucleotide identity and 100% amino acid identity with parapoxvirus strain 3/07 (GenBank: KF478804) detected from cattle in Germany, with strain VR634 (GenBank: GQ329670) detected in humans in the United States and strain B074 (GenBank: KF478803) detected in humans in Germany. The seven ORF 032 gene sequences were obtained from four *R. bursa* pools and three *H. marginatum* pools. The seven sequences showed 99%–100% nucleotide and amino acid identity with each other, 98% and 99.8% nucleotide and amino acid identity, respectively, with strain 3/07 (GenBank: KF478816), and 95% and 99.5% nucleotide and amino acid identity, respectively, with strain VR634 (GenBank: GQ329670). Overall, phylogenetic tree analysis based on amino acid sequences of B2L and ORF 032 genes (Figures 2 and 3) showed that the B2L and the ORF 032 gene of parapoxvirus detected in ticks collected from cattle in Corsica were similar to each other and grouped together with pseudocowpox virus.

4 | DISCUSSION

We report evidence of the detection of parapoxvirus DNA in three main tick species collected from cattle in Corsica. Parapoxvirus DNA was detected at similar rates in pools of *H. marginatum*, *H. scupense*, and *R. bursa* ticks, and throughout the entire 2018–2019 period of collection, showing that parapoxvirus may circulate endemically in Corsica. The results of this study showed that overall parapoxvirus DNA was detected in 6.6% of tick pools collected from 23 cows from 20 farms, demonstrating the wide circulation of poxviruses in bovine herds in Corsica. Sequence analyses showed that at least 19% of the parapoxvirus DNA detected in ticks belonged to pseudocowpox virus. In the phylogenetic reconstruction, all Corsican pseudocowpox viruses clustered with previously published European sequences of pseudocowpox viruses detected in cattle and humans. Although parapoxvirus is reportedly present in cattle worldwide (Cargnelutti et al., 2012; Ohtani et al., 2017; Ziba et al., 2020), there is no published record of the disease at the human or animal health level in Corsica. Therefore, this report marks the first identification of parapoxvirus and pseudocowpox virus in the island. The detection rate of parapoxvirus DNA in 6.6% of tick pools collected in this study was lower than the detection rate (14% parapoxvirus DNA) reported in ticks collected from cattle in Burkina Faso (Ouedraogo et al., 2020), although the percentage of positive pseudocowpox virus was similar (8.2%). No detection of parapoxvirus DNA in ticks collected from the other animal species (horses, wild boar, and sheep) and identification of parapoxvirus in dif-

TABLE 2 Distribution of tick species by host and pools positive for parapoxvirus DNA 2018/2019

Number of pools with <i>n</i> ticks		Number of positive pools detected by real-time Pan-Parapoxvirus PCR												
		Cattle 2019 (<i>n</i> = 456)												
Number of individual ticks or ticks per pool (<i>n</i>)	<i>H. marginatum</i>		<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	Total
	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	Total
1	60	55	30	4	3	12	6	0	170	1	1	2	2	5
2	25	25	6	0	0	2	5	0	63	2	1	3	1	5
3	14	13	6	0	1	4	3	0	41	3	2	1	0	3
4	17	10	4	0	0	0	1	0	32	4	2	1	0	3
5	12	5	2	0	0	4	0	0	23	5	0	0	0	0
6	30	16	11	0	0	5	5	0	67	6	4	2	2	8
Total	158	124	59	4	4	27	20	0	396	Total	10	9	5	24
Number of ticks	460	305	152	4	6	78	59	0	1064	MLE (95%CI)	2.25 (1.13-3.92)	3.04 (1.47-5.42)	3.42 (1.24-7.21)	2.32 (1.52-3.36)
Cattle 2018 (<i>n</i> = 241)														
1	12	25	0	0	0	0	1	0	38	1	0	2	0	2
2	7	9	0	0	0	0	1	0	17	2	2	1	0	3
3	8	14	0	0	0	0	1	0	23	3	2	3	0	5
4	15	5	0	0	0	0	0	0	20	4	0	0	0	0
5	14	5	0	0	0	0	0	0	19	5	0	0	0	0
6	30	1	0	0	0	0	0	0	31	6	2	0	0	2
Total	86	59	0	0	0	0	3	0	148	Total	6	6	0	12
Number of ticks	360	136	0	0	0	0	6	0	502	MLE (95%CI)	1.71 (0.68-3.43)	4.53 (1.83-8.97)	/	2.45 (1.32-4.07)
Cattle 2018/2019 (<i>n</i> = 687)														
1	72	80	30	4	3	12	7	0	208	1	1	4	2	7
2	32	34	6	0	0	2	6	0	80	2	3	4	1	8
3	22	27	6	0	1	4	4	0	64	3	4	4	0	8
4	32	15	4	0	0	0	1	0	52	4	2	1	0	3
5	26	10	2	0	0	4	0	0	42	5	0	0	0	0
6	60	17	11	0	0	5	5	0	98	6	6	2	2	10
Total	244	183	59	4	4	27	23	0	544	Total	16	15	5	36
Number of ticks	820	441	152	4	6	78	65	0	1566	MLE (95%CI)	2.01 (1.18-3.14)	3.5 (2.03-5.55)	3.42 (1.24-7.21)	2.36 (1.68-3.21)

(Continues)

TABLE 2 (Continued)

Number of pools with n ticks		Number of positive pools detected by real-time Pan-Parapoxvirus PCR										
Cattle 2019 (n = 456)												
Number of individual ticks or ticks per pool (n)	H. marginatum	H. scupense	H. punctata	R. sanguineus annulatus	B. I. ricinus	D. marginatus	Total	Pool	R. bursa	H. marginatum	H. scupense	Total
Horses 2019 (n = 538)												
1	15	29	0	0	0	0	44	1	0	0	0	0
2	10	12	0	0	0	0	22	2	0	0	0	0
3	9	12	0	0	0	0	21	3	0	0	0	0
4	9	14	0	0	0	0	23	4	0	0	0	0
5	4	12	0	0	0	0	16	5	0	0	0	0
6	5	16	0	0	0	0	21	6	0	0	0	0
7	5	11	0	0	0	0	16	7	0	0	0	0
8	6	12	0	0	0	0	18	8	0	0	0	0
9	3	7	0	0	0	0	10	9	0	0	0	0
10	32	17	0	0	0	0	49	10	0	0	0	0
Total	98	142	0	0	0	0	240	Total	0	0	0	0
Number of ticks	578	707	0	0	0	0	1285					
Wild boars 2018/2019 (n = 218)												
1	4	1	0	0	0	1	33	1	0	0	0	0
2	1	0	0	0	0	2	25	2	0	0	0	0
3	1	0	0	0	0	0	22	3	0	0	0	0
4	0	0	0	0	0	2	25	4	0	0	0	0
5	0	0	0	0	0	0	19	5	0	0	0	0
6	0	0	0	0	0	0	53	6	0	0	0	0
Total	6	1	0	0	0	5	177	Total	0	0	0	0
Number of ticks	9	1	0	0	0	13	662					
Sheep 2019 (n = 106)												
1	30	0	0	0	0	0	30	1	0	0	0	0
Total	30	0	0	0	0	0	30	Total	0	0	0	0
Number of ticks	30	0	0	0	0	0	30					

Abbreviation: MLE, maximum likelihood estimation.

TABLE 3 Tick species pools positive for parapoxvirus DNA

Pools ID	Tick species	Farms	Cattle ID	Number of pools per cattle	Number of cattle per Farm	Province	Sample	B2L accession number	ORF 032 accession number
2019 75	<i>H. marginatum</i>	CAL1	8520	5	1	Calcatoghju	/	/	/
2019 78	<i>H. marginatum</i>								
2019 79	<i>H. marginatum</i>								
2019 91	<i>H. marginatum</i>	LAV1	7093	7	1	Lavatoghju	PCPVCorsica2019I	MW911454	MW911458
2019 265	<i>H. marginatum</i>	OLM1	2923	4	1	Olmi-Cappella	/	/	/
2019 268	<i>H. marginatum</i>	OLM2	1273	1	1				
2019 259	<i>H. marginatum</i>	OLM3	8821	3	1	Olmu			
2019 74	<i>H. marginatum</i>	NA1	NA4	4	/	Unknown			
2019 96	<i>H. marginatum</i>		NA5	7					
2019 26	<i>H. scupense</i>	CAS1	3186	6	1	Casanova			
2019 27	<i>H. scupense</i>								
2019 25	<i>H. scupense</i>	MOL1	4135	1	1	Moltifau			
2019 24	<i>H. scupense</i>	POP1	3256	2	1	Pulasca			
2019 22	<i>H. scupense</i>	VAL1	4607	2	1	Valle di Rustinu			
2019 306	<i>R. bursa</i>	LAV2	309	4	2	Lavatoghju			
2019 307	<i>R. bursa</i>								
2019 308	<i>R. bursa</i>								
2019 309	<i>R. bursa</i>								
2019 310	<i>R. bursa</i>		310	2					
2019 311	<i>R. bursa</i>								
2019 215	<i>R. bursa</i>	NA2	NA9	4		Unknown			
2019 217	<i>R. bursa</i>								
2019 218	<i>R. bursa</i>								
2019 272	<i>R. bursa</i>	FAR1	2018	1	3	Faringule	PCPVCorsica2019II	MW911455	MW911459
2018 2	<i>R. bursa</i>	FIL1	50	2	1	Filicetu	PCPVCorsica2018III	/	MW911462
2018 3	<i>H. marginatum</i>		50				PCPVCorsica2018I	MW911453	MW911460
2018 9	<i>R. bursa</i>	POR1	5		1	Portivechju	PCPVCorsica2018IV	/	MW911456
2018 10	<i>R. bursa</i>	SAN1	6825	5	1	San Martinu di Lotta	/		/
2018 12	<i>R. bursa</i>		6825				PCPVCorsica2018V		MW911457
2018 13	<i>H. marginatum</i>		6825				PCPVCorsica2018II		MW911462
2018 14	<i>H. marginatum</i>		6825				/		/
2018 15	<i>H. marginatum</i>	MON1	5687	1	1	Monticellu			
2018 101	<i>R. bursa</i>	ZIL1	6924	1	1	Zilia			
2018 102	<i>H. marginatum</i>	LENT1	8523	1	1	Lentu			
2018 103	<i>R. bursa</i>	PIE1	621	1	1	Pietralba			
2018 105	<i>H. marginatum</i>	PIE2	1823	1	2	Nessa			

ferent tick species suggest that ticks became infected through their blood meal from infected cattle and probably do not contribute to virus circulation. No orthopoxvirus DNA was found in ticks collected during this study in Corsica. This could be explained by the capacity for reinfection of the parapoxvirus group and the subsequent permanent

circulation of that virus in the same herd, thereby inhibiting infection with the orthopoxvirus group (Mercer & Weber, 2007). However, coinfections of pseudocowpox virus and orthopoxvirus have been described in samples from lesions in cows and humans during bovine vesicular disease outbreaks in Brazil in 2015 (Abrahão et al., 2010).

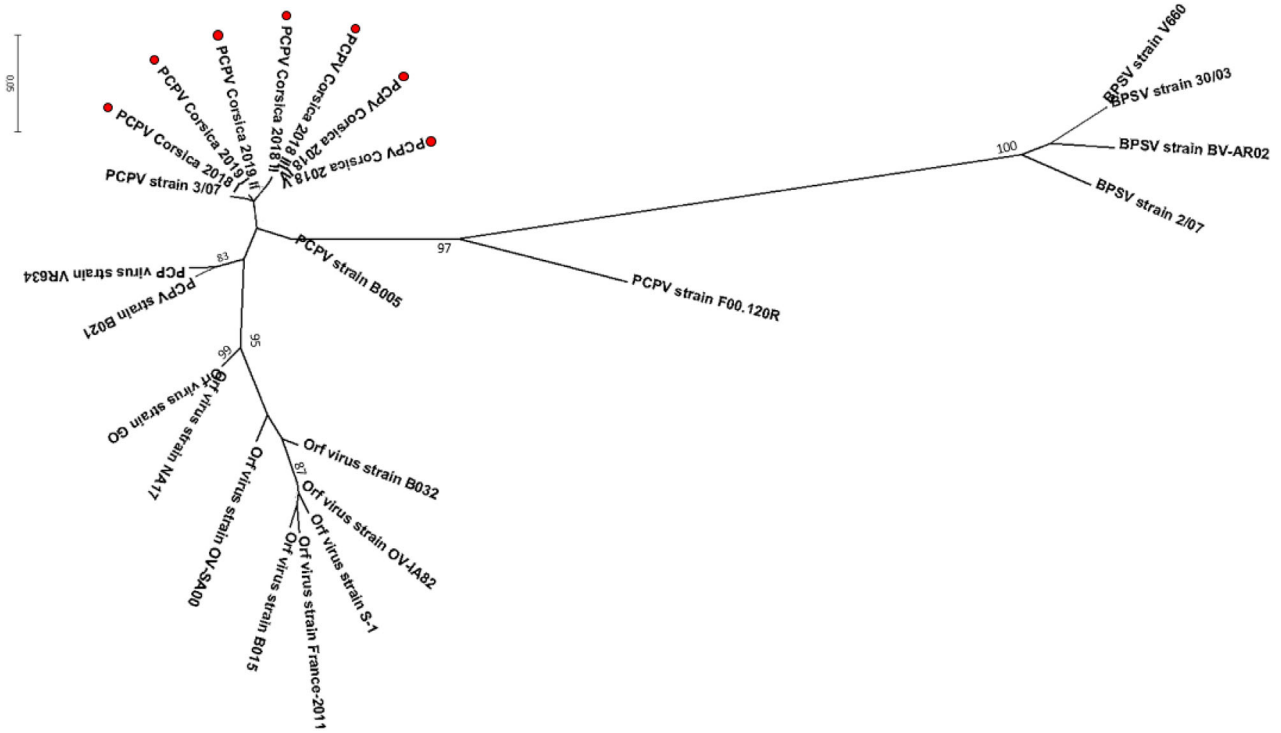


FIGURE 2 Phylogenetic radiation tree of parapoxvirus-group based deduced of 292 amino acid sequences of ORF 032 gene of parapoxvirus. The analysis was performed using a maximum-likelihood method with JTT matrix-based model with 1000 replicates (only values higher than 70% are shown). This analysis involved 24 amino acid sequences

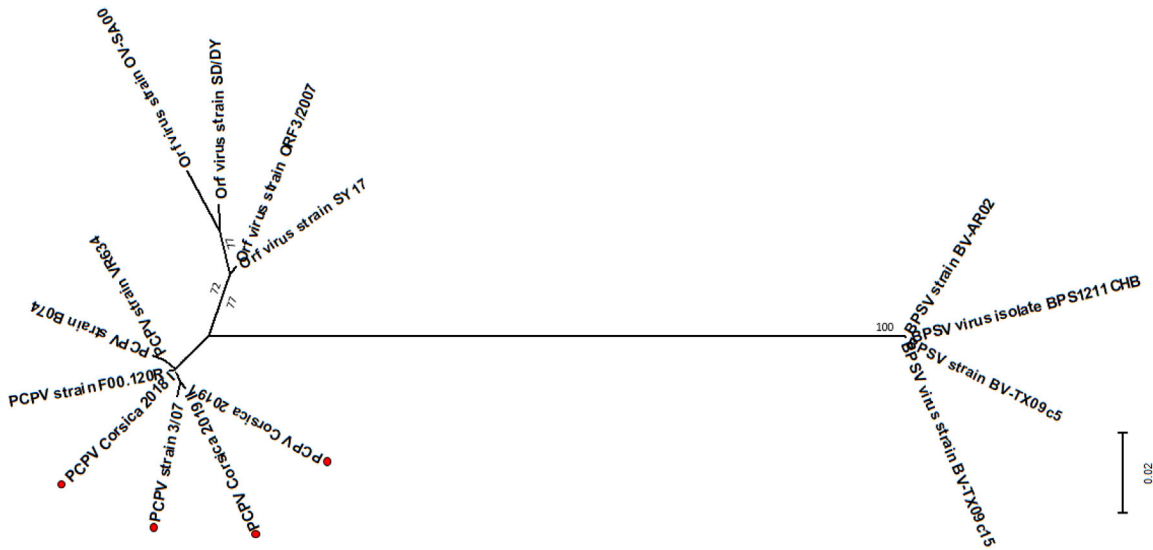


FIGURE 3 Phylogenetic radiation tree of parapoxvirus-group based deduced of 297 amino acid sequences of B2L gene of parapoxvirus. The analysis was performed using a maximum-likelihood method with JTT matrix-based model with 1000 replicates (only values higher than 70% are shown). This analysis involved 15 amino acid sequences

These two viruses have also been detected in milk from affected dairy cows (de Oliveira et al., 2018).

