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Baptiste Defaye

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**Etude des agents pathogènes dans les populations animales de**  
**zones humides en Corse et implications en termes de santé**  
**animale et humaine**

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## Avant-propos

Au cours de ces années, plusieurs publications issues de mes travaux de thèse ont été réalisées. Deux d'entre-elles sont le résultat de recherches bibliographiques qui avaient pour objectif de réaliser un état des lieux sur les agents pathogènes transmis par les tiques dans les populations animales du pourtour méditerranéen. Les trois autres publications obtenues au cours de cette thèse de doctorat résultent de campagnes de terrain et d'analyses dans chacun des groupes d'animaux étudié (populations de sangliers, élevages ovins et bovins et avifaune). Ces trois publications constituent le cœur du travail de cette thèse. Actuellement, plusieurs de ces publications sont parues dans des journaux scientifiques, alors qu'une autre est en cours de soumission.

### Liste des articles publiés

- Defaye, B., Moutailler, S., Pasqualini, V., Quilichini, Y. Distribution of Tick-Borne Pathogens in Domestic Animals and Their Ticks in the Countries of the Mediterranean Basin between 2000 and 2021: A Systematic Review. *Microorganisms* 2022, 10, 1236. <https://doi.org/10.3390/microorganisms10061236>
- Defaye, B., Moutailler, S., Pasqualini, V., Quilichini, Y. Distribution of Tick-Borne Pathogens in Wild Animals and Their Ticks in the Mediterranean Rim between 2000 and 2021: A Systematic Review. *Microorganisms* 2022, 10, 1858. <https://doi.org/10.3390/microorganisms10091858>
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- Defaye, B., Moutailler, S., Pietri, C., Galon, C., Grech-Angelini, S., Pasqualini, V., Quilichini, Y. Molecular Detection of Zoonotic and Non-Zoonotic Pathogens from WildBoars and Their Ticks in the Corsican Wetlands. *Pathogens* 2021, 10, 1643. <https://doi.org/10.3390/pathogens10121643>
- Defaye, B., Moutailler, S., Grech-Angelini, S., Galon, C., Ferrandi, S., Pasqualini, V., Quilichini, Y. Detecting Zoonotic and Non-Zoonotic Pathogens in Livestock and Their Ticks in Corsican Wetlands. *Veterinary Medicine and Science* 2022; 1-16. <https://onlinelibrary.wiley.com/doi/10.1002/vms3.956>

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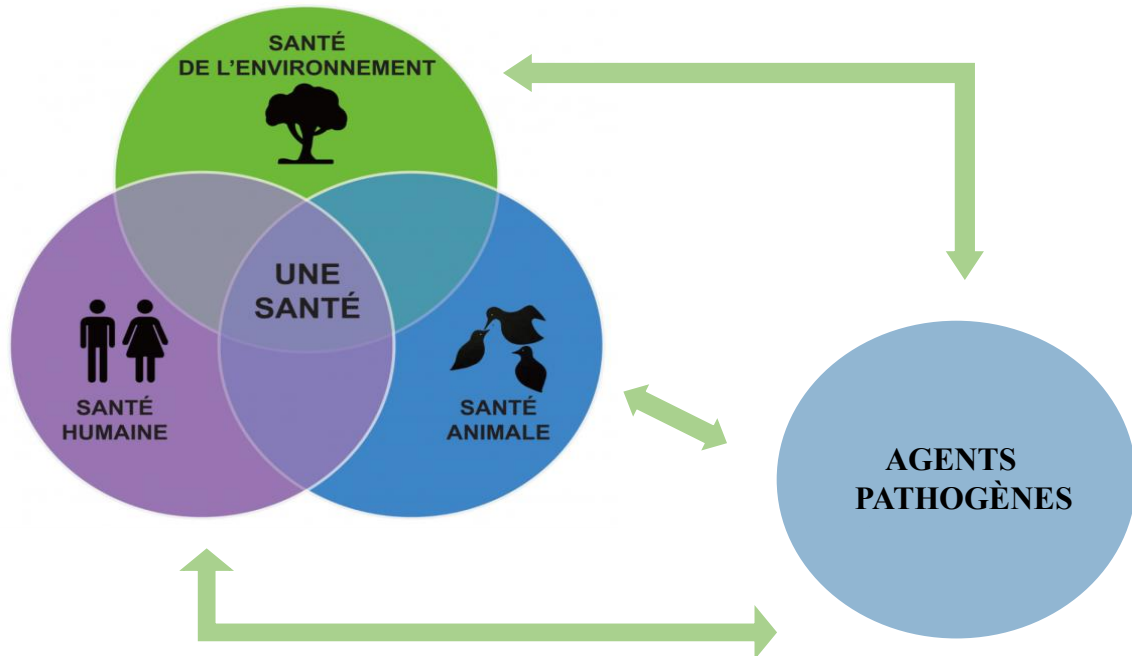
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**Introduction**

## 1. Le concept One Health

Le concept One Health, initié au début des années 2000, repose sur une interconnexion entre les trois santés : humaines, animales et environnementales (Figure 1).



**Figure 1.** Concept One Health (modifié d'après l'Université Paris-Saclay).

Il met en lumière les interactions entre ces trois santés, mais également le fait que la protection de l'une des trois dépend et impacte les deux autres (Inci et al., 2016). Ce concept a pour but d'incorporer et de croiser de nombreuses variables afin d'effectuer une recherche plus efficace des origines et des solutions de troubles sanitaires. Cela est expliqué par le « parapluie » One Health défini par One Health Sweden. C'est un concept regroupant toutes les variables du concept One Health, allant de la santé individuel animal et humaine à la santé de l'environnement en passant par les santés des populations (Lerner and Berg, 2015). Il a pour but de démontrer que tout changement dans une des santés peut trouver son origine dans une autre que ce soit dans le cadre de maladies infectieuses, d'écotoxicité ou de résistance aux antibiotiques (Bordier Bouchot, 2019). C'est le cas dans la situation d'un abus d'antibiotiques en santé humaine qui peut entraîner l'apparition dans l'environnement de nouvelles bactéries résistantes aux antibiotiques pouvant impacter à la fois la santé humaine et animale (Kim and Cha, 2021). Cela peut également impacter la santé animale par interaction avec les activités humaines ou faciliter la circulation de divers agents pathogènes dangereux pour la santé humaine (Daszak et al., 2001; Kelly et al., 2017). Ces concepts ont généré un intérêt grandissant pour les maladies dites « zoonotiques » qui sont des maladies provoquées par des agents pathogènes transmissibles de l'animale à l'homme (Destoumieux-Garzón et al., 2018). Dans le cadre de l'étude de ces maladies, la prise en compte de la santé animale ainsi que des facteurs environnementaux sont nécessaires pour des recherches de diagnostics, d'épidémiologie et de thérapies plus efficaces (Cantas and Suer, 2014). C'est le cas par exemple pour l'épidémiologie du virus West Nile au Nord de l'Italie qui se porte à la fois sur les hôtes cibles du virus que sur le vecteur et les hôtes accidentels (Paternoster et al., 2017). Ces recherches présentent plusieurs avantages comme définir les animaux en tant que « sentinelles » de maladies zoonotiques, créer des modèles prédictifs à partir de données animales ou encore identifier plus précisément des manquements en termes de sécurité sanitaire (Bordier Bouchot, 2019). Un autre élément primordial à prendre en compte dans le contexte

actuel concerne les contraintes que subit l'environnement que ce soit par des changements climatiques mais également par des activités humaines directes telles que la déforestation ou l'urbanisation (Daszak et al., 2001; "One Health, une seule santé," n.d.). Ces perturbations peuvent être à l'origine de mouvements de populations humaines et animales favorisant leurs interactions ainsi que la circulation de divers vecteurs d'agents pathogènes. Cela peut faciliter l'émergence de nouvelles maladies provoquées par divers agents pathogènes au sein de territoires. Cette approche systémique développée dans le concept One Health s'avère pertinente à différentes échelles spatiales et notamment au niveau de territoires subissant d'importants mouvements de populations et soumis à l'influence de différentes masses continentales comme peuvent l'être les îles du bassin méditerranéen.

## 2. Une grande diversité d'agents pathogènes potentiels

Les agents pathogènes sont des agents étiologiques de maladies infectieuses à travers le monde. Ils sont principalement divisés en 4 groupes : Bactéries, Parasites, Virus et Champignons (Shanson, 1989; Theel and Pritt, 2016). La menace qu'ils représentent est à la fois due à leur aire de répartition mondiale ainsi que leur tropisme pour l'intégralité du monde du vivant.

Les bactéries sont des agents pathogènes procaryotes pouvant à la fois être responsables de maladies et également être en symbiose avec leurs hôtes. Elles ont la particularité de pouvoir être présentes dans tous types de milieux y compris les plus extrêmes (Irwin and Baird, 2004). Parmi les bactéries responsables de maladies, il existe différents groupes. Le premier d'entre eux concerne les bactéries détectables via une coloration de Gram. Elles sont divisées en bactéries Gram-négatif et bactéries Gram-positif, des bactéries gastriques telles que *Escherichia coli* et *Staphylococcus aureus* (Ruhai and Kataria, 2021; Shanson, 1989). Le deuxième groupe est celui des bactéries acido-alcooliques, non-détectables à la coloration de Gram, qui inclue notamment des bactéries telles que *Mycobacterium tuberculosis* responsable de la tuberculose. Parmi les autres groupes, il y a celui des spirochètes qui sont des bactéries avec une forme de spirale comprenant des bactéries telles que les agents causatifs de la leptospirose, le genre *Leptospira* spp., le genre *Borrelia* spp. notamment responsable de la maladie de Lyme ou les rickettsiales comprenant des genres de bactéries tels que *Rickettsia* et *Anaplasma* responsable de nombreuses maladies transmises par les tiques (Shanson, 1989).

Les parasites sont des agents pathogènes se reproduisant et vivant au dépend de l'hôte qu'ils infectent de manière bénigne ou maline. Ils sont responsables de nombreuses maladies, parfois mortelles à travers le monde, telles que la maladie de Chagas et du sommeil, causées par des parasites du genre *Trypanosoma* (Shanson, 1989; WHO, 2020). Ils peuvent être divisés en deux groupes : les protozoaires qui sont des parasites intracellulaires pouvant être à la fois intestinaux, sanguins et tissulaires tels que les espèces appartenant au genre *Babesia* responsables de piroplamose ou encore l'agent de la toxoplasmose *Toxoplasma gondii* (Theel and Pritt, 2016). Un grand nombre d'entre eux peuvent être vectorisés tels les *Babesia* par les tiques ou encore l'agent de la Malaria par les moustiques, responsable de plus de 500 000 décès en 2013 (Theel and Pritt, 2016; WHO, 2020). Le deuxième groupe est celui des helminthes qui sont des vers pouvant être responsables de nombreuses maladies telles que la bilharziose, la cysticercose et la trichinellose (Garcia et al., 2020; Guiguen et al., 2013; Zarlenga et al., 2020).

Les virus sont des entités biologiques à ADN ou à ARN pouvant cibler l'intégralité du vivant allant des bactéries, avec les bactériophages, jusqu'à l'humain avec par exemple le virus de la COVID-19 (Aksyuk and Rossmann, 2011; Wu et al., 2020) ou encore Ebola. Au niveau animal nous pouvons citer notamment les épidémies dues au virus de la peste porcine africaine

dans les populations de porcs et de sangliers (Coltart et al., 2017; Dixon et al., 2019; Koonin et al., 2015; Wu et al., 2020).

La plupart des champignons sont peu pathogéniques, mais certains peuvent impacter une part du vivant notamment les plantes pour lesquelles ils sont la principale cause de maladies (Doehlemann et al., 2017). Néanmoins, des champignons peuvent être responsables de maladies chez l'homme tels les genres *Aspergilla* et *Candida* (Köhler et al., 2017).

Parmi ces agents pathogènes, nombres d'entre eux ciblent les animaux. Ces agents pathogènes ont différentes stratégies de transmission que ce soit de manière non vectorielle, comme par contact direct entre individus de la même espèce ou entre individus d'espèces différentes. Dans cette catégorie, rentrent de nombreux agents pathogènes tels que les bactéries du genre *Mycobacterium* responsable de la tuberculose aussi bien dans les populations d'animaux sauvages, domestiques que chez l'homme et le virus d'Aujeszky qui est un virus affectant les populations de sangliers ou de cochons causant des problèmes sanitaires et économiques dans les élevages porcins (Olea-Popelka et al., 2017; Ruiz-Fons et al., 2008b). La deuxième méthode de transmission non vectorielle est par contact indirect via l'environnement ou l'alimentation. Cette deuxième voie de transmission concerne des agents pathogènes tels que *Coxiella burnetii*, agent de la fièvre Q, responsables d'avortements et de morts fœtales dans les élevages d'ovins et caprins ainsi que de problèmes respiratoires chez l'homme (Angelakis and Raoult, 2010). Cela comprend également des parasites tels que *T. gondii*, un parasite du phylum Apicomplexa qui est principalement transmis par les fèces de félinés dans l'environnement (Kochanowsky and Koshy, 2018). Ce parasite est responsable de malformations et morts fœtales lors de l'infection de femmes enceintes. La troisième et dernière voie de transmission implique la participation de divers vecteurs tels que des arthropodes hématophages, comme les moustiques, les tiques ou les phlébotomes, qui peuvent transmettre des agents pathogènes en suçant le sang d'un hôte à un autre (WHO, 2020). Ces vecteurs transmettent un large panel d'agents pathogènes que ce soit des agents pathogènes spécifiques au règne animal (non-zoonotiques) comme le virus de la peste porcine africaine transmis par les tiques et provoquant de nombreuses épidémies mortelles dans les populations de cochons et sangliers ; ou encore des agents pathogènes zoonotiques tels que les bactéries du genre *Borrelia* responsables de la maladie de Lyme transmis par les tiques (Chomel, 2015). Certains agents pathogènes tels que *C. burnetii* peuvent être transmis de manière vectorielle (via les tiques) ou non vectorielle (aérienne).

Ces nombreux agents pathogènes peuvent être détectés par le biais de diverses méthodes dont les principales sont : la microscopie, la culture bactérienne, la sérologie ou la détection d'acides nucléiques Certaines d'entre elles sont spécifiques à une catégorie d'agents pathogènes comme la culture bactérienne, tandis que d'autres peuvent être utilisées pour la détection de plusieurs groupes d'agents pathogènes tel que le criblage à haut-débit micro fluidigm.

### 3. Une large variété de vecteurs et d'hôtes

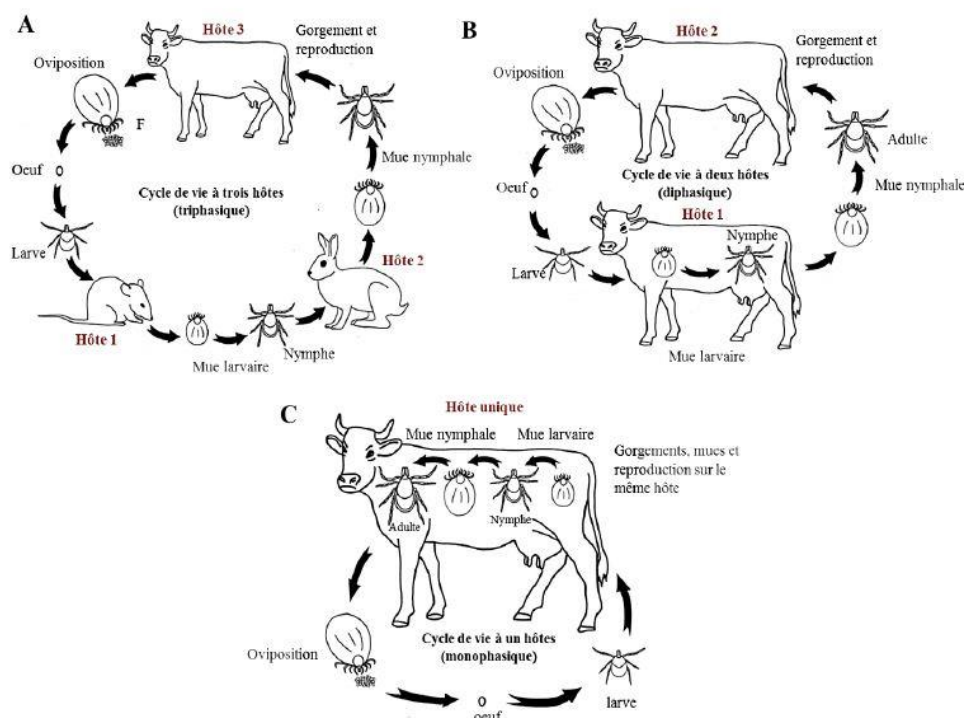
D'après l'OMS, les vecteurs sont des organismes vivants, qui, une fois infectés par un agent pathogène, peuvent transmettre des agents pathogènes infectieux entre humains, entre animaux, ou entre l'animal et l'humain. Les agents pathogènes vectorisés représentent 17 % des maladies infectieuses et sont responsables de plus de 700 000 décès par an (WHO, 2020). De plus, ces différents agents pathogènes sont également responsables de pertes économiques pouvant s'élever à plusieurs millions de dollars dans différents secteurs allant du tourisme au secteur de

l'agriculture comme cela a été le cas en Mongolie avec les agents de la brucellose (McDermott et al., 2013), ainsi que mondialement par la maladie de Lyme (Mattingly and Shere-Wolfe, 2020). Cela montre l'intérêt d'effectuer des recherches sur les potentielles menaces qu'ils représentent dans les zones à fortes activités humaines et animales telles que les zones humides.

Ils peuvent être transmis par de nombreux vecteurs dont 9 types ont été listés par l'OMS (WHO, 2020) :

- Les escargots aquatiques responsables de la circulation des schistosomes provoquant la bilharziose. Ils sont l'un des rares vecteurs de maladies qui ne soient pas hématophages.
- Les simulies qui sont des mouches noires responsables de la circulation de l'espèce de nématode *Onchocerca volvulus* qui est un filaire provoquant l'onchocercose humaine aussi nommée la « cécité des rivières ».
- Les triatominae responsables de la transmission du parasite *Trypanosoma cruzi*, l'agent provoquant la maladie de Chagas. Cette maladie est l'une des plus importantes en Amérique Centrale et du Sud.
- Les glossinae ou mouches Tsétsé sont des mouches noires responsables de la circulation de parasites du genre *Trypanosoma* et notamment de *T. brucei*, responsable de la maladie du sommeil.
- Les puces, connues notamment pour transmettre la bactérie *Yersinia pestis*, l'agent causatif de la peste noire.
- Les poux, responsables de la transmission de maladies telles que le Typhus causé par la bactérie *Rickettsia prowazekii* ou des fièvres récurrentes mondiales pouvant être mortelles causées par la bactérie *Borrelia recurrentis*.
- Les phlébotomes pouvant transmettre des parasites du genre *Leishmania* responsables des leishmanioses cutanée et viscérale à travers le monde ou encore la fièvre pappaticci causée par des arbovirus (virus transmis par des arthropodes).
- Les moustiques qui sont, au niveau mondial, les vecteurs principaux de maladies humaines notamment des maladies virales. Ils sont principalement représentés par trois genres : le genre *Aedes* responsable de la circulation des virus causant les maladies virales les plus importantes telles que les virus du Chikungunya, de la Dengue, de la fièvre jaune et de Zika ; le genre *Anopheles* responsable de la transmission du genre de parasite *Plasmodium* spp., agent causatif de la Malaria ; et pour finir le genre *Culex* responsable de la circulation de virus de maladies virales à encéphalites émergentes tels que les virus du Nil orientale (West Nile virus) ou encore celui de l'encéphalite Japonaise.
- Les tiques (*Acarida*) sont au premier rang des agents pathogènes vectoriels d'importance vétérinaire et au deuxième rang pour les maladies humaines juste après les moustiques (Nicholson et al., 2019; Parola and Raoult, 2001). Les tiques sont des arthropodes hématophages de l'ordre des *Ixodida*. Elles sont composées de trois familles réparties dans le monde entier : *Ixodidae* divisée en Prostriata (sous-famille *Ixodinae*, genre *Ixodes*) et Metastricata (toutes les autres sous-familles, genres : *Anamalohimalaya*, *Cosmiomma*, *Dermacentor*, *Hyalomma*, *Margaropus*, *Nosomma*, *Rhipicentor* et *Rhipicephalus*) également nommées "tiques dures", et comprenant la plupart des espèces ; *Argasidae* divisées en deux sous-familles, les *Argasinae* (genre *Argas*) et les *Ornithodorinae* (genres : *Ornithodoros*, *Otobius* et *Carios*) également nommées "tiques molles" ; et *Nuttalliellidae* composées d'une seule espèce (Beati and Klompen, 2019).

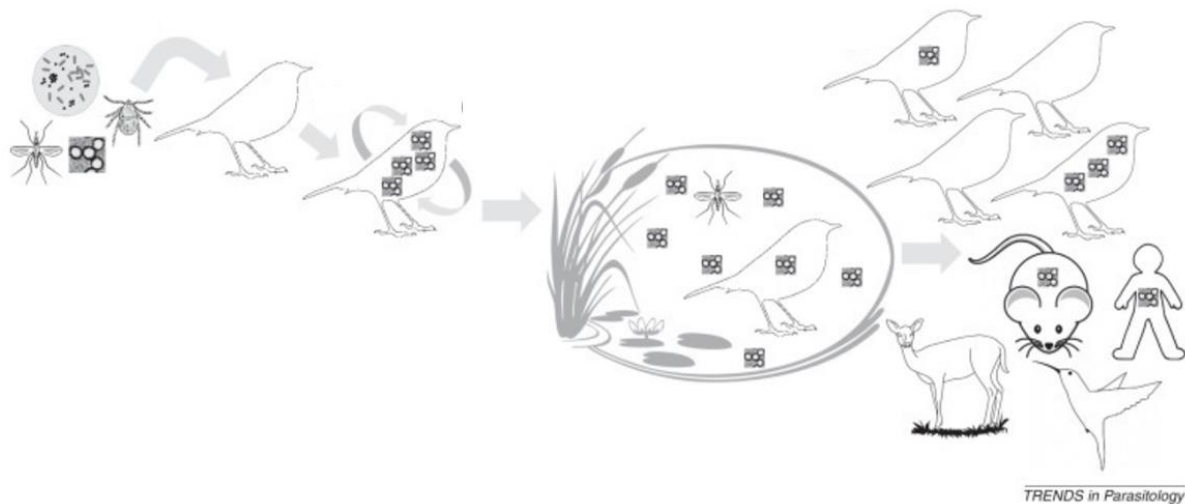
Le cycle de vie de la tique est composé de quatre étapes : un œuf, une larve à six pattes, une nymphe à huit pattes sans organe sexuel et un adulte mâle ou femelle à huit pattes. Le tropisme des tiques dépend de l'espèce et du stade. Elles peuvent être monotropes, ditropes ou téléotropes : respectivement un, deux ou plusieurs hôtes (Figure 2). Elles peuvent transmettre des agents pathogènes en se nourrissant sur un large spectre d'espèces terrestres, notamment les animaux sauvages (mammifères, avifaune, reptiles et amphibiens) ainsi que le bétail et les animaux de compagnie (Inci et al., 2016; Mendoza-Roldan et al., 2019; Pajoro et al., 2018; Sonenshine, 1991). Les agents pathogènes qu'elles abritent peuvent être à la fois d'importance sanitaire animale, humaine ou les deux dans le cadre d'agents pathogènes zoonotiques (Sonenshine, 1991). Elles peuvent transmettre une grande diversité de bactéries, virus et parasites (Nicholson et al., 2019). Comme la maladie de Lyme en Europe, cette zoonose est provoquée par des bactéries spirochètes du groupe *Borrelia burgdoferi sensus lato* du genre *Borrelia*. En Europe, cela inclut *Borrelia burgdoferi sensu stricto*, *Borrelia afzelii* et *Borrelia garinii*, avec environ 65 500 cas chaque année (Chomel, 2015). Un des virus transmis par les tiques est celui de la fièvre hémorragique de Crimée-Congo du genre *Nairovirus*, responsable de nombreuses épidémies dans le monde entier avec un taux de mortalité élevé de 40 % (WHO, 2013). Elle est principalement transmise par les tiques du genre *Hyalomma* qui agissent à la fois comme vecteur et réservoir (Perveen et al., 2021). En ce qui concerne les parasites, les agents pathogènes appartenant au genre *Babesia* sont transmis par les tiques et peuvent constituer des menaces pour la santé vétérinaire des ongulés domestiques et sauvages (Beuglet and Moreau, 2015).



**Figure 2.** Cycles de vie de tiques téléotropiques (A), ditropiques (B) et monotropiques (C) d'après Sonenshine and Roe (2013).

Chacun de ces vecteurs peut avoir une spécificité et une préférence pour un ou plusieurs groupes d'hôtes qui, eux même, peuvent être des hôtes compétents pour la transmission d'agents pathogènes à d'autres hôtes et vecteurs (Nunn et al., 2021). La compétence d'hôte d'un

individu animal ou humain dépend de différents facteurs (Figure 3). Le premier se réfère au comportement des vecteurs et des agents pathogènes qui mène à l'infection de l'hôte par un agent pathogène à travers l'action d'un vecteur. Le deuxième dépend du taux d'infection de l'hôte par l'agent pathogène, ainsi que de la condition physique de l'hôte (vivant ou mort). Cela doit être suivi par une dynamique des populations suffisantes de l'hôte au niveau individuel afin de permettre des transmissions intraspécifiques et interspécifiques avec des prévalences importantes de l'agent pathogène. Cette dynamique dépend de critères biométriques de l'hôte ou du groupe d'hôtes (sexe, âge, population, espèces) qui pourraient faciliter la dynamique d'infection et la propagation d'un agent pathogène (Gervasi et al., 2015).



**Figure 3.** Etape de l'infection d'un hôte compétent d'après Gervasi et al. (2015).

Dans le cadre des maladies à tiques, la majorité du règne animal peut faire office d'hôte. Cela a déjà été démontré par exemple chez la souris *Peromyscus leucopus* pour la bactérie *Borrelia burgdoferi* aux Etats-Unis (Martin et al., 2016).

Parmi ces nombreux hôtes, certains groupes d'animaux sont majoritairement étudiés comme celui des mammifères sauvages. De nombreux agents pathogènes vectorisés ou non peuvent être détectés tels que des bactéries du genre *Borrelia* qui provoque la maladie de Lyme ou encore de la bactérie *Mycobacterium bovis* agent de la tuberculose bovine (Cowie et al., 2016; Hrazdilová et al., 2021). Ils peuvent être porteurs de nombreux agents pathogènes transmissibles par la consommation de viande, tels que des virus comme celui de l'hépatite E, des parasites tels que *Trichinella britovi* responsable de la trichinellose et *Echinococcus canadensis* responsable de l'échinococcose dans des populations de sangliers (Richomme et al., 2010b; Umhang et al., 2014). Ils ont aussi été retrouvés porteurs d'agents pathogènes transmissibles par contact direct et indirect tels que le virus responsable de la maladie d'Aujeszky, et le parasite responsable de la toxoplasmose : *Toxoplasma gondii* (Charrier et al., 2018; Richomme et al., 2009). Ils sont également porteurs d'agents pathogènes transmis par les vecteurs, comme les Rickettsia du groupe de la fièvre boutonneuse (*Rickettsia slovaca* et *Rickettsia aeschlimannii*), *Rickettsia raoultii*, *Rickettsia conorii* (agent de la fièvre boutonneuse méditerranéenne). L'importance des mammifères sauvages en tant qu'hôte d'agents pathogènes vectorisés et non vectorisés, couplée à leur capacité à interagir avec d'autres animaux, en font un élément clef dans la circulation de divers agents pathogènes (Cowie et al., 2016; Hrazdilová et al., 2021).

Le deuxième groupe d'animaux à considérer est l'avifaune, en particulier les passeriformes, appelés plus communément les passereaux, qui regroupent plus de 5 000 espèces (Schmidt et al., 2015). La mer Méditerranée est une des principales zones de migration, automnale de l'Europe du Nord vers l'Afrique du Nord ou le sub-Sahara, et printanière de l'Afrique vers l'Europe. Elle est principalement définie par deux voies de migration : une à travers la France, l'Espagne et le détroit de Gibraltar, et une à travers la Turquie et Israël (Bruderer and Liechti, 1999; Mellone, 2020). Cependant, une partie de cette migration se fait également à travers les îles méditerranéennes (Bruderer and Liechti, 1999; Maggini et al., 2020; Vagliano, 1985). De nombreux agents pathogènes peuvent circuler par le biais de la migration aviaire que ce soit des virus tels que le virus West Nile et le virus Usutu transmis par les moustiques qui sont dangereux pour les oiseaux, les humains et un grand nombre d'autres espèces animales (Levison, 2015; Maquart et al., 2017; Vilibic-Cavlek et al., 2020) ; des bactéries comme *Borrelia burgdoferi* s.l., par la circulation de vecteurs (tiques) (Benskin et al., 2009) ; ou des parasites comme *Trypanosoma* spp., *Haemoproteus* spp., *Hepatozoon* spp. et *Leucocytozoon* spp. transmis par des arthropodes piqueurs (O'Donoghue, 2017). Cette migration peut également favoriser la dissémination d'agents pathogènes non vectorisés tel que *Chlamydia psittacci*, responsable de psittacose dans les populations aviaires (Levison, 2015). L'interaction entre l'avifaune sédentaire et l'avifaune migratrice peut accentuer la propagation et l'établissement de nouveaux ectoparasites et agents pathogènes. Cette circulation d'agents pathogènes, induite par la migration aviaire, peut être favorisée près de zones humides qui sont des lieux de pose et de repos privilégiés des oiseaux (Jourdain et al., 2007).

Le troisième groupe à considérer est celui des animaux d'élevages. Ils sont les hôtes potentiels de nombreux agents pathogènes comme ceux de la brucellose, la tuberculose et la toxoplasmose, ce qui a entraîné en 2013 une dépense de 1,660 milliards d'euros en Europe (Costa and Akdeniz, 2019; Knap and Doeschl-Wilson, 2020; Mévélec et al., 2020). Néanmoins, d'autres agents pathogènes zoonotiques ou non peuvent également être considérés dans les populations d'élevage tels que *Schistosoma bovis* responsable de la Bilharziose et des virus tels que les poxvirus pouvant affecter à la fois les animaux et les humains (Cicculli et al., 2022; Oleaga et al., 2019). Ces agents pathogènes peuvent également être retrouvés dans des vecteurs ectoparasites tels que les tiques : *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Borrelia* spp., *Rickettsia* spp. et *Theileria* spp. Ces derniers ont été détectés en Corse dans des tiques collectées sur des bovins et *Rickettsia* spp. dans des tiques d'ovins (Cicculli et al., 2019a, 2019b; Cicculli et al., 2020; Grech-Angelini et al., 2020). Les mammifères domestiques, tout comme les sauvages, jouent un rôle important dans la transmission de ces agents pathogènes. Ils servent d'hôte de maintenance et contribuent à propager des ectoparasites lors de leurs nombreuses interactions avec d'autres espèces (Cowie et al., 2016).

#### 4. Sites d'intérêt : les zones humides de Corse

La Corse est la plus grande île française de la mer Méditerranée. Elle est située à 15 km du territoire terrestre italien le plus proche (l'île de Sardaigne) et à 160 km de la côte française. Son emplacement géographique se trouve sur le trajet de voies migratoires animales, telle la migration aviaire, et au cœur de mouvements de populations humaines (Jourdain et al., 2007). La Corse est divisée en deux départements administratifs (Haute-Corse et Corse-du-Sud), avec une population de 322 000 habitants, qui est multipliée par 4 pendant la saison touristique estivale avec environ 35 millions de nuitées ("Synthèse - Le poids du tourisme dans l'économie corse – Le poids du tourisme dans l'économie corse | Insee," n.d.). Elle est le lieu de nombreuses activités humaines touristiques ou agricoles. En 2019, le principal élevage en Corse était le mouton, avec un total supérieur à 100 000 têtes, suivi des bovins, du porc et des chèvres (Chambre d'agriculture Corse, 2021). En Corse, la chasse au sanglier est une tradition très



ancienne qui reste très populaire, avec plus de 26 000 sangliers chassés en 2017 (“Oncfs - Grands ongulés - Tableaux de chasse départementaux,” n.d.). La Corse se caractérise par un climat méditerranéen doux et une grande diversité de paysages, tels que des zones humides (principalement sur le littoral), des forêts et des montagnes (dont l'altitude peut atteindre 2706 m) mais également par la présence d'une forte biodiversité dont de nombreuses espèces endémiques. Au sein des différents paysages corses, les zones humides sont des zones qui sont essentielles aux activités humaines et où les interactions avec et entre les animaux sont élevées. Les zones humides de Corse constituent donc des sites d'intérêt pour l'étude des agents pathogènes dans les populations animales et pouvant avoir une incidence sur les populations humaines.

Selon le code de l'environnement français, les zones humides sont des « terrains, exploités ou non, habituellement inondés ou gorgés d'eau douce, salée ou saumâtre de façon permanente ou temporaire ; la végétation, quand elle existe, y est dominée par des plantes hygrophiles pendant au moins une partie de l'année » (Art. [L.211-1](#)) (zones-humides.org, n.d.). Depuis le 2 février 1971, les zones humides sont classifiées pour leur conservation au sein de la Convention de Ramsar grâce à un traité intergouvernemental regroupant actuellement 171 pays. C'est le premier accord multilatéral moderne sur l'environnement, la conservation et l'utilisation durable des ressources naturelles. D'après la convention de Ramsar, les zones humides sont des étendues de marais, de fagnes, de tourbières ou d'eaux naturelles ou artificielles, permanentes ou temporaires, où l'eau est stagnante ou courante, douce, saumâtre ou salée, y compris des étendues d'eau marine dont la profondeur à marée basse n'excède pas six mètres (Article 1.1). Cela inclut des zones de rives ou de côtes adjacentes à la zone humide et des îles ou des étendues d'eau marine d'une profondeur supérieure à six mètres à marée basse, entourées par des zones humides (Article 2.1). D'après la Convention de Ramsar, cinq types principaux de zones humides sont généralement reconnus : les estuaires (comprenant les deltas, vasières et marais côtier et les marécages à mangroves), les palustres (signifiant « marécageux » : marais, marécages et tourbières), les lacustres (zones humides associées à des lacs), les marines (zones humides côtières comprenant les lagunes côtières, des berges rocheuses, des herbiers marins et récifs coralliens ne dépassant pas 6 mètres de profondeur à marée basse) et les riveraines (zones humides bordant les rivières et cours d'eau). En Corse, elles sont divisées en différentes catégories : les lacs majoritairement situés en altitude ; les pozzines correspondant à des pelouses herbeuses à gazon ras sur sol herbeux situées en bordure de lac ou de sources d'eau ; les tourbières composées de tourbes par la présence d'eau stagnante et de micro-organismes provoquant la décomposition et le recyclage de matières organiques ; les forêts alluviales ; les mares temporaires caractérisées par des alternances de phases inondées et de phases sèches ; les lagunes situées sur le littoral ; et les estuaires et micro-estuaires temporaires qui comme les mares temporaires alternent des stades inondés et secs (ORZHC-Observatoire Régional des Zones Humides de Corse, n.d.). D'après l'Office de l'Environnement Corse, les zones humides répertoriées en Corse représentent 33 000 ha sur l'ensemble du territoire, ce qui montre l'importance de ces biotopes au sein du territoire insulaire (ORZHC-Observatoire Régional des Zones Humides de, n.d.) (Figure 4).. Les zones humides sont des zones clef pour les activités humaines et animales. Elles représentent l'une des principales zones d'abreuvement et de repos pour les animaux (Bacon, 1987; Hammer, 1989). Parmi ces animaux, on peut compter les ongulés sauvages tels les sangliers qui vivent à proximité de l'eau, notamment des zones humides, principalement en raison de leur régime alimentaire (Vanschoenwinkel et al., 2008), ainsi que le bétail fréquemment présent dans les zones humides qui peuvent être considérées comme des points d'eau où l'interaction avec les ongulés sauvages peut se faire (Robertson, 1997). Un autre groupe est l'avifaune fréquemment présente dans les zones humides considérées comme des points d'eau pendant leur migration où l'interaction avec les oiseaux sédentaires peut se faire (Jourdain et al., 2007). Ces zones sont également propices aux activités humaines et récréatives (Abraham et al., 2010). Ces habitats ont une grande importance en terme de santé publique compte tenu de leur rôle dans la

prolifération des vecteurs tels que les moustiques et les tiques et la transmission des agents pathogènes (Jourdain et al., 2007; Rabou et al., 2015; Rey et al., 2012). Elles sont des lieux de circulation d'agents pathogènes transmis par des arthropodes tels que des arbovirus. Comme c'est le cas pour le West Nile virus à la fois par la forte présence de moustiques vecteurs du virus au sein de zones humides, mais également par la présence de l'avifaune sédentaire et migratrice dans ces hotspots migratoires obligatoires, comme en France continentale où ce virus a été détecté au sein de populations aviaires dans des zones humides de Camargue et de l'Ain (Jourdain et al., 2007; Lena et al., 2006; Pradier et al., 2014; Rey et al., 2012). Ces risques diffèrent suivant les espèces animales présentes dans la zone humide d'intérêt ainsi qu'en fonction de l'écosystème qui peut provoquer des changements dans la diversité et la prévalence des communautés d'ectoparasites et des agents pathogènes (Dudek, 2014).



**Figure 4.** Carte générale des zones humides de Corse d'après l'Observatoire Régionale des Zones Humides de Corse (ORZHC, n.d.).

## 5. La problématique et les objectifs de l'étude

En Corse, le contexte environnemental devient de plus en plus perturbé. Cela est dû à la fois à une localisation géographique propice à de nombreux mouvements de populations et à une urbanisation croissante, associée à des pressions climatiques. Dans le cadre du concept One Health, mettant en avant les interactions entre les trois santés (humaine, animale et environnementale), ces perturbations pourraient faciliter l'émergence, la prolifération et la circulation de nombreux agents pathogènes et de vecteurs.

Actuellement diverses études ont mis en évidence la présence de divers agents pathogènes zoonotiques ou non de différents groupes : Bactérie, Parasite et Virus ainsi que de nombreux vecteurs tels que des moustiques et des tiques sur le territoire corse. Cela pourrait favoriser leurs circulations au sein des populations d'animaux sauvages et domestiques ainsi qu'à l'Homme. Ces circulations seraient d'autant plus facilitées par le contexte climatique et les activités humaines notamment en zones sensibles. Cela pourrait être le cas des zones humides de Corse qui sont particulièrement soumises à de nombreuses perturbations.