Finding the DNA of parapoxvirus in feeding ticks is only a marker of circulation of this genus in the cattle population; this detection cannot highlight the role of ticks in the transmission or circulation of these

viruses. Implication of ticks in epidemiological cycle of parapoxvirus should be tested in laboratory through vector competence studies to have a comprehensive idea of their real implication. Moreover, we have no data on the impact on animal health of parapoxvirus positive tick hosts. Working with pooled ticks has several advantages but inevitably

poses problems with prevalence estimates. Seven of the 36 positive samples were able to be sequenced and analysis showed the presence of pseudocowpox virus. Hence, it is possible that other viruses of the genus were present.

In conclusion, this study showed that parapoxvirus circulates in cattle in Corsica. Therefore, a broad surveillance is crucial to provide data that elucidate the origin and dissemination dynamics of parapoxvirus to investigate the prevalence of parapoxvirus infections in the cattle population and identify infection risks for other animals and humans.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Cicculi Vincent, Ayhan Nazli, and Falchi Alessandra conceived the study, analyzed data, and drafted the manuscript. Cicculi Vincent, Pezzi Laura, Luciani Léa, Decarreaux Dorine, and Maitre Apolline were involved in microbiological diagnosis. Decarreaux Dorine, Maitre Apolline, and Cicculi Vincent collected ticks. N. de Lamballerie Xavier, Vial Laurence, Paoli Jean-Christophe, and Charrel Remi drafted the manuscript.

ETHICS STATEMENT

No ethical approval was required, as this study does not involve clinical trials or experimental procedures. The cattle inspected were slaughtered for human consumption. Living sheep and Horses were examined with the assistance of their owner. This study did not involve endangered or protected species. The wild boars collected were legally hunted during the hunting season.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.700>.

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3. Jingmen tick virus: First detection of Jingmen Tick virus in Corsica, Development and validation of a real time assay for the detections of Jingmen tick virus group to face a potential emergence.

Contexte

Le groupe Jingmen Tick virus (JMTVs) sont des virus à ARN segmenté identifiés chez les tiques pour la première fois en 2014 dans la région de Jingmen de la province du Hubei en Chine, et simultanément au Brésil. Des études rétrospectives ont montré que des patients présentant des manifestations cliniques graves, notamment de la fièvre, des myalgies et des taux élevé d'aminotransférases hépatiques, ont été testés positifs au JMTV en Chine. En Europe, le JMTV a été détecté dans une co-infection avec le CCHFV dans un cas humain fatal au Kosovo et dans des tiques en Turquie et en Roumanie. Bien que des JMTVs aient été détectés chez l'homme, aucun système de détection efficace n'est disponible pour le JMTV et/ou les virus de type JMTV. La capacité de diagnostic est importante pour la surveillance, la détection précoce et la préparation aux épidémies des agents pathogènes. Elle permet de limiter la propagation des agents pathogènes, en évitant la multiplication de la chaîne de transmission. Un système de détection moléculaire en RT-qPCR JMTV et JMT-like-virus (JMTVs) en temps réel est donc important pour une meilleure connaissance de ce nouveau groupe de virus. L'objectif de cette étude était de concevoir et de valider un système de RT-PCR en temps réel pour la détection des JMTVs et de l'utiliser pour fournir de nouvelles informations sur sa circulation. En effet, les espèces de tiques positives en JMTV présentes au Kosovo et Turquie étant proches de celles de la Corse, la présence de ces virus dans l'île a été évoquée.

Méthode

Les tiques ont été collectées sur des brebis, des bovins, des sangliers et des chevaux de Mai 2018 à Juin 2020 (Figure 12) (Tableau 1). Pour la création des amorces et de la sonde, toutes les séquences des virus appartenant au JMTV group existantes sur GenBank ont été alignées. Un essai en RT-qPCR spécifique du segment 2 a été développé. Le système ciblant le segment 2 a été validé car capable de détecter tous les JMTVs avec une bonne limite de détection. Le système a été utilisé sur des tiques collectées dans différents élevages en Corse. Un séquençage NGS a été utilisé pour le séquençage complet du génome du virus présent dans les échantillons de tiques.

Résultats

Au total, 6269 tiques ont été collectées sur 763 bovins, 538 chevaux, 106 moutons et 218 sangliers et testées pour les JMTVs. Vingt-deux pools sur 1715 étaient positifs à l'ARN de JMTVs. La prévalence la plus élevée a été trouvée dans le genre *Rhipicephalus* avec une prévalence de 0,58% [0,35%-0,9%] chez *R. bursa* et 0,55% [0,03%-2,41%] chez *R. sanguineus*, suivie par *H. marginatum* 0,19% [0,06%-0,45%]. La séquence complète du génome du virus provenant d'un pool tique *R. bursa* collectée en Corse présentait 94,7-95,1% d'identité nucléotidique avec le JMTV collecté au Kosovo et se regroupe avec d'autres souches méditerranéennes du JMTV (Figure 15). La limite de détection du test RT-qPCR du segment 2 était de 345,8 copies/ μ L [224,5-1157,3] avec une bonne linéarité.

Conclusion

Cette étude a permis la création d'un système de diagnostic rapide pour la détection du JMTVs group. Ce système utilisable en diagnostic ou pour la détection dans les vecteurs est un outil efficace pour la surveillance et la préparation en cas d'épidémie dû à un virus appartenant au JMTVs group. Ces résultats montrent aussi que la Corse est une région sentinelle vis-à-vis des virus transmis par les tiques, et qu'elle peut à ce titre, être utilisées pour la création d'outil de maîtrise épidémique.

Les résultats de cette étude sont présentés dans ce manuscrit sous format article scientifique.

First detection of Jingmen Tick virus in Corsica:

Development and validation of a real time assay for the detections of Jingmen tick virus group to face a potential emergence.

Abstracts

Jingmen tick virus (JMTV) is recently discovered segmented RNA virus which identified for the first time in 2014 in China and simultaneously in Brazil. After the first discovery of the JMTV, other genetically close JMT like viruses have been identified in arthropods, reptiles and mammals including human from Asia, America, Africa and Europe. The association between JMTV and febrile illnesses in humans have been shown in several studies. Rapid diagnostic system is important in the control and early detection of epidemics, it allows entomological surveillance of pathogens and hospital diagnosis, both important elements of preparedness for epidemics. After the design and the validation of RT-qPCR Jingmen tick viruses assay, ticks collected from different livestock in Corsica were screened. The aim of this study was to design and validate of a real-time PCR system assay for the detection of JMTVs and used it for provide new information about its circulation in Corsica. In total, 6 269 ticks were collected from 763 cattle, 538 horses, 106 sheep and 218 wild boars and screened to JMTVs. Twenty-two pools of 1715 pools were found positive for JMTVs in field collected ticks in Corsica. The highest prevalence was found in *Rhipicephalus* genus with a maximum-likelihood estimation of 0.58% [0.35%-0.9%] in *R. bursa* and 0.55% [0.03%-2.41%] in *R. sanguineus* followed by *H. marginatum* 0.19% [0.06%-0.45%]. The JMTV complete genome sequence from *R. bursa* tick collected in Corsica had 94.7-95.1% of nucleotide identity with JMTV collected in Kosovo and clusters together with other Mediterranean strains of JMTV. These results shown that Mediterranean islands such as Corsica can play a major role in understanding and the prepariness for future epidemics as a sentinel zone.

Introduction

Jingmen tick virus (JMTV) group is a segmented RNA virus identified in ticks for the first time in 2014 in the Jingmen region of Hubei province in China and simultaneously in Brazil [64, 66]. The JMTV group genome comprise four separate segments positive-sense single-stranded RNA. The segment 1 and segment 3 encode the non-structural proteins, which are genetically and functionally close of NS3 and NS5 of *flaviviruses* of the family Flaviviridae. The remaining segments; segment 2 and 4 encode structural protein and share no homology with any known viral sequences which may due to have origins of uncharacterized ancestral viruses [64, 66, 139]. After the first discovery of the JMTV, other genetically close JMT like viruses have been identified in arthropods, reptiles and mammals including human from Asia, America, Africa and Europe [66, 68, 70, 140]. A number of tick species were found positive for JMTVs such as *Amblyomma variegatum*, *Ixodes ricinus*, *Rhipicephalus microplus*, *Haemaphysalis longicornis*, *Rhipicephalus bursa* and *H. marginatum* [64, 70, 141, 142]. However, their involvement of tick species of JMTV natural circulation is still unclear, in a recent study, *Amblyomma javanense* ticks were experimentally infected by JMTV which was able to replicate and migrate through the midguts to salivary glands, showing that ticks might have a role in the transmission of these viruses [68]. Retrospective studies showed patients with severe clinical manifestations include fever, myalgia an hepatic aminotransferase concentration were tested positive to JMTV in China [68]. Moreover, human febrile illness associated JMT-like virus named Alongshan virus was also described in China and Mongolia [69]. In Europa, JMTV were detected in co-infection with Crimean-Congo Hemorrhagic fever in deadly human case in Kosovo and in ticks in Turkey and Romania [70, 143, 144]. Additionally, two other JMT-like viruses were described; the Yanggou Tick virus identified in ticks from China, and more recently, the Takachi virus identified in ticks from Japan [67].

Due to its recent discovery, very little information is available for JMT viruses. Although, JMTV group have been detected in human cases, no efficient detection system is available for JMTV and/or JMT-like viruses. Diagnostic capacity is important for the surveillance, early detection and epidemic preparedness of pathogens. It helps to limit the spread of the pathogens, avoiding the multiplication of the chain of transmission. In addition, vector diagnostics can be used to determine the spatial distribution and the competent vectors and to detect possible introduction into new territories. JMTV and JMT like-viruses (JMTVs) Real-time PCR assay is primordial for the better understanding of this new virus group.

In Corsica, a Mediterranean island, the presence tick species of the genus *Ixodes*, *Hyalomma*, *Dermacentor*, *Haemaphysalis*, *Rhipicephalus* and *Amblyomma* and their vectorisation of a variety of emerging diseases were known [10, 11, 145]. Circulation of zoonotic diseases in the island is facilitate by an extensive mixed livestock farming, migration avian corridors and the high interactions between livestock, wildlife and human populations [12, 120]. Corsica is therefore, a sentinel area for the study of tick-borne diseases, especially as some species of ticks present in Corsica have already begun to migrate towards south Europe [146]. Through monitoring vectors and vector-borne agents, identifying known and unknown viral agent's diseases on the island and with developing rapid diagnostic systems, we can implement preparedness and response of potential epidemics.

The aim of this study was to design and validate of a real-time PCR system assay for the detection of JMTVs and used it for provide new information about its circulation in Corsica. For this purpose, a JMTVs segment 2 specific RT-qPCR assay was developed, validated and the system was used to screen ticks collected from different livestock in Corsica.

Material and Methods

Primer Design

All the available sequences of Jingmen tick viruses and Jingmen tick like-viruses (Alongshan virus, Mogiana tick virus (JMTV), Yanggou tick virus and Jingmen tick associated virus) from the taxonomy browser of the National Center of Biotechnology Information (NCBI) were used for the alignment on the clustal program (MEGA-X software). Primers and probe were designed to target a highly conserved region of segment 2 of JMTV and Jingmen tick like-viruses (Table 1). JMTVs segment 2 is targeted due to higher homology between JMTVs than the other segments and no homology with genes of other viruses [139].

Generation of in Vitro Transcribed RNAs (IVT RNAs)

Plasmid was synthesized by Genart (ThermoFisher) containing the region of segment 2 of JMTV amplified by the assay of this studies (sequence available in supplementary data). For the detection of eventually possible laboratory contamination, an exogenic NotI hybridization sequence was incorporated. To evaluate the sensitivity of RT-qPCR plasmid in vitro transcribed RNA by using Megashortscript™ T7 transcription kit (Invitrogen-Thermo Fisher) and Turbo DNase was used to remove DNA. RNA transcripts were purified by using MEGAclear™ Purification of transcription reaction kit (Invitrogen-Thermo Fisher Scientific). A Thermo Scientific™ NanoDrop™ was used to determine the RNA concentration and transcripts were diluted from 10^8 to 10^2 copies/ μ L.

RT-qPCR

RT-qPCR was performed with using QuantiFast Probe RT-PCR kit (Ref:204456: QIAGEN), on a QuantStudio™ 3 Real-Time PCR System, software version “xx”. Primers and probe were synthesized by Sigma-Aldrich, Merck KGaA. A volume of 25 μ l reaction was set up containing 12.5 μ l of mix buffer, 0.25 μ L of RT enzyme, 0.5 μ L of Rox, 2 μ L of each Primers (final concentration of primers: 800 nM) and 0.5 μ l of Probe (final concentration probe: 200 nM). The cycling conditions were: 50°C for 10 min; 95°C for 5 min; 45 cycles of 95 °C for 10s and 55°C for 30s. Probe was labeled with Fam Dye and Tamara Quencher.

Analytical Sensitivity

The sensitivity evaluation of the system JMTVs segment 2 assay was performed using the IVT RNA JMTV. Serial dilution of quantified IVT RNAs were prepared containing 28 400 to 10

copy/ μ l. Twelve decreasing concentration were tested using eight replicates for each. Samples with a Ct of >40 were considered as negative. IBM SPSS Statistics software version 24 was used for determined the lower limit of detection (LOD). LOD was defined as a concentration of viral copies, achieving a 95% hit rate (LOD95).

Sample Collection

Ticks were collected between: i) January 2019 to June 2020 from cattle in the Ponte-Leccia slaughterhouse, which is the main active slaughterhouse in Corsica; ii) between March to August in 2019, and May to June in 2020 from horses on farms after they had been used for horse riding in the natural environment across Corsica iii) from August to December, in 2018 and 2019 (hunting season) from wild boars in the northeast of Corsica.

For each animal, all ticks were collected and kept alive until morphological identification and storage. Ticks were identified at species level under a stereomicroscope using an identification key, and immediately stored at -80°C .

Nucleic acid Extraction

Ticks were washed once in 70% ethanol and then twice in distilled water then were grouped individually or using monospecific pools consisting of 2–6 ticks, according to developmental stage and sex, collected from the same animal. Individual or pools of ticks were crushed in minimal essential medium (MEM) containing antibiotics (1% Penicillin-streptomycin, 1% L-glutamine, 1% Kanamycin) and fungicide (5% Amphotericin b), using the TissueLaser II (Qiagen, Hilden, Germany) at 30 cycles for 3 min. Nucleic acid extraction was performed by QIAcube HT (Qiagen) using a QIAamp Cadore Pathogen Minikit, according to the manufacturer's instructions. DNA was eluted in 100 μ l of buffer and stored at -80°C . Extraction was monitored by systematic spiking of each sample with MS2 bacteriophage and subsequent quantitative polymerase chain reaction (qPCR) to assess PCR-inhibitory factors. After extraction quality verification, individual or pooled ticks were tested by designed JMTVs segment 2 RT-qPCR assay

Complete Genome Sequencing

One pool containing 6 males of *R. bursa* ticks positive to JMTVs segment 2 assay (Ct=13) was used for complete genome sequencing using Next Generation Sequencing (NGS). A total of 200 μ L of supernatant was incubated at 37°C for 7h with 25U of Benzonase (Novagen) and MgCl_2 . RNA extraction was performed using the Viral RNA minikit (Qiagen) on the BioRobot

EZ1-XL Advanced (Qiagen). Random two-steps RT-PCR was realised using tagged random primers and ProtoScript® II Reverse Transcriptase kit (New England Biolabs) for reverse transcription and specific primers and Platinum® Taq High Fidelity polymerase enzyme (Thermo Fisher Scientific) for amplification [147]. After quantification using Qubit® dsDNA HS Assay Kit and Qubit 2.0 fluorometer (Thermo Fisher Scientific) amplicons were fragmented (sonication) into fragments of 200bp long. Libraries were built adding barcodes for sample identification, and primers to fragmented DNA using AB Library Builder System (ThermoFisher Scientific). To pool equimolarly the barcoded samples, a quantification step by real time PCR using Ion Library TaqMan™ Quantitation Kit (Thermo Fisher Scientific) was realised. An emulsion PCR of the pools and loading on 520 chip was realised using the automated Ion Chef instrument (ThermoFisher). Sequencing was performed using the S5 Ion torrent technology (Thermo Fisher Scientific) following manufacturer's instructions. Reads were trimmed (reads with quality score <0.99, and length <100pb were removed and the 30 first and 30 last nucleotides were removed from the reads) and De Novo contigs were produced by CLC genomics workbench software v.21 (Qiagen). These contigs were blastn to determine the best reference sequences(s). CLC genomics workbench was used to process reads. Reads longer than 30nts were trimmed using the CLC genomics workbench with 99% quality per base and mapped to references sequences of JMTV on GenBank. Reads mapped to the reference sequence for at least 50% of its length, with a minimum of 80% identity to the reference were used.

Isolation of virus

Two to 3 days old OF1 mice were infected intracerebrally with 2 µl of original homogenate supernatant medium of positive tick pools to JMTV. Mice were observed for signs of disease and sacrificed on day 6 to 10 of the experiment. These animal experiments were performed in accordance with the Animal Experiment Regulations of the Aix-Marseille University under the license A1301309. RNA was extracted from the inoculum and the brain tissues using QIAcube HT with QIAamp 96 Virus QIAcube HT Kit and tested using RT-qPCR described above.

Phylogenetic Analyses

Nucleotide sequence of JMTV of this study was aligned with all the JMTV group virus sequences present in Genbank using the E-INS-I algorithm implemented in MAFFT version 7 [148]. For the four segments, phylogenetic analyses were inferred using the maximum

likelihood estimation method implemented in Mega X [149]. The bootstrap consensus of phylogenetic tree was conducted with 1000 replicates.

Statistical Analysis

RNA of JMTVs segment 2 detected in pools were expressed as the percentage and minimum infection rate (Maximum likelihood estimation (MLE)). MLE calculations are based on the number of pools, pool sizes (number of individuals ticks per pool), and the number of positive pools [150]. Infection rate of JMTV was compared by using Fisher exact test ($p < 0.05$). The analysis was conducted using the R statistical platform (version 3.1.2).

Results

Analytical Sensitivity, Linearity and Signal Intensity of JMTVs Segment 2 Assay.

The limit of detection of JMTVs segment 2 assay was 345,8 copies/ μ L [224.5-1157.3] (Table 2). The results about analytic sensitivity test obtained with JMTVs segment 2 assay evaluate the correlation between Ct values and the six different ITV RNA concentrations tested. The Ct value of the systems was linear, with the correlation coefficient (R^2) close to one (Figure 1). Moreover, a strong signal intensity is provided by the assay, and this even with low copy dilution tested (Figure 1).

Tick Collection and Morphological Identification

In total, 6 269 ticks were collected and 1 715 pools were formed from 763 cattle, 538 horses, 106 sheep and 218 wild boars. Of these, 6 217 (99%) were adult ticks with 1.44 male/female ratio and 52 were nymph (0.8%). Overall, 3 923 ticks were collected from 763 cattle, from which a total of 8 tick species were morphologically identified. The most abundant species was *R. bursa* (n=2 310; 59.9%), followed by *H. marginatum* (n=1 036; 26.4%), *H. scupense* (n=282; 7.1%), *B. annulatus* (n=130; 3.3%), *I. ricinus* (n=75; 1.9%), *R. sanguineus* (n=74; 1.9%), *H. punctate* (n=14; 0.4%) and *D. marginatus* (n=2). 1 682 ticks were collected from 657 horses, the most abundant species was *H. marginatum* (n=1026; 61%), followed by *R. bursa* (n=629; 37.4%) and *R. sanguineus* (n=27;1.6%). A total of 626 ticks were collected from 218 wild boards, the principal species was *D. marginatus* (n=613; 98%), followed by *H. marginatum* (n=12; 1.9%) and *R. bursa* (n=1). 38 ticks were collected from sheep; all the species were identified as *R. bursa* (Table 3).

Detection of Jingmen tick virus

Overall, JMTVs RNA was detected in 21 tick pools collected from three cattle and in one tick pool collected from a sheep with an infection rate (MLE) of 0.35% [0.23%-0.52%] (Table 3). 345 out of the 763 cattle inspected had ticks (infestation rate = 45%), 3 cattle (n=345; 1%) had at least one tick pool positive. One bovine had 2 positive pools to JMTV out of 5 (n=2: 40%), another bovine had 13 positive pools to JMTV out of 27 (n=13; 48%) and the last one had 6 positive pools to JMTV out of 14 (n=6; 43%). The highest prevalence was found in *Rhipicephalus* genus with a MLE of 0.58% [0.35%-0.9%] in *R. bursa* and 0.55% [0.03%-2.41%] in *R. sanguineus* followed by *H. marginatum* 0.19% [0.06%-0.45%]. The MLE of tick positive to JMTV RNA collected in cattle was 0.54% [0.34%-0.81%], more specifically of 0.70% [0.41%-1.11%] for *R. bursa*, 1.16% [0.07%-5%] for *R. sanguineus* and 0.39% [0.12%-0.9%] for *H. marginatum*. The calculation of prevalence by the MLE method showed a significant difference between the prevalence of males and female ticks positive for JMTVs virus. The prevalence of male tick pools (0.79% [0.49%-1.12%]) is statistically higher than that of female tick pools (0.14% [0.02%-0.43%]) (Table 4).

Phylogenetic analysis

The JMTV sequences from *R. bursa* tick collected in Corsica had 94.7-95.1% of nucleotide identity with JMTV collected in Kosovo (Genbank num MH133321), 94.4-94.8% identity with JMTV from Romania (Genbank num:MW561147) and 91-94.8% with JMTV isolate from Turkey (Genbank num:MN486262).

In the phylogenetic trees based on four complete segments (Figure 2-5), the Corsican JMTV forms a monophyletic lineage with JMTVs from Turkey, Romania, Kosovo and Caribbean which was separate from Brazilian, Ugandan and Chinese JMTV isolates clade.

Isolation attempts of virus

After inoculation of the virus into 2-3 days old OF1 mice with original supernatant of positive tick pools to JMTV, no clinical sign was observed, and no virus was detectable by JMTV segment 2 RT-qPCR assay in the new-borne mice brains, six to ten days after inoculation.

Discussion

The JMTVs segment 2 assay have been developed for the detection and quantification of Jingmen tick virus group including Jingmen tick virus, Alongshan virus and Yanggou tick virus. To the best of our knowledge, this is the first RT-qPCR assay specific for these viruses. Segment 2 of JMTVs was chosen for the system design due to its' high homology between target species

and no homology with other viruses or living beings [64]. The Ct value of the systems was linear, with the correlation coefficient (R^2) close to one the assay is therefore particularly effective for the quantification of JMTVs RNA. Moreover, a strong signal intensity is provided even with low copy dilutions by the assay, which is important to discriminate negative and positive samples without ambiguity.

Since there is small number of studies with JMTV many questions remain unanswered about the natural life cycle of the virus. Several factors tend to group them into arboviruses, detection of JMTVs in both engorged and not engorged ticks and not engorged tick larvae can be an evidence of trans-ovarian transmission of JMTVs in ticks. Additionally, replication and migration of JMTV in the salivary glands of ticks and a viremia/tick bite association in humans were showed in previous studies [65-70, 140-142, 151]. The association of two viruses of the JMTVs group with febrile fever in several patients have been showed in China and Mongolia [68, 69]. In addition, the detection of JMTVs RNA has been reported in a number of mammals including humans, monkeys, rodents, bats and cattle. However, there is no association was identified between JMTVs strains and hosts [67, 152].

In this study, we developed a JMTVs segment 2 RT-qPCR assay and used the assay to screen JMTV group in ticks collected in Corsica. Eight species of tick were collected and tested, namely, *R. bursa*, *H. marginatum*, *H. scupens*, *I. ricinus*, *D. marginatus*, *B. anulatus*, *R. sanguineus*, and *H. punctata*. The highest prevalence of JMTV was found in *Rhipicephalus* genus with an MLE of 0.58% [0.35%-0.9%] in *R. bursa* and 0.55% [0.03%-2.41%] in *R. sanguineus* compared with *H. marginatum* 0.19% [0.06%-0.45%]. *R. bursa* and *H. marginatum* were already found positive to JMTV in Turkey and Romania unfortunately the number of ticks and the methodology used in these studies, does not allow doing comparison [142, 153]. Moreover, there are gaps in the knowledge of the prevalence of the JMTV group, some studies were reported a prevalence of over 40%, but these results must be taken with great caution as low number of ticks tested or ticks pooled in large numbers [64, 65, 70]. In our study, 21 pools were found positive from three cattle (Table 5), which means that multiple positive pools were found on the same animal, that can show a potential phenome of co-feeding for these viruses which already was questioned in another study [70]. This may explain the high prevalence rates and the number of tick species found positive reported in some studies when the numbers of ticks tested are too low or collected from few hosts.

We have sequenced and characterized the complete genome of the JMTV present in Corsica from one positive pool of *R. bursa* which identified positive by RT-qPCR JMTVs assay. The

phylogenetic analysis based on four complete segments (Figure 2-5), show that Corsican JMTV forms a monophyletic lineage with JMTVs from Turkey, Romania, Kosovo and Caribbean which is separate from Brazilian, Ugandan and Chinese JMTV clade. Several studies showed that the different strains of JMTV form clades according to their geography, which is reflected in our phylogenetic tree for both four segments [67, 68, 152]. Indeed, the Corsican strain of JMTV forms a clade with the Kosovo, Romanian, Turkish and Caribbean strains. The presence of the Caribbean strains in the Mediterranean JMTV clades can be explained by a massive importation of Senegalese origin cattle and consequently of their main tick species *Amblyomma variegatum*, in the 18th and 19th centuries [154]. It became invasive and the main tick species in all the Caribbean islands within a few years. Infected cattle or ticks of West African origin are probably the source of the Caribbean strains and would explain their strong identity with the Mediterranean strains of JMTV. Moreover, the *Amblyomma variegatum* is known to travel from one continent to another carried by migratory birds [12, 155] which's sporadic detection into Corsica have been previously showed [12].

Our study holds some limitation; Ticks were mostly analysed in pooled samples and not individually. Working with pools can lead to underestimation of the infection rate, which must be interpreted with caution. However, the diversity and number of hosts studied is significant, as is the number of ticks identified and analysed for JMTV.

To our knowledge this is the first evidence of the circulation of the JMTV virus in Corsica and widely in West Mediterranean region and the first one RT-qPCR system validated for the detection of the JMTVs. These results show that Mediterranean islands such as Corsica can play a major role in understanding and the preparedness for future epidemics.

Figure

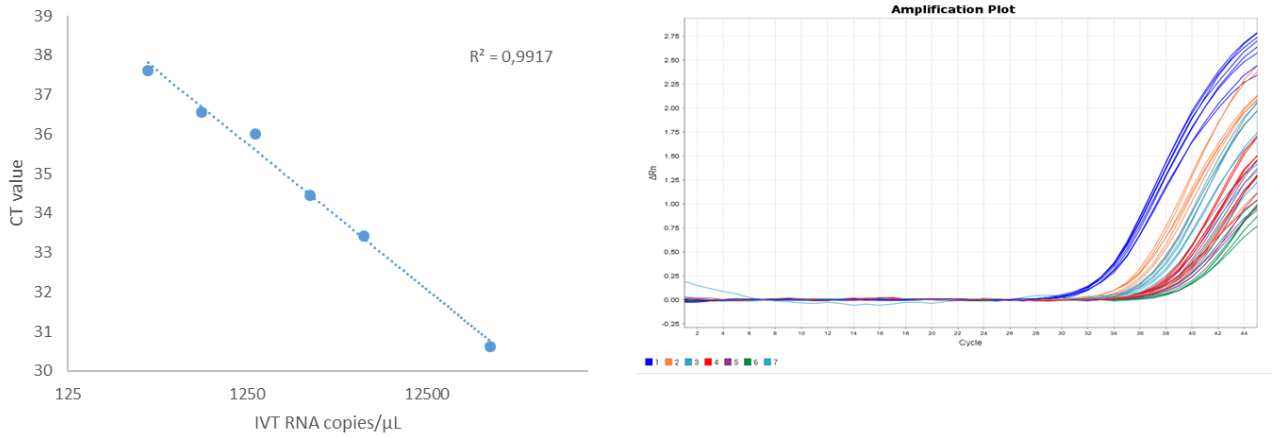


Figure 1: Linearity and signal intensity of JMTV segment 2 assay

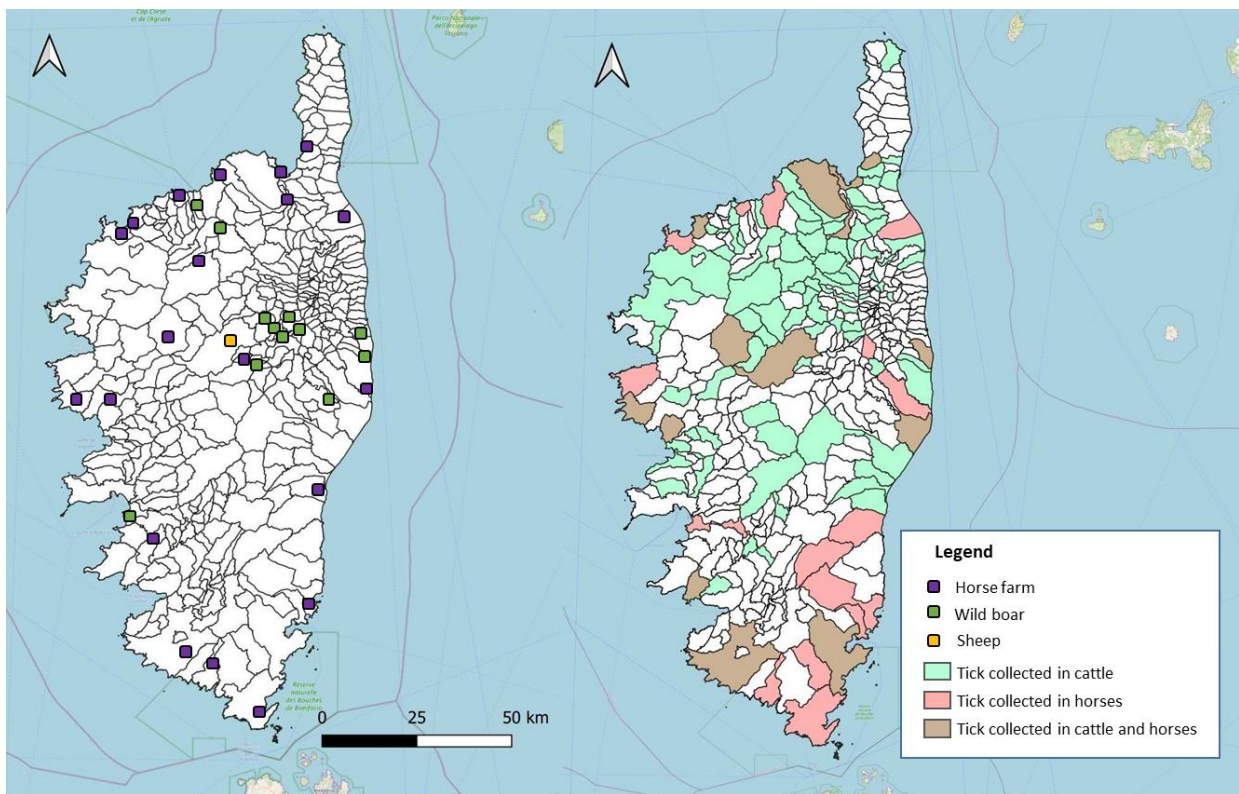


Figure 2: Map of Corsica indicating the tick collection sites by hosts.

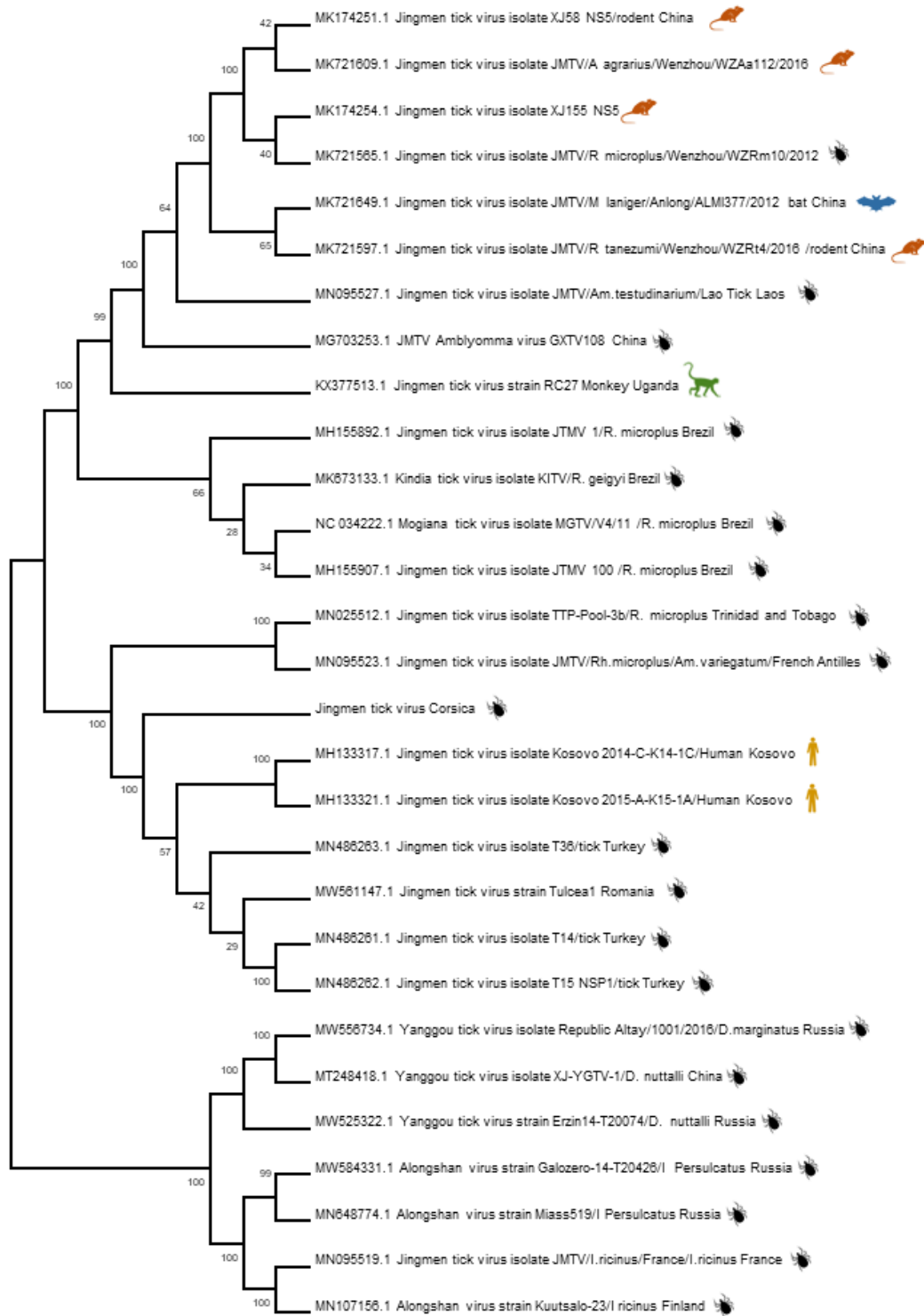


Figure 3: The maximum likelihood analysis of the JMTV complete segment 1. The tree is constructed using the general time reversible (GTR) model, gamma distributed (G) for 1000 replications.