Ces zones humides sont des lieux de fortes diversités animales pouvant être la cible de nombreux agents pathogènes et vecteurs. C'est notamment le cas pour plusieurs groupes représentatifs de la faune corse : les sangliers, les animaux d'élevage et l'avifaune. La circulation des agents pathogènes et vecteurs au sein de ces groupes peut représenter un risque diffus en termes de santé animale et humaine qui nécessite un état des lieux de la diversité des agents pathogènes au sein de ces différents groupes. Ce travail permettrait de combler un manque actuel de données sur la présence de nombreux agents pathogènes au sein de ces zones humides. L'objectif de ce travail vise à effectuer une détection des agents pathogènes zoonotiques et non-zoonotiques, vectorisés et non vectorisés simultanément dans des groupes d'animaux et leurs ectoparasites : sangliers, animaux d'élevage et avifaune au sein de zones humides de Corse.

Le corps principal du manuscrit est organisé sur la base d'articles publiés ou soumis. Il est composé de deux parties :

- La première partie, composée des chapitres 1 et 2, a pour but de répondre à différentes questions : Quel est l'état de l'art à l'échelle du bassin méditerranéen des agents pathogènes transmis par les tiques au sein des animaux domestique et sauvages ? Est-ce que les îles méditerranéennes jouent un rôle particulier dans leurs répartitions ? Les chapitres 1 et 2 visent donc à répertorier et à analyser respectivement les agents pathogènes transmis par les tiques au sein des animaux domestiques et des animaux sauvages dans le bassin méditerranéen entre les années 2000 et 2021 par le biais de deux revues bibliographiques.
- La deuxième partie, composée des chapitres 3, 4 et 5, a pour but de répondre à différentes questions : Quelles sont les principaux agents pathogènes présents au sein de trois grands groupes de la faune des zones humides de Corse et de leurs ectoparasites : les sangliers, les animaux d'élevages et l'avifaune ? Les chapitres 3, 4 et 5 ont pour but de rechercher un panel d'agents pathogènes commun et spécifique à chaque groupe d'animaux.

Ces deux parties seront précédées d'un chapitre sur la méthodologie qui expliquera la méthode PRISMA utilisée pour les chapitres 1 et 2. Elle expliquera par la suite les méthodes utilisées lors des campagnes d'échantillonnages ainsi que la sélection et la technique de détection des agents pathogènes lors des analyses concernant les chapitres 3,4 et 5.

Les chapitres 1 à 5 seront suivies d'une partie discussion, qui portera sur les agents pathogènes et vecteurs détectés présents au sein des différents groupes en zones humides de Corse, ainsi que d'une conclusion.

## **Matériel & Méthodes**

## 1. La méthode PRISMA

La méthode utilisée lors de la recherche bibliographique est la méthode PRISMA (Moher et al., 2015). Elle a pour but de mettre en place un protocole détaillé décrivant les méthodes de recherches et de sélections des publications d'intérêt de manière la plus compréhensive.

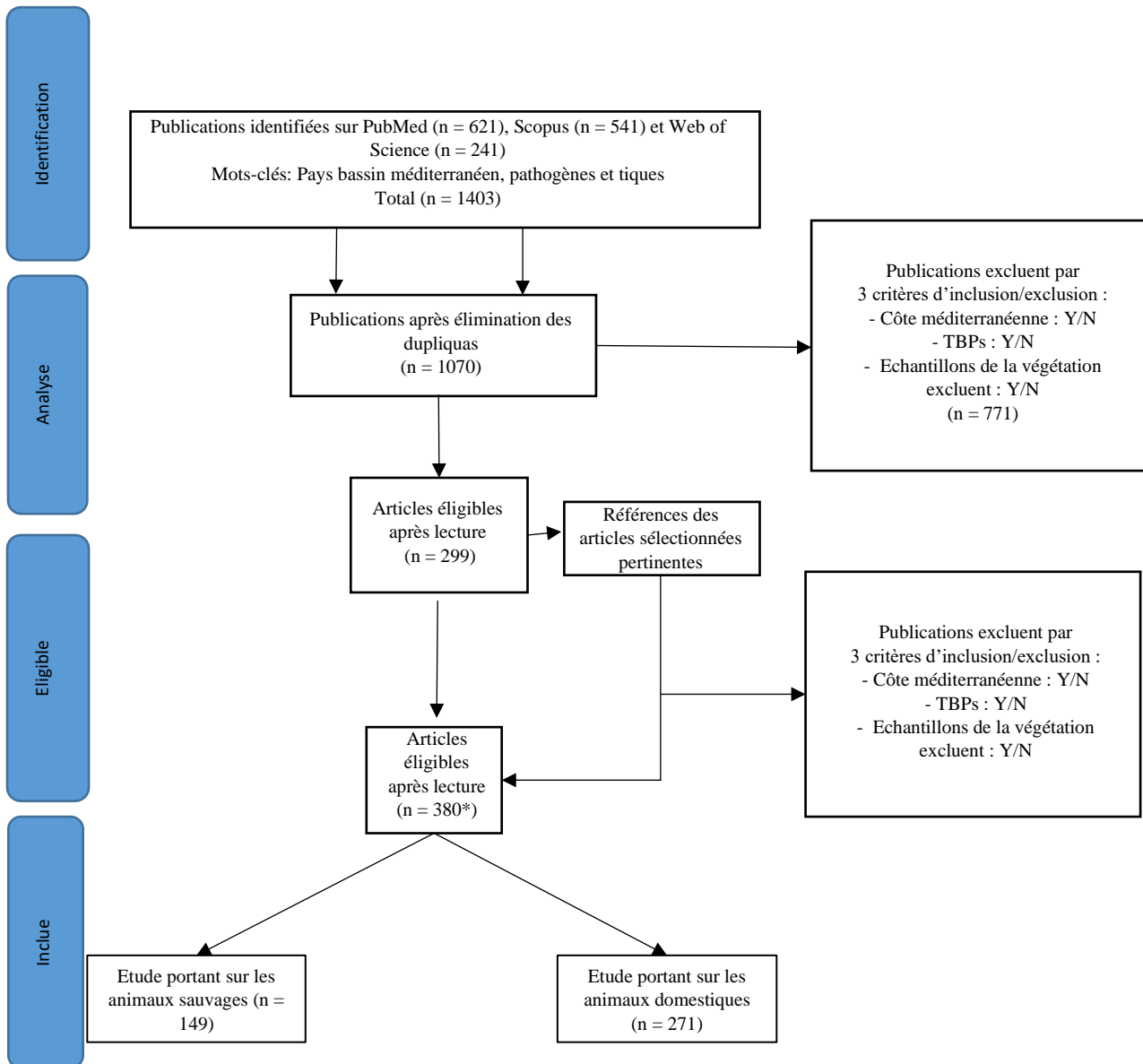
La première étape a été de sélectionner des articles au sein de trois moteurs de recherches répondant à trois mots clés : ticks, pathogènes et les noms des pays méditerranéens (la recherche a été faite séparément pour chaque pays du pourtour méditerranéen). L'étape suivante a été l'exclusion de tout duplicata. La troisième étape a été la sélection ou l'élimination des publications par trois critères de sélection :

- Le pays a-t-il une côte sur la mer Méditerranée ?
- La publication concerne-t-elle les agents pathogènes transmis par les tiques (ou TBPs) ?
- La publication exclue-t-elle les tiques collectées dans la végétation ?

Ce dernier critère a été ajouté car dans cette recherche bibliographique comme dans nos études de terrain, les travaux se sont concentrés à la fois sur les agents pathogènes présents dans les ectoparasites et dans les animaux. Cela avait pour but de déterminer de potentielles corrélations entre le deux.

Si les publications préalablement sélectionnées ont été positives à ces trois critères, elles sont conservées sinon elles sont éliminées. Une fois les articles sélectionnés, des références d'intérêt ont été identifiées dans chaque article et soumises à nouveau aux trois critères de sélection. Cela nous a permis d'obtenir un total de 380 publications qui ont été séparées entre les deux revues bibliographiques réalisées. Le protocole détaillé est décrit en Figure 5.

Pour finir, les données des publications sélectionnées portant sur les caractéristiques agents pathogènes détectés et recherchés, les animaux et les espèces de tiques échantillonnés ainsi que les données bibliographiques ont été répertoriés dans un jeu de données pour analyse.



\*une partie des articles portent à la fois sur les animaux sauvages et les animaux domestiques

**Figure 5.** Schéma PRISMA de sélection des publications portant sur les TBPs présents dans les animaux domestiques, sauvages et leurs tiques dans le bassin méditerranéen.



## 2. Echantillonnage des différents groupes d'animaux

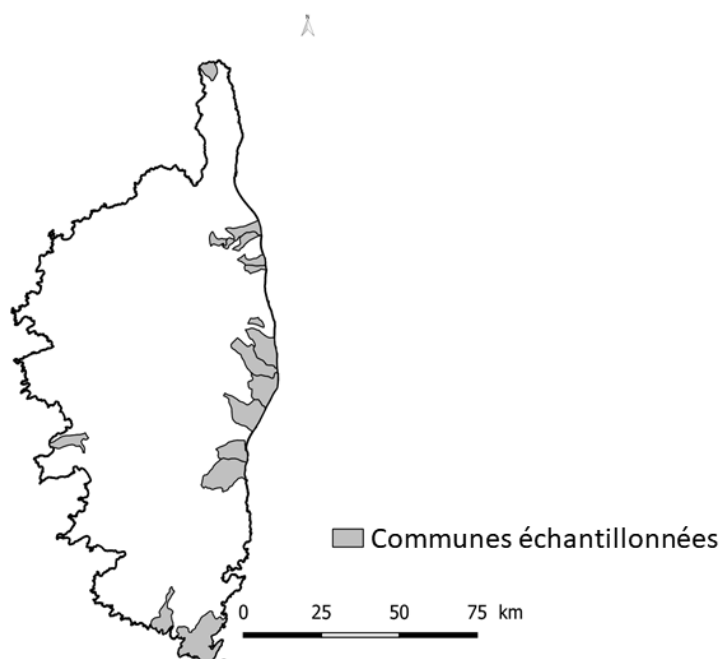
Dans cette section sera présenté les différentes investigations de terrain réalisées pour les trois groupes d'intérêt : sangliers, animaux d'élevages et avifaune, ainsi que les analyses effectuées.

### Les sangliers :

Les sangliers ont été sélectionnés comme groupe représentatif de la faune sauvage corse par sa population importante (26 001 chassés en 2017) ainsi que par sa proximité potentielle avec l'homme et de nombreuses espèces d'animaux sauvages et domestiques partageant des biotopes communs (Cowie et al., 2016).

Cette campagne de terrain a été réalisée en collaboration avec la Fédération de chasse de Haute-Corse et les lieutenants de Louveterie qui ont participé à la mise en place des protocoles d'échantillonnages et à la prise de contact avec les différentes équipes de battue de Haute-Corse. Deux méthodes d'échantillonnages ont été ainsi utilisées. Elles reposent toutes les deux sur la création d'un kit de prélèvement composé de : tire tique, tubes pour les prélèvements d'organes et d'ectoparasites, tubes EDTA pour les prélèvements de sang ainsi qu'une fiche informative.

La première méthode d'échantillonnage a consisté à fournir cinq kits à chaque équipe de battue. Par la suite, une fois ces kits remplis, ils ont été conservés au frais (+4°C) jusqu'à récupération dans les 48h, puis stockés à -80°C après identifications des ectoparasites. La deuxième méthode mis en œuvre a consisté à participer à des battues spécifiques (battues administratives et tirs de nuit). Dans le cadre de ces battues, et après l'autorisation des chefs d'équipes de battue et des lieutenants de louveterie, des prélèvements ont été directement réalisés. Ces campagnes de prélèvements ont débuté à l'hiver 2018-2019 jusqu'à la fin de l'hiver 2019-2020 dans des sites proches de zones humides littorales corses (Figure 6). Un total de 523 échantillons a ainsi été collecté : 100 échantillons de sang, 310 échantillons d'organes et 113 tiques adultes gorgées.

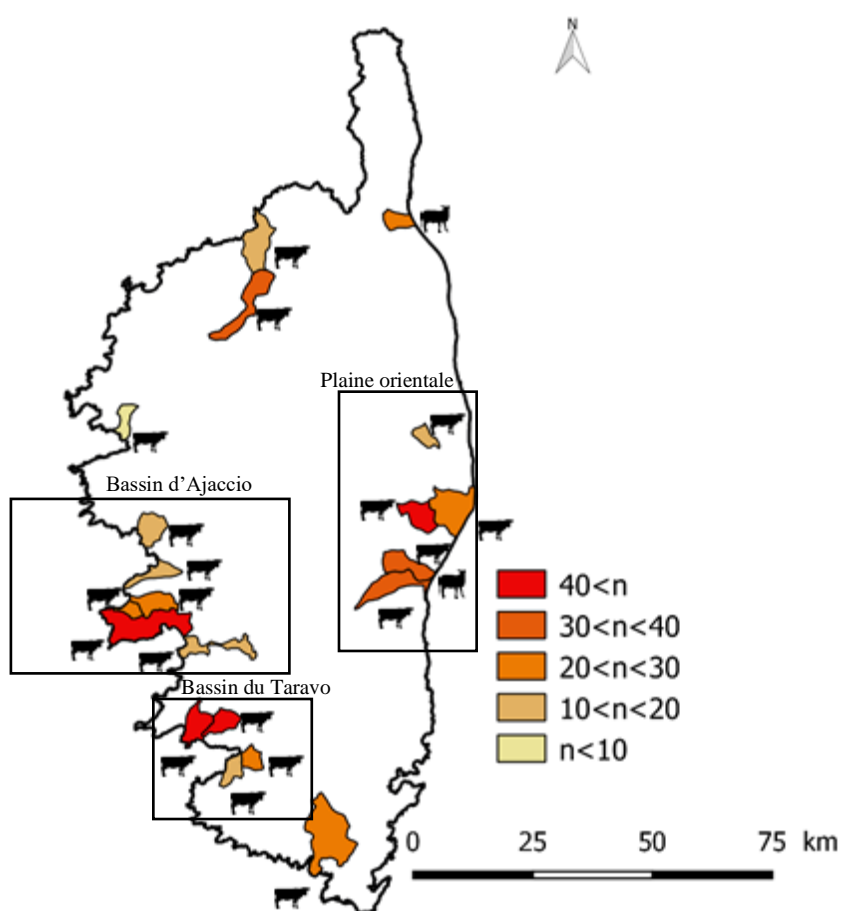


**Figure 6.** Carte des communes où les sangliers ont été échantillonnés.

### Les animaux d'élevages

Au sein des différents élevages présents sur le territoire corse (bovins, ovins, caprins, porcins et apicoles), deux principaux groupes ont été échantillonnés : bovins et ovins.

Cette campagne de terrain a été réalisée en collaboration avec le Groupement Technique Vétérinaire (GTV) de Corse et des vétérinaires responsables des campagnes de prophylaxie pour la tuberculose. Cette campagne a été basée selon la méthodologie suivante : lorsqu'une campagne de prophylaxie était prévue dans un élevage près d'une zone humide, nous nous déplaçons dans l'élevage avec le vétérinaire. Nous effectuons la collecte d'ectoparasites pendant que le vétérinaire réalisait les prélèvements de sang. Un total de 806 échantillons a été récupéré sur une période allant de fin hiver 2018-2019 jusqu'à fin hiver 2019-2020 à savoir 362 échantillons de sang et 444 tiques. L'effort d'échantillonnage s'est avéré centré sur trois microrégions : la plaine orientale, le bassin d'Ajaccio et le bassin du Taravo (Figure 7).

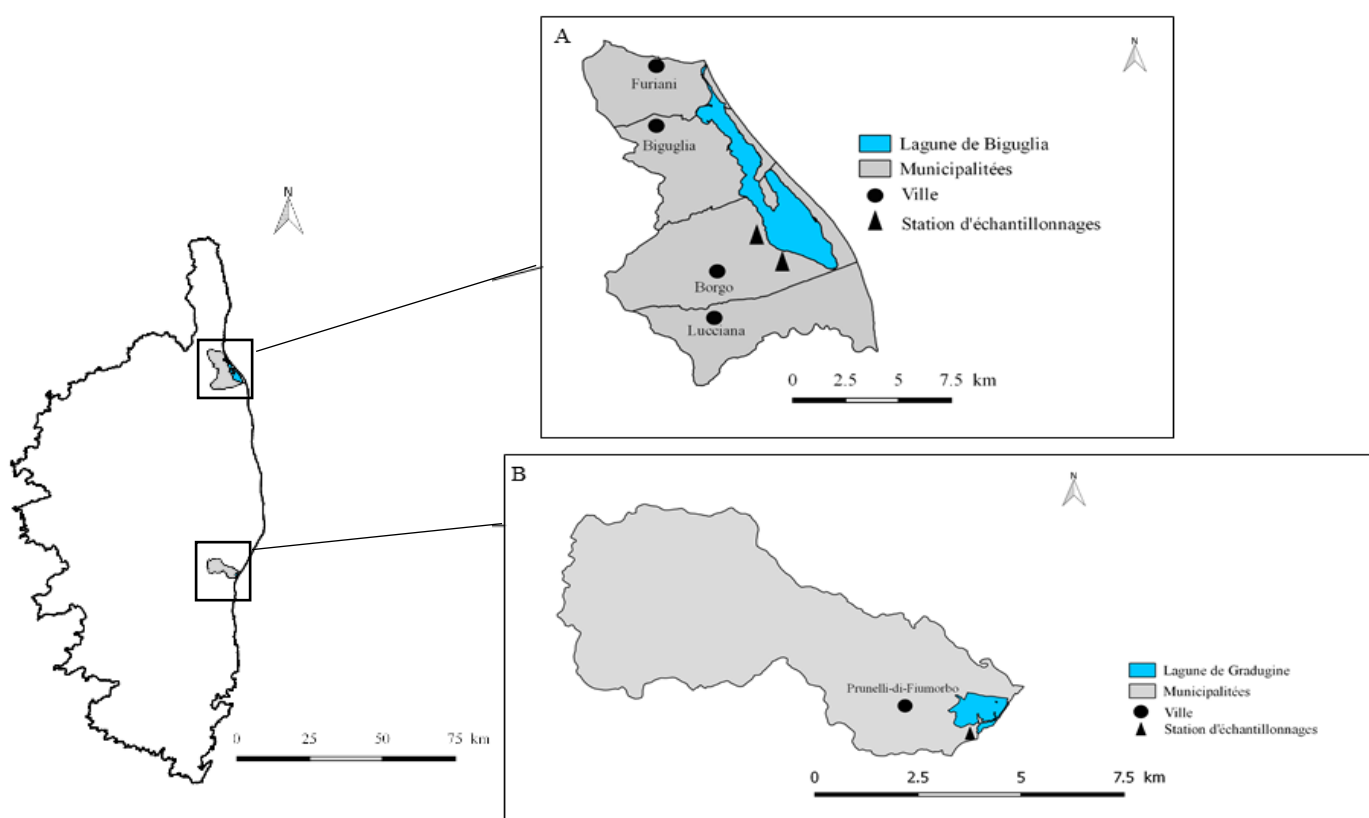


**Figure 7.** Carte des communes où les animaux d'élevages ont été échantillonnés.

## L'avifaune

Le troisième groupe échantillonné est l'avifaune, séparée en deux catégories : l'avifaune migratrice qui est une potentielle entrée d'agents pathogènes et de vecteurs en zones humides corses et l'avifaune sédentaire qui partage un biotope commun avec l'avifaune migratrice et pourrait jouer un rôle de réservoir potentiel.

Cette dernière campagne de prélèvement a été effectuée en collaboration avec un bagueur assermenté du C.R.B.P.O.. La première étape a été de proposer un projet soumis et accepté par le C.R.B.P.O.. Par la suite, des captures ont été effectuées lors des saisons de migrations printanières et automnales. Les captures ont été faites grâce à la disposition de filets de capture. Les oiseaux capturés ont été démaillés toutes les demi-heures ou toutes les heures suivant l'afflux d'oiseaux ou les conditions météorologiques. Le bagueur a effectué ensuite les mesures biométriques, les prélèvements de sang et d'ectoparasites. Les oiseaux ont été libérés cinq minutes après les manipulations. Un total de 834 échantillons a été récupéré dans deux zones humides : étang de Gradugine en plaine orientale (613 échantillons) et l'étang de Biguglia dans le bassin bastiais (230 échantillons avec l'intervention du bagueur assermenté de la Réserve Naturelle de l'Etang de Biguglia) à savoir : 762 échantillons de sangs, 37 mouches plates et 35 tiques (Figure 8). Cette campagne de terrain a eu lieu lors de trois saisons de migration : Automne 2019, Automne 2020 et Printemps 2021. Initialement, la dernière saison devait se réaliser au printemps 2020, mais elle a été décalée en raison de la crise sanitaire liée à la Covid-



19.

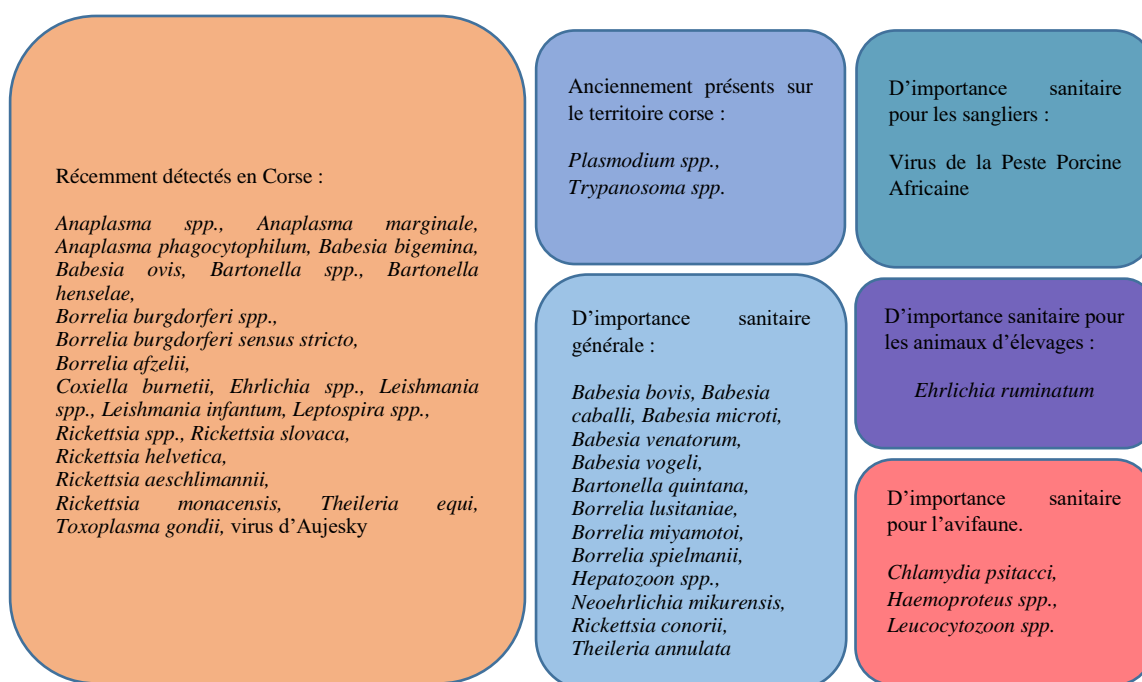
**Figure 8.** Carte des communes où l'avifaune a été échantillonnée : Etang de Biguglia (A) et étang de Gradugine (B).

### 3. Analyses des agents pathogènes

Un total de 42 agents pathogènes a été recherché dans les trois groupes d'animaux. Cela avait pour but d'effectuer une analyse comparative des résultats obtenus entre les trois groupes. Toutefois, il faut préciser qu'une majorité des agents pathogènes sont connus pour être communs aux trois groupes tandis que d'autres sont spécifiques à un groupe. Les agents pathogènes ont été sélectionnés selon les critères suivants (Figure 9) :

- Récemment détectés en Corse.
- Anciennement présents sur le territoire corse.
- D'importance sanitaire générale.
- D'importance sanitaire pour les sangliers.
- D'importance sanitaire pour les animaux d'élevages.
- D'importance sanitaire pour l'avifaune.

Ce panel d'agents pathogènes a été recherché par l'intermédiaire de la méthodologie de screening à haut débit micro fluidique. En complément, deux agents pathogènes spécifiques à l'avifaune ont été recherchés dû à leur importance sanitaire : virus West Nile et virus Usutu.



**Figure 9.** Liste des agents pathogènes recherchés.

## Matériel & Méthode

La méthode utilisée pour l'analyse est le screening à Haut-débit par puce micro fluidique (PCR en temps réel). Cette technique à l'avantage de permettre la détection d'un grand nombre d'agents pathogènes dans un grand nombre d'échantillons de manière simultanée : 45 agents pathogènes dans 45 échantillons pour notre étude. Néanmoins, elle permet de confirmer uniquement la présence des acides nucléotidiques d'un agent pathogène et non la présence de l'agent pathogène vivant. Les analyses sont faites en collaboration avec l'UMR BIPAR du Laboratoire de Santé Animale ANSES de Maisons-Alfort. La méthode d'analyse s'est décomposée en plusieurs étapes, effectuées soit à l'Université de Corse (UMR SPE), soit à l'UMR BIPAR :

- Extraction d'ADN de 2214 échantillons (Université de Corse)
- Pré-amplification de l'ADN et analyse à la puce micro fluidique (UMR BIPAR)
- Confirmation des résultats par Nested PCR et qPCR (UMR BIPAR)
- Envoi à Eurofins© pour séquençage (UMR BIPAR)
- Analyse bio-informatique par le logiciel BioEDit© (UMR BIPAR)

La méthode utilisée est transcrite de manière plus précise dans la partie méthodologie de chaque article ci-après.

**Chapitre 1 : Agents pathogènes chez les animaux domestiques et leurs  
tiques dans le bassin méditerranéen entre 2000 et 2021**

## 1. Résumé de la publication « Distribution of Tick-Borne Pathogens in Domestic Animals and Their Ticks in the Countries of the Mediterranean Basin between 2000 and 2021: A Systematic Review »

Cette revue systématique a fait l'objet d'une publication dans la revue *Microorganisms* : Baptiste Defaye, Sara Moutailler, Vanina Pasqualini, Yann Quilichini (2022). *Distribution of Tick-Borne Pathogens in Domestic Animals and Their Ticks in the Countries of the Mediterranean Basin between 2000 and 2021: A Systematic Review*. *Microorganisms*, 10(6), 1236. <https://doi.org/10.3390/microorganisms10061236>

### Résumé en Français

Cette revue est la première partie d'un travail ayant pour intérêt de lister les différents agents pathogènes transmis par les tiques (TBPs) détectés dans les populations d'animaux domestiques et leurs tiques dans le bassin Méditerranéen entre 2000 et 2021 pour la première fois dans une revue bibliographique. Dans cette revue, nous avons pris en compte un total de 271 publications concernant les TBPs chez les animaux domestiques du bassin méditerranéen entre début 2000 et février 2021. Au cours de ces deux décades, plus de publications ont été trouvées lors de la décade 2010-2020 que lors de la décade 2000-2010. Cela pourrait être dû à différents facteurs, le premier étant un intérêt scientifique accru ainsi que l'utilisation de plus en plus fréquente de méthodes de biologie moléculaire facilitant ainsi les analyses. A cela pourrait s'ajouter une accessibilité aux publications facilitées durant la période 2010-2021.

Parmi les 271 publications analysées, 56,5 % concernaient des bactéries, 37,7 % des parasites et 5,80 % des virus. Un total de 90 agents pathogènes de 18 genres a été détectés chez les animaux domestiques et leurs tiques dans la région méditerranéenne : 11 genres de bactéries, quatre genres de parasites et trois genres de virus. Parmi ces 90 TBPs, 60 % étaient zoonotiques et 40 % non-zoonotiques ou de statut inconnu. Parmi les 90 TBPs détectés, 73,3 % ont été trouvés dans les tiques et 76,7 % dans l'hôte animal, la moitié des TBPs ont été détectés dans les deux (51,1 %). De ces différents genres de pathogènes, les plus reportés sont des genres de bactéries tel qu'*Anaplasma* spp. (25,8 %) genre composé de bactéries intracellulaires Gram-négatives appartenant à la famille des *Anaplasmataceae*. La plupart de ces bactéries sont zoonotiques et ont un impact important sur la santé vétérinaire comme *Anaplasma marginale*, *Anaplasma bovis*, *Anaplasma ovis* et également sur la santé humaine comme *Anaplasma phagocytophilum* et *Anaplasma platys* (Ben Said et al., 2018; Kocan et al., 2015). Le genre *Rickettsia* (25,3 %) qui est également un genre de bactéries intracellulaires composé de deux groupes : le groupe de la fièvre tachetée (SFG) comme *Rickettsia conorii* agent de la fièvre boutonneuse méditerranéenne, et le groupe du typhus (TG) qui sont moins connus comme *Rickettsia typhi* (Abdad et al., 2018; Tomassone et al., 2018). Ainsi que des genres de parasites tel que *Babesia* spp. (17,7 %) qui sont des parasites protozoaires érythrocytaires transmis par les tiques qui provoquent des babésioses chez les animaux et les humains. Les principales tiques responsables de la transmission de *Babesia* sont du genre *Ixodes* et transmettent des espèces de *Babesia* comme *B. divergens*, *B. duncani*, *B. microti*, *B. venatorum*, *B. vogeli* et *B. canis* (responsable de la babésiose canine).

Ces différents agents pathogènes ont été en partie détectés dans 35 espèces et 7 genres de tiques récoltés sur les animaux domestiques du bassin méditerranéen. Parmi ces sept genres, six appartiennent aux tiques dures (*Ixodidae*) : *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* et *Rhipicephalus*. La dernière espèce qui a été collectée en Algérie et en Egypte représente les tiques molles (*Argasidae*) : genre *Argas*. Parmi ces genres de tiques, le genre *Rhipicephalus* abrite la plus grande diversité de de TBPs détectés dans le bassin méditerranéen. Il a une distribution mondiale et est extrêmement présent dans le bassin méditerranéen (Estrada-Peña, 2004). Ce genre cible un large panel d'hôtes et transmet des pathogènes provoquant des maladies ayant un impact sanitaire mondial comme l'anaplasmose,

la babésiose, l'ehrlichiose et la rickettsiose (Estrada-Peña, 2004; Pérez-Eid, 2007). Six espèces de *Rhipicephalus* ont été identifiées positives à 57 agents pathogènes dans un total de 13 pays : *Rh. (Bo.). annulatus*, *Rh. appendiculatus*, *Rh. bursa*, *Rh. (Bo.). microplus*, *Rh. pusillus* et *Rh. sanguineus* s.l.. Ce genre a été suivi par les genres *Ixodes* et *Hyalomma* dans lesquels ont été retrouvés 36 et 25 TBP respectivement.

Ces différents genres de tiques et TBPs ont été reportés sur 12 animaux domestiques, dont neuf d'élevage : Ane, Buffle, Cheval, Chèvre, Cochon, Dromadaire, Mouton, Poule et Vache, ainsi que trois animaux de compagnie : Chat, Chien et Lapin. De ces hôtes, 73,3 % des TBPs ont été détectés dans leurs tiques et 76,7 % directement dans les animaux domestiques. Les données disponibles ont montré que le mouton, la vache et la chèvre étaient respectivement impliqués comme hôte de tiques infectés et/ou de TBPs dans 15, 13 et 13 pays, ainsi que le chien et ses tiques comme hôtes positifs dans 14 pays. À l'inverse, moins de données sont disponibles sur les animaux domestiques comme le porc, le poulet et le lapin, qui ont par ailleurs fait l'objet de moins d'études. Il semble également qu'il y ait une corrélation entre le nombre d'études réalisées chez un hôte et le nombre de TBPs trouvées chez cet hôte.

Afin d'étudier la répartition des TBPs au niveau du bassin méditerranéen, nous avons distingué quatre zones. L'Europe de l'Ouest : France, Italie, Malte, Monaco et Espagne qui représente 45,7 % des publications. Les articles trouvés en Europe de l'Ouest étaient principalement axés sur les chiens, ce qui pourrait expliquer le pourcentage élevé de tiques *Rhipicephalus* trouvées et de TBPs liés au chien tel *Eh. canis* et *B. canis* provoquant respectivement l'ehrlichiose et babesiose canine. Cela peut s'expliquer par la place centrale du chien dans l'activité humaine comme animal de compagnie, chien de chasse ou chien errant. L'Afrique du Nord qui regroupe l'Algérie, l'Égypte, la Libye, le Maroc et la Tunisie avec un pourcentage de 26,1 %. En Afrique du Nord, la recherche semble se concentrer davantage sur la surveillance de la santé vétérinaire du bétail par rapport à l'Europe occidentale avec plus de publications retrouvées chez le mouton et les bovins et la détection de TBPs tels que : *A. marginale* et *A. phagocytophilum* provoquant l'anaplasmose granulocytaire bovine et humaine. Néanmoins, le principal genre de tiques positif retrouvé est également *Rhipicephalus* spp.. Avec un pourcentage de 17,7 %, de publications, le Moyen-Orient qui est composé de Chypre, Israël, Liban, Palestine, Syrie et Turquie, est la troisième région en termes de nombre de publications. Au Moyen-Orient, les données disponibles concernaient la santé vétérinaire des animaux de compagnie et des animaux d'élevage dans des proportions égales avec une forte détection de TBPs appartenant aux piroplasmides et le genre *Anaplasma* transmis par des tiques du genre *Rhipicephalus* spp.. Cependant, elles ont été principalement retrouvées en Turquie (75,5 %). Et enfin, la dernière région est celle des Balkans : Albanie, Bosnie-Herzégovine, Croatie, Grèce, Monténégro et Slovénie avec 10,33 % des publications. Aucun agent pathogène chez les animaux domestiques et leurs tiques n'a été trouvé dans 5 pays : Bosnie-Herzégovine, Libye, Malte, Monaco et Syrie. Comme pour le Moyen-Orient, la majorité des informations ont pu être trouvées à la fois chez le bétail et les animaux de compagnie avec une forte prédominance du genre *Rhipicephalus* spp. transmettant des agents pathogènes comme *Anaplasma* spp., *Rickettsia* spp. et le virus du CCHF.

De l'ensemble des données, celles concernant les îles ont été extraites pour déterminer leur importance dans la détection des TBPs chez les animaux domestiques. Elles représentent 15,9 % des publications mais 77,8 % des TBPs rapportés dans l'étude. Un total de neuf animaux domestiques et six genres de tiques ont été répertoriés. Sur les 44 publications, la plupart d'entre elles proviennent des plus grandes îles du bassin méditerranéen : la Sardaigne, la Sicile, la Corse et Chypre, tandis que peu de données ont été rapportées au niveau des îles de petite taille comme les îles grecques. Nous avons décidé de diviser ces îles en îles de méditerranée occidentale : îles Eoliennes, Corse, Sardaigne, Sicile et Majorque (36 publications) et de méditerranée orientale : Crète, Chypre, Ios, Mykonos, Santorin, Skiathos, Skopelos et Tinos (8 publications). Le chien ainsi que le genre *Rhipicephalus* spp. ont été les hôtes et tiques trouvés dans la plupart



des données dans les îles orientales et occidentales, ce qui montre l'importance de cet animal domestique dans les données.

L'hôte rapporté dans la plupart des études est le chien. Dans les quatre régions (Europe de l'Ouest, Afrique du Nord, Moyen-Orient et Balkans), nous pouvons observer le même schéma suivant la région, avec le chien comme hôte (sauf en Afrique du Nord), le genre *Rhipicephalus* comme genre de tique positif et les bactéries (genres *Anaplasma* et *Rickettsia*) comme TBPs. Cela peut montrer que les données disponibles sur les TBPs et leur diversité sont liées aux animaux domestiques ciblés. Cela pourrait être dû à l'importance vétérinaire et sociale des hôtes animaux domestiques. Les TBPs des animaux domestiques et de leurs tiques sont encore peu étudiés dans les îles (15,9 % des publications). Cependant, une grande diversité d'hôtes (neuf sur 12), de genres de tiques positives (six sur sept) et de genres de TBPs (14 sur 18) a été rapportée dans les îles. Ceci montre l'importance des îles méditerranéennes dans la surveillance des TBPs dans le bassin méditerranéen. L'amélioration de la recherche dans les îles pourrait permettre une meilleure compréhension de leur rôle en tant que point chaud de la circulation et de la migration des tiques et des agents pathogènes transmis par les tiques à travers les mouvements de populations et le commerce des animaux domestiques. Dans les îles, la plupart des données proviennent des plus grandes îles qui sont regroupées dans la partie occidentale du bassin méditerranéen.

# Distribution of tick-borne pathogens in domestic animals and their ticks in the countries of the Mediterranean Basin between 2000 and 2021: a systematic review

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**Abstract:** Tick-borne pathogens (TBPs) include a wide range of bacteria; parasites and viruses that cause a large spectrum of animal; human and zoonotic tick-borne diseases (TBDs). The object of this review was to establish an inventory and an analysis of TBPs found in domestic animals in the countries of the Mediterranean Basin. This geographic area occupies a central position between several continents and is an area of movement for animals; humans; and pathogens of interest and their vectors; which is important in terms of animal and human health. In this systematic review; we included a total of 271 publications between 2000–2021 concerning TBPs in domestic animals. Among this literature; we found a total of 90 pathogen species known as TBPs in the 20 countries of the area; detected both in tick species from domestic animals and directly from domestic animals. In all; 31 tick species were recorded and 12 domestic animal species: nine livestock and three pets. More than 50% of the publications were from Western Europe. Data islands were extracted and assessed as they represent 16% of the publications and 77.8% of the TBPs reported. Our results show the importance of islands in the monitoring of TBPs; despite the low percentage of publications.

**Keywords:** TBPs; pathogens; ticks; domestic animals; Mediterranean Basin; islands

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## 2. Introduction

Pathogens are one of the largest threats to health worldwide. They can be divided into four main groups: bacteria, parasites viruses, and fungi. These groups are presents on all the continents and in the oceans, and target all types of plants and animals, as well as humans, depending on their tropism and life cycle (Nicholson et al., 2019). These pathogens have different transmission strategies: i) direct contact between individuals from the same species or between individuals from different species; ii) indirect contact through the environment or dissemination in the air; and iii) through vectors such as hematophagous arthropods, like mosquitoes, ticks or sandflies that can transmits pathogens via blood-sucking from one host to another (Anderson and Magnarelli, 2008; Bellekom et al., 2021).

Among the different pathogens, many are zoonotic and are transmitted between animals and humans, while others are non-zoonotic and specifically target humans or animals. Zoonotic pathogens account for about 60% of pathogens worldwide and are particularly presents in emerging disease (CDC, 2020). Transmission of these pathogens by hematophagous insects has become one of the main threats to global health in recent decades (CDC, 2020).

Hematophagous arthropod vectors can transmit pathogens called vector-borne pathogens (VBPs), which may be zoonotic or non-zoonotic, from one individual to another. They can transmit bacteria, parasites, and viruses (Rosenberg and Ben Beard, 2011). For example, the role of ticks in the circulation of Lyme disease between animal and human populations in North America has been established (Chomel, 2015). Annually, vector-borne

diseases (VBDs) cause more than 17% of infections and over 700,000 deaths across the globe (WHO, 2020). The two predominant vectors, in order of importance, are mosquitoes and ticks. However, ticks contain also bacterial endosymbionts such as *Rickettsia*-like *Francisella*-like or *Coxiella*-like. They are endosymbiotic intracellular bacteria harmless to mammals which can be required for the tick survival (Ahantarig et al., 2013). In previous research, they could have been misidentified as pathogens harmful for human and animals.

Ticks (Ixodida) rank first for veterinary vector-borne pathogens and second for human pathogens, just after mosquitoes (Nicholson et al., 2019; Parola and Raoult, 2001). Ticks are hematophagous arthropods of the *Ixodida* order. They are composed of three families distributed across the world: *Ixodidae* divided into the Prostriata (subfamily Ixodinae, genus *Ixodes*) and Metastrata (all other subfamilies, genera: *Anamalohimalaya*, *Cosmiomma*, *Dermacentor*, *Hyalomma*, *Margaropus*, *Nosomma*, *Rhipicentor* and *Rhipicephalus*) it also named “hard ticks”, and includes most species; Argasidae divided into two subfamilies, the Argasinae (genus *Argas*) and the Ornithodorinae (genera: *Ornithodoros*, *Otobius* and *Carios*) also named “soft ticks”, and *Nuttalliellidae* composed of just one species (Beati and Klompen, 2019). The tick’s life cycle is divided into 4 stages: egg, six-legged larva, eight-legged nymph without sexual organs, and eight-legged adult male or female. Tick tropism depends on the species and stage. They can be monotropic, ditropic or teleotropic, with one, two or several hosts, respectively. They can transmit pathogens by feeding on a broad spectrum of terrestrial species, including wild animals (mammalian, avifauna, reptilian, and amphibian), livestock, and pets. These pathogens are named tick-borne pathogens (TBPs) and are known to be both pathogens of veterinary importance and zoonotic pathogens harmful for humans (Nicholson et al., 2019).

Ticks can transmit a wide range of bacteria, parasites and viruses (Nicholson et al., 2019). An example is Lyme disease in Europe as this zoonotic disease is caused by spirochetes bacteria from the group *Borrelia burgdorferi sensu lato* and the genus *Borrelia* which could be transmitted by a wide range of ticks (Eisen, 2020). In Europe, this includes *Borrelia burgdorferi sensu stricto*, *Borrelia afzelii* and *Borrelia garinii*, with approximately 65,500 cases every year (Chomel, 2015). One reported tick-borne virus is the Crimean-Congo Hemorrhagic Fever virus from the genus *Orthonairovirus*, which is responsible for many outbreaks internationally, with a high fatality rate of 40% (WHO, 2013). It is mainly transmitted by ticks of the genus *Hyalomma* that act as both vector and reservoir (Perveen et al., 2021). Regarding parasites, causative pathogens such as the *Babesia* genus are transmitted by ticks and can be a human and a veterinary health threats (Hildebrandt et al., 2021).

Given its geographic position between Europe and Africa, the Mediterranean Sea is bordered by a significant number of countries with a high variation of biotopes, ranging from Mediterranean to arid climates. The Mediterranean Basin is an area highly affected by climatic change, animal migration, and human activity (Giannakopoulos et al., 2005; Ochoa-Hueso et al., 2017). While increasing temperatures in Northern Europe favor the development of ticks like *Ixodes ricinus*, in the Mediterranean Basin the development of dry areas with arid types of vegetation favors the development of ticks like *Hyalomma marginatum* (Al-Abri et al., 2017). This development is also supported by feeding of immature stages on birds and the long duration of their attachment to the host during these stages (Hasle et al., 2009). This supports the potential key role of migratory birds in tick dissemination (Estrada-Peña et al., 2015). Other migration, such as movement of dogs, without restriction in the Mediterranean Basin also favors the circulation of *Rhipicephalus sanguineus* s.l. (Gray et al., 2013).

We focused our review on the countries of the Mediterranean Basin where the different TBPs detected in domestic animals and their ticks were screened. Moreover, in order to compare the geographical distribution of these TBPs, and to highlight possible changes in their spread in the future, four areas were considered: Western Europe, composed of France, Italy, Malta, Monaco and Spain; the Balkans, composed of Albania, Bosnia-Herzegovina, Croatia, Greece, Montenegro and Slovenia; the Middle East, composed of Cyprus, Israel, Lebanon, Palestine, Syria, and Turkey; and finally North Africa, composed of Algeria, Egypt, Libya,

Morocco and Tunisia. The different areas were determined by common biotope and geographic proximity. The last part of our review was to investigate the potential role of the western and eastern islands in the monitoring of TBPs in domestic animals and their ticks, according to their geographic position, surface areas, and also potential role in TBP circulation through animal migration. In this part, we focused on the distribution of TBPs in the Mediterranean islands in order to determine a potential role of the islands in the distribution of TBPs.

The aim of this study was to review the existing literature between 2000 and early 2021, following PRISMA guidelines, about the presence of TBPs on domestic animals and their ticks in the countries of the Mediterranean Basin, with the following objectives:

- Perform a bibliometric analysis of TBP studies.
- Review the diversity of TBPs, positive engorged tick species, domestic animal hosts of TBPs, and positive tick species.
- Compare the distribution of TBPs from domestic animals and their ticks in the four main areas defined.
- Focus on the distribution of TBPs in the Mediterranean islands.

### 3. Materials and Methods

We undertook a literature review concerning tick-borne pathogens in all countries of the Mediterranean Basin (n = 20). We followed PRISMA guidelines and used explicit and systematic methods to identify, select, and evaluate the studies that are relevant to the topic [20]. We compiled and evaluated the data from the studies included in this review.

All articles published in English in international journals indexed by PubMed, Scopus and Web of Science were considered (Figure 5.). The date range was from 1 January 2000 through 31 February 2021. We used keywords for each country of the Mediterranean Basin, classified in alphabetical order: Albania OR Algeria OR Bosnia-Herzegovina OR Cyprus OR Croatia OR Egypt OR France OR Greece OR Israel OR Italy OR Libya OR Malta OR Monaco OR Montenegro OR Morocco OR Palestine OR Slovenia OR Spain OR Tunisia OR Turkey AND Pathogens AND Ticks, with the option “all fields” to recover articles in which search items appeared in the title, abstract and keywords. First, all papers considered “grey” literature, literature reviews, cases studies, manuscripts and abstracts of posters from conferences and guides from relevant organizations were excluded. We also discarded clinical descriptions of disease and diagnosis in humans and animals. We selected all papers focusing on the distribution and circulation of tick-borne pathogens and their vectors. We focused on studies dealing with the distribution of these pathogens and ticks in the countries of the Mediterranean Basin and their islands. Second, we excluded duplicate and inaccessible articles (due to language or unavailable full text). We reviewed the titles and abstracts and applied inclusion/exclusion criteria on 1070 articles. Filtering was carried out by responding to the following questions:

1. Did the study include a country with a Mediterranean coast: Yes/No
2. Did the study include tick-borne pathogens: Yes/No
3. Did the study exclude ticks collected in vegetation: Yes/No

Only publications considering TBPs in domestic animals and/or TBPs in engorged tick collected on domestic animals in Mediterranean countries were included. This makes it possible to have an overview of research about TBPs potentially infesting and circulating in the domestic animal population only. The articles were saved if the answers to the three questions were ‘yes’; otherwise they were eliminated. For the next step, we reviewed the full-text and entered the information of interest into a database for 299 articles. We also excluded articles that did not fit the criteria after reading the full-text.

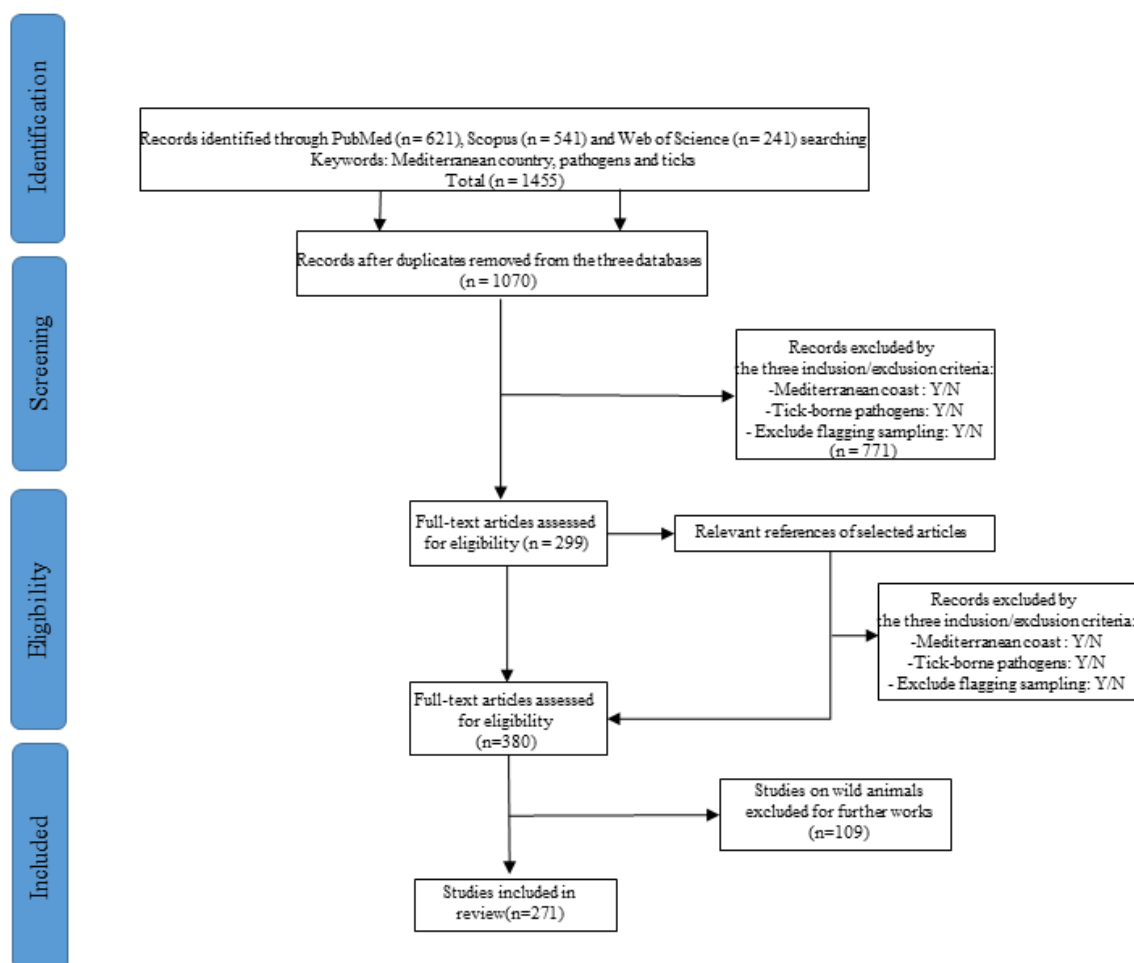
We reviewed the bibliography of each selected article in order to check for new articles to include in the review and relevant articles in the field of research. We followed the same steps previously described for new articles (n=81).

For the last step, we excluded articles dealing with wild animals (n=109) and retained only articles dealing with domestic animals (n=271). The selection steps are summarized in Figure 10, with an explanation of inclusion/exclusion of articles for this review.

The data of interest were captured in an Excel table that was tested before with 15 articles, and included the following information:

1. Main characteristics of the studies: article ID, years, authors, analytical and statistical methodology
2. Pathogen-related information: pathogens screened and detected, species, number of species, zoonotic status, host
3. Tick-related information: species, type, number, stage
4. Host-related information: groups, sedentary or migratory
5. Area of interest: country, type of area, number of sampling sites

The different outputs of the data worksheet were selected following mutual agreement from all the authors.

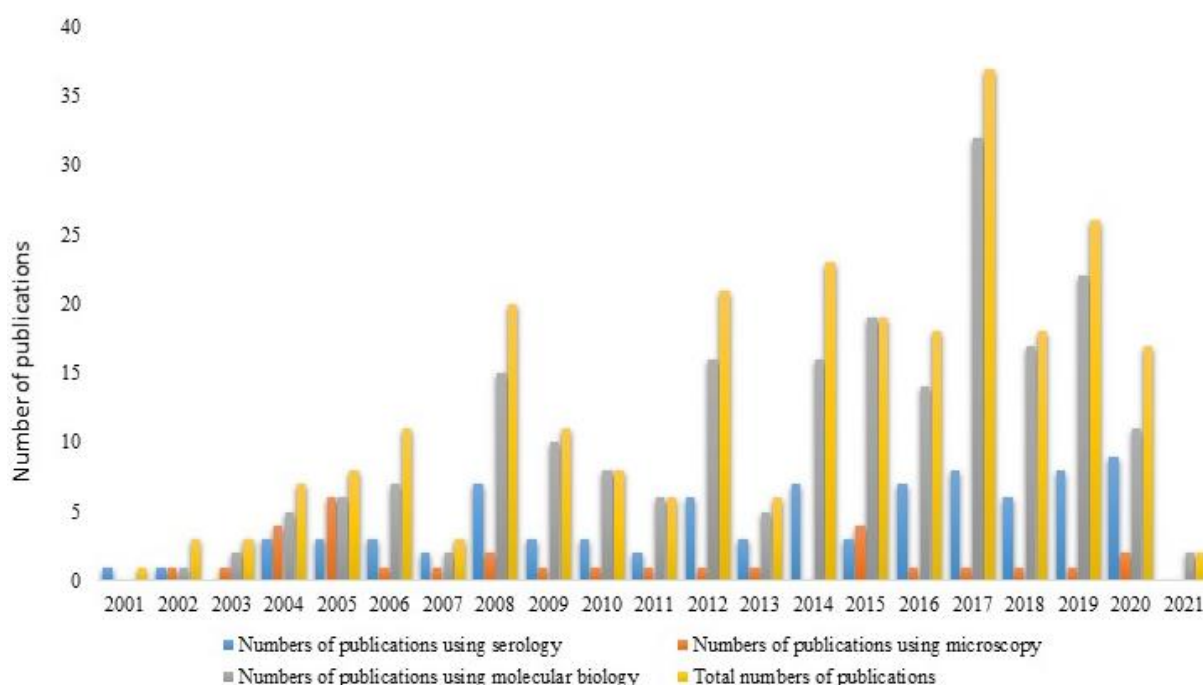


**Figure 10.** Methodological diagram of the bibliographic research following the PRISMA 2009 Flow according to Moher et al., 2015.

## 4. Results

### 4.1. Bibliographic analysis

The distribution of the publications through the years is shown in Figure 11. During the first decade, the number of papers increases slightly until 2008, with a maximum of 20 publications, and decreases in the years 2009 and 2010 (11 and nine papers, respectively). During the second decade, there is first a low rate of publication, except in 2012, with a peak of 21 publications. Between 2014 and 2021, the mean number of published papers per year was approximately 20, accounting for 60% of the published papers about tick-borne pathogens on domestic animals, with a peak of 37 papers in 2017. In 2021, the number of papers was low, because we stopped the research in February 2021. The rate of publication about tick-borne pathogens increases clearly during this period.



**Figure 11.** Number of publications through the years (2000–February 2021). The total number is indicated in yellow, and the other colors indicate the number of publications according to the detection method used (serology; microscopy; molecular biology).

This trend can be explained by different reasons:

The first is increased interest from the scientific community due to the importance of this subject in terms of human and animal health.

The second is improved accessibility of scientific journals and publications.

The third is changes in pathogen detection methodology through the years and particularly developments in the field of molecular biology.

Concerning this last point, tick-borne pathogens were typically detected using three kinds of methods (Figure 6.). The first is serological analysis involving detection of pathogens in blood and tissue samples by the way of antibodies, such as ELISA and immunofluorescence. The second is the microscopy approach, which is more common for parasites. The third is molecular biology, which covers techniques involving the detection of pathogen nucleic acids

in both tick and animal hosts, such as polymerase chain reaction (PCR), next-generation sequencing (NGS), and high-throughput sequencing techniques. Even though researchers may use more than one approach to detect and monitor TBPs, we observed that over the years, molecular biology rapidly became the main analytical approach. The increase in publications over the last ten years seems to be linked to the development and accessibility of molecular biology techniques, and to their ability to simultaneously detect a large number of pathogens.

In addition to changes in the amount of data available depending on the year, we can also note variability of these data according to their geographic origin. In total, 271 publications and 20 countries of the Mediterranean Basin were considered in this review. The mean number of publications per country is about 13, and the number of publications can vary from 0 to 76 according to the country (Figure 12.).



**Figure 12.** Number of publications dealing with TBPs in domestic animals and their ticks in the Mediterranean Basin according to country of origin.

#### 4.2. Tick-borne pathogens in countries of the Mediterranean Basin

A total of 271 publications were analyzed: 56.5% were about bacteria, 37.7% about parasites, and 5.80% about viruses. A total of 90 pathogens from 18 genera were detected in domestic animals and their ticks from the Mediterranean Basin: 11 genera of bacteria, four genera of parasites, and three genera of viruses (Table 1.). Among these 90 TBPs, 60% were zoonotic and 40% were non-zoonotic or of unknown status (Table 1.). Among the 90 TBPs detected, 73.3% were found in ticks and 76.7% were found in animal hosts. 50% of the TBPs reported were detected in both (51.1%).

#### Parasites

##### Nematoda

##### *Cercopithifilaria*

Pathogens in the genus *Cercopithifilaria* are microfilariae parasites that mainly infect wild and domestic animals. Among the three species *Cercopithifilaria grassii*, *Cercopithifilaria*

*spp. sensu* and *Cercopithifilaria binae*, only the latter was reported in our research (Tahir et al., 2019). It was detected in two countries: Greece with a prevalence of 7% from *Rh. sanguineus* s.l. collected from dogs; and Italy at 25.86% from dogs (Latrofa et al., 2017; Ramos et al., 2014). This parasite was found in both ticks and animals, but from two distinct publications. The genus was found in only 0.7% of the publications and 1.9% of the publications concerning parasites.



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**Table 1.** Tick-borne pathogens detected in domestic animals or engorged ticks found on domestic animals in the Mediterranean Basin.

Pathogen	Zoonotic (Yes/No)	Engorged positive ticks collected from hosts	Positive tick hosts	Positive pathogen hosts	Countries	References
<b>Parasites : Nematoda</b>						
<i>Cercopithifilaria</i>						
<i>Cercopithifilaria bainaie</i>	No	<i>Rhipicephalus sanguineus</i> s.l.	Dog	Dog	Greece, Italy	(Latrofa et al., 2017; Ramos et al., 2014)
<b>Parasites : Apicomplexa</b>						
<i>Babesia</i>						
<b><i>Babesia</i> spp.</b>		<i>Hyalomma excavatum, Hyalomma marginatum, Rhipicephalus sanguineus</i> s.l.	Cattle	Cattle, Dog, Donkey, Goats, Pigs, Sheep	Egypt, Italy, Morocco, Turkey	(Aktas et al., 2007, 2012; Munir Aktas and Ozubek, 2017; Aktas and Ozubek, 2018; Al-Hosary et al., 2018; Cassini et al., 2012; Ceci et al., 2014; Guven et al., 2017; Torina et al., 2007b; Ziam et al., 2020)
<i>Babesia bigemina</i>	No	<i>Hyalomma marginatum, Rhipicephalus (B) annulatus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Sheep	Cattle, Pig, Sheep	Algeria, Egypt, Italy, Turkey	(Açici et al., 2016; Al-Hosary et al., 2020; K. Altay et al., 2008; Aydin et al., 2015; Cassini et al., 2012; Chisu et al., 2019; El-Ashker et al., 2015; Elsify et al., 2015; Iori et al., 2010; Toma et al., 2017; Torina et al., 2007b; Ziam et al., 2020)
<i>Babesia bovis</i>	Yes	<i>Dermacentor marginatus, Hyalomma marginatum, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Cattle, Goat, Sheep	Buffalo, Cattle	Algeria, Egypt, Italy, Turkey	(Açici et al., 2016; Al-Hosary et al., 2020; Cassini et al., 2012; Ceci et al., 2014; Cringoli et al., 2002; El-Ashker et al., 2015; Elsify et al., 2015; Iori et al., 2010; Sadeddine et al., 2020; Torina et al., 2007b)
<i>Babesia caballi</i>	No	<i>Rhipicephalus (B) annulatus</i>	Cattle	Dog, Donkey, Horse	Croatia, France, Italy,	(Beck et al., 2009; Leblond et al., 2006; Moretti et al., 2010; Toma et al., 2017; Torina et al., 2007b)
<i>Babesia canis</i>	No	<i>Dermacentor reticulatus, Ixodes hexagonus, Rhipicephalus sanguineus</i> s.l.	Dog	Dog	Croatia, France, Italy, Spain, Turkey	(Munir Aktas and Ozubek, 2017; Aydin et al., 2015; Beck et al., 2009; Camacho et al., 2003; Cassini et al., 2009; Criado-Fornelio et al., 2009; Estrada-Peña et al., 2017; Guo et al., 2017; Iori et al., 2010; Mrljak et al., 2017; Olivieri et al., 2016; Pennisi et al., 2012; René-Martellet et al., 2015; Solano-Gallego et al., 2008, p.; Torina et al., 2007b; Trotta et al., 2012; Veneziano et al., 2018; Zanet et al., 2020)
<i>Babesia capreoli</i>	No	<i>Ixodes hexagonus, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Dog	data not found	Italy	(Zanet et al., 2020)
<i>Babesia divergens</i>	Yes	<i>Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Dog	Cattle	France, Italy	(Agoulon et al., 2012; Devos and Geysen, 2004; Zanet et al., 2020)
<b><i>Babesia equi</i></b>	No	data not found	data not found	Horse	Italy	(Passamonti et al., 2010)
<b><i>Babesia gibsoni</i></b>	No	<i>Dermacentor reticulatus, Ixodes hexagonus, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Dog	Dog	Croatia, Italy, Spain	(Beck et al., 2009; Camacho et al., 2003; Estrada-Peña et al., 2017; Veneziano et al., 2018)
<b><i>Babesia major</i></b>	No	data not found	data not found	Cattle	France, Turkey	(K. Altay et al., 2008; Criado-Fornelio et al., 2009)
<b><i>Babesia microti</i></b>	Yes	<i>Dermacentor marginatus, Ixodes hexagonus, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Dog, Goat, Sheep	Cat, Dog	Italy, Turkey	(Aydin et al., 2015; Iori et al., 2010; Spada et al., 2014; Torina et al., 2007b; Zanet et al., 2020)
<b><i>Babesia motasi</i></b>	No	data not found	data not found	Sheep	Spain	(Nagore et al., 2004)
<i>Babesia occultans</i>	No	data not found	data not found	Cattle	Egypt	(Al-Hosary et al., 2020)

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<i>Babesia ovis</i>	No	<i>Dermacentor marginatus, Haemaphysalis concinna, Haemaphysalis parva, Ixodes ricinus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Dog, Goat, Sheep	Cattle, Goat, Sheep	Algeria, Italy, Palestine, Spain, Turkey	(Aktas et al., 2007; K. Altay et al., 2008; Aouadi et al., 2017; Aydin et al., 2015; Azmi et al., 2016; Bilgic et al., 2017; Iori et al., 2010; Nagore et al., 2004; Ziam et al., 2020)
<i>Babesia venatorum</i>	Yes	<i>Ixodes hexagonus, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Dog	data not found	Italy	(Zanet et al., 2020)
<i>Babesia vogeli</i>	No	<i>Dermacentor marginatus, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Cat, Dog, Goat, Sheep	Dog	Croatia, Cyprus, France, Italy, Palestine, Spain, Turkey	(Munir Aktas and Ozubek, 2017; Attipa et al., 2017b; Azmi et al., 2016; Beck et al., 2009; Bilgic et al., 2019; Cassini et al., 2009; Dyachenko et al., 2012; Estrada-Peña et al., 2017; Iori et al., 2010; Otranto et al., 2010; Pennisi et al., 2015; René-Martellet et al., 2015; Solano-Gallego et al., 2008; Veneziano et al., 2018)
<b>Hepatozoon</b>						
<i>Hepatozoon</i> spp.		<i>Rhipicephalus sanguineus</i> s.l.	Dog	Cat, Dog	Croatia, Cyprus, Italy, Spain, Turkey	(Munir Aktas and Ozubek, 2017; Attipa et al., 2017b; Azmi et al., 2016; Ebani et al., 2015a; Estrada-Peña et al., 2017; Giannelli et al., 2017; Guo et al., 2017; Latrofa et al., 2017; Ortuño et al., 2008; Otranto et al., 2010; Pacifico et al., 2020; Ramos et al., 2014)
<i>Hepatozoon canis</i>	No	<i>Haemaphysalis parva, Ixodes hexagonus, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Dog	Cat, Dog	Croatia, Cyprus, France, Greece, Italy, Palestine, Spain, Turkey	(Munir Aktas and Ozubek, 2017; Attipa et al., 2017b; Azmi et al., 2016; Criado-Fornelio et al., 2009; Ebani et al., 2015b; Estrada-Peña et al., 2017; Giannelli et al., 2017; Guo et al., 2017; Latrofa et al., 2017; Otranto et al., 2010; Pacifico et al., 2020; Ramos et al., 2014; Vojta et al., 2009)
<i>Hepatozoon felis</i>	No	<i>Haemaphysalis concinna, Rhipicephalus sanguineus</i> s.l.	Dog	Cat	Greece, Italy, Turkey	(Aktas et al., 2013; Giannelli et al., 2017; Latrofa et al., 2017)
<b>Theileria</b>						
<i>Theileria</i> spp.		<i>Hyalomma anatolicum, Hyalomma dromedarii, Rhipicephalus sanguineus</i> s.l.	Cattle, Dromedary	Cat, Cattle, Dogs, Donkey, Goat, Pigs, Sheep	Algeria, Egypt, Italy, Spain, Turkey	(Aktas et al., 2004, 2006; Aktas and Ozubek, 2018; Bilgic et al., 2017; Ceci et al., 2014; Estrada-Peña et al., 2017; Hassan et al., 2017; Nagore et al., 2004; Tabar et al., 2008; Torina et al., 2007b; Ziam et al., 2015)
<i>Theileria annae</i>	No	<i>Dermacentor reticulatus, Ixodes hexagonus, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Dog	Cattle, Dog, Donkey, Horse	Croatia, France, Italy, Spain	(Beck et al., 2009; Camacho et al., 2003; Cassini et al., 2012; Giménez Pardo et al., 2009; Moretti et al., 2010; René-Martellet et al., 2015)
<i>Theileria annulata</i>	No	<i>Hyalomma anatolicum, Hyalomma detritum, Hyalomma dromedarii, Hyalomma excavatum, Rhipicephalus (B) annulatus</i>	Cattle, Dromedary, Sheep	Buffalo, Cattle, Dromedary, Donkey, Goat, Pig, Sheep	Algeria, Egypt, Italy, Spain, Tunisia, Turkey	(Açici et al., 2016; Aktas et al., 2002; Al-Hosary et al., 2015; AL-Hosary et al., 2015; Al-Hosary et al., 2018, 2020; K. Altay et al., 2008; Aydin et al., 2015; Dumanli et al., 2005; Elsify et al., 2015; Gargano et al., 2021; Giménez Pardo et al., 2009; Hassan et al., 2017; İnci et al., 2008; Sadeddine et al., 2020; Sallemi et al., 2017; Sayin et al., 2004; Torina et al., 2007b; Ziam et al., 2015, 2020; Ziam and Benaouf, 2004)
<i>Theileria buffeli</i>	No	<i>Dermacentor marginatus, Haemaphysalis punctata, Hyalomma marginatum, Ixodes hexagonus, Ixodes ricinus, Rhipicephalus (B) annulatus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cat, Cattle, Dog, Goat	Cattle, Horse	Algeria, France, Italy, Spain, Turkey	(K. Altay et al., 2008; Chisu et al., 2019; Criado-Fornelio et al., 2009; Giménez Pardo et al., 2009; Sadeddine et al., 2020; Toma et al., 2017; Zanet et al., 2020)
<i>Theileria cervi</i>	No	<i>Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Dog	Data not found	Italy	(Zanet et al., 2020)
<i>Theileria equi</i>	No	<i>Hyalomma marginatum, Ixodes ricinus, Rhipicephalus (B) annulatus, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Horse	Cattle, Dog, Donkey, Horse	Algeria, Croatia, Italy, Spain	(Beck et al., 2009; Chisu et al., 2019; Ebani et al., 2015b; Estrada-Peña et al., 2017; Iori et al., 2010; Laus et al., 2013; Moretti et al., 2010; Passamonti et al., 2010; Sadeddine et al., 2020; Toma et al., 2017; Torina et al., 2007b)

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<i>Theileria lestoquardi</i>	No	data not found	data not found	Buffalo, Sheep	Egypt, Turkey	(Aktas et al., 2004; AL-Hosary et al., 2015)
<i>Theileria luwenshuni</i>	No	data not found	data not found	Goat, Sheep	Turkey	(Bilgic et al., 2017)
<i>Theileria orientalis</i>	No	<i>Ixodes hexagonus, Ixodes ricinus, Rhipicephalus (B) annulatus, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog	Buffalo, Cattle	Algeria, Egypt, Italy	(Elsify et al., 2015; Toma et al., 2017; Zanet et al., 2020; Ziam and Benaouf, 2004)
<i>Theileria ovis</i>	No	<i>Ixodes ricinus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Goat, Sheep	Buffalo, Goat, Sheep	Algeria, France, Greece, Palestine, Spain, Turkey	(Aktas et al., 2006; Altay et al., 2012; Aouadi et al., 2017; Aydin et al., 2015; Azmi et al., 2016; Bilgic et al., 2017; Criado-Formelio et al., 2009; Latrofa et al., 2017; Nagore et al., 2004; Sadeddine et al., 2020)
<i>Theileria uilenbergi</i>	No	data not found	data not found	Buffalo, Goat, Sheep	Egypt, Turkey	(AL-Hosary et al., 2015, p.; Bilgic et al., 2017)
<i>Theileria sergenti</i>	No	<i>Ixodes hexagonus, Ixodes ricinus, Rhipicephalus (B) annulatus, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog	Cattle, Horse	Italy, Spain	(Giménez Pardo et al., 2009; Moretti et al., 2010; Toma et al., 2017; Zanet et al., 2020)
<b>Bacteria</b>						
<i>Anaplasma</i>						
<i>Anaplasma</i> spp.		<i>Argas persicus, Hyalomma excavatum, Hyalomma marginatum, Rhipicephalus spp., Rhipicephalus (B) annulatus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Chicken, Dogs, Dromedary, Goats, Horse, Sheep	Cat, Cattle, Dog, Donkey, Dromedary, Goat, Horse, Pig, Sheep	Algeria, France, Greece, Italy, Morocco, Spain, Tunisia, Turkey, Palestine	(Aktas et al., 2012; Aktas and Ozubek, 2018; Aydin et al., 2019; Ayllón et al., 2011; Belkahia et al., 2015, 2019; Bilgic et al., 2017; Boucheikhchoukh et al., 2018; Dahmani et al., 2015, 2017b; de la Fuente et al., 2005; Diakou et al., 2019; Díaz-Regañón et al., 2020, p.; Elhamiani Khatat et al., 2017; Lbacha et al., 2017; Petrucelli et al., 2020; Zaid et al., 2019)
<i>Anaplasma bovis</i>	No	data not found	data not found	Cattle, Goat, Sheep	Italy, Tunisia	(Belkahia et al., 2019; Ceci et al., 2014)
<i>Anaplasma centrale</i>	No	<i>Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog	Cattle, Goat	Italy, Morocco, Turkey	(Aktas et al., 2012, 2011; Aydın et al., 2019; Belkahia et al., 2019; Ceci et al., 2014; Seng et al., 2009)
<i>Anaplasma marginale</i>	No	<i>Haemaphysalis punctata, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog	Buffalo, Cat, Cattle, Dog, Donkey, Goat, Horse, Pig, Sheep	Algeria, Egypt, France, Italy, Morocco, Tunisia, Turkey	(Açıci et al., 2016; Aktas et al., 2011; Al-Hosary et al., 2020; Aydın et al., 2019; Belkahia et al., 2019; Ceci et al., 2014; Cringoli et al., 2002; Dahmani et al., 2017b; de la Fuente et al., 2005; El-Ashker et al., 2015; Elhariri et al., 2017; Sadeddine et al., 2020; Seng et al., 2009; Torina et al., 2007b, 2008a; Ziam et al., 2020)
<i>Anaplasma ovis</i>	No	<i>Haemaphysalis punctata, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Horse, Goat, Sheep	Cattle, Dog, Goat, Horse, Sheep	Algeria, France, Italy, Morocco, Tunisia, Turkey	(Aktas et al., 2009; Altay et al., 2014; Aouadi et al., 2017; Belkahia et al., 2015, 2019; Bilgic et al., 2017; Cabezas-Cruz et al., 2019; Chisu et al., 2018a; Dahmani et al., 2017b; de la Fuente et al., 2005; Oter et al., 2016; Sadeddine et al., 2020; Selmi et al., 2020b; Seng et al., 2009; Torina et al., 2008a; Zanet et al., 2020)
<i>Anaplasma phagocytophilum</i>	Yes	<i>Haemaphysalis sulcata, Hyalomma marginatum, Ixodes hexagonus, Ixodes ricinus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cat, Dog, Goat, Horse, Sheep	Cat, Cattle, Dog, Donkey, Goat, Horse, Sheep	Algeria, Croatia, Egypt, France, Greece, Italy, Morocco, Spain, Tunisia, Turkey	(Aktas et al., 2011, 2012; Alberti et al., 2005; Altay et al., 2014; Belkahia et al., 2019; Bilgic et al., 2017; Çetinkaya et al., 2016; Chastagner et al., 2014; Dahmani et al., 2015; de la Fuente et al., 2005; Ebani et al., 2008, 2013, 2014, 2015b; Ebani, 2019; Ebani et al., 2020; Estrada-Peña et al., 2017; Ghafar and Amer, 2012; Giudice et al., 2012; Gokce et al., 2008; Huber et al., 2017; Laamari et al., 2020; Laus et al., 2013; Leblond et al., 2006; M'ghirbi et al., 2016; Mrljak et al., 2017; Oter et al., 2016; Palomar et al., 2014; Papa et al., 2017; Passamonti et al., 2010; Pennisi et al., 2012; Persichetti et al., 2016; Satta et al., 2011; Solano-Gallego et al., 2006a; Torina et al., 2007b, 2008a; Vascellari et al., 2016)
<i>Anaplasma platys</i>	Yes	<i>Hyalomma</i> spp., <i>Ixodes hexagonus, Ixodes ricinus, Rhipicephalus (B) annulatus, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Dromedary, Goat, Horse, Sheep	Buffalo, Cattle, Dog, Dromedary	Algeria, Croatia, Cyprus, Egypt, Greece, Italy, Morocco, Palestine, Spain, Tunisia, Turkey	(Aktas et al., 2009, 2015b; Al-Hosary et al., 2020; Attipa et al., 2017b, 2019; Azzag et al., 2015; Belkahia et al., 2015; Çetinkaya et al., 2016; Dahmani et al., 2015; de la Fuente et al., 2006; Dyachenko et al., 2012;

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						Elhamiani Khatat et al., 2017; Estrada-Peña et al., 2017; Hofmann-Lehmann et al., 2016; Huber et al., 2017; Latrofa et al., 2017; Otranto et al., 2010; Ramos et al., 2014; Sadeddine et al., 2020; R. Selmi et al., 2019; Seng et al., 2009; Solano-Gallego et al., 2006a; Sparagano et al., 2003; Zaid et al., 2019; Zanet et al., 2020)
<b>Bartonella</b>						
<i>Bartonella</i> spp.		<i>Rhipicephalus (B) annulatus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Goat	Cat, Dog	Italy, Spain	(Chisu et al., 2018a; Mancini et al., 2019; Otranto et al., 2017; Satta et al., 2011; Tabar et al., 2008)
<i>Bartonella clarridgeiae</i>	Yes	data not found	data not found	Cat	Italy	(Pennisi et al., 2009)
<i>Bartonella henselae</i>	Yes	<i>Ixodes ricinus</i>	Cat, Dog	Cat, Dog	Algeria, Cyprus, Greece, Italy, Spain	(Attipa et al., 2017b; Ayllón et al., 2011; Azzag et al., 2012, 2015; Bessas et al., 2016; Chisu et al., 2020; Diakou et al., 2017; Ebani et al., 2020; Otranto et al., 2017; Pennisi et al., 2009; Persichetti et al., 2016; Pinna Parpaglia et al., 2007; Solano-Gallego et al., 2006a)
<i>Bartonella vinsonii</i>	Yes	data not found	data not found	Cattle, Dog, Goat	Greece, Morocco	(Chochlakis et al., 2020; Henn et al., 2006)
<i>Bartonella vinsonii berkhoffi</i>	Yes	<i>Rhipicephalus sanguineus</i> s.l.	Dog	Dog	Italy, Spain	(Ebani et al., 2015b; Otranto et al., 2010; Solano-Gallego et al., 2006a)
<b>Borrelia</b>						
<i>Borrelia</i> spp.		<i>Argas persicus, Hyalomma aegyptium, Rhipicephalus (B) annulatus</i>	Cattle, chicken, Dog, Goat, Horses, Sheep	Cattle	Algeria, Turkey	(Boucheikhchoukh et al., 2018; Brinkmann et al., 2019; Sadeddine et al., 2020)
<i>Borrelia afzelii</i>	Yes	<i>Ixodes hexagonus, Ixodes ricinus</i>	Dog	data not found	Italy, Spain	(Estrada-Peña et al., 2017; Zanet et al., 2020)
<i>Borrelia burgdoferi sensus lato</i>	Yes	<i>Hyalomma marginatum, Ixodes hexagonus, Ixodes ricinus, Rhipicephalus (B) annulatus, Rhipicephalus bursa</i>	Cattle, Dog	Dog, Horse	Croatia, Italy	(Ebani et al., 2014; Mancini et al., 2019; Mrljak et al., 2017; Petruccelli et al., 2020; Zanet et al., 2020)
<i>Borrelia garinii</i>	Yes	<i>Ixodes ricinus</i>	Dog	data not found	Spain	(Estrada-Peña et al., 2017)
<i>Borrelia theileri</i>	No	data not found	data not found	Goat, Sheep	Algeria	(Aouadi et al., 2017)
<i>Borrelia valaisiana</i>	Yes	<i>Ixodes ricinus</i>	Dog	data not found	Spain	(Estrada-Peña et al., 2017)
<b>Chlamydia</b>						
<i>Chlamydia abortus</i>	Yes	<i>Ixodes ricinus, Rhipicephalus (B) annulatus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cat, Cattle, Dog, Goat, Sheep	data not found	Italy	(Chisu et al., 2020, 2018b)
<b>Chlamydophila</b>						
<i>Chlamydophila psittaci</i>	Yes	<i>Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Cat, Cattle, Dog, Goat, Sheep	data not found	Italy	(Chisu et al., 2020, 2018b)
<b>Coxiella</b>						
<i>Coxiella burnetii</i>	Yes	<i>Argas persicus, Dermacentor marginatus, Haemaphysalis punctata, Haemaphysalis sulcata, Hyalomma</i> spp., <i>Hyalomma dromedarii, Hyalomma marginatum, Ixodes ricinus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Chicken, Dog, Dromedary, Goat, Rabbit, Sheep	Buffalo, Cat, Cattle, Dromedary, Goat, Horse, Sheep	Algeria, Cyprus, Egypt, Greece, Italy, Montenegro, Slovenia, Spain, Tunisia	(Abdullah et al., 2018; Aouadi et al., 2017; Barkallah et al., 2018; Benaissa et al., 2017; Bessas et al., 2016; Chisu et al., 2018a; Chochlakis et al., 2018; Di Domenico et al., 2014; Ebani et al., 2015a, 2015b; Ebani, 2020; Filioussis et al., 2017; Knap et al., 2019; Lausevic, 2001; Loftis et al., 2006; Masala et al., 2004; Pape et al., 2009; Psaroulaki et al., 2006; Ruiz-Fons et al., 2008a; Satta et al., 2011; Selmi et al., 2018; Rachid Selmi et al., 2019; Spyridaki et al., 2002; Torina et al., 2007b)
<b>Ehrlichia</b>						
<i>Ehrlichia</i> spp.		<i>Haemaphysalis parva, Hyalomma</i> spp., <i>Hyalomma excavatum, Hyalomma</i>	Buffalo, Cattle, Dog, Sheep	Cattle, Dog, Goat, Sheep	Egypt, France, Italy, Palestine, Spain, Turkey	(Aktas et al., 2011; Aktas and Ozubek, 2018; Ayllón et al., 2011; Bilgic et al., 2017; Dahmani et al., 2017b; Loftis et al., 2006; Mancini et al., 2019;