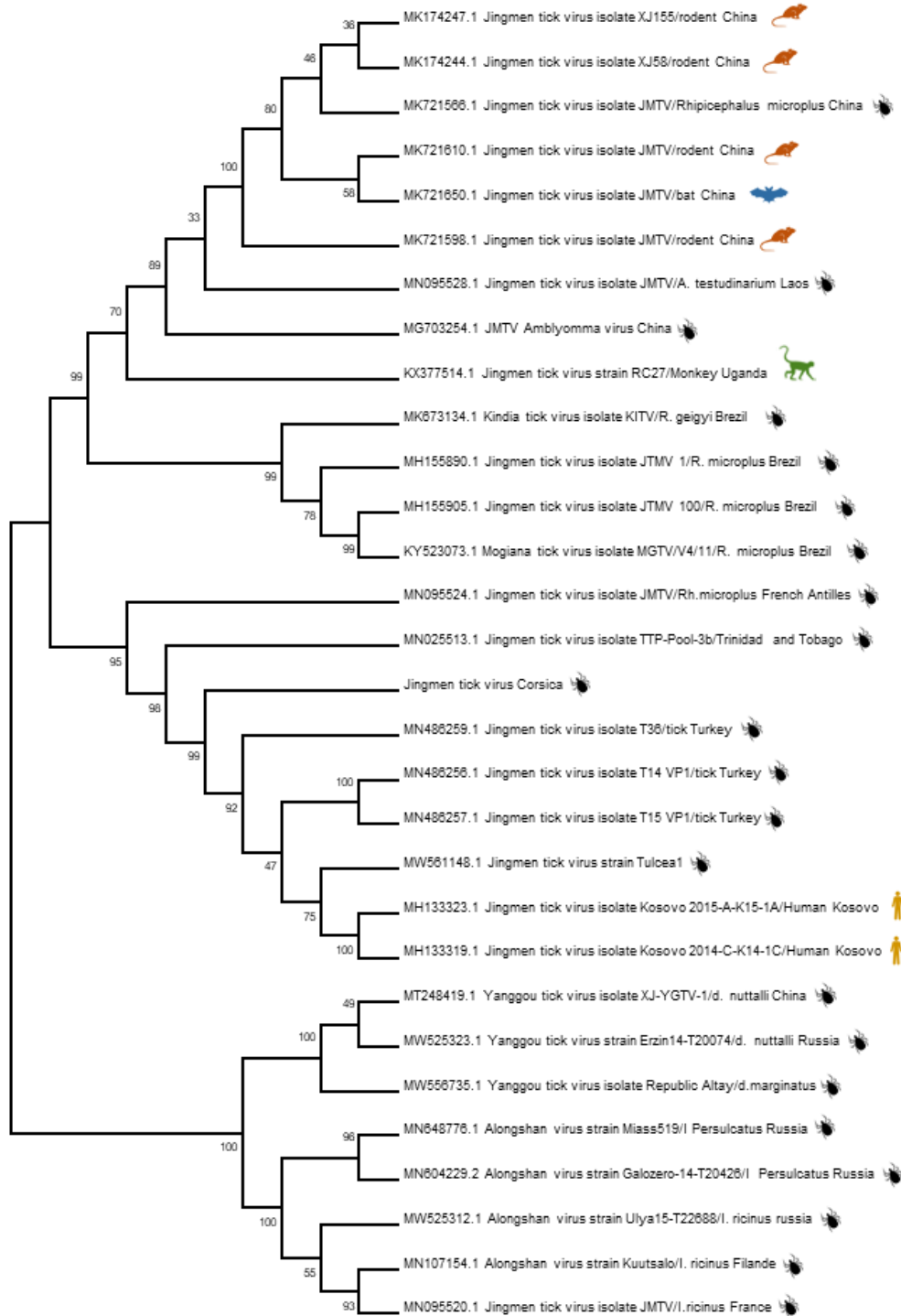


Figure 4: The maximum likelihood analysis of the JMTV complete segment 2. The tree is constructed using the general time reversible (GTR) model, gamma distributed (G) for 1000 replications.

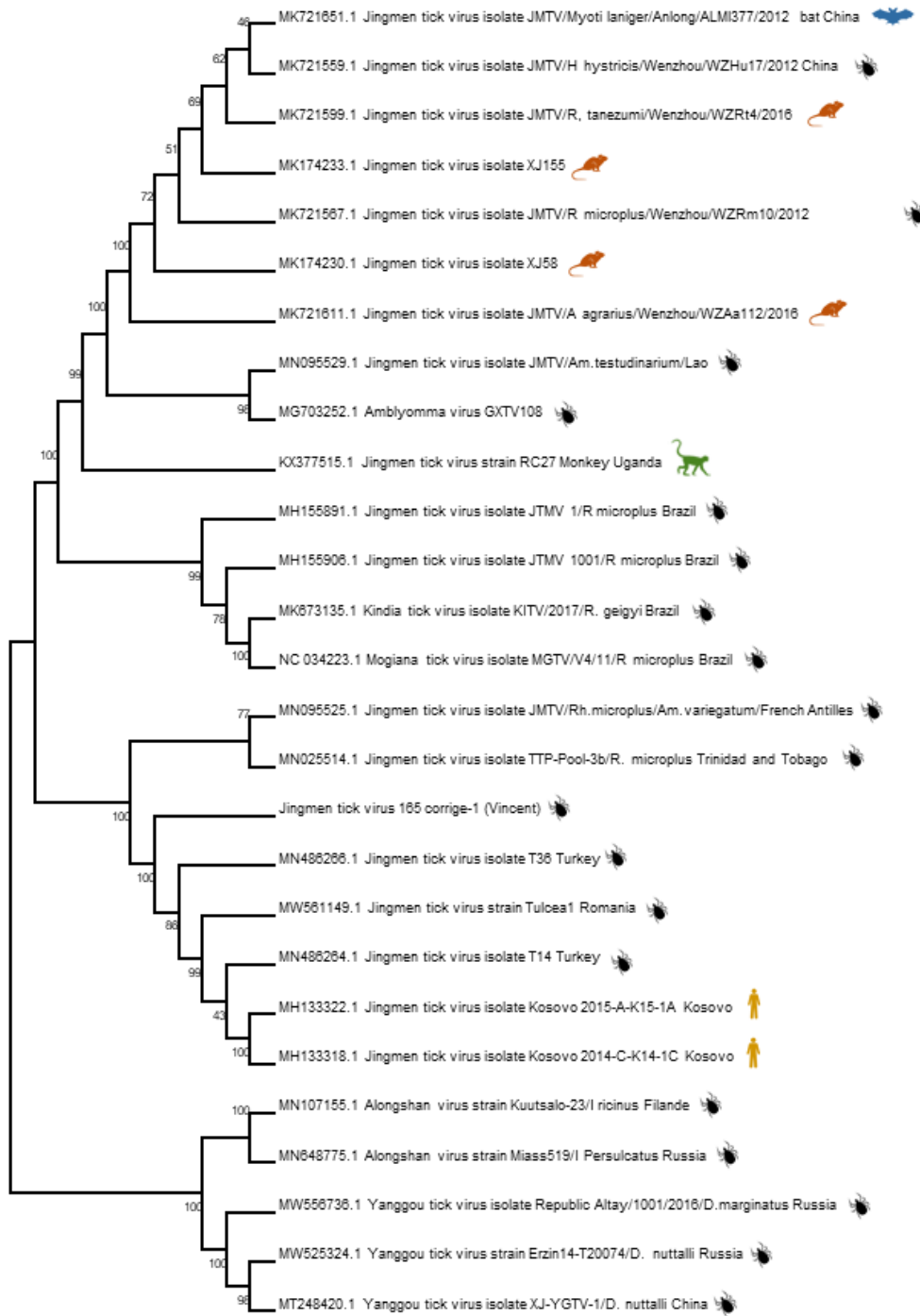


Figure 5: The maximum likelihood analysis of the JMTV complete segment 3. The tree is constructed using the general time reversible (GTR) model, gamma distributed (G) for 1000 replications.

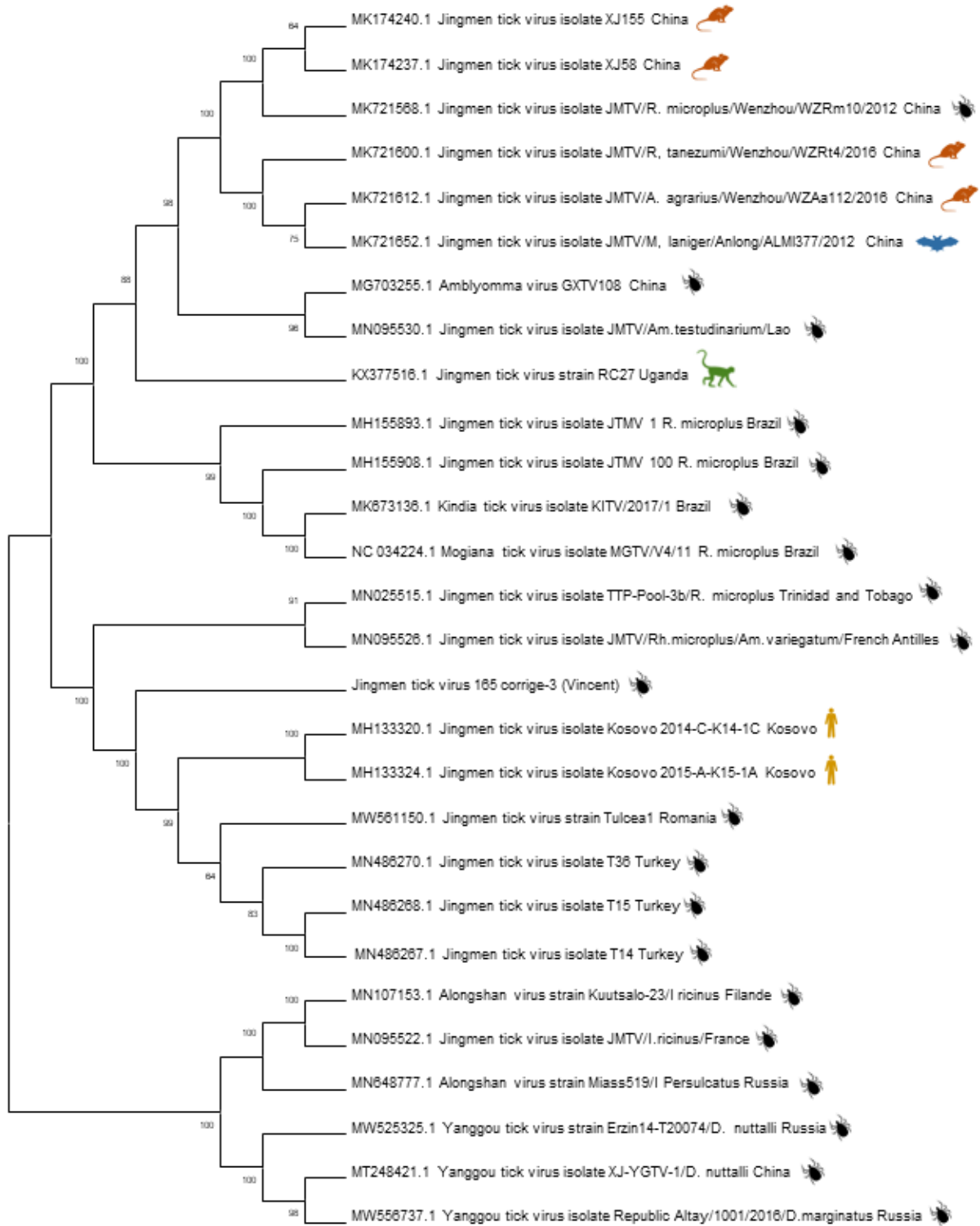


Figure 6: The maximum likelihood analysis of the JMTV complete segment 4. The tree is constructed using the general time reversible (GTR) model, gamma distributed (G) for 1000 replications.

Table 1: Primers and probe of JMTVs segment2 assay.

Primer/Probe	5'-> 3' Sequence	Amplicon Size	Concentration
JMTV segment 2 Foward	TCACAGGAGAYDTYTACMTCAYC	76 pb	400 nM
JMTV segment 2 Reverse	AGC GCC GCI TCC GCC CTA G		400 nM
JMTV segment 2 probe	FAM-TTCAGCGCCATCRCIGCTSTGGG-Tamara		200 nM

Table 2: Analytical sensitivity of JMTVs segment 2 Assay.

IVT RNA copies/ μ L	Total samples tested	Positive samples	% Detected	CT, mean (SD)
2800	8	8	100	34,5 (0.3)
1400	8	8	100	36 (0.5)
700	8	8	100	36,5 (0.4)
350	8	8	100	37,6 (0.6)
175	8	4	50	39,1 (0.9)
87	8	0	0	/
10	8	0	0	/
1	8	0	0	/

Table 4: Tick pools positive for JMTV by sexe.

Number of Pools with <i>n</i> Ticks				Number of positive pools detected by Real- Time JMTV PCR			
Cattle 2019-2020.							
Number of Individual Ticks or Ticks per Pool (<i>n</i>)	<i>Female</i>	<i>male</i>	Total	Pool	Female	Male	Total
1	151	169	320	1	0	1	1
2	73	74	147	2	0	0	0
3	51	61	112	3	1	0	1
4	30	70	100	4	0	1	1
5	25	44	69	5	0	1	1
6	121	242	363	6	1	16	17
Total	451	660	1111	MLE (95% CI)	0.14 (0.02-0.43)	0.79 (0.49-1.12)	0.55 (0.35-0.82)

Table 5: Tick species pools positive for Jingmen tick virus DNA

Host	Host	ID pool	Species	Nbr of tick	sex	commune	Date	JMTV CT	Copies/ μ l
3339		163BOV19	<i>H marginatum</i>	2	M	VENTISERI	22/05/2019	/	/
		164BOV19	<i>R bursa</i>	6	M			CT16.32	2.3 \times 10 ⁸
		165BOV19	<i>R bursa</i>	6	M			CT 30.68	29 808
		166BOV19	<i>R bursa</i>	6	F			/	/
		167BOV19	<i>R bursa</i>	4	F			/	/
2005215529	cattle	526BOV20	<i>R bursa</i>	4	F	GALERIA	26/05/2020	/	/
		527BOV20	<i>R bursa</i>	2	F			/	/
		528BOV20	<i>R bursa</i>	6	F			/	/
		529BOV20	<i>R bursa</i>	6	M			CT 18.1	7.6 \times 10 ⁷
		530BOV20	<i>R bursa</i>	6	F			/	/
		531BOV20	<i>R bursa</i>	6	F			/	/
		532BOV20	<i>R bursa</i>	6	F			/	/
		533BOV20	<i>R bursa</i>	6	F			/	/
		534BOV20	<i>R bursa</i>	6	F			/	/
		535BOV20	<i>R bursa</i>	6	F			/	/
		536BOV20	<i>R bursa</i>	6	F			/	/
		537BOV20	<i>R bursa</i>	6	M			CT 14.76	6.1 \times 10 ⁸
		538BOV20	<i>R bursa</i>	6	M			CT 14.88	5.6 \times 10 ⁸
		539BOV20	<i>R bursa</i>	6	M			CT 19.56	3 \times 10 ⁷
		540BOV20	<i>R bursa</i>	6	F			CT 21.55	8.8 \times 10 ⁶
		541BOV20	<i>R bursa</i>	6	M			CT 21.23	1 \times 10 ⁷
		542BOV20	<i>R bursa</i>	6	M			CT 20.21	2 \times 10 ⁷
		543BOV20	<i>R bursa</i>	4	F			/	/
		544BOV20	<i>R bursa</i>	6	M			CT 22.36	5.3 \times 10 ⁶
		545BOV20	<i>R sanguineus</i>	1	M			CT 20.96	1.2 \times 10 ⁷
		546BOV20	<i>H marginatum</i>	6	M			CT 22.66	4.4 \times 10 ⁶
		547BOV20	<i>H marginatum</i>	6	M			CT 23.35	2.8 \times 10 ⁶
		548BOV20	<i>H marginatum</i>	6	M			/	/
		549BOV20	<i>H marginatum</i>	6	M			/	/
		550BOV20	<i>H marginatum</i>	5	M			CT 21.45	9.4 \times 10 ⁶
551BOV20	<i>H marginatum</i>	4	F	/	/				
552BOV20	<i>H marginatum</i>	3	F	CT 31.78	15 008				
2030850530		579BOV20	<i>I ricinus</i>	1	F	NA	26/05/2020	/	/
		580BOV20	<i>R bursa</i>	6	M			CT 34.51	2 733
		581BOV20	<i>R bursa</i>	6	M			CT 30.15	41 489
		582BOV20	<i>R bursa</i>	6	M			CT 32.46	9 819
		583BOV20	<i>R bursa</i>	6	M			CT 33.29	5 850
		584BOV20	<i>R bursa</i>	6	M			CT 31.18	21 821
		585BOV20	<i>R bursa</i>	6	F			/	/
		586BOV20	<i>D marginatus</i>	1	F			/	/
		587BOV20	<i>R bursa</i>	6	F			/	/
		588BOV20	<i>R bursa</i>	6	F			/	/
		589BOV20	<i>R bursa</i>	4	F			/	/
		590BOV20	<i>R bursa</i>	4	M			CT 30.96	25 031
		591BOV20	<i>H marginatum</i>	6	M			/	/
		592BOV20	<i>H marginatum</i>	3	F			/	/
		Sheep2020/1	Sheep	150SHE20	<i>R bursa</i>			2	f

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4. Nouveau Phébovirus en Corse: Detection of New tick *Phlebovirus* of M deficient segment group in Corsica, France.

Contexte

Parmi les arbovirus qui ont émergé au cours des dernières décennies, les *Phlebovirus* sont parmi les plus importants. Ces virus appartenant à la famille des *Phenuiviridae* de l'ordre des Bunyavirales sont reconnus comme des virus médicalement importants en raison des pathologies fébriles chez l'homme. Les *Phlebovirus* sont transmis par des arthropodes vecteurs tels que les moustiques, les phlébotomes et les tiques. C'est pour ces raisons que l'Unité des Virus Emergents a mis en place une surveillance des *Phlebovirus* à travers la méditerranée. Cette surveillance a permis la caractérisation de nombreux *Phlebovirus* chez les phlébotomes dans de nombreuses zones du bassin méditerranéen [156] et de conduire des études sérologiques en collaboration avec l'UR-bioscope pour quantifier la circulation *Phlebovirus* transmis par ces vecteurs en Corse [116, 157]. Récemment, de nombreux *Phlebovirus* pathogènes transmis par les tiques ont émergé et continuent d'émerger à travers le monde. Ces événements épidémiques et l'expertise dans le domaine de l'UVE et l'UR Bioscope, nous ont poussé à prioriser nos recherches sur ces virus. Le but de cette étude était d'investiguer la présence de *Phlébovirus* transmis par les tiques connus et inconnus à l'aide d'un système Pan-Phlébovirus.

Méthode

Les tiques ont été collectées sur des brebis, des bovins, des sangliers et des chevaux de Mai 2018 à Juin 2020 (Figure 12) (Tableau 1). Du mois d'août à décembre 2018 et 2019 sur des sangliers dans le nord-est de la Corse ; et entre mars et août 2019 sur des chevaux après des promenades à cheval dans le milieu naturel à travers la Corse (figure 13 et 14). Les tiques collectées ont été testés à l'aide des amorces Pan-Tick *Phlebovirus* publié par Keti Matsuno en 2014 pour la détection du gène de la RdRp des Tick-Borne *Phlebovirus* (Tableau 2). Un séquençage NGS a été utilisé pour le séquençage complet du génome du virus présent dans les échantillons de tiques. Un système RT-qPCR a été créé et validé pour la détection d'un nouveau *Phlébovirus* à partir des séquences obtenues. Plusieurs tentatives d'isollements ont été réalisées, notamment sur lignée cellulaire DH-82 et Véro E6.

Résultats

Au total, L'ARN de *Phlébovirus* a été retrouvé dans 112 pools sur 1 531. Tous les pools de tiques positifs étaient des *H. marginatum* avec une prévalence de cette espèce MLE de 6,81 (IC 95 % : 5,66 %-8,1 %). Les caractéristiques génétiques de ce virus ont montré qu'il appartient au groupe des M-déficient *Phlebovirus*, un nouveau groupe de *Phlébovirus* trouvé chez les tiques appartenant au genre *Uukuvirus* et dont le segment M n'a jamais été séquencé. La prévalence de ce nouveau virus provisoirement appelé le Kallisto tick virus, n'avait aucune différence statistiquement significative entre le sexe de la tique ou de l'hôte et une large distribution géographique. L'isolement du virus de manière stable n'a pour l'instant pas été réalisé (figure 15).

Conclusion

Cette étude a permis la découverte et la caractérisation d'un nouveau *Phlébovirus* circulant en Corse avec une spécificité à l'espèce de tiques *H. marginatum*. Ces résultats ont montré encore une fois la diversité des pathogènes retrouvés dans les tiques collectées sur l'île. Cette diversité a permis la caractérisation d'un nouveau virus et a donc montré l'intérêt d'étudier les pathogènes transmis par les tiques en Corse.

Les résultats recueillis lors de la thèse sont présentés dans ce manuscrit sous format article scientifique.

Detection of New tick *Phlebovirus* of M deficient segment group in Corsica, France.

Introduction

Among the arboviruses that have emerged in recent decades, *Phlebovirus* are one of the most important [158]. These viruses belonging to the family *Phenuiviridae* of the order *Bunyavirales* are include medically important viruses associated with febrile and neurological illnesses [158, 159]. *Phlebovirus* genomes are single-stranded RNA with negative polarity, organized into three segments: the large (L) segment encoding RNA-dependent RNA polymerase (RdRp), the medium (M) segment encoding envelope glycoproteins (GPs; Gn and Gc), and the small (S) segment encoding viral nucleocapsid protein (N). Some bunyaviruses encode non-structural proteins (NS) in their M (NSm) and/or S (NSs) segments. Most *Phleboviruses* can be transmitted to their host by an arthropod vector such as mosquitoes, sand flies and ticks [127]. Recently, a number of tick-borne pathogen *Phleboviruses* have emerged and continue to emerge across the globe. These include, Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) with more than 5 000 human cases in China between 2010 and 2026, and more than 400 cases reported in Japan since 2010 [84, 160] and the Heartland virus, discover in United-State in 2009, with more than 30 human cases including deaths [75, 161].

Tick-borne Phleboviruses (TBPV) are divided into four phylogenetically related groups: Uukuniemi group, Dabie Bandavirus (Sever fever with thrombocytopenia syndrome virus) /Heartland virus group, Bhanja group and the Kaisodi group. However, recently the Dabie Bandavirus (SFTSV)/Heartland virus group is taxonomically reclassified in the genus *Banyangvirus* [162]. Recent studies on newly identified tick-borne *Phlebovirus* have shown a potential absence of the M segment, in a group of TBPV whose L and S segment sequences show relatively high similarity [163-170]. These viruses are called as M segment-deficient *Phlebovirus* (MdPV) [163].

In Corsica, a French Mediterranean island, ticks of the genus *Ixodes*, *Hyalomma*, *Dermacentor*, *Haemaphysalis*, *Amblyomma* and *Rhipicephalus* have been identified and can act as vectors for a variety of emerging diseases [10-12, 120, 145, 171-173]. Wild rangelands represent a type of farming particular to Corsica, inevitably involving numerous exchanges and interactions with wild animals, domestic animals and human populations. In addition, cultural practices in Corsica are characteristic of the territory and derive from the rural and autonomous heritage of

the island's ancient peoples. These practices, such as the hunting of feathered or large game, followed by the processing of the meat, such as skinning and evisceration, generally without protection, increase the contact between humans and animals and the environment. The island therefore has all the characteristics of a sentinel region for emerging diseases. Conditions allow for a diversity of vectors and their pathogens, the presence of numerous exchanges between wild and domestic animals and an important interface between humans and animals due to human activities [116].

The aim of this study was to identify and characterize the tick-borne *Phleboviruses* circulating in Corsica to provide new information on their circulation and characteristic. For this purpose, we tested ticks collected in Corsica from different hosts with *Pan-Phlebovirus* RT-PCR system. We detected and sequenced one potential new tick-borne *Phlebovirus* of the M-deficient group *Phlebovirus*. Here we present the discovery and genetic characterization of a novel *Phlebovirus* grouped together with Uukuniemi viruses in Corsica.

Material and methods

Study Area and Ticks collection

Ticks were collected between: i) January 2019 to May 2020 from cattle in the Ponte-Leccia slaughterhouse, the main active slaughterhouse in North-West Corsica. The national cattle identification system, which uses ear tags, allowed the origin of the animals to be tracked and the farm owners to be identified. ii) between March to August in 2019, and May to June in 2020 from horses on farms after they had been used for horse riding in the natural environment across Corsica iii) from August to December, in 2018 and 2019 (hunting season) from wild boars in the northeast of Corsica.

All ticks were collected and kept alive until morphological identification and storage. Ticks were identified at species level under a stereomicroscope using an identification key, and immediately stored at -80°C for further analysis.

Nucleic acid Extraction

Ticks were washed once in 70% ethanol and then twice in distilled water then were grouped individually or using monospecific pools consisting of 2–6 ticks, according to developmental stage and sex, collected from the same animal. Individual or pools of ticks were crushed in

minimal essential medium (MEM) containing antibiotics (1% Penicillin-streptomycin, 1% L-glutamine, 1% Kanamycin) and fungicide (5% Amphotericin B), using the TissueLaser II (Qiagen, Hilden, Germany) at 30 cycles for 3 min. DNA extraction was performed using QIAcube HT (Qiagen) and a QIAamp cadof Pathogen Mini Kit according to the manufacturer's instructions. DNA was eluted in 150 μ L of buffer and stored at -20°C . For each polymerase chain reaction (PCR) reaction, the template DNA was at a final amount of <200 ng.

PCR Detection of Phlebovirus

The Pan-Tick borne *Phlebovirus* RT-PCR assay were performed using the primers and probes previously described for the amplification of 514pb of RNA-dependent RNA polymerases gene of L *Phlebovirus* segment [127]. The reactions were carried out using an Applied Biosystems GeneAmp PCR System 9700 (Applied Biosystems, Courtaboeuf, France). The PCR products were evaluated by electrophoresis in a 2% agarose gels in tris-acetate-ethylenediaminetetraacetic acid (TAE buffer) and were visualized under ultraviolet light after staining with ethidium bromide. A 100-bp DNA ladder (RabBiotechrabbit) was used as a standard marker.

Genome Sequencing

Three Tick borne *Phlebovirus* positive pools were used for complete genome sequencing using Next Generation Sequencing (NGS). A total of 200 μ L of supernatant was incubated at 37°C for 7h with 25U of Benzonase (Novagen) and MgCl_2 . RNA extraction was performed using the Viral RNA minikit (Qiagen) on the BioRobot EZ1-XL Advanced (Qiagen). Random two-steps RT-PCR was realised using tagged random primers and ProtoScript[®] II Reverse Transcriptase kit (New England Biolabs) for reverse transcription and specific primers and Platinum[®] Taq High Fidelity polymerase enzyme (Thermo Fisher Scientific) for amplification [147]. After quantification using Qubit[®] dsDNA HS Assay Kit and Qubit 2.0 fluorometer (Thermo Fisher Scientific) amplicons were fragmented (sonication) into fragments of 200bp long. Libraries were built adding barcodes for sample identification, and primers to fragmented DNA using AB Library Builder System (ThermoFisher Scientific). To pool equimolarly the barcoded samples, a quantification step by real time PCR using Ion Library TaqMan[™] Quantitation Kit (Thermo Fisher Scientific) was realised. An emulsion PCR of the pools and loading on 520 chip was realised using the automated Ion Chef instrument (ThermoFisher). Sequencing was performed using the S5 Ion torrent technology (Thermo

Fisher Scientific) following manufacturer's instructions. Reads were trimmed (reads with quality score <0.99, and length <100pb were removed and the 30 first and 30 last nucleotides were removed from the reads) and De Novo contigs were produced by CLC genomics workbench software v.21 (Qiagen). These contigs were blastn to determine the best reference sequences(s). CLC genomics workbench was used to process reads. Reads longer than 30nts were trimmed using the CLC genomics workbench with 99% quality per base and mapped to reference sequences of JMTV on GenBank. Reads mapped to the reference sequence for at least 50% of its length, with a minimum of 80% identity to the reference were used.

RT-qPCR and primer design

All sequences of positive samples corresponding to New-tick borne *Phlebovirus* and closed *Phlebovirus* from the taxonomy browser of the National Center of Biotechnology Information (NCBI) were used for the alignment on the clustal program (MEGA-X software) [149]. Primers and probe were designed to target a highly conserved region of Lsegment of the *new tick-borne Phlebovirus* (Table 1). RT-qPCR was performed with using QuantiFast Probe RT-PCR kit (Ref:204456: QIAGEN), on a QuantStudio™ 3 Real-Time PCR System, software version "xx". Primers and probe were synthesized by Sigma-Aldrich, Merck KGaA. A volume of 25µl reaction was set up containing 12.5 µl of mix buffer, 0.25 µL of Reverse Transcriptase enzyme, 0.5 µL of Rox, 2µL of each primers (finale concentration of primers: 800 nM) and 0.5µl of probe (finale concentration probe: 200 nM). The cycling conditions were: 50°C for 10 min; 95°C for 5 min; 45 cycles of 95 °C for 10s and 55°C for 30s.

Isolation attempts of the virus

In vitro assays

A volume of 50 µl of homogenized tick material was inoculated into 24 well plate culture tubes (Thermofisher) with 450 µl of Vero cells and DH-82 in enriched MEM: 1 % Penicillin-Streptomycin, 1% [200 mM] l- glutamine, 1% Kanamycin and 3% Fungizone (Amphotericin B) without foetal bovine serum (FBS). The cell culture tubes were incubated at room temperature and after 1 h, 2.5 ml fresh EMEM with 5% FBS was added and cultures were incubated at 37°C in a 5% CO2 atmosphere. The cells were examined daily for the presence of cytopathic effect (CPE) and passaged successively five times. After each passage, 200µl supernatant medium was collected and tested using the specific RT-qPCR described above.

In vivo assays

Two to 3 days old OF1 mice were infected intracerebrally with 2 µl of original homogenate supernatant medium of new tick-Phlebovirus Kallisto Tick virus (KATV). Mice were observed for signs of disease and sacrificed on day 6 to 10 of the experiment. These animal experiments were performed in accordance with the Animal Experiment Regulations of the Aix-Marseille University under the license A1301309. RNA was extracted from the inoculum and the brain tissues using QIAcube HT with QIAamp 96 Virus QIAcube HT Kit and tested using the Pan-generic RT-PCR protocols and RT-qPCR described above.

Phylogenetic analysis

Nucleotide sequence of *Phlebovirus* of this study was aligned with *Phlebovirus* sequences present in Genbank using the L-INS-I algorithm implemented in MAFFT version 7 [148]. For the L and S segments, phylogenetic analyses were inferred using the maximum likelihood estimation method implemented in Mega X [149]. The bootstrap consensus of phylogenetic tree was conducted with 1000 replicates. A matrices of genetic diversity of interest *Phlebovirus* was made with Mega X.

Statistical Analysis

Phlebovirus RNA positive pools were expressed as the percentage and minimum infection rate (Maximum likelihood estimation (MLE)). MLE calculations are based on the number of pools, pool sizes (number of individuals ticks per pool), and the number of positive pools [150]. Infection rate of *Phlebovirus* was compared by using Fisher exact test ($p < 0.05$). The analysis was conducted using the R statistical platform (version 3.1.2).