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		<i>marginatum, Ixodes hexagonus, Ixodes ricinus, Rhipicephalus (B) annulatus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.				Petrucci et al., 2020; Tabar et al., 2008; Zaid et al., 2019; Zanet et al., 2020)
<i>Ehrlichia canis</i>	Yes	<i>Derma-centor marginatus, Haemaphysalis punctata, Haemaphysalis sulcata Hyalomma spp., Hyalomma excavatum, Ixodes hexagonus, Ixodes ricinus, Ixodes ventraloi, Rhipicephalus (B) annulatus, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Buffalo, Cat, Cattle, Dog, Goat, Sheep	Cat, Dog	Algeria, Croatia, Cyprus, Egypt, France, Greece, Italy, Palestine, Spain, Turkey	(Attipa et al., 2017a, 2019; Azzag et al., 2015; Bessas et al., 2016; Çetinkaya et al., 2016; Chisu et al., 2018a; Dahmani et al., 2017b; Diakou et al., 2019; Díaz-Regañón et al., 2020; Ebani et al., 2014, 2015a, 2015b; Ebani, 2019; Ebani et al., 2020; Hofmann-Lehmann et al., 2016; Loftis et al., 2006; Migliore et al., 2020; Miro et al., 2013; Mrljak et al., 2017; Otranto et al., 2010; Pennisi et al., 2012, 2015; Persichetti et al., 2016; Piantedosi et al., 2017; Satta et al., 2011; Solano-Gallego et al., 2006b; Torina et al., 2007b; Zaid et al., 2019; Zanet et al., 2020)
<i>Ehrlichia equi</i>	Yes	data not found	data not found	Horse	Italy	(Torina et al., 2007b)
<i>Ehrlichia ewingii</i>	Yes	data not found	data not found	Dog	Cyprus	(Attipa et al., 2019)
<i>Ehrlichia minancensis</i>	No	<i>Hyalomma marginatum, Rhipicephalus bursa</i>	Cattle, Goat	data not found	France	(V. Cicculli et al., 2019a)
<b>Candidatus</b>						
<i>Candidatus Ehrlichia urmitei</i>	No	<i>Rhipicephalus (B) annulatus</i>	Cattle, Goat, Horse, Sheep	data not found	Algeria	(Sadeddine et al., 2020)
<b>Noehrlichia</b>						
<i>Noehrlichia mikurensis</i>	Yes	<i>Ixodes ricinus</i>	Cattle	data not found	Spain	(Palomar et al., 2014)
<b>Francisella</b>						
<i>Francisella</i> spp.	Yes	<i>Hyalomma marginatum, Rhipicephalus (B) annulatus, Rhipicephalus bursa</i>	Cattle	data not found	Italy	(Mancini et al., 2019)
<b>Leptospira</b>						
<i>Leptospira</i> spp.	Yes	data not found	data not found	Buffalo, Cattle, Dromedary, Sheep	Egypt	(Horton et al., 2014)
<b>Mycoplasma</b>						
<i>Mycoplasma</i> spp.		data not found	data not found	Goat, Sheep	Morocco	(Lbacha et al., 2017)
<i>Mycoplasma haemocanis</i>	No	data not found	data not found	Dog	Greece, Turkey	(Aktas and Ozubek, 2018; M. Aktas and Ozubek, 2017; Guo et al., 2017; Tennant et al., 2011)
<i>Mycoplasma haemofelis</i>	No	data not found	data not found	Cat	Cyprus	(Attipa et al., 2017a)
<b>Candidatus</b>						
<i>Candidatus Mycoplasma haemonutum</i>	No	data not found	data not found	Cat	Cyprus	(Attipa et al., 2017a)
<i>Candidatus Mycoplasma haematoparvum</i>	No	data not found	data not found	Dog	Greece, Turkey	(Aktas and Ozubek, 2018; M. Aktas and Ozubek, 2017; Guo et al., 2017; Tennant et al., 2011)
<i>Candidatus Mycoplasma turicensis</i>	No	data not found	data not found	Cat	Cyprus	(Attipa et al., 2017a)
<b>Rickettsia</b>						
<i>Rickettsia</i> spp.		<i>Derma-centor marginatus, Haemaphysalis sulcata, Hyalomma spp., Hyalomma aegyptium, Hyalomma detritum, Hyalomma dromaderii, Hyalomma impeltatum, Hyalomma marginatum, Ixodes ricinus, Rhipicephalus spp., Rhipicephalus (B)</i>	Cat, Cattle, Chicken, Dog, Donkey, Dromedary, Goat, Horse, Sheep	Cat, Dog, Horse	Algeria, Egypt, Israel, Italy, Palestine, Spain, Tunisia, Turkey	(Abdullah et al., 2016; Ayllón et al., 2011; Azagi et al., 2017; Beninati et al., 2005; Boucheikhchoukh et al., 2018; Brinkmann et al., 2019; Ereqat et al., 2016a; Khrouf et al., 2014; Loftis et al., 2006; Mancini et al., 2019; Márquez, 2008; Oteo et al., 2006; Persichetti et al., 2016; Satta et al., 2011; Tabar et al., 2008)

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		<i>annulatus, Rhipicephalus bursa, Rhipicephalus pusillus, Rhipicephalus sanguineus</i> s.l.				
<i>Rickettsia aeschlimannii</i>	Yes	<i>Hyalomma dromedarii, Hyalomma impeltatum, Hyalomma marginatum, Hyalomma rufipes, Ixodes ricinus</i>	Cattle, Dromedary, Goat	Dromedary, Horse	Egypt, France, Israel, Spain, Tunisia	(Abdel-Shafy et al., 2012; V. Cicculi et al., 2019a; Demoncheaux et al., 2012; Kleinerman et al., 2013; Loftis et al., 2006; Matsumoto et al., 2004; Oteo et al., 2006; Rachid Selmi et al., 2019)
<i>Rickettsia africae</i>	Yes	<i>Amblyomma variegatum, Hyalomma</i> spp., <i>Hyalomma anatolicum, Hyalomma dromedarii, Hyalomma excavatum, Hyalomma impeltatum, Hyalomma marginatum, Hyalomma turanicum</i>	Cattle, Donkey, Dromedary, Goat, Sheep	Dromedary	Egypt, France, Israel, Italy, Lebanon, Palestine, Tunisia, Turkey	(Abdel-Shafy et al., 2012; Beninati et al., 2005; V Cicculi et al., 2019; Ereqat et al., 2016a; Kleinerman et al., 2013; Orkun et al., 2014; Selmi et al., 2020a)
<i>Rickettsia conorii</i>	Yes	<i>Hyalomma marginatum, Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Donkey, Goat, Sheep	Cat, Dog	Greece, Italy, Spain, Tunisia	(Beninati et al., 2005; Chisu et al., 2018a; Diakou et al., 2019; Espejo et al., 2016; Khrouf et al., 2014; Migliore et al., 2020; Morganti et al., 2019; Ortuño et al., 2009; Persichetti et al., 2016; Psaroulaki et al., 2006, 2003; Solano-Gallego et al., 2015, 2006a, 2006b; Vascellari et al., 2016)
<i>Rickettsia conorii israelensis</i>	Yes	<i>Rhipicephalus sanguineus</i> s.l.	Dog, Goat	data not found	Italy	(Chisu et al., 2018a, 2014)
<i>Rickettsia felis</i>	Yes	<i>Rhipicephalus sanguineus</i> s.l.	Cat, Dog, Sheep	data not found	Italy, Spain	(Raele et al., 2018; Toledo et al., 2009)
<i>Rickettsia helvetica</i>	Yes	<i>Hyalomma impeltatum, Ixodes ricinus, Ixodes ventralloii</i>	Cat, Cattle, Dromedary	Dromedary	Algeria, Italy, Tunisia	(Chisu et al., 2020; Dib et al., 2018; Pennisi et al., 2015; Selmi et al., 2020a)
<i>Rickettsia hoogstraalii</i>	Unknown	<i>Haemaphysalis parva, Haemaphysalis sulcate</i>	Dog, Sheep	data not found	Greece, Italy	(Chisu et al., 2018a; Latrofa et al., 2017)
<i>Rickettsia massiliae</i>	Yes	<i>Haemaphysalis adleri, Haemaphysalis parva, Rhipicephalus (B) annulatus, Rhipicephalus bursa, Rhipicephalus pusillus, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Goat, Horse, Sheep	Cat, Dog, Dromedary	Algeria, Cyprus, France, Greece, Israel, Italy, Lebanon, Palestine, Spain, Tunisia	(Bessas et al., 2016; Bitam et al., 2006; Chisu et al., 2014; Chochlakis et al., 2012; V. Cicculi et al., 2019b; Dib et al., 2018; Ereqat et al., 2016a; Estrada-Peña et al., 2017; Khrouf et al., 2014; Latrofa et al., 2017; Leulmi et al., 2016; Márquez, 2008; Márquez and Millán, 2009; Mura et al., 2008; Oteo et al., 2006; Papa et al., 2017; Psaroulaki et al., 2003, 2006; Sadeddine et al., 2020; Scarpulla et al., 2016; Selmi et al., 2020a; Toledo et al., 2009; Trotta et al., 2012; Waner et al., 2014)
<i>Rickettsia monacensis</i>	Yes	<i>Hyalomma impeltatum, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Cat, Cattle, Dog, Dromedary, Goat	Dromedary	Algeria, France, Greece, Italy, Spain, Tunisia	(V. Cicculi et al., 2019b; Dib et al., 2018, 2009; Estrada-Peña et al., 2017; Papa et al., 2017; Pascucci et al., 2019; Scarpulla et al., 2016; Selmi et al., 2020a)
<i>Rickettsia raoultii</i>	Yes	<i>Dermacentor reticulatus, Ixodes ricinus, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog	data not found	Algeria, Spain, Turkey	(Dib et al., 2018; Orkun et al., 2014; Vila et al., 2019)
<i>Rickettsia rickettsii</i>	Yes	data not found	data not found	Dog	Italy	(Vascellari et al., 2016)
<i>Rickettsia rhipicephali</i>	Unkown	<i>Rhipicephalus bursa, Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Goat	data not found	Greece	(Psaroulaki et al., 2006, 2003)
<i>Rickettsia sibirica mongolotimonae</i>	Yes	<i>Hyalomma excavatum</i>	Goat, Sheep	data not found	Cyprus	(Chochlakis et al., 2012)
<i>Rickettsia slovacica</i>	Yes	<i>Dermacento marginatus, Haemaphysalis punctata</i>	Cattle, Dog, Donkey, Sheep, Pig	Cattle, Goat, Sheep	France, Italy, Spain, Turkey	(Beninati et al., 2005; Chisu et al., 2017; V. Cicculi et al., 2019b; Márquez, 2008; Orkun et al., 2014; Ortuño et al., 2012; Oteo et al., 2006; Toledo et al., 2009)
<i>Rickettsia typhi</i>	Yes	data not found	data not found	Cat	Greece	(Diakou et al., 2017)
<i>Candidatus</i>						

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<b><i>Candidatus Rickettsia goldwasserii</i></b>	Unknown	<i>Haemaphysalis adleri</i> , <i>Haemaphysalis parva</i> , <i>Rhipicephalus sanguineus</i> s.l.	Dog, Goat, Horse, Sheep	data not found	Palestine	(Erekat et al., 2016a)
<i>Candidatus Rickettsia barbariae</i>	Unknown	<i>Hyalomma dromedarii</i> , <i>Rhipicephalus (B) annulatus</i> , <i>Rhipicephalus bursa</i> , <i>Rhipicephalus sanguineus</i> s.l.	Cattle, Dog, Goat, Horse, Sheep	data not found	Cyprus, France, Italy, Lebanon, Palestine	(Chisu et al., 2018a; Chochlakis et al., 2012; V. Cicculi et al., 2019b; Erekat et al., 2016a)
<b>Viruses</b>						
<b><i>Capripoxvirus</i></b>						
Lumpy skin disease virus	Yes	<i>Amblyomma</i> spp., <i>Amblyomma hebraeum</i> , <i>Hyalomma truncatum</i> , <i>Rhipicephalus (B) annulatus</i> , <i>Rhipicephalus appendiculatus</i> , <i>Rhipicephalus (B) microplus</i>	Cattle	data not found	Egypt	(Tuppurainen et al., 2015)
<b><i>Flavivirus</i></b>						
Tick-borne encephalitis	Yes	<i>Ixodes ricinus</i>	Goat	Goat	Greece, Italy	(Alfano et al., 2020; Papa et al., 2008)
<b><i>Orthonairovirus</i></b>						
Crimean-Congo Hemorrhagic Fever	Yes	<i>Dermacentor marginatus</i> , <i>Haemaphysalis parva</i> , <i>Hyalomma anatolicum</i> , <i>Hyalomma dromedarii</i> , <i>Hyalomma excavatum</i> , <i>Hyalomma lusitanicum</i> , <i>Hyalomma marginatum</i> , <i>Ixodes ricinus</i> , <i>Rhipicephalus</i> spp., <i>Rhipicephalus bursa</i> s.l., <i>Rhipicephalus sanguineus</i> s.l.	Buffalo, Cattle, Dromedary, Dog, Goat, Sheep	Buffalo, Cattle, Goat, Sheep	Albania, Greece, Egypt, Spain, Turkey	(Albayrak et al., 2010; Chisholm et al., 2012; Dinçer et al., 2017; Horton et al., 2014; Lugaj et al., 2014a, 2014c, 2014b; Mohamed et al., 2008; Negro et al., 2019; Schuster et al., 2016; Tonbak et al., 2006)

## Apicomplexa

*Babesia*

*Babesia* is a genus of erythrocytic protozoal parasites transmitted by ticks that cause babesiosis in both animals and humans. The main *Babesia* species transmitted by ticks are *B. divergens*, *B. duncani*, *B. microti*, *B. venatorum*, *B. vogeli* and *B. canis* (responsible for canine babesiosis). *Babesia vogeli* was the species found in the largest range of countries among the 15 *Babesia* species found in domestic animals. *Babesia vogeli* was found in seven countries (Croatia, Cyprus, France, Italy, Palestine, Spain, and Turkey) and was not detected in countries of North Africa. Among these seven countries, the highest prevalence of *B. vogeli* in animals was 14% from dogs in Italy and 10.5% in *Rh. sanguineus* s.l. ticks collected from dogs in France (René-Martellet et al., 2015; Veneziano et al., 2018). The next most commonly found species were *B. canis* and *B. ovis*. *Babesia canis* was found in five countries (Croatia, France, Italy, Spain, and Turkey). The highest prevalence was 71.4% from dogs in Italy and 5.65% from *Rh. sanguineus* s.l. and *Dermacentor reticulatus* from dogs in France. *Babesia ovis* was found in five countries (Algeria, Italy, Palestine, Spain, and Turkey). The genus *Babesia* is one of the most frequently screened or found pathogens, along with *Rickettsia* and *Anaplasma*: 17.7% of the publications. It was the most commonly screened or found of the parasites: 46.2% of the publications concerning parasites. It showed the highest species diversity, just after the genus *Rickettsia*.

*Hepatozoon*

Parasites in the genus *Hepatozoon* are intracellular protozoa belonging to the phylum Apicomplexa that infect amphibians, birds, mammals and reptiles (Ewing and Panciera, 2003). Two species were found in domestic animals from countries in the Mediterranean Rim. *Hepatozoon canis* was found in eight countries in cats or dogs (Croatia, Cyprus, France, Greece, Italy, Palestine, Spain, and Turkey). The highest prevalence was 22.3% in dogs and 20.58% in *Rh. sanguineus* s.l. from dogs in Turkey (Aktas et al., 2015a, 2013). The second species is *Hepatozoon felis*, with the highest prevalence found at 5.1% from cats in Italy and 1.7% from *Rh. sanguineus* s.l. from dogs in Turkey (Aktas et al., 2013; Giannelli et al., 2017). The genus *Hepatozoon* was the third most commonly found or screened parasite in a total of 7% of the overall publications and 18.2% of the publications concerning parasites. Pathogens in the genus *Hepatozoon* were found only in two pet hosts (cats and dogs).

*Theileria*

Like *Babesia* and *Hepatozoon*, the genus *Theileria* belongs to the phylum Apicomplexa and to the piroplasmids group with *Babesia*. Species in this genus infect mammals and have an obligatory cycle in ticks. They cause benign to fatal theileriosis in breeding animals (Mans et al., 2015). A total of 11 species were found in domestic animals from the Mediterranean Basin: *T. annae*, *T. annulata*, *T. buffeli*, *T. cervi*, *T. equi*, *T. lestoquardi*, *T. luwenshuni*, *T. orientalis*, *T. ovis*, *T. uilenberg*, and *T. sergenti*. The species found in most countries were *T. ovis*, *T. annulata*, and *T. buffeli*. They were found in six countries for *T. ovis* (Algeria, France, Greece, Palestine, Spain, and Turkey) and *T. annulata* (Algeria, Egypt, Italy, Spain, Tunisia, and Turkey), and five countries for *T. buffeli* (Algeria, France, Italy, Spain, and Turkey). The highest prevalence of *T. ovis* was 53.3% in goats and sheep from Algeria and 37.35% in ticks from the genus *Rhipicephalus* taken from goats and sheep in Algeria (Aouadi et al., 2017; Sadeddine et al., 2020). For *T. annulata*, it was found in 64% of cattle from Turkey and in 5% of *Rh. (Bo.) annulatus* collected from cattle, goats and sheep from Algeria (Inci et al., 2008; Sadeddine et al., 2020). The highest prevalence of *T. buffeli* was 11.56% from cattle in Turkey and 2.8% in three tick species: *I. hexagonus*, *I. ricinus* and *Rh. sanguineus* s.l. from cattle in Italy (Kursat Altay et al., 2008; Zanet et al., 2020). Like *Babesia*, the genus *Theileria* has



widespread distribution in a large number of countries. It was found in 17.3% of the overall publications and 45.2% of the publications concerning parasites.

### *Bacteria*

#### *Anaplasma*

The genus *Anaplasma* includes intracellular Gram-negative bacteria belonging to the family *Anaplasmataceae* from the Rickettsiales order. Most of these bacteria are zoonotic and have a high impact in veterinary health, such as *Anaplasma marginale*, *Anaplasma bovis*, and *Anaplasma ovis*, and also in human health, such as *Anaplasma phagocytophilum* and *Anaplasma platys* (Ben Said et al., 2018; Kocan et al., 2015). The most common species is *A. platys*, responsible for canine cyclic thrombocytopenia, which was found in 11 countries out of 20 in the Mediterranean Basin (Algeria, Croatia, Cyprus, Egypt, Greece, Italy, Morocco, Palestine, Spain, Tunisia, and Turkey). Six different tick species were found to be positive and to transmit this pathogen, with *Rhipicephalus sanguineus* s.l. as the main tick species, and with the highest prevalence in Morocco at 6.25%. The most common host was the dog, with a prevalence of 40.8% and 33% positive blood samples in Italy (Otranto et al., 2010; Seng et al., 2009; Sparagano et al., 2003). The second most common species is *A. phagocytophilum*, the causative agent of human granulocytic anaplasmosis, found in 10 countries (Algeria, Croatia, Egypt, France, Greece, Italy, Morocco, Spain, Tunisia, and Turkey). Out of six tick species found to be positive for this bacterium, *Rh. sanguineus* s.l. was the main tick species, especially on dogs. The highest prevalence of *A. phagocytophilum* in *Rh. sanguineus* s.l. was 13.7% in Egypt. The main pathogen host was cattle, with a prevalence of 40.6% in blood samples in Algeria (Dahmani et al., 2015; Ghafar and Amer, 2012).

As seen above, the *Anaplasma* genus is both highly present in the Mediterranean Basin and also highly screened, with detection in more than 25.8% of the publications analyzed and 44.9% of the publications concerning bacteria. This can be explained by the number of countries (12) and hosts affected (11) and shows the public health and veterinary importance of this genus in the countries of the Mediterranean Basin, especially for the two zoonotic species: *A. phagocytophilum* and *A. platys*.

#### *Bartonella*

*Bartonella* are Gram-negative bacteria belonging to the family *Bartonellaceae* from the Rhizobiales order, and half of them are known to be zoonotic. They are responsible for diseases such as trench fever and cat-scratch disease, and frequently cause endocarditis (Houpikian and Raoult, 2001). The most commonly detected species in domestic animals is *Bartonella henselae*, responsible for cat-scratch disease, detected in five countries (Algeria, Cyprus, Greece, Italy, and Spain). *Bartonella henselae* were mainly found in *Ixodes ricinus*, with the highest prevalence in Italy at 5.4% (Chisu et al., 2020). The ticks reported positives were mainly collected on cat. The host reported with the highest prevalence was the cat, with 83.5% in Italy (Pennisi et al., 2009). The other four *Bartonella* species detected were observed in only one or two countries, mainly Italy and Spain.

The *Bartonella* genus is less often screened for and less often found than the previous genus: 7.7% of the publications and 13.5% of the publications concerning bacteria. It is mainly screened for in pets (cats and dogs). This genus is mainly transmitted by biting flies and by fleas; however, we can see here that ticks can also be involved (Álvarez-Fernández et al., 2018). This shows both unequal presence and screening for the *Bartonella* genus in the countries of the Mediterranean Basin. Veterinary interest in the genus focuses on pets.

#### *Borrelia*

The *Borrelia* genus includes spirochetes bacteria belonging to the family *Spirochaetaceae*. It is divided into two groups: the Lyme borreliosis group responsible for Lyme

disease mainly caused by bacteria of the *Borrelia burgdorferi sensu lato* group, and the relapsing fever group which includes *Borrelia miyamotoi* (Margos et al., 2018). The main species found in our study were from the *B. burgdorferi sensu lato* group, with the highest prevalence in animal hosts at 1.47% in dogs from Italy; and in ticks at 53% and 26% in *Rh. sanguineus* s.l. and *Rh. annulatus*, respectively from cattle in Italy. Concerning *B. afzeeli*, *B. garinii* and *B. valaisiana*, the highest prevalences in ticks were 4.3%, 4.3% and 6.4%, respectively from *Ixodes ricinus* collected from dogs in Spain (Ebani et al., 2014; Estrada-Peña et al., 2017; Mrljak et al., 2017; Zanet et al., 2020). The last species detected in the Mediterranean Basin from domestic animals is *Borrelia theileri*, which is a relapsing fever bacteria responsible for bovine borreliosis (Kernif et al., 2016). It was detected in goats and sheep with 10.8% and 5.8% prevalence, respectively in Algeria (Aouadi et al., 2017).

*Borrelia* found in domestic animals from the Mediterranean Basin were mainly from the Lyme Borreliosis group, except for *Bo. theileri*. They were detected in only 4.1% of the publications analyzed and in 7.1% of the publications concerning bacteria. Among these publications, they were more commonly found in ticks (72.7%) than in animal hosts (27.8%). On the basis of these results, research seems to be more focused on the possible circulation of infested ticks than on the potential role of domestic animals as reservoirs of the pathogen. *Borrelia* is either poorly screened or not common in domestic animals from countries of the Mediterranean Basin.

#### *Chlamydia/Chlamydophila*

The genera *Chlamydia* and *Chlamydophila* belong to the *Chlamydiales*, which are Gram-negative bacteria responsible for a wide range of diseases in nearly the entire animal realm (Chisu et al., 2018b). Recently, a few studies carried out in Italy showed that ticks could be vectors of *Chlamydia/Chlamydophila* (Chisu et al., 2020, 2018b). Two bacterial species have been found. The first is *Chlamydia abortus*, from the genus *Chlamydia*, with the highest prevalence at 40.5% in *Ixodes ricinus* from cats in Italy. The second, belonging to the genus *Chlamydophila*, is *Chlamydia psittaci*, with the highest prevalence at 4.4% in *Rh. sanguineus* s.l. from dogs and breeding animals in Italy. No bacteria were found in animal hosts. The *Chlamydia* genus was found in 0.7% of the overall publications and in 1.3% of the publications concerning bacteria. This shows a possible role of ticks in the circulation of *Chlamydia* species; however, transmission and circulation in the domestic animal population in the countries of the Mediterranean Rim cannot be confirmed.

#### *Coxiella*

Only one pathogen is representative of this genus: *Coxiella burnetii*, responsible for Q fever which is transmitted by ticks and affects both humans and animals. This disease has a worldwide distribution and can cause febrile illness, endocarditis, meningoenzephalitis or pneumonia in humans, while it is mainly asymptomatic in animals with sporadic cases of abortion in pregnant animals (Angelakis and Raoult, 2010). It has been found in nine countries of the Mediterranean Basin (Algeria, Cyprus, Egypt, Greece, Italy, Montenegro, Slovenia, Spain, and Tunisia), with the highest prevalence in animals at 71.2% from camels in Algeria and 10.2% in *Rh. sanguineus* s.l. from goats and sheep in Cyprus (Benaissa et al., 2017; Spyridaki et al., 2002). This genus was found in 8.9% of the publications and in 15.4% of the publications concerning bacteria. For *C. burnetii*, most of the publications investigate both ticks and their hosts, which showed public health and veterinary interest.

#### *Ehrlichia*

*Ehrlichia* spp. is a genus closely related to *Anaplasma* spp., and is responsible for human monocytotropic ehrlichiosis (*Ehrlichia chaffeensis* and *Ehrlichia canis*) and for canine ehrlichiosis (*Ehrlichia canis*) (Ismail et al., 2010). The main species in domestic animals from

the Mediterranean Basin is *Ehrlichia canis*, responsible for disease in both humans and dogs. It has been detected in 10 countries (Algeria, Croatia, Cyprus, Egypt, France, Greece, Italy, Palestine, Spain, and Turkey) and has the highest prevalence at 48.5% in dogs from Italy and 6.6% in *Rh. sanguineus* s.l. collected from dogs in Turkey (Çetinkaya et al., 2016; Solano-Gallego et al., 2015). The other four species found in our review, *E. equi*, *E. ewingii*, *E. minacensis* and *Candidatus E. urmitei*, have a minor health impact and were found only in one country each. The *Ehrlichia* genus was the third most common bacterial genus found or screened for after *Anaplasma* and *Rickettsia*: 11.8% of the overall publications and 20.5% of the publications concerning bacteria. Among the publications dealing with the *Ehrlichia* genus, 93.5% concerned *E. canis*.

#### *Neoehrlichia*

Of the four bacteria in this genus, only one is a human pathogen: *Neoehrlichia mikurensis*, which causes chronic lymphocytic leukemia, for example. Its vectors are from the *Ixodes* genus and rodents are the most well-known host (Portillo et al., 2018). In domestic animals, it has been detected only in Spain with 1% prevalence in *I. ricinus* from cattle (Palomar et al., 2014). This species was rarely found or poorly screened for, with only 0.4% of the publications and 0.6% of the publications concerning bacteria.

#### *Francisella*

Bacteria from the genus *Francisella* are Gram-negative bacteria with one important species, *Francisella tularensis*, among other species, responsible for tularemia in humans and animals (Rohani et al., 2019). In the domestic animals of the Mediterranean Basin, only the genus *Francisella* spp. level was identified. It was found only in Italy, with a prevalence of 66%, 21% and 8% in *Hyalomma marginatum*, *Rh. bursa* and *Rh. (Boophilus) annulatus* from cattle, respectively. Like the *Neoehrlichia* genus, no *Francisella* was detected directly in animal hosts (Mancini et al., 2019).

#### *Leptospira*

The genus *Leptospira* includes spirochetes and zoonotic bacteria that are responsible for leptospirosis worldwide. The first case was documented over 100 years ago. The bacteria are usually transmitted by direct or indirect contact with a contaminated element, but can also be found in ticks (Levett, 2001; Wójcik-Fatla et al., 2012). Again, only the genus *Leptospira* spp. was detected. It was found in Egypt with a prevalence of 50%, 41%, 40% and 29% from camel, sheep, cattle and buffalo, respectively (Horton et al., 2014). Unlike the two previous genera, *Leptospira* spp. was only found in domestic animals and not in ticks. However, like the two previous genera, it was found in 0.4% of the overall publications and in 0.6% of publications concerning bacteria. Nevertheless, the *Leptospira* genus has already been found in ticks in Europe (Wójcik-Fatla et al., 2012), but not in the present review.

#### *Mycoplasma*

The genus *Mycoplasma* is composed of commensal and pathogenic bacteria that can cause anemia in a wide range of mammals (Jambhekar et al., 2019; Mascarelli et al., 2014; Parker et al., 2018). *Mycoplasma haemocanis*, *My. haemofelis*, *Candidatus My. haemonutum*, *Candidatus My. haematoparvum* and *Candidatus My. turicensis* were found, but only *My. haemocanis* and *Candidatus My. haematoparvum* were found in two countries (Greece and Turkey). The prevalence of *My. haemocanis* was 5.6% in dogs from Greece and 26.2% in dogs from Turkey. For *Candidatus My. haematoparvum*, the prevalence was 4.2% from dogs in Greece and 6.7% from dogs in Turkey (M. Aktas and Ozubek, 2017; Tennant et al., 2011). The other three species were found only in one country each. Like the *Leptospira* genus, the

*Mycoplasma* genus was found only in animals in this review. It was found in 2.2% of the publications and in 1.3% of the publications concerning bacteria.

### *Rickettsia*

The genus *Rickettsia* is one of the most important tick-borne pathogen genera. It is divided into two groups: the spotted fever group (SFG), like *Rickettsia conorii*, causative agent of Mediterranean spotted fever, and the typhus group (TG), which is less well-known and includes for example *Rickettsia typhi* (Abdad et al., 2018; Tomassone et al., 2018). Seventeen species were detected in domestic animals in this study, 16 from the SFG: *R. aeschlimannii*, *R. africae*, *R. conorii*, *R. conorii israelensis*, *R. felis*, *R. helvetica*, *R. hoogstraalii*, *R. massiliae*, *R. monacensis*, *R. raoultii*, *R. rickettsia*, *R. rhipicephali*, *R. sibirica mongolotimonae*, *R. slovacica*, *Candidatus R. barbariae* and *Candidatus R. goldwasserii*; and one from the TG: *R. typhi*. Of these *Rickettsia* spp., *Rickettsia massiliae* (SFG group) was the most widespread bacterium and was detected in 10 countries (Algeria, Cyprus, France, Greece, Israel, Italy, Lebanon, Palestine, Spain, and Tunisia). Its highest prevalence was 40.4% in *Rh. sanguineus* s.l. collected from cattle, dogs and sheep from Algeria, and 2.7% from camel blood in Tunisia (Leulmi et al., 2016; Rachid Selmi et al., 2019). The second most common bacterium was *R. africae* (SFG group), found in eight countries (Egypt, France, Israel, Italy, Lebanon, Palestine, Tunisia, and Turkey), with the highest prevalence at 26.7% in *Hyalomma impeltatum* collected from cattle and camels in Egypt, and 0.3% in camel blood from Tunisia (Abdel-Shafy et al., 2012, p.; Selmi et al., 2020a). *Rickettsia typhi* was the only bacterium from the TG group, detected with a prevalence of 29.7% from cats in Greece (Diakou et al., 2017). In the Mediterranean Rim, the genus *Rickettsia* is widespread and diversified in domestic animals and their ticks and widely screened: it was found in 25.3% of the publications overall, and in 44.2% of the publications concerning bacteria. This genus is the second most commonly studied after *Anaplasma* spp. However, the SFG group (98.6% of the publications) was of greatest scientific interest compared to the TG group (1.4% of the publications).

### *Viruses*

#### *Capripoxvirus*

This genus includes species that affect a broad range of domestic ruminants and that have a considerable economic impact, especially in Africa and the Middle East. It covers three species: lumpy skin disease virus (LSDV), goatpox virus (GTPV) and sheeppox virus (SPV) (Tuppurainen et al., 2017). Among these three species, only LSDV was found in ticks from domestic animals in this review. It was found in ticks from the genera *Amblyomma*, *Hyalomma* and *Rhipicephalus* from cattle in Egypt, with a prevalence of 65.5% (Tuppurainen et al., 2015). It was the least commonly found virus (0.4% of the overall publications and 7.2% of the publications concerning viruses). This genus does not represent a major threat in veterinary health in the countries of the Mediterranean Rim. Nonetheless, it should still be considered a potential threat considering the range of ticks in which it has been found.

#### *Flavivirus*

The flaviviruses are an important part of the arboviruses. They are transmitted by both mosquitoes and ticks. They mainly infect mammals and account for a large proportion of the recent outbreaks of public health and veterinary concern in terms of morbidity and mortality (Holbrook, 2017). The most well-known tick-borne flavivirus, tick-borne encephalitis virus (TBEV), was found in two countries in domestic animals. In Greece with a prevalence of 1.4% from *I. ricinus* ticks from goats, and 16.43% in goats from Italy (Alfano et al., 2020; Papa et al., 2008). TBEV was the second most common virus species, found in a total of 0.7% of publications and 14.3% of the publications concerning viruses.

### *Orthonairovirus*

Globally, this genus is mainly represented by one species: Crimean-Congo hemorrhagic fever virus (CCHFV). This virus is transmitted by ticks, mainly from the genus *Hyalomma*, causes severe or even fatal human disease nearly all across the Old World, and its range has expanded with climate change (Portillo et al., 2021). In domestic animals from countries of the Mediterranean Basin, the virus has been found in five different countries (Albania, Greece, Egypt, Spain, and Turkey). The highest prevalences were 90% from sheep in Albania and 6.88% from three tick genera (*Hyalomma* spp., *Rhipicephalus* spp. and *Ixodes* spp.) collected from buffalo, cattle, goats and sheep in Turkey (Albayrak et al., 2010; Lugaj et al., 2014c). CCHFV was the most commonly screened or found virus in about 78.5% of the publications concerning viruses and 4.1% of the overall publications. In order to determine the expansion of the virus, numerous research studies have been performed in the tick species that are potential vectors of the virus.

### 4.3. Positive ticks to tick-borne pathogens from domestic animals in the Mediterranean Basin

In all, 35 tick species from seven genera found on domestic animals in the Mediterranean Basin were positive for TBPs. Out of the seven genera, six belongs to the hard ticks (Ixodidae): *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*. They are widely distributed in this region. The last genus, which was collected in Algeria and Egypt, represents the soft ticks (Argasidae): *Argas* (Table S1). The diversity of tick species positive for TBPs varies between these genera (Figure 13.).

#### *Ixodidae*

##### *Genus Rhipicephalus*

The top ranking genus in terms of TBP diversity detected in the Mediterranean Basin was the genus *Rhipicephalus* (Table S1). It has a worldwide distribution and is very broadly present in the Mediterranean Basin where it can target a large range of wild and domestic animals (Estrada-Peña, 2004; Vayssier-Taussat et al., 2015). This genus transmits pathogens causing diseases with global health impact, such as anaplasmosis, babesiosis, ehrlichiosis and rickettsiosis (Estrada-Peña, 2004; Pérez-Eid, 2007). However, only six species were identified for the detection of 57 pathogenic species in a total 13 countries: *Rh* (*Bo.*) *annulatus*, *Rh. appendiculatus*, *Rh. bursa*, *Rh* (*Bo.*) *microplus*, *Rh. pusillus* and *Rh. sanguineus* s.l.. Out of these 57 TBP species, 47 were detected in *Rh. sanguineus* s.l., which is the tick species with the highest number of pathogenic species found in our review (Figure 13.). It is a tick species known for the transmission of TBPs such as *E. canis*, *R. conorii* and *B. canis* found in our review. This tick species is highly present in domestic animals and has a tropism for both pets, such as dogs, and livestock, such as cattle (Estrada-Peña, 2004). This tropism and the TBPs transmitted by this species could be the reason underlying both the scientific interest in this species and the amount of TBPs found in domestic animals.