Results

Tick Collection and Morphological Identification

In total, 5 439 ticks and 1 531 pool were formed from 734 cattle, 657 horses, 106 sheep and 218 wild boars.

From cattle, 3 093 ticks were collected and 8 species were identified morphologically. The most abundant species was *R. bursa* (n=1 700; 55%), followed by *H. marginatum* (n=838; 27%), *H. scupense* (n=282;9%), *B. annulatus* (n=130; 4%), *I. ricinus* (n=74; 2%), *R. sanguineus* (n=54; 1.7%), *H. punctate* (n=14) and *D. marginatus* (n=1).

From Horses, 1 682 ticks were collected, the most abundant species was *H. marginatum* (n=1026; 61%), followed by *R. bursa* (n=629; 37.4%) and *R. sanguineus* (n=27;1.6%).

From Wild boards, 626 ticks were collected, the principal tick species was *D. marginatus* (n=613; 98%), followed by *H. marginatum* (n=12; 1.9%) and *R. bursa* (n=1). From sheep, 38 ticks were collected; all the tick species were identified as *R. bursa* (table 2).

Detection of Phlebovirus

Overall, *Phlebovirus* RNA was detected in 112 pools of 1531. All *Phlebovirus* positive tick pools were containing *H. marginatum*. In total, 67 tick pools positives were from cattle (n=947), 44 from horses (n= 390) and, one from wild boards (n=162). The infection rate (MLE) of *H. marginatum* ticks collected in cattle was 9.34 (95% CI: 7.35-11.64%), 8.55 (95% CI: 5.59-12.31%) in 2019 and 9.93 (95% CI: 7.28-12.85%) in 2020 and 9.31 (95% CI: 7.05-11.96) for males *H. marginatum* and 9.45 (95% CI: 5.65-14.49) for females. For Horses, the infection rate (MLE) was 4.81 (95% CI: 3.54-6.33%), 4.12 (95% CI: 2.75-5.86%) in 2019, 5.11 (95% CI: 3.13-7.75%) in 2020 and 5.31 (95% CI: 3.51-7.35) for *H. marginatum* males and 4.29 (95% CI: 2.58-6.59) for female (Table 2) (Figure 1). For wild boards, one pool of *H. marginatum* was found positive with the infection rate of 2.38 (95% CI: 0.1-10). There are no statistically significant differences between the prevalence of hosts, years or sex of the ticks (Table 3).

Isolation of virus

In vitro assays

Twenty-eight available tick homogenates were used for Vero cell and Dh-82 cell inoculations. However, no cytopathic effects were observed during five consecutive blind passages. Supernatants lost one to two Ct by RT-qPCR screening between passages, until all was negative at the 5 passage screening assays (Table 4).

In vivo assays

Available aliquots from two tick pools were used for intracerebral inoculation of suckling mice. During the observation period of 0–14 days, the mice did not exhibit any symptoms. They were sacrificed on day 14 and brain specimens were obtained aseptically. No gross pathology was visible during necropsy. Aseptically-obtained brain homogenates were negative in screening RT-qPCRs.

Phylogenetic analysis

The *Phlebovirus* sequences of complete L and S segments of three positive pools from collected ticks in Corsica show 99% to 100 % of nucleotide identity each other with almost 100 % of amino acid due to mutation on third nucleotide between two populations of the same virus. The new tick *Phlebovirus* designed as “Kallisto tick virus” segment L shows 89% nucleotide and 98% amino acid identity with the Balambala Tick virus (GenBank Accession num: MW561966) sequences from *H. truncatum* in Kenya and Limansky tick *Phlebovirus* (GenBank Accession num: MN542375) sequenced from *H. anatolicum* of Astrakhan region of Russia (Figure 2). The segment S shown 94% of nucleotide identity and 95% of amino acid identity with Balambala Tick virus (GenBank Accession num: MW561971) (Figure 3). The KATV is belonging to the group of M-deficient segment tick *Phleboviruses* of the genus Uukuvirus. The closer virus present on the International Committee on Taxonomy of Virus (ICTV) and belonging to the same cluster are Lihan Tick virus with 50% of amino acid identity (GenBank accession num: NC055423), 55 % of amino acid identity for Pacific Coast Tick virus (GenBank accession num: KU933936) and the Tacheng Tick virus 2 (GenBank accession num: MN567189) with 50% of identity amino acid identity. Despite our efforts, we could not assemble any genomic segment sequence that corresponded to the M segment. According to the latest ICTV criteria for species delineation suggest that Balambala tick virus, Limansky tick virus and the new Kallisto tick virus are the same virus (Figure S1) [174].

Discussion

In this study, we characterized a new *Phlebovirus* from *H. marginatum* ticks in Corsica. The genetic characteristics of this virus show that it belongs to the M-deficient *Phlebovirus* group (MdPV), a new group of *Phleboviruses* found in ticks whose RdRps and nucleoproteins showed relatively high sequence similarity to *Uukuvirus* and where the M-segment has never been sequenced.

The Kallisto tick virus was identified only in *H. marginatum* ticks with a prevalence MLE of 6.81 (95% CI: 5.66%-8.1%), with not statistical significant difference between sex of the tick, host or geographic distribution. *H. marginatum* positive tick were collected from cattle, horses and wild board and despite the large number of tick species collected on these hosts throughout the study, only *H. marginatum* was found to be positive, which indicates that the virus is specific to the tick species and not to any particular parasitized host. *H. marginatum* geographical distribution is mainly south of the Mediterranean. However, this presence is limited in climates with a mild winter and no frost. In its adult stage the ticks of this species mainly parasitize large ungulates with a strong tropism. The immature stages only parasitize rodent lagomorphs and birds, the last of which provides them with a high dispersal capacity through the migratory avian corridor [175]. In Corsica the *H. marginatum* tick is present throughout the territory, mainly on the coast, with a seasonality in summer from May to July [11, 173]. Logically, the seasonality and geographical distribution of the Kallisto Tick virus follows that of *H. marginatum* without significant difference. In the south of the island, only ticks collected from horses were found to be positive, this is explained by the low number of cattle examined in the south of the island due to the closure of a slaughterhouse. The collection of ticks from horses complements the collection from cattle to cover the whole territory, which explains the difference between the collection areas, as well as the difference between the regions with KATV positive ticks between the two hosts.

Several studies showed a high prevalence (up to 54%) rate of these *M-deficient Phleboviruses* in sampled ticks, may suggest some virus of this group can be viral endosymbionts, while other viruses in the group show lower prevalence, especially the Tacheng virus 2 (9.6%) associated with a febrile symptom in humans [166, 176]. If that the sequences of MdPV are present as Endogenous viral elements (EVEs) in the tick genome are suggest, some studies shown the RNA form and not EVEs in this group [163, 164].

The challenges of isolating this group of viruses makes it difficult to characterise them properly. Indeed, among different studies, only one virus of this group, the Tacheng virus 2, associated with febrile symptoms in humans has been isolated on Human Hepatoma Cell Line (SMMC-7721), after several isolation attempts on different cell lines (Vero, BHK21, human foreskin fibroblasts, Cos7, C6/36, 297 cell lines, CCL-141 cells). The attempts to isolate Kallisto Tick virus on Vero E6 and DH-82 cells were failed despite the virus was still detectable after several passages with a decreased viral load indicating that the virus may well adapted to insect-specific cells and likely is more common in ticks than in mammals.

In recent years, a number of new M- deficient *Phleboviruses* have been discovered from ticks in many parts of the world, for example in Africa, America, Asia, Australia and Europe [65, 127, 140, 163, 164, 166, 168, 169, 177-185]. Several hypotheses are cited, to try to explain this phenomenon, such as the absence of the segment or high genetic distance in segment M. These two hypotheses are depending on the studies, document recombination events involving the glycoproteins of highly divergent virus groups, within negative-sense RNA viruses and between negative- and positive-sense RNA viruses, and even between negative-sense RNA and DNA viruses. Moreover, gene loss is most often involved the glycoproteins, although several viruses within the Bunya–Arena clade may lack both glycoprotein genes [164, 186]. Recently, isolated Tacheng virus 2 electron microscopy photos showed that this virus may harbor a glycoprotein [176].

The Kallisto tick virus share close genetic homology with two other described viruses, the Limansky tick *Phlebovirus* and Balambala tick *Phlebovirus* that suggest they are the same viral species according to ICTV. The Balamabala tick virus has been found in ticks of the species *H. tracanum*, *H. rupifex*, *H. dromaderii* and *Amblyoma* spp. in Kenya and Ghana and the Limansky virus has been found in the ticks *H. anatolicum* in Russia. These viruses are genetically close and belonging to the same viral species, thus showing a specificity for ticks of the genus *Hyalomma* and a distribution that could follow the air of repartition of this genus of tick. It is interesting to note that other ticks of the genus *Hyalomma*, (*H. scupens*) collected during this study were negative to KATV. This may indicate the tick species specify of the KATV. For the other viruses close to KATV, as Bole tick virus 1, Xinjiang tick virus, Tick *Phlebovirus* Anatolia and brown tick *Phlebovirus* none of them have such a marked specificity for a particular tick species. Indeed, these viruses are detected in several tick species without specificity. These viruses were found in ticks sometimes in regions close to Corsica (Romania and Turkey) such as *R. bursa* and *H. marginatum* both collected in this study but no other

Phleboviruses could be detected apart from KATV. This shows a very complex ecology of this group of *Phleboviruses* and may even be due to the specificity of the insularity of Corsica.

The genetic identity matrix of the M-deficient segment group shows that several viruses have been described in the literature as new viruses although they belong to the same virus species according to ICTV. For example, *Kharabali Tick Phlebovirus* and *Xinjiang Tick Phlebovirus* have a difference in amino acid content of the RdRp gene of 0.6%, and therefore belong to the same virus species according to ICTV. Similar results are found with *Brown dog tick Phlebovirus* and *Tick Phlebovirus Anatolia* (2.9%), *Rhipicephalus associated Phlebovirus* and *Lihan tick Phlebovirus* (2.6%), *Khasan virus* and *Huangpi Tick virus* (1.1%), *FinV707 virus* and *Zaliv Terpeniya virus* (3.7%) and *Chize virus* and *EgAN 1825-61 virus* (1%). These results show that viruses belonging to M-deficient segment are still not-well characterized and require further analysis of taxonomy, allowing a better understanding of the natural biology of these viruses.

Recently, *H. marginatum* ticks, species in which the Kallisto tick virus has been found, originally from the south of the Mediterranean, have been spreading to northern Europe, where they have become permanently established in new areas due to global change [146]. The discovery of new viruses in this species of ticks, notably a vector of the Crimean-Congo Haemorrhagic Fever virus, shows the potential risk of implantation of foreign pathogens in previously unscathed territories, increasing the risk of tick-borne pathogens in public health. It is therefore necessary to investigate potential uncharacterised viruses in ticks before an emergence, in order to be able to anticipate it.

This study allowed the discovery and characterisation of a novel *Phlebovirus* circulates in Corsica with specificity to *H. marginatum* tick species. The virus is belonging to *Uukuvirus*, requires further analysis to a better understand the characteristics and natural cycle of these viruses

Figure 1: Maps of *H. marginatum* collected and positive sample; A) tick collected from horses and B) for tick collected from cattle

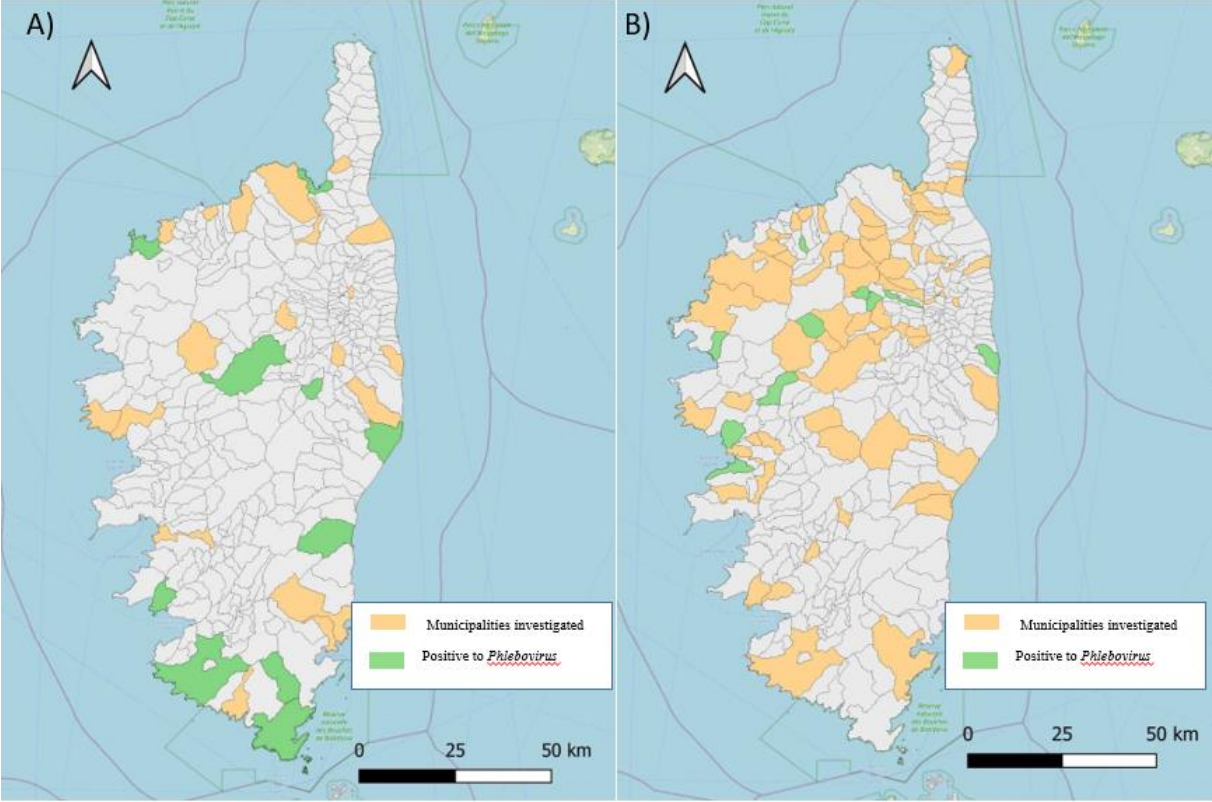




Figure 2: Phylogenetic tree of *New-tick Phlebovirus* L segment using Maximum Likelihood method with General Time Reversible model and Gamma Distributed with Invariant Sites (G+I), 500 bootstrap Replication.

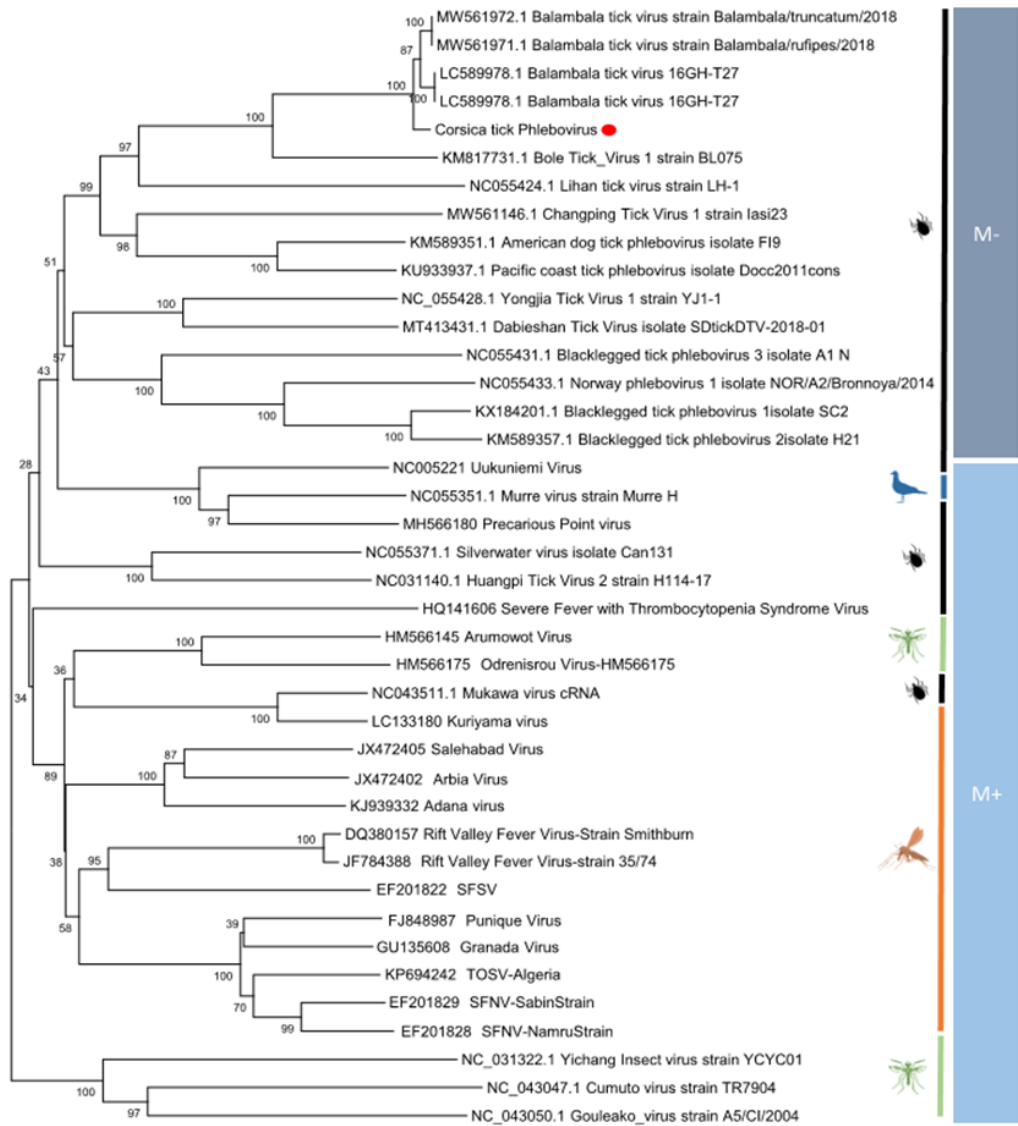


Figure 3: Phylogenetic tree of *New-tick Phlebovirus* S segment using Maximum Likelihood method with General Time Reversible model and Gamma Distributed with Invariant Sites (G+I), 500 bootstrap Replication.