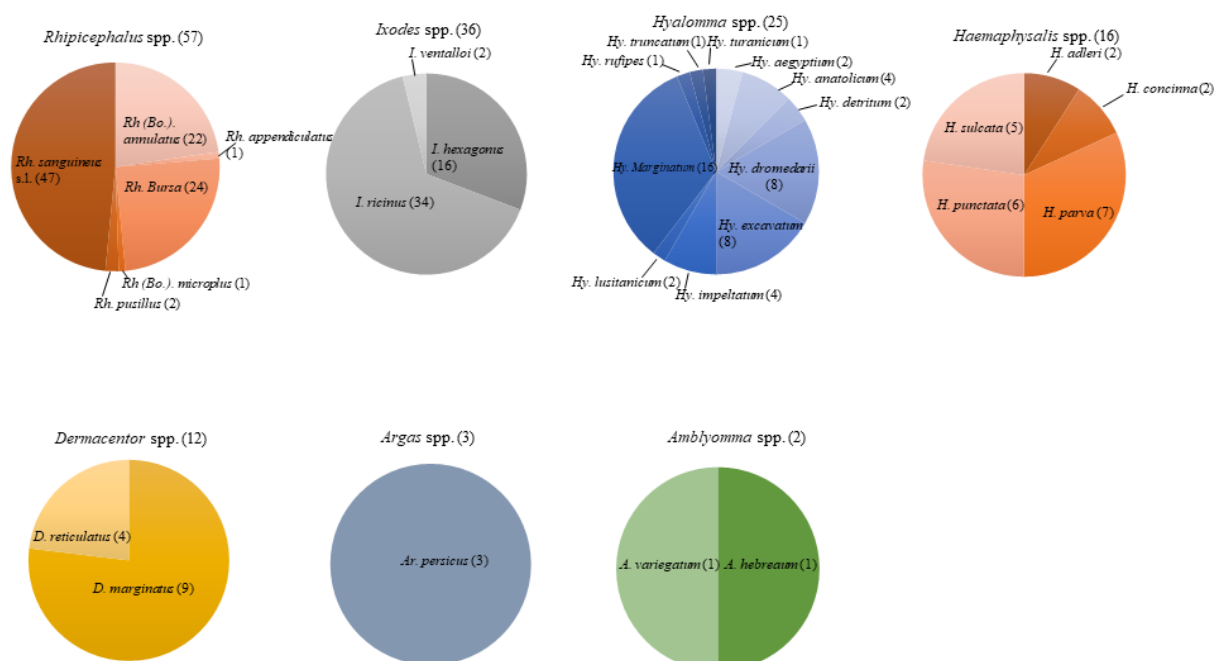
##### *Genus Ixodes*

The second ranking genus in terms of pathogen diversity was the genus *Ixodes* (Table S1). This genus is mainly known for the transmission of Lyme disease. The ticks are present mainly in Europe where due to their pleiotropic feeding, they are responsible for the spread of numerous TBPs (Vayssier-Taussat et al., 2015). They target a broad range of hosts (ubiquity) and have a high impact on human and animal health (Estrada-Peña, 2004; Pérez-Eid, 2007). Only three species were detected in countries of the Mediterranean Basin: *I. hexagonus*, *I. ricinus*, and *I. ventalloi*. A total of 36 pathogens were found on six animal hosts. This genus has the highest number of pathogens detected for the lowest number of tick species, with a total of 36 pathogen species for just three tick species: *I. hexagonus*, *I. ricinus*, and *I. ventalloi*. The

main species is *I. ricinus* in which 34 TBP were found out of the 36 found in the genus (Figure 13.). This species is ubiquitous and can be found on mammals, reptiles and avifauna. *Ixodes ricinus* is mainly known for the transmission of *B. burgdorferi* s.l., the causative agent of Lyme disease. However, as shown in our review, it can be positive for a wide range of pathogens. This could be due to the large array of hosts on which this species can feed.

### Genus *Hyalomma*

The genus *Hyalomma* ranks third in terms of TBPs detected. It is known for the transmission of the CCHFV and bacteria such as *Rickettsiae*. The ticks are large in size (5–6 mm) with tropism for large mammals for the adult forms, and for small mammals and birds for the immature stages. *Hyalomma* species are mainly distributed in the southern part of the Mediterranean Basin, with a slow increase in range in Western Europe and the Balkans (Estrada-Peña, 2004; Pérez-Eid, 2007). In the Mediterranean Basin, a total of 11 species were found, which is the highest number of species among all the tick genera. They were found on nine hosts in 12 countries and were found to be infected by a total of 25 TBPs (Table S1). The number of hosts, countries and pathogens associated with this genus shows its public health importance and the considerable threat it poses. Among the 11 species of the genus *Hyalomma*, the species found positive for the largest amount of TBPs was *Hy. marginatum* (Figure 13.). A total of 16 TBPs were found out of 25 species found in the genus. Concerning domestic animals, *Hy. marginatum* affects a large range of livestock and can be responsible for TBDs of veterinary importance, such as infections with *Rickettsia* spp., CCHFV, *Babesia* spp., and *Theileria* spp. These characteristics, along with the tropism of the immature stages for birds, could underpin the public health importance of this species.



**Figure 13.** Engorged ticks collected from domestic animals and positive for TBPs (the number of pathogens identified per taxon is indicated in brackets).

### Genus *Haemaphysalis*

The genus *Haemaphysalis* is the fourth most common genus in terms of pathogens detected (Table S1). The ticks are small in size and target mammals for the adult stage, and a wide array of hosts for the immature stage. They are known to have veterinary importance in

livestock and are present in Asia (Pérez-Eid, 2007). On domestic animals from the Mediterranean Basin, the genus *Haemaphysalis* was detected in seven different countries and ticks were positive for 16 TBPs. Five different species were found: *H. adleris*, *H. concinna*, *H. parva*, *H. punctata* and *H. sulcata*. Among these, the species found to be positive for the highest number of TBPs was *H. parva* (Figure 13.). This species is mainly distributed in the Mediterranean Basin, with key tropism for domestic ungulates during the adult stage. It can transmit a wide range of TBPs, such as *Rickettsia* spp., *C. burnetii* and *F. tularensis* (Vatansever, 2017). Unlike the genus *Hyalomma*, the pathogen distribution in the different species of *Haemaphysalis* is more uniform, especially between *H. parva*, *H. punctata*, and *H. sulcata*. This could confirm overall interest in the genus *Haemaphysalis* on domestic animals across the countries of the Mediterranean Basin.

#### *Genus Dermacentor*

The genus *Dermacentor* ranked fifth in terms of TBPs detected (Table S1). Like the genus *Ixodes*, it is mainly present in Europe where it transmits a broad range of TBPs (Estrada-Peña, 2004; Vayssier-Taussat et al., 2015). The genus is represented by two common species in the Mediterranean Basin: *D. marginatus* and *D. reticulatus*. They transmit a wide array of pathogens, including *Rickettsia* spp. and *Babesia* spp., which are of veterinary and human health importance. All the development stages were found in six different domestic animals from eight different countries. Twelve pathogen species have been identified in two both tick species. The main species was *D. marginatus*, which was found to be positive for 9 TBPs (Figure 13.). This species is known for its tropism on wild and domestic ungulates, but also for transmission of *B. canis* and *R. slovacica* (Estrada-Peña, 2004).













#### *Genus Amblyomma*

The genus *Amblyomma* was the last ranking genus of hard ticks in terms of TBPs detected (Table S1). It can be found in nearly all terrestrial animals and occurs mainly in the tropical and sub-tropical areas of Asia, Africa, and Oceania (Yonow, 1995). Recently, it appeared in the Mediterranean Basin, for instance in Corsica (V Cicculli et al., 2019; Hornok et al., 2020b). Due to the geographical distribution of this genus, it was rarely found in the Mediterranean Basin and only two species (*A. hebraeum* and *A. variegatum*) have been identified. Positive ticks were only found on cattle. The virus and bacteria detected were: lumpy skin disease virus and *Rickettsia africae* (Table S1, Figure 13.).

#### *Genus Argasidae*

Among the soft ticks, only one species was found: *Argas persicus*. This species is mainly found on domestic birds. It is mainly found in the Mediterranean Basin but is primarily involved in the transmission of bird-related pathogens (Estrada-Peña, 2004; Hasan, 2019; Pérez-Eid, 2007). In our review, it was found on two hosts and three pathogens species were found (Table S1, Figure 13.). This genus is one of the less commonly collected ticks in the countries of the Mediterranean Basin, along with the genus *Amblyomma* of the hard ticks group.

**Table 2.** TBP diversity in domestic animals or engorged ticks collected on these animals from the Mediterranean Basin.

Categories of domestic animals				Livestock									Pets		
Animal names (Number of countries with data available)	Cattle (13) ( <i>Bos taurus</i> ) 	Goat (13) ( <i>Capra hircus</i> ) 	Sheep (15) ( <i>Ovis aries</i> ) 	Dromedary (6) ( <i>Camelus dromedarius</i> ) 	Horse (7) ( <i>Equus caballus</i> ) 	Buffalo (1) ( <i>Bubalus spp.</i> ) 	Chicken (2) ( <i>Gallus gallus</i> ) 	Donkey (1) ( <i>Equus asinus</i> ) 	Pig (1) ( <i>Sus domesticus</i> ) 	Dog (14) ( <i>Canis lupus familiaris</i> ) 	Cat (5) ( <i>Felis silvestris</i> ) 	Rabbit (1) ( <i>Oryctolagus sp.</i> ) 			
<b>Pathogens found in ticks</b>															
Number of studies	51	42	42	20	14	5	4	1	0	63	15	1			
<b>Number of TBPs found in ticks</b>	Bacteria: 29 Parasite: 10	Bacteria: 25 Parasite: 5 Virus: 2	Bacteria: 21 Parasite: 7 Virus: 1	Bacteria: 8 Parasite: 2 Virus: 1	Bacteria: 12 Parasite: 1	Bacteria: 2 Virus: 1	Bacteria: 3	Bacteria: 4 Parasite: 4	0	Bacteria: 26 Parasite: 17 Virus: 1	Bacteria: 9 Parasite: 2	Bacteria: 1			
<b>Percentage of TBPs in ticks</b>	59.1%	48.5%	43.9%	16.7%	19.7%	4.5%	4.5%	12.1%	0%	66.7%	16.7%	1.5%			
<b>Pathogens found in animals</b>															
Number of studies	51	33	39	15	20	8	0	4	5	77	24	0			
<b>Number of TBPs found in animals</b>	Bacteria: 13 Parasites: 13 Viruses: 2	Bacteria: 12 Parasites: 5 Viruses: 2	Bacteria: 11 Parasites: 10 Viruses: 1	Bacteria: 9 Parasites: 1	Bacteria: 9 Parasites: 6	Bacteria: 4 Parasites: 6 Viruses: 1	0	Bacteria: 3 Parasites: 2	Parasites: 2	Bacteria: 19 Parasites: 13	Bacteria: 15 Parasites: 5	0			
<b>Percentage of TBPs in animals</b>	40.6%	27.5%	21.7%	14.5%	21.7%	15.9%	0%	7.2%	2.9%	46.4%	29%	0%			



#### 4.4. Domestic animal hosts of both positives ticks and TBPs in the countries of the Mediterranean Basin

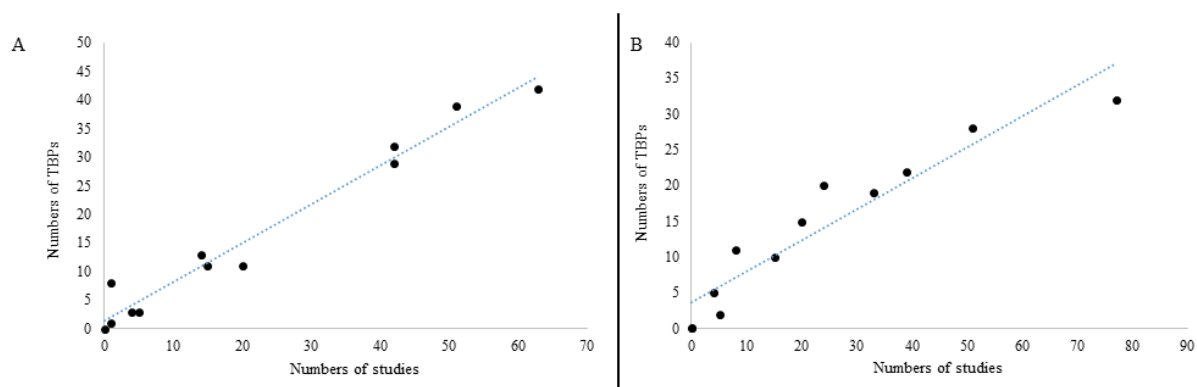
All the information in this part brings together data about domestic animals infested by TBPs and about domestic animal hosts of positive engorged ticks. However, the data about positive engorged ticks do not confirm the vector character of the ticks for the different TBPs. The domestic animals from countries of the Mediterranean Basin identified in this review can be divided into two groups: livestock animals and pets.

Livestock was composed of nine species (Table 2.), which have been more or less well studied. It appears that cattle, sheep, and goats have been studied in more than 13 countries and have been the subject of the highest number of studies. This can be explained by a wide range of tick-borne pathogen genera targeting these species (Table 2.), and their economic importance in the countries of this region (de Rancourt and Mottet, 2006). Among the 90 TBPs identified in this review, about 59% were found in positive ticks from cattle, with more than 40% found directly on the animal. On the other hand, some species (buffalo, chickens, donkeys and pigs) have been less well studied and available data were found in only one or two countries. The number of TBPs detected in these animals or in their ticks is much lower (Table 2.).

The pet group is composed of only three species: cats, dogs, and rabbits. Among these three species the dog is clearly the more commonly studied pet. Available data about the detection of TBPs in dogs (77 studies) and their ticks (63 studies) were found for 14 countries, while cat and rabbit data were found in only five and one countries, respectively (Table 2.).

Among the 90 TBPs listed in this review (in livestock or pets), 66 were detected in ticks from animals and 69 directly in the animals. The highest number of pathogens was found in ticks on dogs (66.7%) (Table 2.). The list of TBPs reported in each domestic animal host and their ticks was summarized in the Table S2.

The research effort, expressed by the number of publications, varies greatly from one animal to another. This is why it is difficult to conclude that an animal, or its ticks, are more or less susceptible to be infected by TBPs. In this sense, Figure 8 shows a positive correlation between the number of studies and the number of TBPs found in animals (Figure 14.B) or in their ticks (Figure 14.A). The very high correlation coefficient (0.95 and 0.98, respectively) expresses a strong correlation between these variables. If we assume that correlation does not imply causation, we can nevertheless observe a correlation between the number of TBPs found and the amount of publications focusing on the different domestic animal species, and conclude what may be obvious: the more we seek, the more we find.



**Figure 14.** ScatterPlot diagrams showing a strong linear correlation between (A) the number of studies and the number of TBPs from positive ticks by domestic animal (correlation coefficient  $R = 0.98$ ), and (B) between the number of studies and the number of TBPs by positive domestic animal (correlation coefficient  $R = 0.95$ ).

#### 4.5. Biogeography, diversity and distribution in the Mediterranean Basin

### *Overall analysis of the four main regions in the Mediterranean Basin*

In this section, we divide the countries of the Mediterranean Basin into four different areas to allow for better comparison of the data found. Western Europe is made up of France, Italy, Malta, Monaco and Spain, which represent 45.7% of the publications. North Africa, which includes Algeria, Egypt, Libya, Morocco and Tunisia, has a percentage of 26.1% of the total publications included in this review. The Middle East, which is composed of Cyprus, Israel, Lebanon, Palestine, Syria and Turkey, represents 17.7%. The Balkans area is made up of Albania, Bosnia-Herzegovina, Croatia, Greece, Montenegro and Slovenia and has 10.33% of the publications. No pathogens on domestic animals and their ticks were found in five countries: Bosnia-Herzegovina, Libya, Malta, Monaco and Syria.

#### *Western Europe*

In the five countries of this area, tick-borne pathogens on domestic animals were detected in Italy, France and Spain. These are also the three countries with the highest number of publications in the Mediterranean Basin. A total of eight domestic animal species were found to be carriers of positive ticks and pathogens: cats, cattle, dogs, donkeys, goats, horses, sheep and pigs. From our data, six tick genera from domestic animals were found to be positive for TBPs: *Amblyomma* spp., *Dermacentor* spp., *Haemaphysalis* spp., *Hyalomma* spp., *Ixodes* spp. and *Rhipicephalus* spp. The most commonly found host is the dog (Figure 15.A) which is the main target of the genus *Rhipicephalus*. In addition, the most predominant tick genus found out of the seven genera collected was *Rhipicephalus* spp. (Figure 15.B), vector of pathogens, for example *B. canis* and *E. canis*. The other main tick genera are *Ixodes*, a ubiquitous genus and *Hyalomma*, mainly on livestock. Of 68 pathogens detected in this region, five are included in nearly 60% of the publications in Western Europe. They belong to the bacteria and parasite groups: *E. canis*, *A. phagocytophilum*, *R. conorii*, *B. canis* and *R. massiliae* (Figure 15.C). It appears that the papers found in Western Europe mainly focused on dogs, which could explain the high percentage of *Rhipicephalus* ticks found and TBPs related to dogs. This can be explained by the central place of the dog in human activities, as pets, hunting dogs or stray dogs.

#### *North Africa*

The North Africa area has the largest diversity of domestic animal hosts of pathogens and positive ticks reported in the countries of the Mediterranean Basin, with a total of 10 domestic animal hosts: cats, cattle, chickens, buffalo, dogs, dromedaries, goats, horses, rabbits and sheep. The main animals reported were from the livestock group: cattle, sheep, goats and dromedaries (Figure 15.A). From these hosts, five tick genera were found to be positive to TBPs: *Argas* spp., *Haemaphysalis* spp., *Hyalomma* spp., *Ixodes* spp., and *Rhipicephalus* spp. Even though the main tick genus reported was the same as in Western Europe (genus *Rhipicephalus*), it can be found both in livestock and pets, with equal importance in terms of health. The other main tick genus was *Hyalomma* spp. which is mainly found on livestock (Figure 15.B). The main TBPs reported belong to the bacteria and parasite groups: *A. marginale*, *A. phagocytophilum*, *A. platys*, *C. burnetii*, and *T. annulata* (Figure 15.C). They are mainly related to livestock rather than pets. In North Africa, research seems to be more focused on livestock veterinary health monitoring, compared to Western Europe.

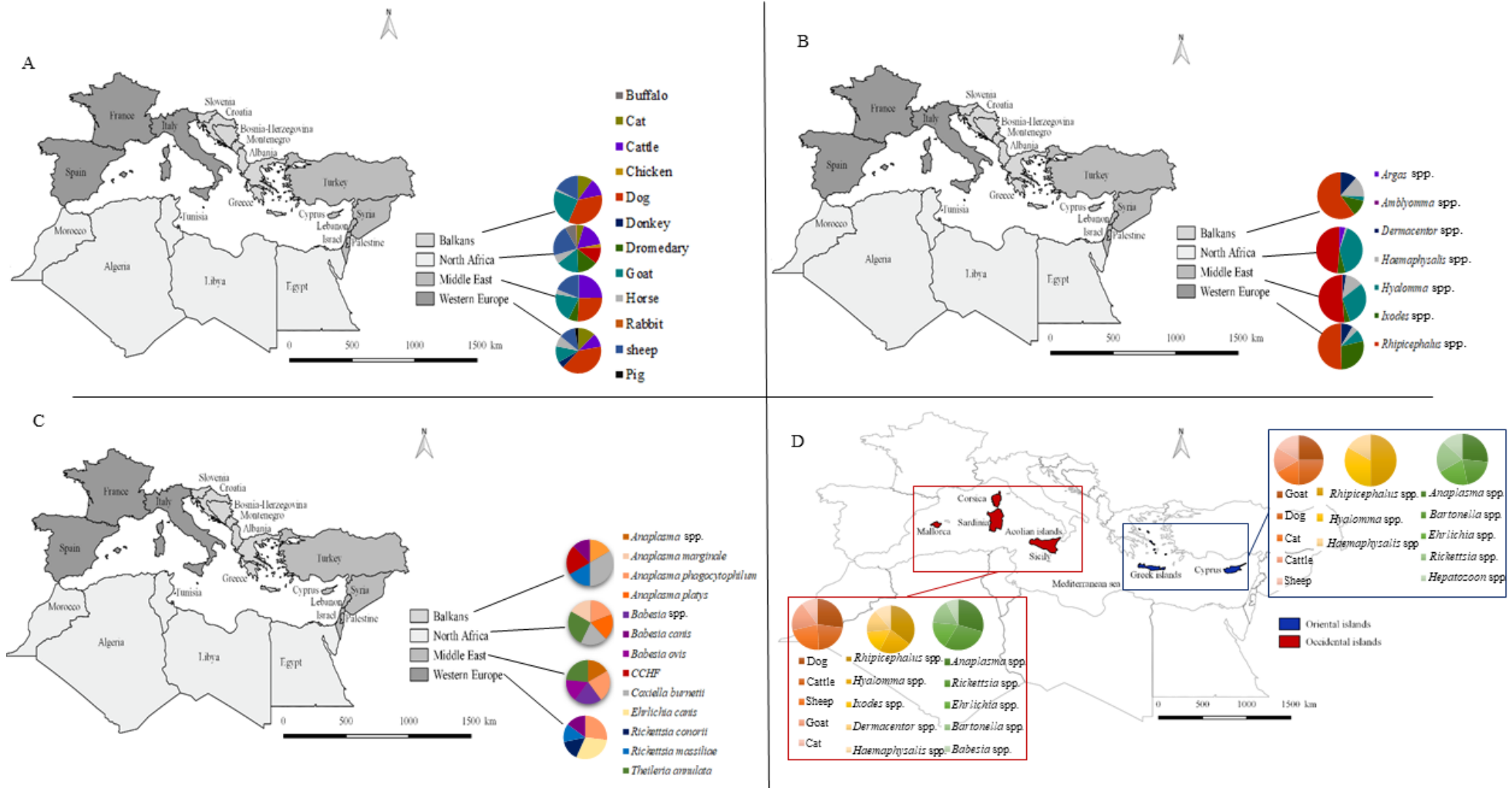
#### *The Middle East*

In this area, a total of six domestic animals were reported to be carriers of positive ticks and TBPs: cattle, dogs, dromedaries, goats, horses and sheep. Like Western Europe, the main domestic animal host found was the dog, closely followed by cattle, with 66.7% and 62.5% of the total publications in the area, respectively (Figure 15.A). From the domestic animal hosts, five tick genera were reported to be positive for TBPs: *Dermacentor* spp., *Haemaphysalis* spp.,

*Hyalomma* spp., *Ixodes* spp. and *Rhipicephalus* spp. Like the two previous areas, *Rhipicephalus*, which can be found both on livestock and pets, is the main tick genus found. The other main tick genera were *Hyalomma* and *Haemaphysalis*, which also infest livestock (Figure 15.B). Of the 45 pathogens found in the Middle East, the most predominant were: *Anaplasma* spp., *A. phagocytophilum*, *Babesia* spp., *B. ovis* and *T. annulata*. They were identified in 62.5% of the publications in the area (Figure 15.C). In the Middle East, the data available were related to veterinary health of both pets and livestock to equal extents. However, they were mainly from Turkey (75.5%).

### *The Balkans*

A total of six domestic animal hosts were reported to be carriers of positive ticks and TBPs: cats, cattle, dogs, goats, horses and sheep. Of the six domestic animal hosts reported, the main host was the dog, followed by the goat, which both seem to be the focus of veterinary research in the four areas, probably due to their economic and social importance (Figure 15.A). Five tick genera were reported to be positive for TBPs: *Dermacentor* spp., *Haemaphysalis* spp., *Hyalomma* spp., *Ixodes* spp., and *Rhipicephalus* spp. The main positive tick genus collected on domestic animals was *Rhipicephalus* spp., followed by the genera *Haemaphysalis*, *Ixodes* and *Dermacentor* out of the five genera found in the Balkans (Figure 15.B). A total of 32 pathogens were found in the seven countries of the Balkans area. The most frequently detected were: the bacteria *C. burnetii*, *A. platys* and *R. massiliae*, the parasite *B. canis*, and the CCHF virus (Figure 15.C). They were reported in 90% of the publications. As for the Middle East, these TBPs can be found in livestock and pets.



**Figure 15.** Map of the main domestic animal hosts (A), main positive tick genera (B), main TBPs in the four areas of the Mediterranean Basin (C); and main domestic animal hosts, positive tick genera and TBP genera in the western and eastern islands (D).

*Focus on insular tick-borne pathogens in domestic animals and their ticks*

The Mediterranean islands are an important place for animal migrations, human activities and pathogen circulation due to their geographical situation. A total 44 publications were reported on islands, which represent 16% of the publications taken into account in this review. These 44 publications were considered separately from the previous dataset. Out of these 44 publications, most came from the largest islands of the Mediterranean Basin: Sardinia, Sicily, Corsica and Cyprus, while few data were reported from smaller islands, such as the Greek islands. According to their geographic location between the western and eastern parts of the Mediterranean Sea, we decided to split the islands in the Western basin, including the Aeolian islands, Corsica, Sardinia, Sicily and Mallorca (36 publications), and the Eastern basin, including Crete, Cyprus, Ios, Mykonos, Santorini, Skiathos, Skopelos and Tinos (8 publications) (Table 3.).

On the islands, a total of nine domestic animal hosts have been studied: cats, cattle, dogs, donkeys, goats, horses, pigs, rabbits and sheep. Despite this host diversity, the majority of the islands follow the same schema as in the rest of the Mediterranean Basin with a predominance of the dog as host of positive ticks and TBPs (43.2% of the island publications), followed by cattle, sheep and goats. We can also see higher diversity of domestic animal hosts (eight species) in the data reported in the Western islands: cats, cattle, dogs, donkeys, goats, horses, pigs and sheep; than in the Eastern islands (five species): cats, cattle, dogs, goats and sheep (Table 3.). In the Western islands, most of the publications found were about dogs (44.4%), followed by cattle and sheep (36.1% of the publications each). In the Eastern islands, the main domestic animal hosts reported were goats and dogs, with 37.5% of the publications each (Figure 15.D).

A total of six hard tick genera were found to be positive, and no soft ticks, in the data from the islands: *Amblyomma* spp., *Dermacentor* spp., *Haemaphysalis* spp., *Hyalomma* spp., *Ixodes* spp. and *Rhipicephalus* spp. (Table 3.). Of these genera, the main positive tick genus reported was *Rhipicephalus* spp. with 43.2% of the island publications. This follows the same schema as the overall data. Nevertheless, the second most common genus reported was *Hyalomma*. This corresponds to the observations made in North Africa and the Middle East and differs from Western Europe and the Balkans. This finding could be due to its wide distribution in the Mediterranean Basin, especially on the South and East border and some species on the North border (Estrada-Peña, 2004). This could also testify to the extension of the distribution area of this genus, through animal migration and facilitated by climate change. This may result in the spread of TBPs, such as the CCHF virus transmitted by these ticks (Capek et al., 2014; De Liberato et al., 2018; Estrada-Peña et al., 2015). In this dissemination, islands may play an important role as places of transition between the different borders of the Mediterranean Basin (Bruderer and Liechti, 1999). This suggests a potential sentinel role of islands when monitoring dissemination of this tick genus and their TBPs on a continental scale (Figure 15.D).

In the Western islands, TBPs were screened and found in ticks from domestic animals in 63.9% of the papers. Meanwhile, the value was only 37.5% of the publications in the Eastern islands. For both groups of islands, the main positive tick genus found was *Rhipicephalus* spp. in 69.6% and 100% of the publications, respectively. This shows the importance of this genus on islands, like across the whole Mediterranean Basin. For both Western and Eastern islands, it was followed by the genus *Hyalomma* (Figure 15.D). From the data analyzed, it seems that there is a stronger focus on TBPs in ticks from domestic animals in the Western islands than in the Eastern islands.

**Table 3.** Tick-borne pathogens from insular domestic animals or engorged ticks collected from these animals in the Mediterranean Basin.

Country	Island	Surface area	Western basin/Eastern basin	Pathogen (found in <sup>A</sup> animal and/or <sup>T</sup> tick)	Positive ticks	Positive tick hosts	Positive pathogen hosts	References
Cyprus	Cyprus	9251 km <sup>2</sup>	Eastern	<i>A. platys</i> <sup>A</sup> , <i>B. vogeli</i> <sup>A</sup> , <i>Ba. henselae</i> <sup>A</sup> , <i>C. burnetii</i> <sup>T</sup> , <i>E. canis</i> <sup>A</sup> , <i>E.H. punctata</i> , <i>H. sulcata</i> , <i>ewingii</i> <sup>A</sup> , <i>H. canis</i> , <i>H. felis</i> <sup>A</sup> , <i>M. haemofelis</i> <sup>A</sup> , <i>Candidatus M. turicensis</i> <sup>A</sup> , <i>R. aeschlimanii</i> <sup>T</sup> , <i>R.Hy. marginatum</i> , <i>Hy. excavatum</i> , <i>haemotomum</i> <sup>A</sup> , <i>Candidatus M. sibirica mongolotimonae</i> <sup>T</sup> and <i>Candidatus R.gibbosus</i> , <i>Rh. bursa</i> and <i>Rh. barbariae</i> <sup>T</sup>	<i>Rh. bursa</i> and <i>Rh. sanguineus</i> s.l.	Cattle, Goat and Sheep	Cat and Dog	(Attipa et al., 2019, 2017b, 2017a; Chochlakis et al., 2012; Spyridaki et al., 2002)
France	Corsica	8722 km <sup>2</sup>	Western	<i>Anaplasma spp.</i> <sup>A,T</sup> , <i>A. ovis</i> <sup>A</sup> , <i>A. marginale</i> <sup>A,T</sup> , <i>A. phagocytophilum</i> <sup>T</sup> , <i>B. bigemina</i> <sup>T</sup> , <i>B. canis</i> <sup>A,T</sup> , <i>B. vogeli</i> <sup>A,T</sup> , <i>Ba. henselae</i> <sup>T</sup> , <i>Bo. afzelii</i> <sup>T</sup> , <i>Bo. burgdoferi</i> s.l. <sup>T</sup> , <i>Bo. miyamotoi</i> <sup>T</sup> , <i>E. canis</i> <sup>T</sup> , <i>E. minancensis</i> , <i>R. aeschlimanii</i> <sup>T</sup> , <i>R. africae</i> <sup>T</sup> , <i>R. helvetica</i> <sup>T</sup> , <i>R. massiliae</i> <sup>T</sup> , <i>R. monacensis</i> <sup>T</sup> , <i>R. slovacca</i> <sup>T</sup> , <i>Candidatus R. barbariae</i> <sup>T</sup> , <i>T. annae</i> <sup>A</sup> and <i>T. equi</i> <sup>T</sup>	<i>A. variegatum</i> , <i>D. marginatus</i> , <i>D. reticulatus</i> , <i>I. ricinus</i> , <i>Hy. aegyptium</i> , <i>Hy. marginatum</i> , <i>R. rufipes</i> , <i>Rh. bursa</i> and <i>Rh. sanguineus</i> s.l.	Cat, Dog and Sheep	Cattle, Cattle, dog, Horse, goat and sheep	(V. Cicculi et al., 2019a, 2019b; V Cicculi et al., 2019; Dahmani et al., 2017b; Grech-Angelini et al., 2020; Matsumoto et al., 2004; René-Martellet et al., 2015)
	Crete	8450 km <sup>2</sup>	Eastern	<i>Ba. henselae</i> <sup>A</sup> , <i>Ba. vinsonii</i> <sup>T</sup> , <i>R. felis</i> <sup>A</sup> and <i>R. Typhi</i> <sup>A</sup>	<i>Rh. bursa</i> and <i>Rh. sanguineus</i> s.l.	Goat, Cattle	Cat	(Chochlakis et al., 2020; Diakou et al., 2017)
Greece	Ios	109 km <sup>2</sup>	Eastern	<i>Anaplasma spp.</i> <sup>A</sup> , <i>B. canis</i> <sup>A</sup> , <i>Bo. burgdoferi</i> <sup>A</sup> , <i>E. canis</i> <sup>A</sup> and <i>R. conorii</i> <sup>A</sup>	data not found	data not found	Dog	(Diakou et al., 2019)
	Mykonos	85.5 km <sup>2</sup>	Eastern	<i>Ba. henselae</i> <sup>A</sup> and <i>Rickettsia spp.</i> <sup>A</sup>	data not found	data not found	Cat	(Diakou et al., 2017)
	Santorini	76.19 km <sup>2</sup>	Eastern	<i>Anaplasma spp.</i> <sup>A</sup> , <i>B. canis</i> <sup>A</sup> , <i>Bo. burgdoferi</i> <sup>A</sup> , <i>E. canis</i> <sup>A</sup> and <i>R. conorii</i> <sup>A</sup>	data not found	data not found	Dog	(Diakou et al., 2019)
	Skiathos	49.9 km <sup>2</sup>	Eastern	<i>Anaplasma spp.</i> <sup>A</sup> , <i>B. canis</i> <sup>A</sup> , <i>Bo. burgdoferi</i> <sup>A</sup> , <i>E. canis</i> <sup>A</sup> and <i>R. conorii</i> <sup>A</sup>	data not found	data not found	Dog	(Diakou et al., 2019)
	Skopelos	96.3 km <sup>2</sup>	Eastern	<i>Ba. henselae</i> <sup>A</sup> and <i>Rickettsia spp.</i> <sup>A</sup>	data not found	data not found	Cat	(Diakou et al., 2017)
	Tinos	197 km <sup>2</sup>	Eastern	<i>Anaplasma spp.</i> <sup>A</sup> , <i>B. canis</i> <sup>A</sup> , <i>Bo. burgdoferi</i> <sup>A</sup> , <i>E. canis</i> <sup>A</sup> and <i>R. conorii</i> <sup>A</sup>	data not found	data not found	Dog	(Diakou et al., 2019)
Italy	Aeolian Island	114.7 km <sup>2</sup>	Western	<i>Anaplasma spp.</i> <sup>A</sup> , <i>B. vogeli</i> <sup>A</sup> , <i>Bartonella spp.</i> <sup>A</sup> , <i>Ba. clarridgeae</i> <sup>A</sup> , <i>Ba. henselae</i> <sup>A</sup> , <i>Ehrlichia canis</i> <sup>A</sup> , <i>H. canis</i> <sup>A</sup> , <i>H. felis</i> <sup>A</sup> , <i>R. helvetica</i> <sup>A</sup> and <i>R. monacensis</i> <sup>A</sup>	<i>I. ricinus</i> , <i>I. ventalloi</i> , <i>Rh. pusillus</i> and <i>Rh. sanguineus</i> s.l.	Cat	Cat and Dog	(Otranto et al., 2017; Pennisi et al., 2015)
	Sardinia	24090 km <sup>2</sup>	Western	<i>A. phagocytophilum</i> <sup>A</sup> , <i>A. ovis</i> <sup>A</sup> , <i>B. bigemina</i> <sup>T</sup> , <i>Bartonella spp.</i> <sup>T</sup> , <i>Ba. henselae</i> <sup>A,T</sup> , <i>Chlamydia abortus</i> <sup>T</sup> , <i>Chlamydomphila psittaci</i> <sup>T</sup> , <i>C. burnetii</i> <sup>A,T</sup> , <i>E. canis</i> <sup>T</sup> , <i>Rickettsia spp.</i> <sup>T</sup> , <i>R. aeschlimanii</i> <sup>T</sup> , <i>R. conorii israelensis</i> <sup>T</sup> , <i>R. helvetica</i> <sup>T</sup> , <i>R. hoogstralii</i> <sup>T</sup> , <i>R. massiliae</i> <sup>T</sup> , <i>R. slovacca</i> <sup>T</sup> , <i>Candidatus R. barbariae</i> <sup>T</sup> , <i>T. buffeli</i> <sup>T</sup> , <i>T. equi</i> <sup>T</sup> , <i>T. orientalis</i> <sup>T</sup> and <i>T. sergenti</i> <sup>T</sup>	<i>D. marginatus</i> , <i>H. punctata</i> , <i>H. sulcata</i> , <i>Hy. marginatum</i> , <i>I. festai</i> , <i>Rhipicephalus spp.</i> , <i>Rh. annulatus</i> , <i>Rh. bursa</i> , <i>Rh. sanguineus</i> s.l.	Cat, Dog, Horse and Sheep	Cattle, Goat, Cat, Dog, Goat, Horse and Sheep	(Alberti et al., 2005; Chisu et al., 2019, 2018a, 2018b, 2017, 2014; Masala et al., 2004; Mura et al., 2008; Pinna Parpaglia et al., 2007; Satta et al., 2011)
	Sicily	25711 km <sup>2</sup>	Western	<i>Anaplasma spp.</i> <sup>A</sup> , <i>A. marginale</i> <sup>A</sup> , <i>A. phagocytophilum</i> <sup>A</sup> , <i>A. platys</i> <sup>A</sup> , <i>A. ovis</i> <sup>A</sup> , <i>Babesia spp.</i> <sup>A</sup> , <i>B. bigemina</i> <sup>A</sup> , <i>B. bovis</i> <sup>A</sup> , <i>B. caballi</i> <sup>A</sup> , <i>B. canis</i> <sup>A</sup> , <i>B. microti</i> <sup>A</sup> , <i>B. vogeli</i> <sup>T</sup> , <i>Ba. claridgeae</i> <sup>A,T</sup> , <i>Ba. henselae</i> <sup>A</sup> , <i>Bo. burgdoferi</i> s.l. <sup>A</sup> , <i>Cercophifilaria spp.</i> <sup>A,T</sup> , <i>C. burnetii</i> <sup>A</sup> , <i>Ehrlichia spp.</i> <sup>A</sup> , <i>E. canis</i> <sup>A</sup> , <i>E. equi</i> <sup>A</sup> , <i>Hepatozoon spp.</i> <sup>A,T</sup> , <i>Rickettsia spp.</i> <sup>A,T</sup> , <i>R. aeschlimanii</i> <sup>T</sup> , <i>R. africae</i> <sup>T</sup> , <i>R. conorii</i> <sup>A,T</sup> , <i>R. felis</i> <sup>A</sup> , <i>R. helvetica</i> <sup>T</sup> , <i>R. monacensis</i> <sup>T</sup> , <i>R. rickettsii</i> <sup>A</sup> , <i>R. slovacca</i> <sup>T</sup> , <i>Theileria spp.</i> <sup>A</sup> , <i>T. annulata</i> <sup>A</sup> and <i>T. equi</i> <sup>A</sup>	<i>D. reticulatus</i> , <i>D. marginatus</i> , <i>H. punctata</i> , <i>Hy. lusitacum</i> , <i>Hy. marginatum</i> , <i>Ixodes spp.</i> , <i>I. ricinus</i> , <i>I. ventalloi</i> , <i>Rh. bursa</i> , <i>Rh. pusillus</i> , <i>Rh. sanguineus</i> s.l.	Cat, Dog, Donkey and Sheep	Cattle, Cat, Cattle, Dog, Donkey, Goat, Horse, Pig and Sheep	(Beninati et al., 2005; de la Fuente et al., 2005, 2006; Galluzzo et al., 2020; Gargano et al., 2021; Giudice et al., 2012; Hofmann-Lehmann et al., 2016; Migliore et al., 2020; Otranto et al., 2012; Pennisi et al., 2009; Persichetti et al., 2016; Torina et al., 2007b, 2008a)
Spain	Mallorca	3640 km <sup>2</sup>	Western	<i>A. phagocytophilum</i> <sup>A</sup> , <i>Ba. henselae</i> <sup>A</sup> , <i>Ba. vinsonii berkhoffi</i> <sup>A</sup> , <i>E. canis</i> <sup>A</sup> and <i>R. conorii</i> <sup>A</sup>	data not found	data not found	Cat and Dog	(Solano-Gallego et al., 2006b, 2006a)

Of the 18 genera of pathogens reported in the data, 14 genera were reported in the islands, which represents 77.8% of the total genera (Table 3.). The main TBP genera detected were *Anaplasma* spp. and *Rickettsia* spp., reported in 43.2% and 40.9% of the publications concerning islands, respectively. This follows the same schema as the overall data. In the Western islands, the 14 TBP genera were reported (66.7% bacteria and 33.3% parasites), while only nine were reported (77.8% bacteria and 22.2% parasites) in the Eastern islands. In both groups, the main reported genus was *Anaplasma*, followed by *Rickettsia* (Figure 15.D). They were both reported in 41.7% of the Western Island publications, and in 50% and 37.5% of the Eastern islands publications respectively. No viruses were reported in either of the island groups.