Table 1:

Primer/Probe	5'→3' Sequence	Amplicon Size	Concentration
KATV Forward	ACYCTCACAAACCCAGATCAAAA	119 pb	400 nM
KATV 2 Reverse	GGTCATCAGARGTCCCAACTGT		400 nM
KATV probe	FAM-TCATGGCCGTAGAGCAAAAGC- TAMRA		200 nM

Table 2: Number of ticks, pools positive pools and prevalence to the *New-Phlebovirus*

Number of Pools										Number of positive pools detected		
Cattle 2019 (n=456)												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	158	124	59	4	4	27	20	0	396	Total	23	23
Number of ticks	460	305	152	4	6	78	59	0	1064	MLE (95% CI)	8.55 (5.59-12.31)	2.23 (1.45-3.26)
Number of Pools										Number of positive pools detected		
Cattle 2020 (n=278)												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	287	173	37	6	19	20	8	1	551	Total	44	44
Number of ticks	1240	533	130	10	48	52	15	1	2029	MLE (95% CI)	9.93 (7.28-12.85)	2.26 (1.66-2.98)
Number of Pools										Number of positive pools detected		
Cattle 2019-2020 (n=734)												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	445	297	96	10	23	47	28	1	947	Total	67	67
Number of ticks	1 700	838	282	14	54	130	74	1	3 093	MLE (95% CI)	9.34 (7.35-11.64)	2.75 (2.15-3.45)
Number of Pools										Number of positive pools detected		
Horse 2019 (n=538)												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	98	142	0	0	0	0	0	0	240	Total	26	26
Number of ticks	578	707	0	0	0	0	0	0	1285	MLE (95% CI)	4.12 (2.75-5.86)	2.15 (1.43-3.07)
Number of Pools										Number of positive pools detected		
Horse 2020 (n=119)												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	35	105	0	0	10	0	0	0	150	Total	18	18
Number of ticks	51	319	0	0	27	0	0	0	397	MLE (95% CI)	5.11 (3.13-7.75)	4.18 (2.55-6.35)
Number of Pools										Number of positive pools detected		
Horse 2019-2020 (n=657)												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	133	247	0	0	10	0	0	0	390	Total	44	44
Number of ticks	629	1026	0	0	27	0	0	0	1682	MLE (95% CI)	4.81 (3.54-6.33)	2.8 (2.06-3.69)
Number of Pools										Number of positive pools detected		
Wild boars 19-2020 (n=218)												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	1	6	0	0	0	0	0	155	162	Total	1	1
Number of ticks	1	12	0	0	0	0	0	613	626	MLE (95% CI)	2.38 (0.1 10)	0.16 (0.01-0.7)
Number of Pools										Number of positive pools detected		
Sheep 2019-2020 (n=107)												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	32	0	0	0	0	0	0	0	32	Total	0	0
Number of ticks	38	0	0	0	0	0	0	0	38	MLE (95% CI)		
Number of Pools with <i>n</i> Ticks										Number of positive pools detected by Real- Time		
Ticks 2019-2020												
Species	<i>R. bursa</i>	<i>H. marginatum</i>	<i>H. scupense</i>	<i>H. punctata</i>	<i>R. sanguineus</i>	<i>B. annulatus</i>	<i>I. ricinus</i>	<i>D. marginatus</i>	Total	Pool	<i>H. marginatum</i>	Total
Pool	611	550	96	10	33	47	28	156	1531	Total	112	112
Number of ticks	2368	1876	282	14	81	130	74	614	5 439	MLE (95% CI)	6.81 (5.66-8.1)	2.15 (1.78-2.56)

Table 3: Tick species pools positive for New *Phlebovirus* by sex.

<i>H. marginatum</i>						
	Number of Pools			Number of positive pools detected by Real- Time		
Horse 2019						
	Male	Female	TOTAL	Male	Female	TOTAL
TOTAL pools	70	72	142	15	11	26
			MLE (95% CI)	4.41 (2.56-6.96)	3.78 (1.96-6.39)	4,12 (2,75-5,86)
Horse 2020						
TOTAL pools	60	45	105	12	6	18
			MLE (95% CI)	6,71 (3.64-11.02)	5.72 (3.31-1.13)	5.11 (3.13-7.75)
Horse 2019-2020						
TOTAL pools	130	117	247	27	17	44
			MLE (95% CI)	5.21 (3.51-7.35)	4.29 (2.58-6.59)	4,81 (3,54-6,33)
Bovin 2019						
TOTAL pools	117	56	173	33	11	44
			MLE (95% CI)	9.72 (6.85-13.23)	10,14 (5.39-16.8)	9,83 (7,28-12,85)
Bovin 2020						
TOTAL pools	117	56	173	33 (28,21)	11 (19,64)	44 (25,43)
			MLE (95% CI)	9.72 (6.85-13.23)	10,14 (5.39-16.8)	9,83 (7,28-12,85)
Bovin 2019-2020						
TOTAL pools	205	92	297	51	16	67
			MLE (95% CI)	9.31 (7.05-11.96)	9.45 (5.65-14.49)	9,34 (7,35-11,64)

Table 4: CT of the *New-Phleovirus* cell culture after inoculation.

Number	Samples ID	CT first passage		CT second pasage		Delta CT	
		Vero E6	DH-82	Vero E6	DH-82	Vero E6	DH-82
1	58	23,6	23	24,9	24,9	-1,3	-1,9
2	84	26,7	25	28,5	28	-1,8	-3
3	91	23,5	22	24,7	24,2	-1,2	-2,2
4	92	23	23,1	24,9	25,4	-1,9	-2,3
5	122	27,4	25,9	29,7	28,6	-2,3	-2,7
6	127	23,8	22	24,6	24,2	-0,8	-2,2
7	49	23	22,2	23,7	23,7	-0,7	-1,5
8	5	25	23,7	25,6	26	-0,6	-2,3
9	64	29,6	26,2	29,4	27,6	0,2	-1,4
10	65	27	27,6	29,2	29,5	-2,2	-1,9
11	76	26	24,3	26,7	26,2	-0,7	-1,9
12	62	27,6	25,8	28,3	32,8	-0,7	-7
13	63	0	37	0	0	0	37
14	19	23,9	24,1	26,1	25,4	-2,2	-1,3
15	89	25,3	25,4	27,5	27,4	-2,2	25,4
16	81	25,4	29,3	27,5	27,6	-2,1	1,7
17	4	27,7	27,3	30,7	29	-3	-1,7
18	1	29	29	30,6	31,5	-1,6	-2,5
19	2	28,4	28,9	29,4	29,7	-1	-0,8
20	33	31,3	30,1	33,6	31,7	-2,3	-1,6
21	35	26,6	25,8	29,1	33,6	-2,5	-7,8
22	36	31,7	29	34,2	31,3	-2,5	-2,3
23	37	29,6	28,3	30,1	30,4	-0,5	-2,1
24	38	29	27,5	29,4	29,1	-0,4	-1,6
25	39	31	25,9	30,7	27,1	0,3	-1,2
26	41	29,3	29	28,6	31,1	0,7	-2,1
27	128	27,1	24,4	27,6	26,3	-0,5	-1,9
28	18	24,7	24,6	26,5	26,2	-1,8	-1,6

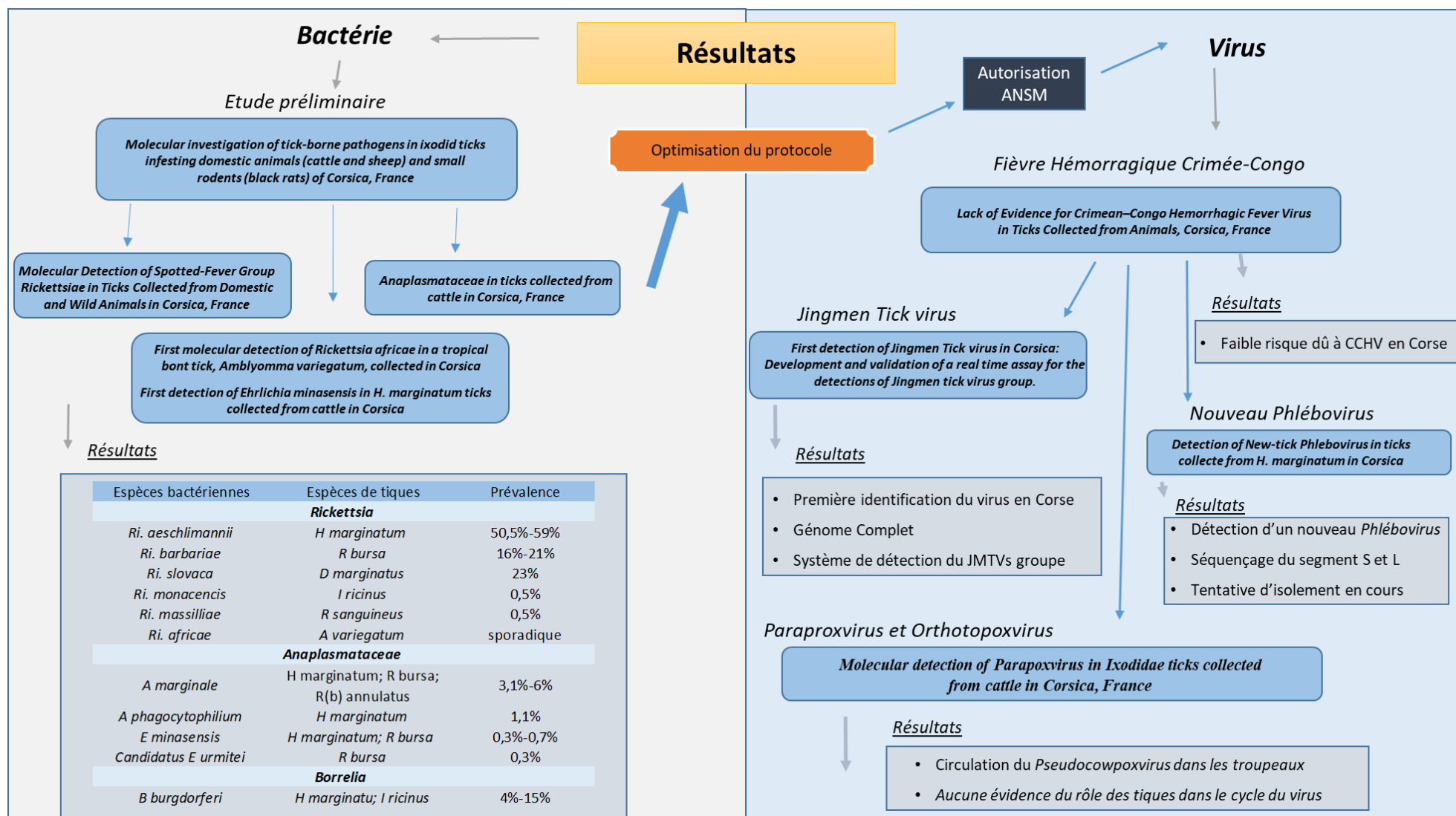
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Figure 15 : Schéma récapitulatif des différents résultats de la thèse



Chapitre 6 :
Discussion, conclusion et perspectives

1. Identification des tiques et pathogènes bactériens présents en Corse

De 2016 à 2021, nous avons pu identifier 8 espèces de tiques : *H. marginatum*, *H. scupense*, *R. bursa*, *Rhipicephalus sanguineus*, *Dermacentor marginatus*, *I. ricinus*, *Boophilus annulatus* et *Haemaphysalis punctata*. Un mâle d'une espèce d'origine sub-tropicale, *Amblyomma variegatum* a été aussi identifié.

Les tiques *H. marginatum*, *R. bursa*, *Rhipicephalus sanguineus*, *Dermacentor marginatus* et *Haemaphysalis punctata* sont des espèces typiquement méditerranéennes. Les espèces *Boophilus annulatus* et *H. scupense* sont retrouvées dans les pays du nord de l'Afrique alors que la tique *I. ricinus* est principalement retrouvée en Europe [14, 173]. Il n'est donc pas surprenant de retrouver ces espèces en Corse dont les microclimats permettent des environnements propices à chaque espèce [173]. En 2014, une étude réalisée en Corse a signalé la présence de tiques de l'espèce *Hyalomma aegyptium* collectées sur des sangliers en Corse. Si la présence de la tique sur l'île est fortement probable dû à la présence de son hôte spécifique *Testudo hermanni*, tortue endémique de Corse, la collecte de plusieurs tiques adultes de cette espèce sur sangliers est peu probable et doit être dûe à une erreur d'identification [187]. On peut citer la présence de l'espèce *Boophilus annulatus* en Corse alors que celle-ci n'est pas retrouvée en France continentale malgré son introduction récurrente par les transferts d'animaux de rente des régions où la tique est endémique vers la France continentale. Ceux-ci peuvent s'expliquer par un climat et des hivers plus doux en Corse, permettant la survie de l'espèce, qui est retrouvée sur l'île plutôt en automne [14].

Nous avons retrouvé plusieurs bactéries notamment *Ri. aeschlimannii*, *Ri. slovacica*, *Rickettsia barbariae*, *Ri. monacensis* et *Ri. massiliae* ou encore *A. phagocytophilum* et *Anaplasma marginale*. Ces bactéries sont responsables de cas sporadiques humains, principalement pour la bactérie *A. phagocytophilum* responsable de l'Anaplasmose granulocytaire humaine [188]. Les bactéries *Ri. monacensis* et *Ri. massiliae* ont été associées à des cas humains avec des symptômes similaires à la fièvre boutonneuse de méditerranée (*Ri. conorii*); la bactérie *Ri. slovacica* est responsable du syndrome TIBOLA (Tick-borne Lymphadenopathy, maladie sporadique et saisonnière [189-191]. Une mise en lumière peut être faite sur la bactérie *E. minasensis*. En effet, à l'époque de notre publication, il s'agissait de sa première détection en Europe. La découverte de la tique *Amblyomma variegatum* adulte sur un bovin de l'île positive à *Ri. africae* montre que la Corse se trouve sur des voies de migration aviaire propices à l'introduction de nouvelles espèces de tiques et par conséquent de nouveaux pathogènes en

Corse. Cependant, l'introduction d'une espèce de tiques dans un milieu n'est pas systématique car elle ne retrouve pas forcément toutes les conditions favorables à son installation. L'introduction de pathogènes par l'intermédiaire d'une tique « voyageuse » n'est pas lui aussi systématique car le pathogène importé doit trouver un vecteur potentiel installé sur le territoire et une espèce réservoir qui n'est pas forcément présente en dehors de leur géographie d'origine.

En conclusion les espèces de tiques présentes en Corse sont diversifiées avec des espèces typiquement méditerranéennes comme *H. marginatim*, *R. bursa*, *D. marginatum*, des espèces plutôt africaines comme *H. scupense* et *B. annulatus* et des espèces plus Européennes comme *I. ricinus*. Cette diversité est aussi retrouvée dans les pathogènes bactériens détectés dans les tiques. En effet, chaque espèce de tiques est vectrice de pathogènes qui lui sont propres, il est donc logique de retrouver une diversité de pathogènes tout aussi importante.