Even though few data were reported for the islands (16%), high diversity of TBPs was reported. This may indicate the importance of islands in the monitoring of TBPs in domestic animals in the Mediterranean Basin.

## 5. Conclusion

In this study, 90 TBPs from 18 genera of bacteria, parasites and viruses were reported from domestic animals and their ticks in the countries of the Mediterranean Basin. Most pathogens were bacteria, followed by parasites and viruses. The main genera detected were bacteria: *Anaplasma* spp. and *Rickettsia* spp. The data collected reflect high diversity of TBPs in domestic animals and their ticks in the countries of the Mediterranean Basin, which shows their importance in veterinary and human health. A wide range of pathogens was reported from seven positive tick genera (six hard ticks and one soft tick) and 31 tick species. The most reported was *Rhipicephalus* spp., a genus found in a large range of domestic animals from livestock to pets. This genus is also known for the transmission of TBPs from the genera *Anaplasma* and *Rickettsia*. These TBPs and positive tick genera were reported in 12 different domestic animal hosts divided into two groups: livestock and pets. The main domestic animal hosts were both dogs and cattle, from which the highest diversity of TBPs was reported. This seems linked to the amount of data reported for these hosts. In the four areas of the Mediterranean Basin (Western Europe, North Africa, the Middle East and the Balkans), the main studied host is the dog (except in North Africa) and the main positive tick genus is *Rhipicephalus*. Depending on the area, the second most important genera were *Ixodes* or *Hyalomma*. This could be due to the distribution area of *Ixodes* spp. in Europe and the distribution area of *Hyalomma* spp. on the South border of the Mediterranean Basin and expansion through the islands [263,270]. The diversity of the TBPs identified in this review is linked to the domestic animal targeted in the studies and depend on their veterinary and social importance. In all, 16% of the publications concerned TBPs from domestic animals and their ticks on islands, but high diversity of domestic animal hosts (nine of 12), positives tick genera (six of seven) and TBP genera (14 of 18) was reported. This shows the importance of the Mediterranean islands in the monitoring of TBPs in this region as sentinel territories. The development of research on the islands could provide a better understanding of their role as a hotspot for the circulation of ticks and tick-borne pathogens.

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6. Supplementary data

**Table S1.** Positive engorged ticks for TBPs collected on domestic animals and their distribution in the Mediterranean Basin.

Tick genus	Number of tick species and tick species	Stage	Number of countries with data and countries	Number of tick host species with data and host species	Number of pathogenic genera/species and pathogens detected in ticks ( <sup>b</sup> bacteria, <sup>p</sup> parasite, <sup>v</sup> virus)
<i>Rhipicephalus</i>	<i>Rh (Bo). annulatus, Rh. appendiculatus, Rh. bursa, Rh. pusillus, Rh. sanguineus</i> s.l.	Larva, nymph and adult	Algeria, Cyprus, Egypt, France, Greece, Italy, Lebanon, Morocco, Palestine, Spain, Tunisia, Turkey	Buffalo, Cat, Cattle, Dog, Donkey, Goat, Horse and Sheep	13/57 <i>Anaplasma</i> spp. <sup>b</sup> , <i>A. centrale</i> <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. bigemina</i> <sup>p</sup> , <i>B. bovis</i> <sup>p</sup> , <i>B. canis</i> <sup>p</sup> , <i>B. capreoli</i> <sup>p</sup> , <i>B. divergens</i> <sup>p</sup> , <i>B. gibsoni</i> <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>B. venatorum</i> <sup>p</sup> , <i>B. vogeli</i> <sup>p</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Ba. caballi</i> <sup>b</sup> , <i>Ba. vinsonii</i> <sup>b</sup> , <i>Ba. vinsonii berkhoffi</i> <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Cercopithifilaria bainae</i> <sup>p</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Chlamydomphila psittaci</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>E. minacensis</i> <sup>b</sup> , <i>Candidatus E. urmitei</i> <sup>b</sup> , <i>Francisella</i> spp. <sup>b</sup> , <i>Hepatozoon</i> spp. <sup>p</sup> , <i>Hepatozoon canis</i> <sup>p</sup> , <i>Hepatozoon felis</i> , lumpy skin disease virus <sup>v</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. conorii</i> <sup>b</sup> , <i>R. conorii israelensis</i> <sup>b</sup> , <i>R. felis</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. rhipicephali</i> <sup>b</sup> , <i>Candidatus Rickettsia goldwasserii</i> <sup>b</sup> , <i>Candidatus R. barbariae</i> , <i>Theileria</i> spp. <sup>p</sup> , <i>Theileria annae</i> <sup>p</sup> , <i>T. annulata</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. cervi</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup> , <i>T. sergenti</i> <sup>p</sup>
<i>Ixodes</i>	<i>I. hexagonus, I. ricinus</i> and <i>I. ventraloi</i>	Larva, nymph and adult	Algeria, France, Greece, Italy, Morocco, Slovenia, Spain, Turkey	Cat, Cattle, Dog, Goat, Horse and Sheep	12/36 <i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia bovis</i> <sup>p</sup> , <i>B. canis</i> <sup>p</sup> , <i>B. capreoli</i> <sup>p</sup> , <i>B. divergens</i> <sup>p</sup> , <i>B. gibsoni</i> <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>B. venatorum</i> <sup>p</sup> , <i>B. vogeli</i> <sup>p</sup> , <i>Bartonella henselae</i> <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Bo. afzelii</i> <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Bo. garinii</i> <sup>b</sup> , <i>Bo. valaisiana</i> <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Chlamydomphila psittaci</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>Noehrlichia mikurensis</i> <sup>b</sup> , <i>Hepatozoon canis</i> <sup>p</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>Theileria annae</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. cervi</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup> , <i>T. sergenti</i> <sup>p</sup> , Tick-borne encephalitis virus <sup>v</sup>
<i>Hyalomma</i>	<i>Hy. aegyptium, Hy. anatolicum, Hy. deritum, Hy. dromedarii, Hy. excavatum, Hy. impeltatum, Hy. lusitanicum, Hy. marginatum, Hy. rufipes, Hy. truncatum</i> and <i>Hy. turanicus</i>	Larva, nymph and adult	Algeria, Cyprus, Egypt, France, Greece, Israel, Italy, Lebanon, Palestine, Spain, Tunisia, Turkey	Buffalo, Cat, Cattle, Dog, Donkey, Dromedary, Goat, Horse and Sheep	11/25 <i>Anaplasma</i> spp. <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. bigemina</i> <sup>p</sup> , <i>B. bovis</i> <sup>p</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>E. minacensis</i> <sup>b</sup> , <i>Francisella</i> spp. <sup>b</sup> , lumpy skin disease virus <sup>v</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. africae</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. sibirica mongolotimonae</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>Theileria annulata</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup>
<i>Haemaphysalis</i>	<i>H. adleris, H. concinna, H. parva, H. punctatanymp</i> and <i>H. sulcata</i>	Larva, nymph and adult	Algeria, Greece, Italy, Palestine, Slovenia, Spain, Turkey	Cat, Cattle, Dog, Donkey, Horse, Goat, Pig and Sheep	8/16 <i>Anaplasma marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>B. ovis</i> <sup>p</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>Hepatozoon canis</i> <sup>p</sup> , <i>H. felis</i> <sup>p</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. hoogstraalii</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup> , <i>Candidatus Rickettsia goldwasserii</i> <sup>b</sup> , <i>Theileria buffeli</i> <sup>p</sup>
<i>Dermacentor</i>	<i>D. marginatus</i> and <i>D. reticulatus</i>	Larva, nymph and adult	Algeria, France, Greece, Italy, Slovenia, Spain, Turkey	Cattle, Dog, Donkey, Goat, Pig and Sheep	6/12 <i>Babesia bovis</i> <sup>p</sup> , <i>B. canis</i> <sup>p</sup> , <i>B. gibsoni</i> <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>B. vogeli</i> <sup>p</sup> , Crimean-Congo hemorrhagic fever virus, <i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup> , <i>Theileria annae</i> <sup>p</sup>
<i>Argas</i>	<i>Ar. persicus</i>	Adult	Algeria, Turkey	Chicken and Rabbit	3/3 <i>Anaplasma</i> spp. <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup>
<i>Amblyomma</i>	<i>A. hebreum</i> and <i>A. variegatum</i>	Adult	Egypt, France, Israel	Cattle	2/2 Lumpy skin disease virus <sup>v</sup> , <i>R. africae</i> <sup>b</sup>

**Table S2.** TBP species reported in domestic animals or engorged ticks collected on these animals from the Mediterranean Basin.

Categories of domestic animals	Animal names	Pathogens found in ticks ( <sup>b</sup> bacteria, <sup>p</sup> parasite, <sup>v</sup> virus)	Pathogens found in animals ( <sup>b</sup> bacteria, <sup>p</sup> parasite, <sup>v</sup> virus)
Livestock	Cattle	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. centrale</i> <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. bigemina</i> <sup>p</sup> , <i>B. bovis</i> <sup>p</sup> , <i>B. caballi</i> <sup>p</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Chlamydomphila psittaci</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>E. minancensis</i> <sup>b</sup> , <i>Candidatus</i> <i>E. urmitei</i> <sup>b</sup> , <i>Francisella</i> spp. <sup>b</sup> , <i>Neoehrlichia mikurensis</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. africana</i> <sup>b</sup> , <i>R. conorii</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. rhipicephali</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup> , <i>Candidatus</i> <i>R. barbariae</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. annulata</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. sergenti</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. bovis</i> <sup>b</sup> , <i>A. centrale</i> <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia bigemina</i> <sup>p</sup> , <i>B. bovis</i> <sup>p</sup> , <i>B. divergens</i> <sup>p</sup> , <i>B. major</i> <sup>p</sup> , <i>B. occultans</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>Bartonella vinsonii</i> <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>Leptospira</i> spp. <sup>b</sup> , Lumpy skin disease virus <sup>v</sup> , <i>Rickettsia slovacica</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. annae</i> <sup>p</sup> , <i>T. annulata</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. sergenti</i> <sup>p</sup>
	Goat	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia bovis</i> <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>B. vogeli</i> <sup>p</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Chlamydomphila psittaci</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia canis</i> <sup>b</sup> , <i>E. minancensis</i> <sup>b</sup> , <i>Candidatus</i> <i>E. urmitei</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. africana</i> <sup>b</sup> , <i>R. conorii</i> <sup>b</sup> , <i>R. conorii israelensis</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. rhipicephali</i> <sup>b</sup> , <i>R. sibirica mongolotimonae</i> <sup>b</sup> , <i>Candidatus</i> <i>R. goldwassenii</i> <sup>p</sup> , <i>Candidatus</i> <i>R. barbariae</i> <sup>b</sup> , <i>Theileria buffeli</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup> , Tick-borne encephalitis virus <sup>v</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. bovis</i> <sup>b</sup> , <i>A. centrale</i> <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia</i> spp., <i>Bartonella vinsonii</i> <sup>b</sup> , <i>Borrelia theileri</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>Mycoplasma</i> spp. <sup>b</sup> , <i>Rickettsia slovacica</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. annulata</i> <sup>p</sup> , <i>T. luwenshuni</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup> , <i>T. uilenberg</i> <sup>p</sup> , Tick-borne encephalitis virus <sup>v</sup>
	Sheep	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia bigemina</i> <sup>p</sup> , <i>B. bovis</i> <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>B. vogeli</i> <sup>p</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Chlamydomphila psittaci</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>Candidatus</i> <i>E. urmitei</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. africana</i> <sup>b</sup> , <i>R. conorii</i> <sup>b</sup> , <i>R. felis</i> <sup>b</sup> , <i>R. hoogstraalii</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. sibirica mongolotimonae</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup> , <i>Candidatus</i> <i>R. goldwassenii</i> <sup>p</sup> , <i>Candidatus</i> <i>R. barbariae</i> <sup>b</sup> , <i>Theileria annulata</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. bovis</i> <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. bigemina</i> <sup>p</sup> , <i>B. motasi</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>Borrelia theileri</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>Leptospira</i> spp. <sup>b</sup> , <i>Mycoplasma</i> spp. <sup>b</sup> , <i>Rickettsia slovacica</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. annulata</i> <sup>p</sup> , <i>T. lestoquardi</i> <sup>p</sup> , <i>T. luwenshuni</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup> , <i>T. uilenbergi</i> <sup>p</sup>
	Dromedary	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. africana</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. annulata</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Leptospira</i> spp. <sup>b</sup> , <i>Rickettsia aeschlimannii</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. africana</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>Theileria annulata</i> <sup>p</sup>
	Horse	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Candidatus</i> <i>Ehrlichia urmitei</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>Candidatus</i> <i>R. goldwassenii</i> <sup>b</sup> , <i>Candidatus</i> <i>R. barbariae</i> <sup>b</sup> , <i>Theileri equi</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia caballi</i> <sup>p</sup> , <i>B. equi</i> , <i>Borrelia burgdoferi</i> s.l. <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia equi</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>Theileria annae</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup> , <i>T. sergenti</i> <sup>p</sup>
	Buffalo	Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup>	<i>Anaplasma marginale</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia bovis</i> <sup>p</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Leptospira</i> spp. <sup>b</sup> , <i>Theileria annulata</i> <sup>p</sup> , <i>T. lestoquardi</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. ovis</i> , <i>T. uilenbergi</i> <sup>p</sup>
Chicken	<i>Anaplasma</i> spp. <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup>	-	
Donkey	<i>Rickettsia</i> spp. <sup>b</sup> , <i>R. africana</i> <sup>b</sup> , <i>R. conorii</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. annae</i> <sup>p</sup> , <i>T. annulata</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. caballi</i> <sup>p</sup>	
Pig		<i>Babesia</i> spp. <sup>p</sup> , <i>Theileria</i> spp. <sup>p</sup>	
Pet	Dog	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. centrale</i> <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia bigemina</i> <sup>p</sup> , <i>B. canis</i> <sup>p</sup> , <i>B. capreoli</i> <sup>p</sup> , <i>B. divergens</i> <sup>p</sup> , <i>B. gibsoni</i> <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>B. venatorum</i> <sup>p</sup> , <i>B. vogeli</i> <sup>p</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Ba. henselae</i> <sup>b</sup> , <i>Ba. vinsonii berkhoffi</i> <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Bo. afzelii</i> <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Bo. garinii</i> <sup>b</sup> , <i>Bo. valaisiana</i> <sup>b</sup> , <i>Cercopithifilaria bairae</i> <sup>p</sup> , Crimean-Congo hemorrhagic fever virus <sup>v</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Chlamydomphila psittaci</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>Hepatozoon</i> spp. <sup>p</sup> , <i>H. canis</i> <sup>p</sup> , <i>H. felis</i> <sup>p</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. conorii</i> <sup>b</sup> , <i>R. conorii israelensis</i> <sup>b</sup> , <i>R. felis</i> <sup>b</sup> , <i>R. hoogstraalii</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. rhipicephali</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup> , <i>Candidatus</i> <i>R. goldwassenii</i> <sup>b</sup> , <i>Candidatus</i> <i>R. barbariae</i> <sup>b</sup> , <i>Theileria annae</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. sergenti</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. caballi</i> <sup>p</sup> , <i>B. canis</i> <sup>p</sup> , <i>B. gibsoni</i> <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. vogeli</i> <sup>p</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Ba. henselae</i> <sup>b</sup> , <i>Ba. vinsonii</i> <sup>b</sup> , <i>Ba. vinsonii berkhoffi</i> <sup>b</sup> , <i>Borrelia burgdoferi</i> s.l. <sup>b</sup> , <i>Cercopithifilaria bairae</i> <sup>p</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>E. ewingii</i> <sup>b</sup> , <i>Hepatozoon</i> spp. <sup>p</sup> , <i>H. canis</i> <sup>p</sup> , <i>Mycoplasma haemocanis</i> <sup>b</sup> , <i>Candidatus</i> <i>M. haematoparvum</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. conorii</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. rickettsia</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. annae</i> <sup>p</sup> , <i>T. cervi</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup>
	Cat	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Babesia vogeli</i> <sup>p</sup> , <i>Bartonella henselae</i> <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Chlamydomphila psittaci</i> <sup>b</sup> , <i>Ehrlichia canis</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. felis</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>Theileria buffeli</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia microti</i> <sup>p</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Ba. clarridgeae</i> <sup>b</sup> , <i>Ba. henselae</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia canis</i> <sup>b</sup> , <i>Hepatozoon</i> spp. <sup>p</sup> , <i>H. canis</i> <sup>p</sup> , <i>H. felis</i> <sup>p</sup> , <i>Mycoplasma haemofelis</i> <sup>b</sup> , <i>Candidatus</i> <i>M. haemonotum</i> <sup>b</sup> , <i>Candidatus</i> <i>M. turicensis</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. conorii</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. typhi</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup>
	Rabbit	<i>Coxiella burnetii</i> <sup>b</sup>	

**Chapitre 2 : Agents pathogènes chez les animaux sauvages et leurs tiques dans le bassin méditerranéen entre 2000 et 2021**

## 1. Résumé de la publication « Distribution of tick-borne pathogens in wild animals and their ticks in the Mediterranean Rim between 2000 and 2021: a systematic review »

Cette revue systématique a été acceptée avec révision majeure dans la revue *Microorganisms* : Baptiste Defaye, Sara Moutailler, Vanina Pasqualini, Yann Quilichini (in prep). Distribution of tick-borne pathogens in wild animals and their ticks in the Mediterranean Rim between 2000 and 2021: a systematic review.

### Résumé en Français

Cette revue est la deuxième partie d'un travail ayant pour intérêt d'inventorier les différents agents pathogènes transmis par les tiques (TBPs) détectés dans les populations d'animaux et leurs tiques au niveau du bassin Méditerranéen entre 2000 et 2021 pour la première fois dans une revue bibliographique. Cette deuxième revue a pour but de faire un premier bilan sur les TBPs dans les populations d'animaux sauvages. Dans cette revue, 148 publications ont été prises en compte. Comme pour les animaux domestiques la plupart des publications reportées l'ont été entre 2014 et 2020 (57,4 % des publications). Cela pourrait être dû à un intérêt scientifique plus important lors de ces dernières années, l'évolution des techniques de biologie moléculaire, mais également l'intérêt de plus en plus important dans certains pays notamment de l'Europe de l'Ouest. Cela pourrait également être dû à une accessibilité facilitée des publications entre 2010 et 2020 par rapport à 2000-2010.

Parmi les 148 publications analysées, 66,7 % portaient sur des bactéries, 21 % sur des parasites et 12,3 % sur des virus. Au total, 85 agents pathogènes ont été recensés dans les animaux sauvages et leurs tiques au niveau du bassin méditerranéen : neuf genres de bactéries, huit genres de parasites et quatre genres de virus. Parmi ceux-ci, 51,1 % étaient zoonotiques et 48,9 % non-zoonotiques ou de statut inconnu. Le pourcentage de publications sur les agents pathogènes détectés chez les tiques était de 72,2 %, alors que celui des agents pathogènes détectés chez l'hôte était de 61,1 %. Comme pour les animaux domestiques, les genres les plus détectés ont principalement été des genres de bactérie comme *Rickettsia* spp. (30,4 %), *Anaplasma* spp. (19,6 %) et *Borrelia* spp. (18,2 %) qui sont des bactéries spirochètes appartenant aux *Spirochaetaceae*. Elle se divise en deux groupes : le groupe des borrélioses de Lyme responsable de la maladie de Lyme principalement provoquée par les bactéries du groupe *Borrelia burgdoferi sensus lato*, et le groupe de la fièvre récurrente comme *Borrelia miyamotoi* (Margos et al., 2018). Cependant, contrairement aux animaux domestiques, les genres de parasites ont été plus faiblement reportés. Les virus ont quant à eux été reportés de manière plus importante comme pour le CCHF (5,4 %), qui est un virus transmis par les tiques, principalement du genre *Hyalomma*, provoquant des maladies humaines graves, voir même mortelles dans presque tout l'Ancien Monde et dont l'expansion s'est accrue avec le changement climatique (Portillo et al., 2021).

Parmi les agents pathogènes, 71,8 % pourraient être transmis par l'intermédiaire de 30 espèces de tiques provenant de huit genres trouvés sur les animaux domestiques du bassin méditerranéen. Parmi ces huit genres, six appartiennent aux tiques dures (*Ixodidae*) : *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* et *Rhipicephalus*. Les deux dernières espèces qui ont été collectées en Algérie et en Egypte représentent les tiques molles (*Argasidae*) : *Argas* et *Ornithodoros*. Parmi ces différents genres, le plus reporté était le genre *Ixodes*. C'est un genre de tique dure qui est principalement connu pour la transmission de la maladie de Lyme (Chomel, 2015). Il se trouve principalement dans la partie supérieure du bassin méditerranéen et a un tropisme ubiquitaire ainsi qu'un impact élevé en termes de santé humaine et vétérinaire (Estrada-Peña, 2004 ; Pérez-Eid, 2007). Dans nos données, il a été signalé dans sept pays (Chypre, France, Grèce, Italie, Slovénie, Espagne et Turquie). Huit espèces du genre *Ixodes* ont été reportées : *I. acuminatus*, *I. festai*, *I. frontalis*, *I. hexagonus*, *I. ricinus*, *I. simplex*, *I. ventalloi*, *I. vespertilionis* dans le bassin méditerranéen sur un total de 23

hôtes. De ces huit espèces de tiques collectées, 63,9 % des TBPs détectés dans les tiques ont été reportés. Néanmoins, la forte détection du genre *Ixodes* positifs pourrait être due à son aire de répartition principale en Europe de l'Ouest où plus de 65 % des publications ont été reportées.

Ces différents TBPs et genres de tiques ont été recensés chez un total de 17 groupes d'animaux sauvages, hôtes à la fois de tiques et d'agents pathogènes. Parmi ces hôtes, 61 TBPs ont été rapportés dans les tiques et 50 TBPs l'ont été directement dans les animaux sauvages. Les principaux groupes d'animaux sauvages signalés comme porteurs de tiques ou positifs aux TBPs étaient des animaux de chasse comme le sanglier et le cerf rouge, ou de petits animaux comme les rongeurs et les oiseaux. Il semble que la quantité de données rapportées des animaux sauvages comme hôte de tiques positives et hôte de TBPs diffère selon le statut de conservation des animaux. Les animaux sauvages comme les animaux chassables et les rongeurs sont signalés dans un certain nombre d'études pour les hôtes de tiques positives et les hôtes de TBPs. D'un autre côté, les animaux sauvages éventuellement protégés comme les chauves-souris, les oiseaux, les hérissons et les tortues sont principalement signalés comme hôtes de tiques positives. Il semble également qu'il y ait une légère corrélation entre le nombre d'études réalisées chez un hôte et le nombre de TBPs trouvées à partir de tiques positives avec un coefficient de corrélation de 0,84 et d'animaux sauvages positifs avec un coefficient de corrélation de 0,87.

Lors de cette revue, le bassin Méditerranéen a été également divisé en 4 zones. L'Europe occidentale qui regroupe la France, l'Italie, Malte, Monaco et l'Espagne. C'est dans cette partie que l'on trouve le plus grand nombre d'études, avec 102 articles, soit 68,9 % des publications prises en compte. Sur les 17 animaux hôtes, 16 ont été trouvés en Europe occidentale. Parmi ceux-ci, le plus représenté était le cerf dans 32 % des publications. Parmi les sept genres de tiques détectés dans la zone, le plus important était *Ixodes* spp., un genre de tique ubiquiste très répandu en Europe (Estrada-Peña, 2004; Pérez-Eid, 2007). Parmi les TBPs rapportés chez ces animaux sauvages et leurs tiques, les trois pathogènes les plus trouvés étaient *A. phagocytophilum*, l'agent responsable de l'anaplasmose granulocytaire humaine (HGA), des bactéries du genre *Rickettsia* comme *R. slovaca*, une bactérie du groupe de la fièvre tachetée (SFG) provoquant des lymphadénopathies et *C. burnetti*, la bactérie responsable de la fièvre Q. Le Proche-Orient, composé de Chypre, Israël, le Liban, la Palestine, la Syrie et la Turquie, est la deuxième région la plus importante en terme de publications (23), ce qui représente 15,5 % du nombre total de publications. De ces publications, 12 animaux hôtes ont été trouvés, soit 70,6 % des animaux hôtes intégrés dans cette étude. Parmi ces hôtes, celui reporté le plus souvent était la tortue dans 29,2 % des publications du Proche-Orient. Les genres de tiques les plus souvent retrouvés ont été cette fois *Hyalomma* spp., un genre très répandu en Méditerranée et dont certaines espèces ont un tropisme pour les tortues. Trente-neuf agents pathogènes ont été détectés chez les animaux sauvages et dans leurs tiques au Moyen-Orient, soit 51,3 % du total des agents pathogènes. Parmi eux, les plus signalés sont : le genre *Rickettsia* spp. responsable de nombreuses maladies comme la fièvre boutonneuse Méditerranéenne et le TIBOLA (Abdad et al., 2018), le virus de la fièvre hémorragique de Crimée-Congo principalement transmis par les tiques du genre *Hyalomma* et *R. massiliae* une bactérie SFG. Les Balkans composés de l'Albanie, la Bosnie-Herzégovine, la Croatie, la Grèce, le Monténégro et la Slovaquie, comptent un total de 15 publications, ce qui représente 8,8 % du total des publications de l'étude. Les principaux hôtes retrouvés ont été le cerf et les rongeurs chacun dans 26,7 % des publications concernant les Balkans. De ces hôtes, les genres principaux de tiques collectés positifs ont été *Hyalomma* spp. dont les stades adultes ciblent les ongulés et les stades immatures ciblent les micros vertébrés. Les principaux agents pathogènes trouvés sont : *A. phagocytophilum*, le genre *Babesia* et le virus de l'encéphalite à tiques dans 7,5 % des publications. Dans les Balkans, comme en Europe occidentale, les recherches scientifiques se sont concentrées sur les animaux chassés, comme le cerf et le sanglier, et sur les micromammifères, même si les deux principaux genres de tiques trouvés sont *Hyalomma* spp.

et *Rhipicephalus* spp.. L'Afrique du Nord qui regroupe l'Algérie, l'Égypte, la Libye, le Maroc et la Tunisie avec 10 publications trouvées. Les principaux hôtes en Afrique du Nord ont été les tortues et les hérissons. Sur les hôtes, le principal genre de tiques retrouvés positifs a été le genre *Hyalomma*. Les principaux TBPs retrouvés appartenaient tous au genre *Rickettsia* : *R. aeschlimannii*, *R. massiliae* et *R. slovacica*. L'Afrique du Nord a le plus faible nombre de publications.

Dix-neuf publications ont été réalisées au niveau des îles méditerranéennes, ce qui représente 12,8 % des publications. Sur ces 19 publications, la plupart d'entre elles proviennent des plus grandes îles du bassin méditerranéen : Sardaigne, Corse, Chypre ; et le reste provenant de petites îles grecques. Nous avons décidé de comparer les îles occidentales : Asinara, Capris, Columbretes, Corsica, Isla Grossa, Majorque, Medes, Pianosa, Ponza, Sardaigne, Sicile, Ustica, Ventotene et Zanone (16 publications) et les îles orientales : Antikythira et Chypre (4 publications). Une publication a été rapportée à la fois dans les publications occidentales et orientales. Le groupe d'hôtes le plus souvent retrouvé a été l'avifaune (50 % des publications dans les îles occidentales et 25 % dans les îles orientales). Cela pourrait être dû à la position stratégique des îles sur le chemin de migration de certains oiseaux (Jourdain et al., 2007; Vagliano, 1985). Le genre de tique le plus souvent retrouvé a été le genre *Hyalomma* comme pour les régions : Proche-Orient, Balkans et Afrique du Nord. Les principales causes pourraient être le tropisme des stades immatures de ce genre pour l'avifaune, mais également l'aire de répartition de genre plus présent dans le Sud du Bassin Méditerranéen contrairement au genre *Ixodes*. 36,8 % du total des TBPs a été rapporté au niveau des îles. Même si un faible pourcentage de données a été rapporté dans les îles, un pourcentage important des TBPs a été rapporté, ce qui montre l'importance des TBPs dans les îles méditerranéennes. Parmi ces genres, le principal rapporté était *Rickettsia* spp., dans 68,8 % des publications (îles occidentales) et 100% (îles orientales). Toutefois il est à noter que 80 % des publications dans les îles ont été trouvées dans les îles d'Europe occidentale.

Un total de 148 publications a été rapporté sur les TBPs chez les animaux sauvages et leurs tiques. Parmi elles, 68,9 % ont été rapportées dans les pays d'Europe occidentale, 15,5 % au Moyen-Orient, 8,8 % dans les Balkans et 6,8 % en Afrique du Nord. Cela montre que la recherche scientifique sur les TBPs provenant d'animaux sauvages se concentre principalement sur l'Europe de l'Ouest par rapport à d'autres régions du bassin méditerranéen. Parmi ces publications, 85 agents pathogènes ont été signalés : neuf genres de bactéries, huit genres de parasites et quatre genres de virus, ce qui montre la grande diversité des TBPs. Le principal genre positif rapporté était *Ixodes* spp.. Cependant, il s'agissait du principal genre positif signalé uniquement en Europe occidentale. L'importance de ce genre dans l'ensemble des données pourrait être due au taux de publications plus important rapporté en Europe occidentale (68,9 %). Dans les autres zones et îles, les principaux genres de tiques positifs étaient *Hyalomma* spp.. Parmi les 17 animaux sauvages hôtes signalés, les principaux étaient des ongulés sauvages et des micros vertébrés comme les rongeurs, hôtes des tiques immatures. Cet intérêt pourrait être dû à la relation étroite entre les ongulés sauvages, en tant qu'animaux de chasse ainsi qu'au rôle des rongeurs et des oiseaux dans le cycle de vie des tiques. De même, la quantité de données sur les oiseaux dans les îles (63,2 % des publications), pourrait être due à l'importance de ce groupe dans la dissémination des tiques et des TBPs dans les îles et entre les zones du bassin méditerranéen. Cela montre l'importance de l'animal sauvage dans la conservation et la circulation des agents pathogènes transmis par les tiques. De plus, avec seulement 12,8 % des publications, les îles présentent 36,8 % des TBPs recensés dans cette étude. Cela montre l'importance des îles dans la surveillance des TBPs chez les animaux sauvages au niveau du bassin méditerranéen.

# Distribution of tick-borne pathogens in wild animals and their ticks in the Mediterranean Rim between 2000 and 2021: a systematic review

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## Abstract

Tick-borne pathogens included three groups: bacteria, parasites and virus transmitted by a wide range of tick species and cause a large spectrum of human, animal and zoonotic disease. A total of 148 publications were found about tick-borne pathogens in wild animals with the report of 85 pathogen species from 35 tick species and 17 wild animals host between 2000 and February 2021. From those TBPs, the main reported were from the bacteria group as *Anaplasma* spp. and *Rickettsia* spp.. A total of 72.2% of the TBPs were reported from infected tick genera collected on wild animals. The main genera positive was the genus *Ixodes* mainly reported in Western Europe where most part of the publications was performed (66.9%). It was followed by the *Hyalomma* spp., that we found mainly reported in the other area of Mediterranean Rim. Those TBPs and positive tick genera were reported from a total of 17 wild animal hosts. The main wild animal hosts reported were hunt animals as red deer and wild boar and micro vertebrates as birds and rodents. Out of the 148 publications, 12.8% were found to be reported in the islands, 36.8% of the total TBPs were reported in seven tick genera and eleven wild animal hosts. The main positive wild animals and tick genera reported in the islands were birds and *Hyalomma* spp.. Despite the low percentage of publications reported, it shows the importance of islands in the TBPs monitoring in wild animals especially in wild birds which could be responsible of the dissemination of TBPs and their ticks through their migration path.

**Keywords:** Islands, Mediterranean Rim, TBPs, Ticks, Wild Animals

## 2. Introduction

Pathogens are one of the largest threats to worldwide health. They can be divided into four main groups: bacteria, viruses, parasite and fungi. Among these pathogens, many are zoonotic and are transmitted between animals and human. meanwhile non-zoonotic pathogen target specifically humans or animals. Zoonotic pathogens account for about 60% of pathogens worldwide and are particularly presents in emerging disease (CDC, 2020). Some of bacteria, parasite and virus can be transmitted by hematophagous arthropod vectors (Rosenberg and Ben Beard, 2011) such as mosquitoes, ticks or sandflies via blood-sucking from one host to another. Annually, vector-borne disease cause more than 17% infection and over than 700,000 deaths across the globe (WHO, 2020). The pathogens transmitted by ticks are called Tick-Borne Pathogens (TBPs) are known to be both pathogens of veterinary importance and zoonotic pathogens harmful for human (Sonenshine, 1991).

Ticks (Ixodida) rank first for veterinary vector-borne pathogens and second for human pathogens, just after mosquitoes (Nicholson et al., 2019; Parola and Raoult, 2001). Ticks are hematophagous arthropods of the Ixodida order. They are composed of three families distributed across the world: Ixodidae divided into the Prostriata (subfamily Ixodinae, genus *Ixodes*) and Metastrata (all other subfamilies, genera: *Anamalo Himalaya*, *Cosmiomma*, *Dermacentor*, *Hyalomma*, *Margaropus*, *Nosomma*, *Rhipicentor* and *Rhipicephalus*) it also named “hard ticks”, and includes most species; Argasidae divided into two subfamilies, the Argasinae (genus *Argas*) and the Ornithodorinae (genera: *Ornithodoros*, *Otobius* and *Carios*) also named “soft ticks”, and Nuttalliellidae composed of just one species (Beati and Klompen, 2019). The tick’s cycle of life is separated in 4 stages: the eggs, a six-legged larva, eight-legged nymph without a sexual organ and eight-legged adult male or female. Tick tropism depends of the species and stage, they can be monotropic, ditropic or teleotropic, with: one, two or several host, respectively. They can transmit pathogens a various specter of bacteria, virus and parasites (Nicholson et al., 2019), on a broad range of wild animals (mammalian, reptilian, amphibian and avifauna) (Inci et al., 2016; Klaus et al., 2016; Mendoza-Roldan et al., 2019; Pajoro et al., 2018; Sonenshine, 1991).

Ticks and tick-borne disease can spread by the way of migratory birds. Their long-distance migration allows migratory birds to carry infected immature argasid and ixodid ticks to Europe from Africa or in the other sense (Georgopoulou and Tsiouris, 2008). They can be one of the dissemination ways of ticks and tick-borne pathogens during their migration thanks to the seasonality of their migration and of the presence of stopover places which are mainly wetlands (Georgopoulou and Tsiouris, 2008).

Given its geographic position between Europe and Africa, the Mediterranean Sea is bordered by a significant number of countries with a high variation of biotopes, ranging from Mediterranean to arid climate. The Mediterranean Rim is one of the area affected by climatic change, animal migration and human activity (European commission, 2017; Giannakopoulos et al., 2005). While increasing temperatures in Northern Europe favor the development of ticks like *Ixodes ricinus*, in the Mediterranean Rim the development of dry area with arid types of vegetation favors the development of ticks like *Hyalomma marginatum* (Al-Abri et al., 2017). This development is also supported by feeding of immature stages on birds and the long duration of their attachment to the host during these stages. This supports the dissemination of the tick by the migratory birds (Estrada-Peña et al., 2015).

We focused our review on the countries of the Mediterranean Rim where the different TBPs detected in wild animals and their ticks were detected. Moreover, in order to compare the geographical distribution of these TBPs and to highlight possible changes in their spread in the future, four areas were considered: Western Europe composed of the France, Italy, Malta, Monaco and Spain; the Balkans composed of Albania, Bosnia-Herzegovina, Croatia, Greece,



Montenegro and Slovenia; the Middle East composed of Cyprus, Israel, Lebanon, Palestine, Syria and Turkey; and finally North Africa composed of Algeria, Egypt, Libya, Morocco and Tunisia. The different areas were determined by common biotope and geographic proximity. The last part of our review was to realize focus on the western and the eastern Mediterranean islands in the monitoring of TBPs in wild animals and their ticks. Especially in the avifauna, for which the islands could have a role of hotspots during their migrations.

The aim of this study was to review the existing literature between 2000 and early 2021 following PRISMA guideline about the presence of TBPs on wild animals and their ticks in the Mediterranean Rim with the following objectives:

- Perform a bibliometric analysis of TBP studies
- Review the diversity of TBPs from wild animals and their ticks, positive engorged tick species and wild animals TBPs host in The Mediterranean Rim.
- Compare the distribution of TBPs from wild animals and their ticks in the four main areas defined
- Focus on the distribution of the TBPs in the Mediterranean islands

### 3. Methods

We undertook a literature review concerning ticks-borne pathogens in all the countries of the Mediterranean Rim (n = 22). We followed PRISMA guidelines (Moher et al., 2015) and used explicit and systematic methods to identify, select and evaluate the studies that are relevant to the topic. We compiled and evaluated the data from the studies included in this review.

All articles published in international journals indexed by PubMed, Scopus and Web of Science were considered (Figure 11.). Only English was considered. The date range was from 1 January 2000 through 31 February 2021. We used keywords for each country of the Mediterranean Rim, classified in alphabetical order: Albania OR Algeria OR Bosnia-Herzegovina OR Cyprus OR Croatia OR Egypt OR France OR Greece OR Israel OR Italy OR Lebanon OR Libya OR Malta OR Monaco OR Montenegro OR Morocco OR Palestine OR Slovenia OR Spain OR Syria OR Tunisia OR Turkey AND Pathogens AND Ticks with the option “all fields” to recover articles in which search items appeared in the title, abstract and keywords. First, all paper considered “grey” literature, literature review, cases studies, manuscripts and abstracts of posters from conferences and guides from relevant organizations were excluded. We also discarded clinical descriptions of disease and diagnosis in humans and animals. We selected all papers focusing on the distribution and circulation of tick-borne pathogens and their vectors. We focused on studies dealing with the distribution of these pathogens and ticks in the Mediterranean Rim and their islands. Secondly, we excluded every duplicate and inaccessible articles (due to the languages or unavailable full text). We reviewed the title and abstracts and applied inclusion/exclusion criteria for 1070 articles. Filtering was carried out by responding to the following questions:

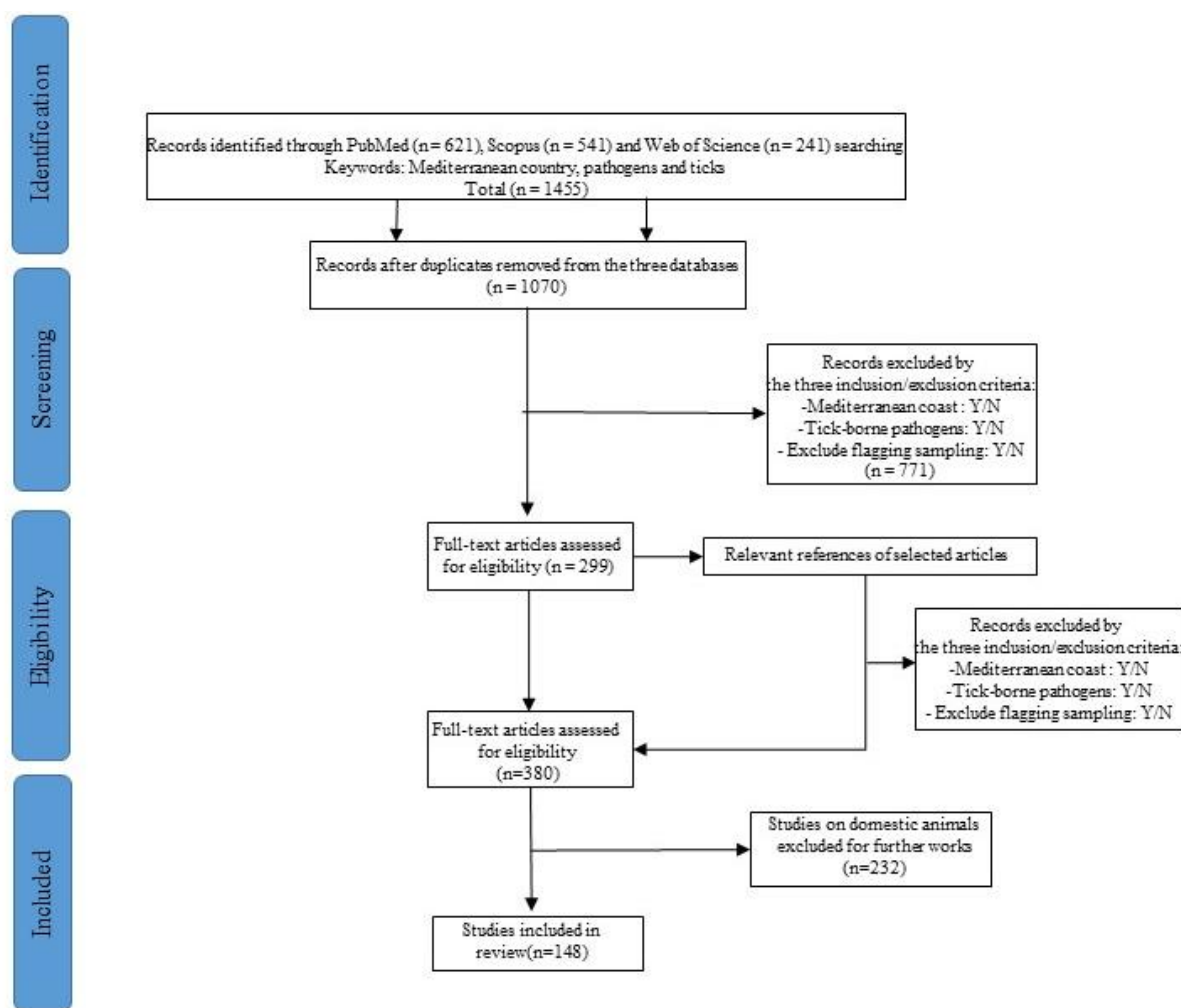
- Did the study include a country with a Mediterranean coast: Yes No
- Did the study include tick-borne pathogens: Yes No
- Did the study exclude ticks collected in vegetation: Yes No

Only publications considering TBPs in wild animals and/or TBPs in engorged ticks collected on wild animals in Mediterranean countries were included. This make it possible to have an overview of research about TBPs potentially infesting and circulating in the wild animal population only. The articles were saved if the answers to the three questions were “yes” otherwise they were eliminated. For the next step, we reviewed the full-text and entered the

information of interest into a database for 299 articles. We also excluded articles that did not fit the criteria after reading the full-text.

Then, we reviewed the bibliography of each selected article in order to check for new articles to include in the review and relevant articles in the fields of research. We followed the same steps previously described for new articles. For the last step, we excluded articles dealing with domestic animals (n=232) and retained only articles dealing with wild animals (n=148).

All the selection steps are summarized in the Figure 16 with an explanation of the inclusion/exclusion of articles for this review.



**Figure 16.** Methodological diagram of the bibliographic research following the PRISMA 2009 Flow according to Moher et al., 2015.

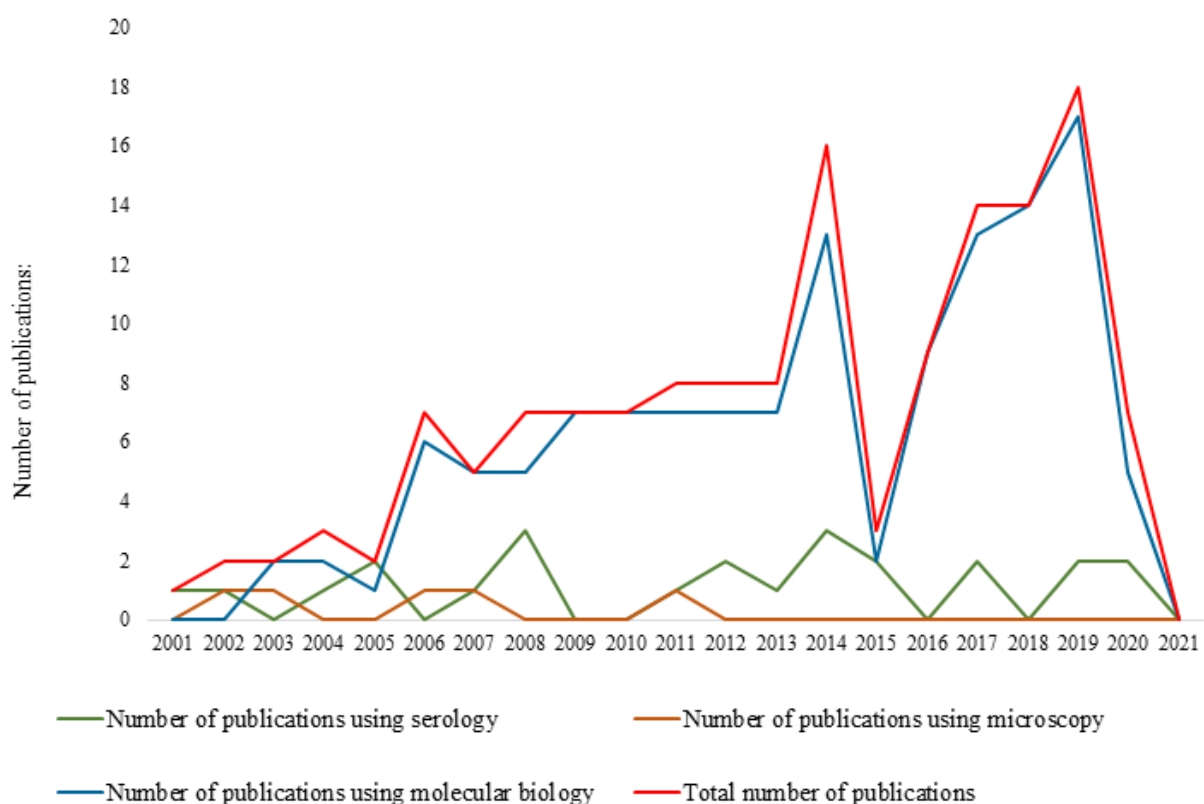
The data of interest were captured in an Excel table that was tested before with 15 articles, and included the following information:

- Main characteristics of the studies: article ID, years, authors, analytical and statistical methodology.
- Pathogen-related information: pathogens screened and detected, species, number of species, zoonotic status, host.
- Tick-related information: species, type, number, stage
- Host-related information: groups, sedentary or migratory, and domestic or wild
- Area of interest: country, type of area, number of sampling sites

The different outputs of the data worksheet were selected following mutual agreement from all the authors.

#### 4. Bibliographic analysis

The first article considered was published in 2001 and was the only paper this year. During the first decade, the number of paper is low between 2001 and 2005 with the highest peak of three paper in 2004. It starts to increase between 2006 and 2013 with mean of 7.1 publication per years (range from 5 to 8 papers per years). Between 2014 and 2020, it gathers 57.4% of the paper about TBPs on wild animals from the Mediterranean Rim. The rate of paper published double to eight per years in 2013 to 16 per years in 2014. However, it is followed by a fall to four publications in 2015. Between 2016 and 2020, the number of paper published per years continued to increase until reaching the highest peak of the both decade in 2019 of 18 publications in the year. No paper was found in the two first month of 2021 (Figure 17.).



**Figure 17.** Number of publications through the years (2000-February 2021). The total number is indicated in yellow, and the other indicate the number of publications according to each detection method used (Serology, Microscopy and Molecular biology).

This trend can be explained by three reasons:

- the increase of scientific interest about TBPs from wild animals and their ticks
- the increase of the accessibility to scientific journal and publication full-text
- the evolution of the pathogen detection methodology through the years and the development of the molecular biology.

About this last point, TBPs were detected by using three types of methodology. The serological analysis involving the detection of pathogens in blood, tissue and ticks by the way of antibodies as ELISA and immunofluorescence. The microscopic analysis which was lowly found even if it the most accurate methods to confirmed the presence of TBPs in samples. The

molecular analysis which gather techniques involving in the detection of TBPs nuclear acids in both ticks and animals such as polymerase chain reaction (PCR), next-generation sequencing (NGS) and high sequencing techniques. These molecular techniques are the most used, even if more than one technique could be used for the monitoring of TBPs. The increase of publications over the years seems to be linked with the gain of popularity of the molecular approach (Figure 17.).

## 5. Tick-Borne pathogens from wild animals and their tick in countries of the Mediterranean Rim

A total of 148 publications were analyzed. Among them, 66.7% were about bacteria, 21% about parasites and 12.3% about viruses. A total of 85 pathogens were detected in wild animals and their ticks from the Mediterranean Rim: nine genera of bacteria, eight genera of parasites and four genera viruses (Table 4.). From those, 51.1% were zoonotical and 48.9% were non-zoonotical or of unknown status (Table 4.). The percentage of publications about pathogens detected in ticks was 72.2% and quite higher than the one about TBPs detected in host: 61.1%.

### *Parasites*

#### Nematoda

##### *Cercopithifilaria*

Parasites of the genus *Cercopithifilaria* are tick-borne filaroids cutaneous parasites which were mainly described in dogs with two species: *Cercopithifilaria bainaie* and *Cercopithifilaria grassi* (Angelou et al., 2020). In the wild animals of the Mediterranean Rim, only one species was reported: *Cercopithifilaria rugosicauda*, a roe deer parasite transmits by *Ixodes ricinus* ticks. It was found in a dead roe deer subcutaneous samples from Italy (Ramos et al., 2013) in only one animal and never detected in ticks collected on wild animals. The few informations of the presence of those parasite can be explaining by the low interest and impact of those parasite on the wild life health. This genus is one of the three less reported of the whole data (0.7%) and of the data concerning parasites (1%).

#### Apicomplexa

##### *Babesia*

*Babesia* spp. is genus of erythrocytic protozoal parasites transmits by ticks that cause babesiosis in both animals and human. The zoonotic species are principally transmitted by ticks belonging to the genus *Ixodes*. The genus *Babesia* is composed of a varied number of species which were detected in wild animals and their ticks in the Mediterranean Rim, however each species were only found between one and four countries. Among the 13 species found, *Babesia microti*, zoonotic specie responsible of babesiosis in both human and animals, was detected in the largest range of countries: Croatia, Italy, Spain and Turkey. The highest prevalence in ticks was found in *Ixodes hexagonus* from red fox from Spain (55.6%); and in wild animals in Italy from red fox (54%) (Checa et al., 2018; Da Rold et al., 2018). The others *babesia* species were found in a large panels of host as birds, wild ungulates and rodents and mainly in ticks of the genus *Ixodes*. This genus was the most found parasite genus reported in 10.8% of the whole data and 51.6% of the data concerning parasites.

**Table 4.** Tick-borne pathogens distribution from wild animals and their ticks in the Mediterranean Rim countries.

Pathogens	Positives Ticks	Positives Ticks host	Pathogens host	Country	References
<b>Parasites: Nematoda</b>					
<i>Cercophthifilaria</i>					
<i>Cercophthifilaria rugosicauda</i>			Roe deer	Italy	(Ramos et al., 2013)
<b>Parasites: Apicomplexa</b>					
<i>Babesia</i>					
<i>Babesia</i> spp.	<i>Dermacentor marginatus</i> , <i>Hyalomma</i> spp., <i>Hyalomma marginatum</i> , <i>Ixodes ricinus</i> and <i>Rhipicephalus bursa</i>	Deer, Roe Deer and Wild boar	Deer, Badger, Hare, Roe deer, Rodent and Wolf	Croatia, France, Italy and Spain	(Bonnet et al., 2007; de la Fuente et al., 2004; Iori et al., 2010; Orkun and Karaer, 2017; Remesar et al., 2019; Rocchigiani et al., 2018; Santoro et al., 2019; Tadin et al., 2012)
<i>Babesia annae</i>			Red fox	Italy	(Da Rold et al., 2018)
<i>Babesia bigemina</i>			Red deer, Roe deer and Wild boar	Italy and Spain	(Remesar et al., 2019; Zanet et al., 2014)
<i>Babesia canis</i>			Wolf	Croatia	(Beck et al., 2017)
<i>Babesia capreoli</i>	<i>Ixodes ricinus</i>	Bird	Chamois, Red deer and Roe deer	Italy and Spain	(Baráková et al., 2018; Remesar et al., 2019; Zanet et al., 2014)
<i>Babesia crassa</i>	<i>Haemaphysalis parva</i>			Turkey	(Orkun and Karaer, 2017)
<i>Babesia divergens</i>			Red deer	Italy	(Ebani et al., 2016b)
<i>Babesia microti</i>	<i>Ixodes hexagonus</i> and <i>Ixodes ricinus</i>	Red fox, Rodent and Roe deer	Red fox and Rodent	Croatia, Italy Turkey	(Baráková et al., 2018; Beck et al., 2011; Checa et al., 2018; Da Rold et al., 2018; Usluca et al., 2019)
<i>Babesia occultans</i>	<i>Hyalomma marginatum</i>	Wild boar		Turkey	(Orkun and Karaer, 2017)
<i>Babesia ovis</i>	<i>Ixodes ricinus</i>	Roe deer		Italy	(Iori et al., 2010)
<i>Babesia rodaini</i>	<i>Ixodes ricinus</i> and <i>Rhipicephalus sanguineus</i> s.l.	Roe deer and Wild boar		Italy	(Iori et al., 2010)
<i>Babesia rossi</i>	<i>Haemaphysalis parva</i>	Wild boar		Turkey	(Orkun and Karaer, 2017)
<i>Babesia venatorum</i>	<i>Ixodes ricinus</i>	Bird and Rodent	Roe deer	Italy and Spain	(Baráková et al., 2018; Remesar et al., 2019; Zanet et al., 2014)
<i>Babesia vulpes</i>			Red Fox	Italy and Turkey	(Da Rold et al., 2018; Orkun and Karaer, 2017; Santoro et al., 2019)
<i>Cytauxzoon</i>					
<i>Cytauxzoon</i> spp.			Lynx	Spain	(Millán et al., 2007)
<i>Hemolivia</i>					
<i>Hemolivia mauritanica</i>			Tortoise	Palestine	(Paperma, 2006)
<i>Hepatozoon</i>					
<i>Hepatozoon</i> spp.			Lizard	Palestine	(Paperma et al., 2002)
<i>Hepatozoon canis</i>			Red fox	Croatia, Israel and Italy	(Deždek et al., 2010; V. V. Ebani et al., 2017b; Fishman et al., 2004)
<i>Hepatozoon kisrae</i>			Lizard	Palestine	(Paperma et al., 2002)
<i>Theileria</i>					
<i>Theileria</i> spp.	<i>Dermacentor marginatus</i> , <i>Hyalomma marginatum</i> , <i>Ixodes ricinus</i> and <i>Rhipicephalus bursa</i>	Fallow deer, Red deer and Wild boar	Chamois, Hare, Fallow deer, Red deer, Roe deer and Wild boar	Italy and Spain	(de la Fuente et al., 2004; Galuppi et al., 2011; García-Sanmartín et al., 2007; Remesar et al., 2019; Rocchigiani et al., 2018; Zanet et al., 2014)
<i>Theileria annae</i>	<i>Ixodes hexagonus</i> and <i>Ixodes ricinus</i>	Red Fox and Rodent	Badger and Red Fox	Croatia, Italy and Spain	(Da Rold et al., 2018; Deždek et al., 2010; V. V. Ebani et al., 2017b; Giménez Pardo et al., 2009; Lledó et al., 2010; Zanet et al., 2014)
<i>Theileria buffeli</i>	<i>Hyalomma marginatum</i> , <i>Rhipicephalus bursa</i> and <i>Rhipicephalus sanguineus</i> s.l.	Bird, Red fox and Mouflon		Italy	(Chisu et al., 2019)
<i>Theileria capreoli</i>			Wolf	Croatia	(Beck et al., 2017)
<i>Theileria orientalis</i>	<i>Hyalomma marginatum</i> , <i>Rhipicephalus bursa</i> and <i>Rhipicephalus sanguineus</i> s.l.	Birds, Red fox and Mouflon		Italy	(Chisu et al., 2019)
<i>Theileria ovis</i>	<i>Rhipicephalus ovis</i>	Mouflon	Chamois	Italy and Spain	(García-Sanmartín et al., 2007)
<i>Theileria sergenti</i>	<i>Hyalomma marginatum</i> , <i>Rhipicephalus bursa</i> and <i>Rhipicephalus sanguineus</i> s.l.	Birds, Red fox and Mouflon		Italy	(Chisu et al., 2019)
<b>Bacteria</b>					
<i>Anaplasma</i>					

<i>Anaplasma</i> spp.	<i>Haemaphysalis punctata</i> , <i>Ixodes ricinus</i> , <i>Rhipicephalus bursa</i> and <i>Rhipicephalus sanguineus</i> s.l.	Chamois and Hedgehog	Bird	Algeria, Cyprus and Italy	(Borgni et al., 2019; Boucheikhchoukh et al., 2018; Ioannou et al., 2009)
<i>Anaplasma capra</i>			Red deer and Swamp deer	France	(Jouglin et al., 2019)
<i>Anaplasma marginale</i>	<i>Dermacentor marginatus</i> , <i>Hyalomma marginatum</i> , <i>Ixodes ricinus</i> and <i>Rhipicephalus bursa</i>	Red deer and Wild boar		Italy	(de la Fuente et al., 2004)
<i>Anaplasma phagocytophilum</i>	<i>Boophilus kohlsi</i> , <i>Dermacentor marginatus</i> , <i>Haemaphysalis punctata</i> , <i>Hyalomma marginatum</i> , <i>Ixodes ricinus</i> , <i>Rhipicephalus bursa</i> and <i>Rhipicephalus sanguineus</i> s.l.	Bird, fallow deer, Red deer, Roe, deer, Rodent and Wild boar	Fallow deer, Porcupine, Red fox, Roe deer, Rodent, Swamp deer and Wild boar	France, Greece, Israel, Italy, Slovenia and Spain	(Baráková et al., 2014, 2018; Beninati et al., 2006; Carpi et al., 2009; Chastagner et al., 2016; Da Rold et al., 2018; de la Fuente et al., 2004; Dugat et al., 2016; Ebani et al., 2007, 2008, 2015a, 2016b; V. V. Ebani et al., 2017a, 2017b; Hoffman et al., 2020; Jouglin et al., 2017, 2019; Keysary et al., 2007; Palomar et al., 2012; Perez et al., 2017; Petrovec et al., 2002; Remesar et al., 2020; Rosso et al., 2017; Smrdel et al., 2009; Torina et al., 2008b)
<i>Anaplasma ovis</i>	<i>Haemaphysalis punctata</i> , <i>Haemaphysalis sulcata</i> , <i>Ixodes festai</i> , <i>Rhipicephalus bursa</i> , and <i>Rhipicephalus sanguineus</i> s.l.	Hedgehog, Marten, Mouflon and Red fox	Mouflon	Cyprus and Italy	(Chisu et al., 2018a; Ioannou et al., 2011)
<b>Bartonella</b>					
<i>Bartonella</i> spp.	<i>Argas vespertilionis</i> , <i>Ixodes vespertilionis</i> , <i>Ixodes simplex</i> , <i>Rhipicephalus sanguineus</i> s.l.	Bat, Red fox	Bird and Rodent	Israel and Italy	(Chisu et al., 2018a; Ebani et al., 2016a; Hornok et al., 2019; Morick et al., 2009)
<i>Bartonella acomodis</i>	<i>Ixodes ricinus</i>	Red deer		Italy	(Ebani et al., 2015a)
<i>Bartonella bacilliformis</i>	<i>Haemaphysalis punctata</i> and <i>Ixodes ricinus</i>	Fallow deer and Red deer		Italy	(Ebani et al., 2015a)
<i>Bartonella bovis</i>	<i>Dermacentor marginatus</i> , <i>Haemaphysalis punctata</i> and <i>Ixodes Ricinus</i>	Fallow deer and Red deer		Italy	(Ebani et al., 2015a)
<i>Bartonella chomelii</i>	<i>Dermacentor marginatus</i> and <i>Ixodes ricinus</i>	Red deer and Roe deer		Italy	(Ebani et al., 2015a)
<i>Bartonella elizabethae</i>			Rodent	Israel	(Morick et al., 2009)
<i>Bartonella tamiae</i>	<i>Ixodes vespertilionis</i>	Bat		Algeria	(Leulmi et al., 2016)
<i>Bartonella tribocorum</i>	<i>Ixodes ricinus</i>	Red deer		Italy	(Ebani et al., 2015a)
<i>Bartonella vinsonnii berkoftii</i>	<i>Ixodes ricinus</i>	Red deer		Italy	(Ebani et al., 2015a)
<b>Borrelia</b>					
<i>Borrelia</i> spp.	<i>Hyalomma aegyptium</i>	Turtle	Rodent	Algeria, Morocco, Spain and Turkey	(Barandika et al., 2007; Boucheikhchoukh et al., 2018; Diatta et al., 2012; Güner et al., 2003)
<i>Borrelia afzelii</i>	<i>Ixodes ricinus</i>	Bird, Lizard and Rodent	Rodent	France, Italy, Spain and Turkey	(Barandika et al., 2007; Gil et al., 2005; Güner et al., 2005; Marchant et al., 2017; Marsot et al., 2013; Martello et al., 2019; Pajoro et al., 2018; Tomassone et al., 2017)
<i>Borrelia bavariensis</i>			Rodent	France	(Marchant et al., 2017)
<i>Borrelia burgdoferi</i> s.l.	<i>Dermacentor marginatus</i> , <i>Ixodes acuminatus</i> , <i>Ixodes ricinus</i> and <i>Rhipicephalus sanguineus</i> s.l.	Fallow deer, Lizard, Red deer, Rodent and Roe deer	Bird, Rodent, Roe deer and Wild boar	France, Italy and Spain	(Barandika et al., 2007; V. Ebani et al., 2017; Ebani et al., 2016a, 2016b, 2015a; V. V. Ebani et al., 2017a; Marchant et al., 2017; Martello et al., 2019; Pascucci et al., 2015; Pato et al., 2013; Perez et al., 2017; Tomassone et al., 2017)
<i>Borrelia burgdoferi</i> s.s.	<i>Ixodes acuminatus</i> and <i>Ixodes ricinus</i>	Rodent and Roe deer	Rodent	France, Italy and Spain	(Gil et al., 2005; Marchant et al., 2017; Marsot et al., 2013; Martello et al., 2019; Pascucci and Cammà, 2010; Pisanu et al., 2014)
<i>Borrelia crocidurae</i>	<i>Ornithodoros erritacus</i>	Rodent		Morocco	(Diatta et al., 2012)
<i>Borrelia garinii</i>	<i>Haemaphysalis punctata</i> , <i>Ixodes</i> spp., <i>Ixodes frontalis</i> and <i>Ixodes ricinus</i>	Bird, Lizard and Rodent	Rodent	France, Italy and Spain	(Gil et al., 2005; Marsot et al., 2013; Martello et al., 2019; Pajoro et al., 2018; Palomar et al., 2012; Tomassone et al., 2017)
<i>Borrelia hispanica</i>	<i>Ornithodoros erritacus</i>	Rodent		Morocco	(Diatta et al., 2012)
<i>Borrelia lusitaniae</i>	<i>Ixodes ricinus</i>	Bird, Lizard Rodent and Roe deer	Rodent	Italy	(Amore et al., 2007; Martello et al., 2019; Mendoza-Roldan et al., 2019; Mori et al., 2018; Pajoro et al., 2018; Pascucci and Cammà, 2010; Tomassone et al., 2017)
<i>Borrelia merionesi</i>	<i>Ornithodoros erritacus</i>	Rodent	Rodent	Morocco	(Diatta et al., 2012)
<i>Borrelia spielmanii</i>	<i>Ixodes acuminatus</i>	Bird		Italy	(Tomassone et al., 2013)
<i>Borrelia valaisiana</i>	<i>Ixodes</i> spp., <i>Ixodes ricinus</i> and <i>Ixodes ventralis</i>	Bird, Lizard and Rodent	Rodent	Italy and Spain	(Amore et al., 2007; Gil et al., 2005; Martello et al., 2019; Pajoro et al., 2018; Palomar et al., 2012; Tomassone et al., 2017, 2013)
<i>Borrelia turdi</i>	<i>Ixodes frontalis</i>	Bird		Spain	(Palomar et al., 2012)
<b>Chlamydia</b>					
<i>Chlamydia</i> spp.			Bird	Italy	(Ebani et al., 2016a)
<i>Chlamydia abortus</i>	<i>Haemaphysalis sulcata</i> , <i>Rhipicephalus bursa</i> and <i>Rhipicephalus sanguineus</i> s.l.	Hedgehog, Marten, Mouflon, Red deer and Red fox		Italy	(Chisu et al., 2018b)
<i>Chlamydia psittaci</i>			Bird	Italy	(Ebani et al., 2016a)
<b>Coxiella</b>					

<i>Coxiella burnetii</i>	<i>Haemaphysalis punctata</i> , <i>Haemaphysalis sulcata</i> , <i>Hyalomma anatolicum excavatum</i> , <i>Ixodes</i> spp., <i>Ixodes acuminatus</i> , <i>Ixodes ricinus</i> , <i>Ixodes ventraloi</i> , <i>Ixodes vespertilionis</i> <i>Rhipicephalus bursa</i> and <i>Rhipicephalus sanguineus</i> s.l.	Bat, Bird, Hare, Marten, Mouflon, Roe deer, Rodent and wild boar	Bird, Mouflon, Red deer, Red fox and Rodent	Algeria, Cyprus Italy, Slovenia and Spain	(Astobiza et al., 2011; Barandika et al., 2007; Chisu et al., 2018a; Ebani et al., 2019, 2016a, 2016b; V. V. Ebani et al., 2017b, 2017a; González-Barrio et al., 2016; Ioannou et al., 2011, 2009; Knap et al., 2019; Leulmi et al., 2016; Pascucci et al., 2015)
<b>Ehrlichia</b>					
<i>Ehrlichia</i> spp.	<i>Am. marmoreum</i> , <i>Dermacentor marginatus</i> , <i>Hyalomma</i> spp., <i>Hyalomma marginatus</i> , <i>Hyalomma rufipes</i> and <i>Rhipicephalus bursa</i>	Bird, Red deer and Wild boar		Spain	(de la Fuente et al., 2004; Toma et al., 2014)
<i>Ehrlichia canis</i>	<i>Dermacentor marginatus</i> , <i>Haemaphysalis punctata</i> and <i>Rhipicephalus sanguineus</i> s.l.	Mouflon, Red fox and Wil boar	Otter, Red fox and wolf	Israel and Italy	(Chisu et al., 2018a; V. V. Ebani et al., 2017b; Fishman et al., 2004; Santoro et al., 2017, 2016; Satta et al., 2011)
<b>Leptospira</b>					
<i>Leptospira</i> spp.			Rodent	Croatia	(Tadin et al., 2012)
<i>Leptospira borpetersenii</i>			Rodent	Croatia, France	(Izquierdo-Rodríguez et al., 2020; Turk et al., 2003)
<i>Leptospira interrogans</i>			Rodent	Croatia, France	(Izquierdo-Rodríguez et al., 2020; Tadin et al., 2012; Turk et al., 2003)
<i>Leptospira kishneri</i>			Rodent	Croatia	(Turk et al., 2003)
<i>Leptospira santarorai</i>			Rodent	Croatia	(Turk et al., 2003)
<b>Noehrlichia</b>					
<i>Neoehrlichia mikurensis</i>	<i>Ixodes ricinus</i>	Rodent	Rodent	France and Italy	(Baráková et al., 2018; Vayssier-Taussat et al., 2012)
<b>Rickettsia</b>					
<i>Rickettsia</i> spp.	<i>Argas transgarepinus</i> , <i>Argas vespertilionis</i> , <i>Dermacentor marginatus</i> , <i>Haemaphysalis</i> spp., <i>Haemaphysalis punctata</i> , <i>Haemaphysalis sulcata</i> , <i>Hyalomma aegyptium</i> , <i>Hyalomma marginatum</i> , <i>Ixodes frontalis</i> , <i>Ixodes ricinus</i> , <i>Ixodes ventraloi</i> , <i>Rhipicephalus bursa</i> , <i>Rhipicephalus pusillus</i> , <i>Rhipicephalus sanguineus</i> s.l. and Unknown	Bat, Bird, Hare, Hedgehog, Lizard, Mouflon, Red deer, roe deer, Turtle, Tortoise and Wild boar	Bird, Mouflon and Otter	Algeria, Cyprus, Italy, Spain and Turkey	(Borgni et al., 2019; Boucheikhchoukh et al., 2018; de la Fuente et al., 2004; Ebani et al., 2016a; V. V. Ebani et al., 2017a; Hornok et al., 2019; Ioannou et al., 2011, 2009; Kar et al., 2011; Maioli et al., 2012; Oteo et al., 2006; Palomar et al., 2012; Santoro et al., 2017; Satta et al., 2011; Tomassone et al., 2017; Wallménius et al., 2014)
<i>Rickettsia aeschlimannii</i>	<i>Am. marmoreum</i> , <i>Haemaphysalis</i> spp., <i>Hyalomma</i> spp., <i>Hyalomma aegyptium</i> , <i>Hyalomma marginatum</i> , <i>Hyalomma rufipes</i> , <i>Ixodes frontalis</i> and unknown	Bird, Roe deer Tortoise and Wild boar		France, Greece, Israel and Italy	(Azagi et al., 2017; Battisti et al., 2020; Bitam et al., 2009; V. Cicculi et al., 2019b; Keysary et al., 2011; Matsumoto et al., 2004; Scarpulla et al., 2016; Toma et al., 2014; Wallménius et al., 2014)
<i>Rickettsia africae</i>	<i>Hyalomma</i> spp., <i>Hyalomma aegyptium</i> , <i>Hyalomma detritum</i> , <i>Hyalomma marginatum</i> , <i>Hyaloma rufipes</i> , <i>Ixodes ricinus</i> and Unkown	Bird, Tortoise and Wild boar		Algeria, Greece, Israel, Italy and Turkey	(Azagi et al., 2017; Battisti et al., 2020; Boucheikhchoukh et al., 2018; Orkun et al., 2014; Toma et al., 2014; Wallménius et al., 2014; Waner et al., 2014)
<i>Rickettsia helvetica</i>	<i>Ixodes acuminatus</i> , <i>Ixodes festai</i> , <i>Ixodes ricinus</i> and <i>Ixodes ventraloi</i>	Bird, Chamois, Fallow deer, Hedgehog, Lizard, Lynx, Red deer, Red fox, Roe deer and Rodent		France, Italy and Spain	(Baráková et al., 2018; Chisu et al., 2018a; Maioli et al., 2012; Márquez, 2008; Márquez and Millán, 2009; Pajoro et al., 2018; Palomar et al., 2012; Tomassone et al., 2017, 2013)
<i>Rickettsia hoogstraalii</i>	<i>Haemaphysalis punctata</i> , <i>Haemaphysalis sulcata</i> and <i>Ixodes ricinus</i>	Lizard and Mouflon		Cyprus and Italy	(Chisu et al., 2018a, 2017; Chochlakis et al., 2012; Tomassone et al., 2017)
<i>Rickettsia massiliae</i>	<i>Haemaphysalis erinacei</i> <i>Rhipicephalus pusillus</i> , <i>Rhipicephalus sanguineus</i> s.l.	Hare, Hedgehog, Golden Jackal, Lynx, Mongoose, Red fox, Roe deer and wild boar		Algeria, Cyprus, Israel, Italy and Spain	(Bitam et al., 2006; Chisu et al., 2018a, 2017; Chochlakis et al., 2012; Keysary et al., 2011; Leulmi et al., 2016; Márquez, 2008; Márquez and Millán, 2009; Waner et al., 2014)
<i>Rickettsia monacensis</i>	<i>Haemaphysalis punctata</i> , <i>Ixodes ricinus</i> and <i>Ixodes ventraloi</i>	Badger, Bird, Fallow deer, Genet, Hare, Lizard, Lynx, Mongoose, Red deer, Red fox Roe deer and Rodent		Spain and Italy	(Baráková et al., 2018; Ebani et al., 2015a; Maioli et al., 2012; Márquez, 2008; Márquez and Millán, 2009; Martello et al., 2019; Mori et al., 2018; Pajoro et al., 2018; Palomar et al., 2012; Tomassone et al., 2017)
<i>Rickettsia raoultii</i>	<i>Dermacentor marginatus</i> and <i>Hyalomma</i> spp.	Bird, Fallow deer, Rodent and Wild boar		Italy and Spain	(Battisti et al., 2020; Maioli et al., 2012; Márquez, 2008; Martello et al., 2019; Selmi et al., 2009)
<i>Rickettsia sibirica</i>	<i>Ixodes ricinus</i>	Bird		Spain	(Palomar et al., 2012)
<i>Rickettsia sibirica mongolitimonae</i>	<i>Rhipicephalus</i> (Bo.) <i>annulatus</i>	Fallow deer		Israel	(Waner et al., 2014)
<i>Rickettsia slovaca</i>	<i>Dermacentor marginatus</i> , <i>Haemaphysalis punctata</i> , <i>Ixodes ricinus</i> and <i>Rhipicephalus bursa</i>	Fallow deer, Red deer, Rodent and Wild boar	Rodent and Wild boar	Algeria, France, Italy and Spain	(Chisu et al., 2017, 2014; V. Cicculi et al., 2019b; Ebani et al., 2015a; Leulmi et al., 2016; Maioli et al., 2012; Márquez, 2008; Martello et al., 2019, 2013; Masala et al., 2012; Ortuño et al., 2006; Oteo et al., 2006; Raelle et al., 2018; Selmi et al., 2009; Zeroual et al., 2018)
<i>Candidatus Rickettsia barbariae</i>	<i>Rhipicephalus sanguineus</i> s.l.	Hare, Mouflon, Red fox and Wolf		Cyprus, Italy and Palestine	(Chisu et al., 2018a; Chochlakis et al., 2012; Ereقات et al., 2016a)

<b>Candidatus Rickettsia goldwasserii</b>	<i>Haemaphysalis adleri</i> , <i>Haemaphysalis parva</i> and <i>Rhipicephalus sanguineus</i> s.l.	Golden Jackal and Wolf		Israel and Palestine	(Erekat et al., 2016a; Keysary et al., 2011)
<i>Candidatus Rickettsia africa septentrioalis</i>	<i>Ornithodoros normandi</i>	Rodent		Tunisia	(Buysse and Duron, 2020)
<b>Viruses</b>					
<b>Flavivirus</b>					
Louping ill			Roe deer and Wild boar	France	(Bournez et al., 2019)
Maeban/Maeban-like virus	<i>Ornithodoros maritimus</i>	Birds	Birds	Algeria, France and Spain	(Arnal et al., 2014)
TBEV			Rodent, Roe deer and Wild boar	France and Slovenia	(Bournez et al., 2020a, 2020b, 2019; Knap et al., 2012)
<b>Orthonairovirus</b>					
<b>Tamdy orthonairovirus</b>	<i>Hyalomma</i> spp. and <i>Hyalomma marginatum</i>	Rodent		Turkey	(Brinkmann et al., 2018)
<b>CCHF</b>	<i>Hyalomma</i> spp., <i>Hyalomma aegyptium</i> , <i>Hyalomma lusitanicum</i> , <i>Hyalomma marginatum</i> , <i>Hyalomma rufipes</i> and <i>Ixodes</i> spp.	Bird, Red deer, Tortoise	Tortoise	Algeria, Italy, Morocco, Spain and Turkey	(Cajimat et al., 2017; Estrada-Peña et al., 2012b; Kar et al., 2020; Kautman et al., 2016; Leblebicioglu et al., 2014; Mancuso et al., 2019; Negro et al., 2019; Palomar et al., 2012)



### *Cytauxzoon*

Parasites from the genus *Cytauxzoon* are piroplasmids infecting wild and domestic felines and their ticks in both the New World and the Old World (Panait et al., 2021). In wild animals of the Mediterranean Rim the genus was only found in Lynx from Spain with a prevalence of 15%. These pathogens were only identified at the genus level and it's seems to give rise a low interest of the scientific. This genus is one of the three less reported of the whole data (0.7%) and of the data concerning parasites (1%).

### *Hemolivia*

This genus belongs to the apicomplexan family and its composed of haemogragines parasites with a tropism for “reptiles” as intermediates host and hard ticks as final host (Kvičerová et al., 2014). In wild animals of the Mediterranean Rim only one specie was found: *Hemolivia mauritanica*. This specie is closely related to tortoises and especially to the genus *Tetsudo* where it was found with a prevalence of 17.2% in Palestine (Paperna, 2006). The low research and detection of this genus is probably related to the specific tropism of this genus for the “reptiles”. This genus is the last of the three less reported in of the whole data (0.7%) and of the data concerning parasites (1%).

### *Hepatozoon*

As the precedent genus, the genus *Hepatozoon* belongs also to the apicomplexan family and its closely related to the genus *Hemolivia* however its mainly focused wild and domestic carnivores. Only two species from wild animals were found in the publication is the Mediterranean Rim: *Hepatozoon canis* found in fox in three countries with a higher prevalence of 49% in Italy; and *Hepatozoon kisrae* found in lizard with a prevalence of 33.3% in Palestine (V. V. Ebani et al., 2017b; Paperna et al., 2002). As the previous genus, *Hepatozoon* pathogens were found mainly in animal's samples. This genus was poorly reported: in 2.7% of the total publications and in 4% of the publications concerning parasites.

### *Theileria*

Like *Babesia* and *Hepatozoon*, the genus *Theileria* belongs to the *Apicomplexa* phylum and to the piroplasmids group as *Babesia*. They infect mammals with and obligatory cycle in ticks. They cause benign to fatal theileriosis in breeding animals (Mans et al., 2015). A total of six *Theileria* species were detected in wild animals. Out of them three species were only found in ticks, one specie only in animals and two species in both. *T. annae* was the most detected. It was found in a highest prevalence of 9.1% in *Ixodes ricinus* collected on rodents in Italy and 14% in fox from Spain (Giménez Pardo et al., 2009; Lledó et al., 2010). This genus was the second most reported parasite genus in 8.8% of the whole data and in 13.1% of the data concerning parasites.