Nous avons pris la décision de donner priorité à nos recherches sur les virus. En effet, les virus transmis par les tiques à l'exception du TBEV et de CCHF sont moins étudiés que les virus transmis par d'autres arthropodes et moins étudiés que les bactéries transmises par les tiques. Pourtant de nombreux virus transmis par les tiques continuent d'émerger, de re-emerger et même d'être découverts [4, 192]. De plus, l'UVE est spécialisée depuis plus de 15 ans dans les arboviroses et offre une expertise, des techniques et des moyens performants dans la gestion et l'étude de ces virus.

2. Identification des virus présents dans les tiques en Corse, outil de maîtrise et perspective scientifique.

Au cours de ce travail, 7 systèmes moléculaire pan-genre et 8 systèmes moléculaires spécifiques par RT-qPCR ont été utilisés pour l'identification des virus présents dans les tiques collectées sur les animaux en Corse.

Les systèmes Pan-genre permettent de détecter tous les membres d'un genre viral. Dans ce travail, ils ont permis de découvrir un nouveau phlebovirus. Leur utilisation est particulièrement adaptée aux démarches exploratoires, plutôt qu'aux activités diagnostiques. Elle peut s'avérer difficile car la présence d'amplification parasite due à l'hybridation des amorces sur l'ADN des tiques n'est pas à exclure, notamment lorsque le système a été validé par son auteur sur d'autres matrices. Cette technique est souvent moins sensible que les techniques spécifiques ce qui exclut son utilisation en diagnostic et demande une analyse prudente des résultats. Enfin,

l'identification du virus détecté fait appel au séquençage ce qui retarde l'identification et augmente les coûts.

La validation d'un système moléculaire requiert du temps, des outils spécifiques (contrôles positifs naturels ou synthétiques) et une approche rigide. L'utilisation de système de RT-qPCR cible une espèce virale, elle est donc très spécifique et plus sensible que les techniques moléculaires conventionnelles. C'est la technique de choix pour le diagnostic médical ou vétérinaire. De plus, elle permet déterminer la charge virale.

L'utilisation de système de détection par la technique PCR (PCR conventionnelle et RT-qPCR) permet de manière efficace de poser les bases d'une surveillance de virus connus ou inconnus. Cependant malgré les nombreux avantages qu'offre la technique de la PCR et ses variantes, celle-ci reste basé sur une approche de détection avec a priori. Si cette approche peut être amélioré par l'utilisation de système Pan-genre, elle ne reste cependant pas corrigée. Pour pallier à cela, un séquençage par NGS apporterait des informations supplémentaires grâce à sa capacité de détecter sans a priori tous les micro-organismes présents dans un échantillon ce qui a déjà été démontré avec les tiques. C'est notamment grâce à cette technique, que la famille des Flavi-like segmented virus dont fait partie les JMTVs a été découverte. La technique reste cependant toujours couteuse en temps d'analyse. C'est pour ces raison que l'utilisation des techniques PCR, couplé au séquençage NGS doit être considéré dans les programmes de surveillance des arbovirus et des virus zoonotiques.

La Fièvre Hémorragique Crimée-Congo

Une partie du travail de thèse s'est concentré sur la surveillance du virus CCHF. En effet, une récente étude a montré la présence d'anticorps anti-CCHFV dans du sérum de bovins à hauteur de 13 % et dans du sérum de petits ruminants à hauteur de 2 %, bien qu'aucun cas humain n'ait été décrit en Corse et dans le reste de la France. [193]. Afin de savoir si CCHFV est présent dans les tiques corses, nous avons décidé de mettre en place une surveillance systémique dans des tiques collectées sur différents vertébrés. Nous avons testé plus de 8000 tiques appartenant à 8 espèces différentes, dont 2000 *H. marginatum*. Aucun positif ! Cette absence est significative et elle pose la question des résultats contrastant avec ceux de l'étude sérologique. Pour expliquer l'absence du virus dans les tiques, il existe plusieurs hypothèses: (i) une espèce de tiques différente de celles testées serait vectrice du virus; cependant les tiques testées dans cette étude ont été collectées sur bovins qui se sont aussi révélés porteurs d'anticorps; (ii) les bovins prélevés à l'abattoir étaient jeunes (car système d'élevage dit de « vache allaitante » en

Corse) et ont pâturé dans des zones dénuée de tiques. Cependant, les tiques testées avaient été collectées sur des animaux dans les communes où les sérologies étaient élevées. Nous avons aussi collecté des tiques sur chevaux lors de randonnées à travers des circuits traversant différents biotopes qui rendent cette hypothèse peu probable. Reste une troisième hypothèse: la présence d'un virus CCHFV-like responsable des sérologies positives mais non détecté par nos outils moléculaires; elle devrait être balayée par l'utilisation d'un système *Pan-Nairovirus*. Néanmoins, notre prochaine étape réside dans l'utilisation de la technique NGS. Cette approche sans a priori permettrait alors, peut-être de séquencer en éventuel CCHFV-like.

Dans le but de conclure sur la présence de CCHFV en Corse, nous allons également mener une étude de séroprévalence en population humaine. En Espagne, la survenue de cas humains est passée inaperçue dans un premier temps [194]. Il est donc possible que la même situation s'applique en Corse. Pour cela, une enquête sérologique en population générale et chez les populations à risques (éleveur, vétérinaire, personnel d'abattoir) est en cours d'organisation. Pour les populations à risques, le prélèvement de sérum sera associé à un auto-questionnaire qui nous permettra de mettre en évidence les facteurs et pratiques favorisant une infection.

Le Jingmen Tick virus

Cette thèse a permis la création et la validation d'un système de détection pour les virus du Jingmen Tick virus group et de mettre en évidence de la circulation d'une souche du Jingmen Tick virus proche d'une souche détectée chez l'homme au Kosovo [143]. Ce virus a une répartition mondiale avec l'existence de lignages génétique plus ou moins dictés par des aspects géographiques. L'analyse génétique des différentes souches de JTMV montre une grande diversité génétique. Il est probable qu'il existe plusieurs espèces virales n considérant la définition actuelle de l'ICTV (>5% de diversité entre polymérase virales). Pour compléter la taxonomie des JMTV nous avons décidé de pousser l'analyse taxonomique de ce groupe. Une fois réalisée, la partie taxonomique sera rajoutée à la version de l'article qui est présentée dans ce manuscrit et l'article sera soumis pour publication.

Pour la suite des travaux sur le Jingmen Tick virus, nous travaillerons à développer des outils de diagnostic sérologiques qui seront appliquées à diverses collections de sérums d'animaux en Corse et dans d'autres régions du monde. Nous allons tester des sérums de bovins du Kosovo où le virus a déjà été retrouvé chez l'homme et dans les tiques. Dans un second temps des sérums humains de personnes à risques en Corse, associés à un questionnaire portant sur les données sociodémographiques, ainsi que les activités à risque seront testées. Cette étude

permettra de mettre en évidence l'impact de ce virus en santé humaine mais aussi de comprendre les pratiques à risques vis-à-vis de ce virus en population humaine.

Le nouveau phlébovirus transmis par les tiques

Grâce à l'utilisation d'un système pan-*Phlébovirus*, nous avons pu détecter et caractériser un *Phlebovirus* associé à la tique de l'espèce *H. marginatum* collecté sur différents hôtes de l'île. Le virus que nous avons décidé d'appeler *Kallisto tick virus* (KATV), fait partie d'un nouveau groupe de *Phlebovirus* appelé M-déficient *Phlebovirus* du genre *Uukuvirus*. Les virus de ce groupe sont de plus en plus nombreux à être découverts [163]. Cependant le segment M des virus n'a jamais pu être séquencé. De plus, seul le virus Tacheng 2 a pu être cultivé dans une lignée cellulaire de carcinome humain, le virion était bien visible au microscope électronique mais le segment M n'a pas pu être séquencé. D'autres lignées cellulaires ont été utilisées pour tenter d'isoler les virus du groupe sans résultat, mais jamais des cellules de tiques.

Lors de nos tentatives d'isolement, nous avons utilisé les lignées cellulaires de mammifères Véro E6 et DH-82, communes pour l'isolement des phlebovirus de moustiques et de tiques. Nous avons observé une diminution de la charge virale à chaque passage avec disparition au 5^{ème} passage. Nous avons émis plusieurs hypothèses, notamment que la réplication de ce virus n'est pas optimale dans ces deux lignées cellulaires et nécessite plus de temps pour sa réplication ou des conditions de température moins importantes. Dans un premier temps, sur ces lignées cellulaires (Véro E6 et DH-82) avec des conditions de température plus basses, et en allongeant les temps entre chaque passage. Ensuite, nous allons utiliser la lignée cellulaire de carcinome humain, sur laquelle le virus Tacheng 2, virus appartenant au même groupe que le virus Kallisto, se réplique. Pour finir, nous allons tenter d'isoler le virus sur des lignées cellulaires de tiques.

Parapoxvirus

La récente découverte de *Parapoxvirus* au Burkina-Faso, nous a conduit à mener une recherche de ce genre de virus dans les tiques collectées en Corse [124]. Nous avons pu identifier après séquençage, le virus Pseudocowpox dans les tiques corses. Ce virus est bénin pour les humains et a pour principal réservoir les bovins [195]. Les résultats montrent qu'hormis les tiques collectées sur bovins, aucune autre tique de la même espèce mais collectée sur d'autres espèces de vertébrés ne s'est révélée positive. Ce n'est pas la tique qui est infectée, elle est seulement colonisée à l'occasion de son repas sanguin sur individus virémiques. Ces résultats sont intéressants car ils montrent qu'une détection de pathogènes dans une espèce de tiques ne

signifie pas qu'il s'agit d'un arbovirus ou même que la tique est vectrice. De ce fait, les résultats d'indentification de pathogènes sur tiques gorgées ou collectées sur hôte doivent être pris avec précaution et ne sont témoins que d'une circulation d'un pathogène.

3. Conclusion

La thèse *Holis-tick : identification et maîtrise des maladies vectorielles* commence avec la thématique des maladies transmises par les tiques dans le laboratoire UR Bioscope et l'Unité des Virus Emergents. La Corse, qui au fil de ce travail s'est révélée être une région sentinelle vis-à-vis du risque épidémique, offrait un environnement de choix pour l'étude de ces pathogènes. Nous avons commencé une étude ciblée sur les pathogènes bactériens qui ont détecté plusieurs espèces bactériennes dont certaines sont des pathogènes humains. Cette étude a permis de mettre en place et d'optimiser le protocole des collectes de tiques et leur manipulation au laboratoire. La Corse possède une grande diversité de tiques et de pathogènes bactériens endémiques. Nous y avons décrit (i) *E. minasensis* pour la première fois, (ii) une tique non endémique en Corse (*A. variegatum*) porteuse de *Rickettsia africa*, un pathogène importé démontrant les risques d'introduction et l'importance de la Corse comme zone de surveillance des importations. Ces résultats renforcent le statut d'espace sentinelle de l'île, notamment pour les maladies transmises par les tiques.

Pour la recherche des pathogènes viraux, nous avons concentré nos efforts sur le virus de la Fièvre Hémorragique Crimée-Congo car la présence de son vecteur, la découverte récente d'anticorps dans le bétail et sont risque en santé humaine est une question de santé publique. Bien qu' aucune tique n'ait été détectée porteuse de l'ARN de CCHFV, la description d'autres virus pathogènes ou potentiellement pathogènes souligne l'importance de poursuivre ces études sur les virus détectés dans ce travail et l'élargir à d'autres pathogènes viraux d'intérêt médical ou vétérinaire.

La découverte du virus Jingmen Tick et du virus Kallisto, un nouveau virus appartenant au genre *Phlebovirus* sont des pistes qu'il convient de continuer à explorer suivant l'approche One-Health mise en œuvre au cours de ce travail en combinant les outils technologiques classiques et innovants et en renforçant les collaborations avec des experts de différents horizons. Parmi ces outils, notre capacité à développer rapidement des techniques de détection moléculaire pour des microorganismes nouvellement décrits est un atout majeur autant pour la surveillance de la circulation de ces agents en Corse que pour les capacités de réponse rapide et adaptée applicable localement comme cela a été démontré dans la cadre de la crise du COVID-19 avec la mise en

place d'un plateau de détection autonome du SARS-CoV-2 au niveau insulaire. Les travaux que j'ai mené pendant cette thèse ont démontré la faisabilité et l'efficacité d'une approche One-Health associé à une organisation collaborative dans la cadre de la découverte, de la surveillance et de la mise en place de contre-mesures face a des situations à potentiel épidémique en santé humaine et animale. Pour terminer, ces travaux souligne l'importance stratégique de la Corse, au même titre que les autres îles de la Méditerranée, dans l'alerte et la prévention vis-à-vis des microorganismes et des vecteurs en situation d'invasion en Europe.

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Résumé

Les dernières années témoignent du fardeau en santé publique de l'émergence des maladies vectorielles à tiques. Ces arthropodes parasites hématophages stricts sont aujourd'hui, les premiers vecteurs de maladies chez les humains dans l'hémisphère nord, et les premiers chez les animaux dans le monde avant les moustiques. La Corse possède un environnement de choix pour l'étude des maladies vectorisées par les tiques, notamment un climat chaud subtropical de façade ouest, des microclimats dus à de nombreuses stratifications géologiques et végétales, de nombreux corridors de migration aviaire, ainsi que de nombreux échanges dus à son héritage culturel et au tourisme de masse, entre la faune sauvage, les animaux domestiques et les populations humaines. Ces caractéristiques font de l'île un environnement favorable à une diversité de tiques importante. De plus, des espèces de tiques présentes en Corse, ont déjà commencé à migrer vers l'Europe grâce au changement climatique.

L'objectif est d'utiliser la Corse comme espaces sentinelles pour se préparer aux épidémies des maladies transmises par les tiques. En effet, en identifiant et en se préparant aux épidémies que peuvent causer les pathogènes transmis par les tiques en Corse, notamment par la création d'outils de diagnostic déployable rapidement.

Nos résultats ont montré que la Corse possède une diversité de tiques importante, qui se répercute sur la diversité de pathogènes bactérien et viral retrouvé. Nous avons identifié 8 espèces de tiques différentes porteuses d'une dizaine de pathogènes bactériens des genres *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. et *Borrelia* spp.. La collecte de plus de 8 000 tiques testées au virus de la Fièvre Hémorragique Crimée-Congo, nous a permis de conclure que la Corse n'est pas un Hotspot pour ce virus, malgré la présence de sa tique vectrice. Nous avons détecté le Jingmen Tick virus, nouveau virus associé avec des symptômes fébriles chez les humains et créé un système de détection rapide capable de détecter tous les virus du groupe Jingmen Tick virus group. Nous avons pu caractériser un nouveau virus, appartenant lui-même à un nouveau groupe des *Phlebovirus*, dans le genre *Uukuvirus*, des analyses sont en cours pour comprendre ces mécanismes et sa pathogénicité.

Grace à cette thèse nous établissons les bases d'une réponse générique qui pourra être mise en œuvre dans la lutte contre les épidémies futures à l'échelle Européenne.

Mots clés : Tique - Pathogène - Epidémiologie - Corse - PCR en temps réel – Préparation

Abstract

Recent years have witnessed the public health burden of the emergence of tick-borne diseases. These strict hematophagous arthropod parasites are currently the primary vectors of disease in humans in the northern hemisphere, and the primary vectors of diseases in animals worldwide before mosquitoes. Corsica has a privileged environment for the study of tick-borne diseases, including a warm subtropical climate on the western side, micro-climates due to numerous geological and vegetation stratification, numerous avian migration corridors, as well as numerous exchanges due to its cultural heritage and mass tourism, between wild fauna, domestic animals and human populations. These characteristics provide a favourable environment for a wide variety of ticks. Moreover, some tick species present in Corsica have already started to migrate to Europe due to global climate change.

The objective of this initiative is to make use of Corsica as a sentinel area to prepare for epidemics of tick-borne diseases. Indeed, by identifying and preparing for epidemics that may be caused by tick-borne pathogens in Corsica, notably by creating rapidly deployed diagnostic tools.

Our results showed that Corsica possesses a high diversity of ticks, which is reflected in the diversity of bacterial and viral pathogens found. We recognised 8 different species of ticks carrying about 10 bacterial pathogens of the genus *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* spp.. The collection of more than 8,000 ticks tested for Crimean-Congo Haemorrhagic Fever virus, allowed us to conclude that Corsica is not a Hotpost for this virus, despite the presence of its vector tick. We have detected the Jingmen Tick virus, a new virus associated with febrile symptoms in humans, and created a rapid detection system able to detect all the viruses of the Jingmen Tick virus group.

We were therefore able to characterise a new virus, itself belonging to a new group of Phleboviruses, in the genus Uukuvirus, and analyses are underway to understand its mechanisms and pathogenicity.

Through this thesis we are establishing the basis for a generic response that can be implemented in the fight against future epidemics on a European scale.

Keywords: Epidemiology - Corsica - Real-time PCR - Preparedness - Ticks - Pathogen