## *Bacteria*

### *Anaplasma*

The genus *Anaplasma* includes intracellular Gram-negative bacteria belonging to the family *Anaplasmataceae* from the *Rickettsiales* order. These bacteria are zoonotic and have a high impact in the veterinary health such as *Anaplasma marginale*, *Anaplasma bovis* *Anaplasma ovis* and also in human health such as *Anaplasma phagocytophilum* and *Anaplasma platys* (Ben Said et al., 2018; Kocan et al., 2015). The most reported species in wild animals was *Anaplasma phagocytophilum*, the causative agent responsible of the Human granulocytic anaplasmosis, detected in six countries out of 22 countries of the Mediterranean Rim (France, Greece, Israel, Italy, Slovenia and Spain). Eight different tick's species were found positives to

the pathogens with *Ixodes ricinus* with a highest prevalence of 11% collected on Red and Roe deer from Spain (Baráková et al., 2018). Across the Mediterranean Rim, the main host of positive ticks to *A. phagocytophilum* were wild ungulate (Table 4.). In animals, *A. phagocytophilum* was mainly found in rodent with a prevalence of 22.8% in France. The second most distributed specie was *Anaplasma ovis*, responsible of the ovine anaplasmosis in goat and sheep but also in wild ungulate, found in two countries in wild animals: Cyprus and Italy. The highest prevalence was found in the tick *Haemaphysalis sulcata* collected on mouflon in Cyprus (24%) and in *Ixodes festai* collected on hedgehog in Italy (33.3%). In animals it was only found in mouflon from Cyprus with a prevalence of 10% (Chisu et al., 2018a; Ioannou et al., 2011). Across the Mediterranean Rim, the genus *Anaplasma* is widely distributed and searched. It was reported in 19.6% of the whole data and 29.3% of data concerning bacteria. It was the second most reported bacteria genus. However, most part of the researches were reported on the zoonotic species (*Anaplasma phagocytophilum*) and a fewer part of the researches are done on the animal specific species. Nevertheless, an equal part of the researches was reported in ticks and animals.

### *Bartonella*

*Bartonella* are Gram-negative bacteria belonging to the family *Bartonellaceae* from the Rhizobiales order and half of them are known to be zoonotic. They are responsible of disease such as trench fever and cat-scratch and frequently cause endocarditis. In these genus a total of eight species were found in three countries: Algeria, Israel and Italy. The only species found in Algeria was *Bartonella tamiae*, a zoonotic specie primarily found in human in Thailand, reported in *Ixodes vespertilionis* collected on bat with a prevalence of 63.2% (Kosoy et al., 2008; Leulmi et al., 2016). Six species were detected in Italy; they were all detected in ticks (Table 4.). The highest prevalence in ticks was for *Bartonella bovis* from the species *Dermacentor marginatus*, *Haemaphysalis punctata* and *Ixodes ricinus* collected on Fallow deer and Red deer (Ebani et al., 2015a). The last species detected was *Bartonella elizabethae*, it was the only reported on animals with a prevalence of 25% in rodent in Israel (Morick et al., 2009). For this genus, the research was mainly performed in ticks and in a low number of countries. It was reported in 4.1% of the whole publications and in 6.1% of the publications concerning bacteria.

### *Borrelia*

The genus *Borrelia* includes spirochetes bacteria belonging to the family *Spirochaetaceae*. It is divided in two groups: the Lyme borreliosis group responsible for Lyme disease mainly caused by bacteria of the *Borrelia burgdoferi sensus lato* group, and the relapsing fever group which includes *Borrelia miyamotoi* (Margos et al., 2018). A total of 12 *Borrelia* species were found in the Mediterranean Rim. Out of them, the most part of the *Borrelia* species detected were from the *Borrelia burgdoferi* s.l. group, responsible of the Lyme disease, and are the most widely distributed (Table 1). Out of this species, *Borrelia afzelii* was the most reported in four countries: France, Italy, Spain and Turkey. In ticks, it was only detected in the species *Ixodes ricinus* with the highest prevalence of 4.8% in ticks collected on Birds from Italy. In animals, *Bo. afzelii* was only detected in rodent with a highest prevalence of 4.5% from Spain (Barandika et al., 2007; Pajoro et al., 2018). Among the other *Borrelia* species, the one reported with highest prevalence in *Ornithodoros erraticus* s.l. was *Borrelia crociduræ* from Morocco (41%) and *Borrelia merionesi* in rodent from Morocco (4%) (Diatta et al., 2012). In the Mediterranean Rim, the *Borrelia* species of the Lyme disease group were most searched and found in ticks and animals probably linked to the sanitary importance of the Lyme disease. However, the presence of other species with a possible sanitary importance was confirmed in two countries: Morocco and Spain. The genus *Borrelia* was reported in 18.2% of the whole data and 27.2% of the data concerning bacteria. It was the third most reported bacteria genus.

### *Chlamydia/Chlamydophila*

The genera *Chlamydia* and *Chlamydophila* belongs to the *Chlamydiales* which are Gram-negative bacteria responsible for a wide range of diseases in nearly the entire animal realm (Chisu et al., 2018b). Few studies showed that ticks could be vector of *Chlamydia/Chlamydophila* (McKercher et al., 1980). Two species were found: *Chlamydia abortus* and *Chlamydia psittaci*. The first one responsible of abortive chlamydiosis in small ruminants, was found only in ticks with a highest prevalence of 21.4% in *Haemaphysalis sulcata* in hedgehog from Italy (Arif et al., 2020; Chisu et al., 2018b). The second one, responsible of avian psittacosis and human pneumonia, was found in animals with a highest prevalence of 11.9% in pigeon from Italy (Ebani et al., 2016a; Knittler and Sachse, 2015). These genera were only found in Italy in both animals and ticks which probably shown a potential role of ticks in their circulation. It was reported in only 1.4% of the whole publication and 2% of the publications concerning bacteria.

### *Coxiella*

Only one pathogen is representative of this genus: *Coxiella burnetii* responsible for the Q fever which is transmitted by ticks and affects both human and animals. This disease has a worldwide distribution and can cause febrile illness, endocarditis, meningoencephalitis or pneumonia in human and mainly asymptomatic in animals with sporadic case of abortion in pregnant animals (Angelakis and Raoult, 2010). *C. burnetii* was detected in wild animals of five countries. The highest prevalence in ticks and animals were observed in *Ixodes ricinus* collected on rodent in Italy, and in Red deer in Spain with respectively a prevalence of 17.3% and 48.9% (González-Barrio et al., 2015; Pascucci et al., 2015) (Table 4.). It was reported and researched in a large panel of countries (Algeria, Italy, Italy, Slovenia and Spain), ticks and hosts, that shows the importance of *C. burnetii* in the Mediterranean Rim. It was reported in 9.5% of the whole data and 14.1% of the publications concerning bacteria.

### *Ehrlichia*

*Ehrlichia* spp. is a genus closely related to *Anaplasma* spp. and is responsible of the human monocytotropic ehrlichiosis (*Ehrlichia chaffeensis* and *Ehrlichia canis*) and canine ehrlichiosis (*Ehrlichia canis*) (Ismail et al., 2010). This last species was the only one found in wild animals in the Mediterranean Rim (Israel and Italy). The highest prevalence in ticks was found in *Dermacentor marginatus* collected on wild boar (7.8%). The highest prevalence in animals was in fox both from Italy (52%) (Santoro et al., 2016; Satta et al., 2011) (Table 4.). This genus was reported in 4.7% of the whole data and 7.1% of the data concerning bacteria.

### *Neoehrlichia*

Of the four bacteria composing this genus only one was reported in our study. It and *Noehrlichia mikurensis*, a zoonotic pathogen, which causes chronic lymphocytic leukemia. Its vectors are from the *Ixodes* genus and rodents are the well-known host (Portillo et al., 2018). It was found in two countries: France with a prevalence of 1.8% in rodent and Italy with a prevalence of 5.3% in *Ixodes ricinus* collected on rodent (Baráková et al., 2018; Vayssier-Taussat et al., 2012) (Table 4.). It was only reported in 1.4% of the whole publications and 2% of publications concerning bacteria.

### *Leptospira*

The genus *Leptospira* represents spirochetes and zoonotic bacteria which is responsible of leptospirosis all over the world. The first case was notice over 100 years ago. It's usually transmitted by direct or indirect contact with a contaminated element but it can be also found in ticks (Levett, 2001; Wójcik-Fatla et al., 2012). A total of 4 bacteria were reported in our research. Two of them were reported only in Croatia in rodent; and *L. interrogans* and *L.*

*borpetersenii* were reported in Croatia and France with the higher prevalence of 30% for *Leptospira interrogans* in rodents in Croatia (Tadin et al., 2012) (Table 4.). As the previous genus, it was reported in 1.4% of the whole data and 2% of the bacteria data.

### *Rickettsia*

The genus *Rickettsia* is one of the most important economical and health-threat tick-borne pathogens genera with a varied number of species. It is divided into two groups: the spotted fever group (SFG) like *Rickettsia conorii*, causative agent of Mediterranean spotted fever, and the typhus group (TG) which is less well-known and includes for example *Rickettsia typhi* (Abdad et al., 2018; Tomassone et al., 2018). Out of the 13 *Rickettsia* species or *Candidatus* species found *Rickettsia africae* and *Rickettsia massiliae* were reported in a total of five countries each: Algeria, Greece, Israel, Italy and Turkey for *R. africae*, and Algeria, Cyprus, Israel, Italy and Spain for *R. massiliae*. The first one, responsible of the African tick-bite fever, was found with highest prevalence of 25% from *Hyalomma detritum* collected on wild boar in Israel (Waner et al., 2014). *Rickettsia massiliae* is one of the rickettsia responsible of the Mediterranean spotted fever and it was highly detected in ticks from the *Rhipicephalus sanguineus* s.l. group on wild boar in Algeria (88.9%) (Leulmi et al., 2016). However, none of these rickettsias were found in animal's samples. The only specie found in animals was *Rickettsia slovaca* an agent of the Tick born lymphadenitis (TIBOLA), with a prevalence of 5.4% in wild boar from Algeria and 6% in rodent from Italy (Martello et al., 2019; Zeroual et al., 2018). This genus was the one detected in the Mediterranean Rim with the highest diversity of species during our research, and reported in the most whole data (30.4%) and the most data concerning bacteria (45.5%). This may transcript a high concern of the scientific research on this genus. Unlike most part of the bacteria genera cited above, the genus *Rickettsia* was rather reported in ticks than in wild animals. Only one species was reported in wild animals.

### *Viruses*

#### *Flavivirus*

The flaviviruses are an important part of the arboviruses. They are transmitted by both mosquitoes and ticks. They mainly infect mammals and account for a large proportion of the recent outbreaks of public health and veterinary concern in terms of morbidity and mortality (Holbrook, 2017). Among this genus, three viruses were detected in wild animals and their ticks in the Mediterranean Rim: Tick-borne encephalitis virus (TBEV), Louping ill virus and the Maeban virus. The two first virus are well-known virus transmitted by ticks responsible of many encephalitis in the world. The TBEV was detected in France and Slovenia in animals with a highest prevalence of 5.9% in rodents from Slovenia (Knap et al., 2012). Like the TBEV, the Louping ill virus was only detected in animals however only in France with a prevalence of 0.9% in wild boar and 0.1% in roe deer (Bournez et al., 2019). Few informations are known about the Maeban virus. It was detected in seagulls eggs in four countries: Algeria, France, Italy and Spain. The highest prevalence was observed in Spanish seagulls eggs (17.5%). In ticks, it was found only in Spain in *Ornithodoros maritimus* with a highest prevalence of 5.9% (Arnal et al., 2014). The flavivirus was the less reported virus genus which was reported in 4.7% of the whole data and 38.9% of the data concerning viruses.

#### *Orthonairovirus*

Generally, in ticks, this genus is represented by a wide range of tick-borne viruses. One of the most known is the Crimean-Congo hemorrhagic fever virus. This virus is transmitted by ticks mainly from the genus *Hyalomma* causes severe or even fatal human disease nearly all across the Old World and its range has expanded with climate change (Portillo et al., 2021). In wild animals of the Mediterranean Rim, it was found in five countries: Algeria, Italy, Morocco, Spain and Turkey. In animals, the highest prevalence was found in Tortoise from Turkey (9.5%) and in the tick *Hyalomma aegyptium* collected from tortoises in Algeria (28.6%) (Kar et al.,

2020; Kautman et al., 2016). The CCHF was the most reported virus in 61.1% of the data concerning viruses and 5.4% of the whole data.

## 6. Ticks involved in positive Tick-Borne disease in wild animals in countries of the Mediterranean Rim

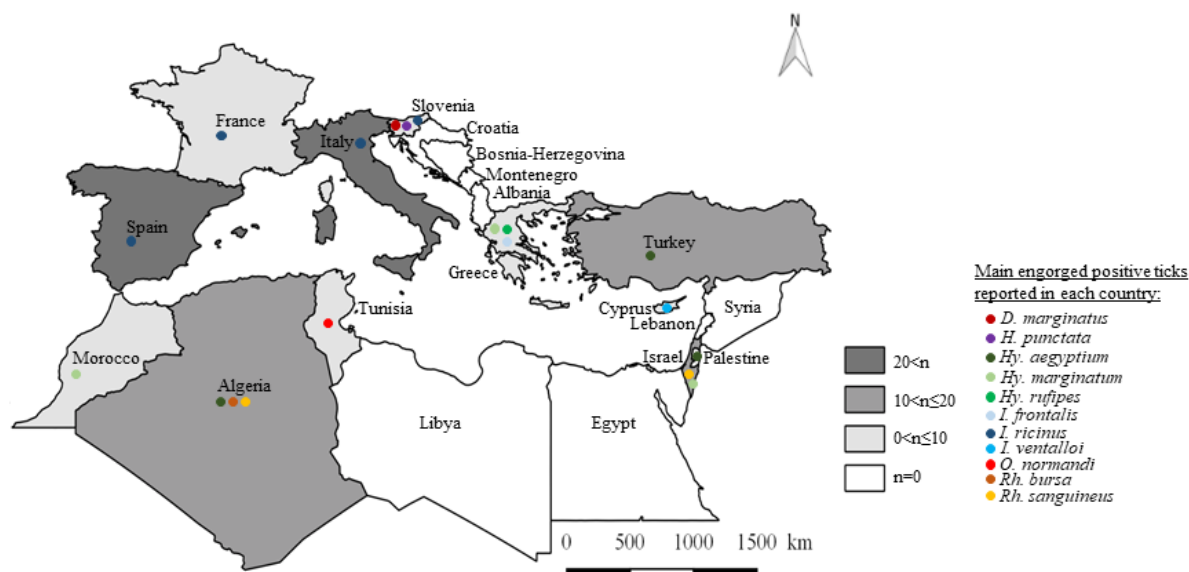
All the previous pathogens could be transmitting by the way of 32 species from eight genera reported on wild animals from the Mediterranean Rim. However, it important to remember that ticks can also contain bacterial endosymbionts such as *Rickettsia*-like *Francisella*-like or *Coxiella*-like. They are endosymbiotic intracellular bacteria harmless to mammals which can be required for the tick survival (Ahantarig et al., 2013). In previous research, they could have been misidentified as pathogens harmful for human and animals.

Out of the eight genera, six belongs to the hard ticks (Ixodidae): *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*. They were widely distributed in this region. The two last genus which was collected in Algeria and Egypt represents the soft ticks (Argasidae): *Argas* and *Ornithodoros* (Table S3.). In this part, the engorged positive tick species we found reported in the highest number of publication in a country was define as main tick species in the country.

### *Ixodidae*

#### *Genus Ixodes*

The most important genus collected on wild animals in terms of TBPs reported was the genus *Ixodes* (Table S3). This genus is mainly known for the transmission of Lyme disease (Chomel, 2015). The ticks are mainly present in the North part of the Mediterranean Rim and have an ubiquity tropism and a high impact on the human and animal health (Estrada-Peña, 2004; Pérez-Eid, 2007). In our data they were reported in eight countries (Cyprus, France, Greece, Italy, Slovenia, Spain and Turkey). Eight species were detected: *I. acuminatus*, *I. festai*, *I. frontalis*, *I. hexagonus*, *I. ricinus*, *I. simplex*, *I. ventalloi*, *I. vespertilionis* in the Mediterranean Rim on a total of 23 hosts. Among TBPs detected in ticks, 63.9% of them were detected in these eight tick species. The main species found is *I. ricinus* in which 32 TBPs were found out of the 39 found in the genus (Table S3.) and it was also the tick main species reported Italy, France, Slovenia and Spain in our review (Figure 18.). This species is an ubiquitous species mainly known for the transmission of *Bo. burgdoferi* s.l., the causative agents of the Lyme disease. However, it can be positive to a wide range of pathogens in wild animals. In complement of *I. ricinus*, two other *Ixodes* species were define as main tick species: *I. frontalis* in Greece and *I. ventalloi* in Cyprus despite the low number of TBPs reported in thoses species (Table S3, Figure 18.) It shows the main importance of *I. ricinus* in the monitoring of TBPs especially in European countries in the genus *Ixodes* however the other species were also considered.



**Figure 18.** Repartition of main engorged positive tick species in the countries of countries of the Mediterranean Rim (n= number of engorged positive tick species by country).

### *Genus Rhipicephalus*

The second ranking genus in term of pathogens diversity reported on wild animals from the countries of the Mediterranean Rim was the genus *Rhipicephalus* (Table S3.). It main distribution occurs in Africa and Europe excepted for *Rhipicephalus sanguineus* s.l. which is also present in America and Oceania (Dantas-Torres, 2008). As the genus *Ixodes*, this genus transmits diseases with a global health impact as anaplasmosis, babesiosis, ehrlichiosis and rickettsiosis (Estrada-Peña, 2004; Pérez-Eid, 2007). A total of six species, *Rh. (B). annulatus*, *Rh. bursa*, *Rh. (B). kohlsi*, *Rh. ovis*, *Rh. pusillus* and *Rh. sanguineus* s.l., were reported in seven countries (Cyprus, Greece, Israel, Italy, Palestine, Spain and Turkey). 36.1% (22 species) of the TBPs in ticks have been reported from this genus. It was defined as a main tick species in Algeria and Israel (Figure 18.). Among them, 18 were detected in *Rh. sanguineus* s.l. (Table S3.). It is a tick specie known for the transmission of *Eh. canis*, *R. conorii* and *B. canis*, found in our review. This tick species is usually highly present in domestic animals (Estrada-Peña, 2004). Among the other *Rhipicephalus* species, *Rh. bursa* was also a considered species with the detection of 14 TBPs and it was one of the main tick species in Algeria (Figure 18.). This show the predominance of these two species in the monitoring of *Rhipicephalus* species.

### *Genus Haemaphysalis*

The *Haemaphysalis* genus ticks have small size and target mammals for the adult stage, and wide array of hosts for the immature stage (Pérez-Eid, 2007). On wild animals from the Mediterranean Rim, five species were detected: *H. adleri*, *H. erinacei*, *H. parva* *H. punctata*, *H. sulcata* in seven countries (Cyprus, Greece, Israel, Italy, Slovenia, Spain and Turkey). They were collected on 13 wild animals with a total of 34.4% of the TBPs reported from ticks (Table S3.). The main *Haemaphysalis* species reported was *H. punctata* which was reported for 11 species out of the 21 reported in the genus and it was one of the main tick species in Slovenia (Figure 18.). It's a species mainly responsible of the distribution of parasite from the genera *Babesia* and *Theileria* and it can be also positive to a large panel of bacteria in wild animals (Estrada-Peña, 2004) (Table S3.). The large variety of TBPs present in this species could explain the importance of this species in our data.

### *Genus Hyalomma*

The *Hyalomma* genus is known for the transmission of the CCHFV virus and bacteria such as *Rickettsia*. They are big size ticks with a tropism on large mammals for the adult forms, and for small mammals and bird for the immature stages. *Hyalomma* species are mainly distributed in the southern part of the Mediterranean Basin with a low migration in the upper part (Estrada-Peña, 2004; Pérez-Eid, 2007). A total of six species (*Hy. aegyptium*, *Hy. anatolicum excavatum*, *Hy. detritum*, *Hy. lusitanicum*, *Hy. marginatum*, *Hy. rufipes*) were collected on twelve wild animal hosts. This genus was the one found in the highest diversity of countries (Algeria, Cyprus, Greece, France, Israel, Italy, Morocco, Palestine, Spain and Turkey). It was found positive to 26.2% of the TBPs recorded in ticks. The main *Hyalomma* species reported for the largest amount of TBPs was *Hy. marginatum* (Table S3.). A total of 13 TBPs were found in this species out of 16 species found in the genus and it was defined as a main tick species in Greece and Morocco (Figure 18.). On wild animals, *Hy. marginatum* was reported in a large amount of countries and can be responsible of TBPs of sanitary importance as *Rickettsia* spp., CCHF, *Babesia* spp. and *Theileria* spp.. These characteristics in complement of the immature stage's tropism for birds, could be in cause of the sanitary importance of this species (Capek et al., 2014). This confirm the importance of *Hy. marginatum* in the TBPs monitoring. However, as the genus *Ixodes*, other species were highly considered as *Hy. aegyptium* in Algeria and Palestine, and *Hy. rufipes* in Greece (Figure 18.).

#### *Genus Dermacentor*

The genus *Dermacentor* targets principally mammals and have a nearly worldwide occurrence. The genus is represented by two common species in the Mediterranean Rim: *D. marginatus* and *D. reticulatus* (Estrada-Peña, 2004). They transmit a wide array of pathogens as *Rickettsia* spp. and piroplasmids which have an animal and human health importance (Estrada-Peña, 2004). *Dermacentor marginatus* was found positives to 12 TBPs on wild animals in five countries of the Mediterranean Rim (Algeria, France, Italy, Slovenia and Spain) where it was defined as a main tick species in Slovenia (Table S3., Figure 18.). All the stages were reported on 10 wild animal's species. This species is mainly found in wild ungulate, as the wild boar, and responsible of the circulation of TBPs from the genera *Babesia* and *Rickettsia* (Selmi et al., 2017). Its main tropism for the wild ungulate during the adult stage and micromammals and birds for the immature stages could explain the high percentage of data found for this species.

#### *Genus Amblyomma*

The genus *Amblyomma* transmits pathogens responsible of disease as Rickettsiosis, and is associated with nearly all terrestrial animals. It occurs mainly in the tropical and sub-tropical areas of Asia, Africa and Oceania (Chi-Yen et al., 1973; Eldin et al., 2011; Kennedy and Marshall, 2021). In cause of the geographically distribution of this genus, it was poorly found in the Mediterranean Rim. It was only found at the genera level in two countries (Greece and Israel) and the species *Am. marmoreum* in Italy from birds, mainly migratory, where two bacteria genus were detected: *Ehrlichia* spp. and *Rickettsia* spp. with the species *R. aeschlimannii* (Table S3.).

### *Argasidae*

#### *Genus Ornithodoros*

The genus *Ornithodoros* is involved in the transmission of diseases as CCHF and the Relapsing Fever in Middle East and Africa (Galindo and Alonso, 2017; Moradi-Asl and Jafari, 2020). In the Mediterranean Rim, it was collected on two wild animals' species from three

countries (Morocco, Spain and Tunisia). Three *Ornithodoros* species were reported (*O. erritacus*, *O. maritimus*, *O. normandi*) with detection of 8.2% of the TBPs detected in ticks. The tick species reported with the highest amount TBPs was *O. erritacus* with three TBPs reported (Table S3.). This could be explained by its main tropism for rodent and micro mammals (Estrada-Peña, 2004). However, it was not defined as a main tick species unlike *O. normandi* which was define as a main tick species in Tunisia (Figure 18.). This shown that the number of TBPs reported in a tick species is not necessarily related with the amount of publications about this tick species.

#### *Genus Argas*

The genus *Argas* is mainly found in the Mediterranean Rim but mainly involve in transmission of birds and bats related diseases (Pérez-Eid, 2007). The *Argas* species was the less reported tick genus with two species reported: *A. transgaripepinus* (one TBPs reported) and *A. vespertilionis* (Two TBPs reported). It was only in Italy on bats for the detection of two bacteria genera: *Bartonella* spp. and *Rickettsia* spp.. This genus is the less collected genus in Mediterranean Rim with the genus *Amblyomma* of the hard ticks (Table S3.).

### **7. Wild animals host of positives ticks and tick-borne pathogens in countries of the Mediterranean Rim**

All the information in this part bring together data about wild animals infested by TBPs and about wild animal hosts of positives engorged tick. However, the data about positive engorged ticks do not confirm the vector character of the ticks for the different TBPs.

A total of 17 wild animal groups host of both ticks and pathogens were reported in our data. From these wild animal hosts, 61 TBPs were reported from ticks and 50 TBPs were reported directly from the wild animals (Table 5.). The complete list of TBPs found reported in our study from engorged positives ticks by animal host and from positives animal host is shown in the supplementary Table S4. In this part, the wild animal hosts of TBPs reported in the highest number of publications in a country was define as a main TBPs host in the country and the wild animal reported as a host of engorged positive tick in the highest number of publication in a country as main engorged positive tick host in the country. The main groups reported positive to TBPs was the rodents in which 46% of the TBPs in wild animals were reported and it was defining as main TBPs host in Croatia, France, Italy, Israel and Morocco. It was followed by the Roe deer (20%), Red fox (16%) define as a main host in Turkey, Wild boar (14%) define as a main TBPs host in Algeria and Turkey and wild carnivores (14%). The next wild animal host were the Red deer (10%) define as a main host in Spain, Turkey and Slovenia and birds (10%) define as a main host in Cyprus and Turkey. Those found with the fewest percentage of TBPs were Mouflon (6%) define as a main host in Cyprus, Chamois (6%), Hare (4%) define as a main host in Turkey, Lizard (4%) define as a main host in Palestine, Tortoise (4%) define as a main host in Palestine and Turkey, Swamp deer (4%), Fallow deer (2%) and Porcupine (2%) (Table 5.).



**Table 5.** TBPs diversity in wild animals and their ticks from the Mediterranean Rim.

Animal name (Number of countries with data available)	Wild boar (6)	Red deer (5)	Birds (4)	Rodent (7)	Wild carnivores* (6)	Red fox (5)	Turtle/ Tortoise (2)	Fallow deer (2)	Roe deer (1)	Hedgehog (4)	Mouflon (2)	Lizard (2)	Chamois (2)	Hare (3)	Bat (1)	Porcupine (2)	Swamp deer (1)
<b>Pathogens found in ticks</b>																	
<b>Percentage of TBPs</b>	27.9%	26.2%	36.1%	34.4%	13.1%	21.3%	9.9%	16.4%	22.9%	9.9%	18%	14.8%	3.3%	8.2%	6.6%	0%	0%
<b>Number of studies</b>	26	24	21	18	13	12	12	11	8	8	7	4	3	2	2	0	0
<b>Pathogens found in ticks</b>																	
<b>Number of pathogens</b>	Bacteria: 9 Parasite: 5	Bacteria: 15 Parasite: 1 Virus: 1	Bacteria: 16 Parasite: 4 Virus: 2	Bacteria: 17 Parasite: 3 Virus: 1	Bacteria: 8	Bacteria: 8 Parasite: 5	Bacteria: 4 Virus: 1	Bacteria: 9 Parasite: 1	Bacteria: 10 Parasite: 4	Bacteria: 6	Bacteria: 7 Parasite: 4	Bacteria: 9	Bacteria: 2	Bacteria: 5	Bacteria: 4		
<b>Pathogens found in wild animals</b>																	
<b>Percentage of TBPs</b>	14%	12%	10%	46%	14%	16%	4%	2%	20%	0%	6%	4%	6%	4%	0%	2%	4%
<b>Number of studies</b>	11	22	4	28	11	13	2	6	12	0	1	3	3	3	0	3	2
<b>Pathogens found in animal</b>																	
<b>Number of pathogens</b>	Bacteria: 3 Parasite: 2 Virus: 2	Bacteria: 2 Parasite: 4	Bacteria: 5	Bacteria: 20 Parasite: 2 Virus: 1	Bacteria: 2 Parasite: 5	Bacteria: 3 Parasite: 5	Parasite: 1 Virus: 1	Bacteria: 1	Bacteria: 2 Parasite: 6 Virus: 2	0	Bacteria: 3	Parasite: 2	Parasite: 3	Parasite: 2	0	Bacteria: 1	Bacteria: 2

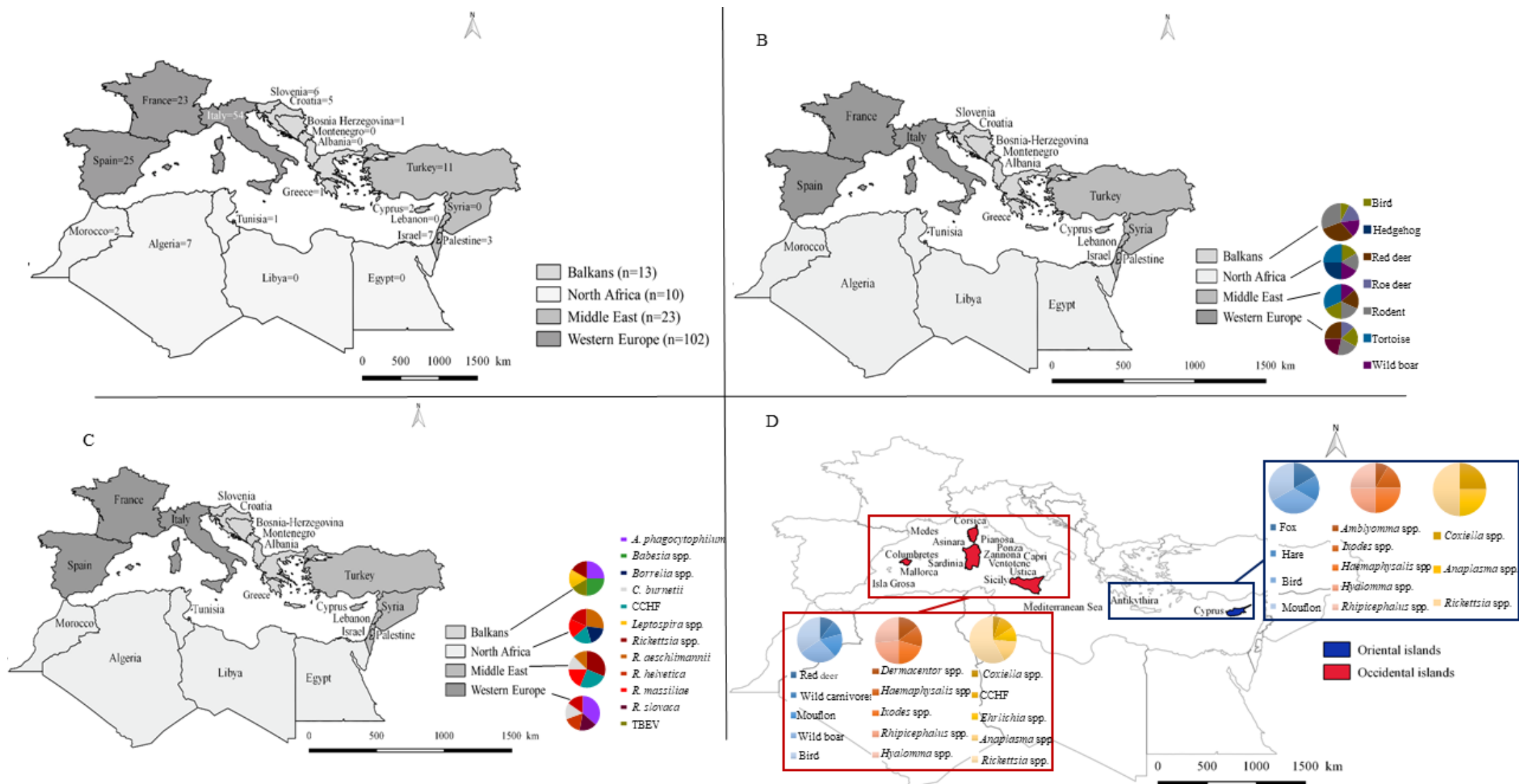
The main wild animal's groups reported for the highest diversity of TBPs in positive tick were small animals as rodent (36.1%), birds (34.4%); hunting mammals as the wild boar (27.9% of TBPs), the red deer (26.2%) the roe deer (22.9%) (Table 5.). The amount of data found on rodents and birds can be explained by their role as host of the tick immature stage and as their potential role in tick dissemination of the migratory birds (Georgopoulou and Tsiouris, 2008; Slowik and Lane, 2009). The hunt mammals's host are well-known wild animals and can also interact with livestock which coupled with the accessibility of the sample due to their hunt status, can explain the scientific interest on these animals (Barasona et al., 2014; Cowie et al., 2016). They are followed by the Red fox (21.3%), this could be due to its worldwide distribution and close relationship with human facilities. A certain amount of data was found in another wild carnivore: Badger, Genet, Golden jackal, Lynx, Marten, Mongoose, Otter and Wolf (13.1%) where the data were sporadically obtained from animal captures for veterinary reasons or from samples directly on the corpse after natural or accidental death. The rest of wild ungulates are composed of species as Mouflon (18%), Fallow deer (16.4%) and Chamois (3.3%) which are also hunt mammals and probably search for the same reason than the red deer and wild boar but at a lower level. The last part of wild animal host is composed of immature stage of tick host: Lizard (14.8%), Hedgehog (9.9%), Tortoise (9.9%), Hare (8.2%) and Bat (6.6%) (Table 5). They are also host of specific tick species as *Hy. aegyptium* on Tortoise. As the main TBPs host, it's impossible to say if there is correlation between the number of TBPs reported in engorged positive tick carrier by a wild animal host and the number of countries where it's the main engorged positive tick host. For some wild animal host we can see a high percentage of TBPs reported in their ticks and a large number of countries where the host is a main host as the birds however for other host as rodents there is a high percentage of TBPs reported in their ticks but it is a main host in only one country or as the Fallow deer where a relatively high percentage of TBPs was reported in their ticks but it was defining as a main host in any countries. Nevertheless, this shows the high level both of research performed on host of immature stage tick as birds and rodent, and hunt animal which are close to human as wild boar and of TBPs reported in those wild animal. In another hand, the high amount of countries (five) where the bird was defined as a main engorged positive tick host could be explained by the scientific interest about the role of birds in ticks and their TBPs dissemination (Capek et al., 2014; Georgopoulou and Tsiouris, 2008).

## 8. Biogeography, diversity and distribution in the Mediterranean Rim

The distribution of the publications through countries in the Mediterranean Rim is shown in Figure 19.A. It gives a total of 148 publications in 22 countries, with a mean number of seven articles per country. We can note a variability of these data according to their geographical origin. The number of publications can vary from zero to 54 according to the countries. The most part of the articles reported in our review were from: Italy (54), Spain (25) and France (23). The rest of the publications are dispatched between the other countries of the Mediterranean Rim excepted in six countries: Albania, Egypt, Lebanon, Libya, Malta, Montenegro and Syria where zero publications were reported in our dataset.

### *Overall analysis of the four region in the Mediterranean Rim*

In this part, we divide the countries Mediterranean Rim into four areas: Western Europe which includes France, Italy, Malta, Monaco and Spain (68.8% of the publication); the Balkans composed of Albania, Bosnia-Herzegovina, Croatia, Greece, Montenegro and Slovenia (8.8% of the publication); Middle East composed of Cyprus, Israel, Lebanon, Palestine Syria and Turkey (15.5% of the publication); and North Africa which includes Algeria, Egypt, Libya, Morocco and Tunisia (6.8% of the publication) (Figure 19.A).



**Figure 19.** Map of the publications by countries and Mediterranean area (n=number of publication) (A), of the main wild animal host (B), main TBPs in the four area of the Mediterranean Rim (C); and Main wild animal host, positives tick genera and TBPs genera in the western islands and eastern islands (D).

### Western Europe

Western Europe is divided in five countries: France, Italy, Malta, Monaco and Spain. Out of them, publications about pathogens on wild animals were only found in France, Italy and Spain. These countries represent the highest amount of research done in the Mediterranean rim with 102 papers done which represent 68.9% of the of the publications found. Of the 17 animal hosts, 16 were found in Western Europe. From those, five animals were highly representing: the red deer in 32% of the publication in Western Europe, the wild boar in 27% of the publication, the rodent in 26% of the publication, birds in 25% and the roe deer in 17% of the publications (Figure 19.B). From the 16 hosts, a total of 31 ticks species were found in Western Europe which represent 97% of the total amount of tick species found in the Mediterranean Rim in our review. The seven tick genera reported in the area in our study were: *Ixodes* an ubiquitous tick genus followed by the genera *Hyalomma* and *Dermacentor* both targeting ungulates (Estrada-Peña, 2004; Pérez-Eid, 2007). The high prevalence of *Ixodes* spp. in Western Europe compare to the other area is due to repartition area which is mainly in Europe (Vayssier-Taussat et al., 2012). Meanwhile, the high prevalence of *Hyalomma* in this area could be due to the recent expansion of this genus from the other areas (Estrada-Peña et al., 2015; Wallménius et al., 2014). The immature stages of these three genera can be found on animal hosts as rodents and birds (Battisti et al., 2020; Slowik and Lane, 2009). The other tick genera reported were: *Argas* spp., *Amblyomma* spp., *Haemaphysalis* spp., *Orniyhodoros* spp. and *Rhipicephalus* spp.. Among the TBPs reported from those wild animal hosts and ticks, the three most found pathogens were *A. phagocytophilum* the causative agents of the human granulocytic anaplasmosis (HGA) found in more than 30% of the publications in Western Europe; bacteria from the genus *Rickettsia* as *R. slovaca* a bacterium from the spotted fever group (SFG) found in 14% of the publication in the area or *R. Helvetica* in 13% of the publications in the area and *C. burnetti* found in the 13% of the publications (Figure 19.C). In Western Europe, the data reported where mainly from research focusing on hunt animals as wild ungulates (red deer and wild boar) and their ticks. They are also focusing on animals host of immature stage ticks and important for ticks and their pathogens circulation (rodent). This can be explained by the proximity of this two wild animals group with human facilities and their importance in the tick life cycle.

### Middle East

The Middle east is the second most important area in term of publications with 23 publications found on wild animals (15.5% of the total amount of publication found). From those publication, 12 animal hosts were found which equal of 70.6% of the animal host found in the Mediterranean Rim. From these 14 animal hosts, five were mainly found: Tortoise, Birds, Rodent, Red deer and Wild boar respectively reported in 29.2%, 16.7%, 16%, 16% and 12.5% of the publication in Middle East (Figure 19.B). From the animal host, 29 tick species from six genera where reported which represents 93.5% of the ticks found in the Mediterranean Rim. From them, the main genera reported was *Hyalomma* spp. followed by *Rhipicephalus* spp., *Haemaphysalis* spp, *Ixodes* spp., *Dermacentor* spp. and *Argas* spp.. Thirty-nine pathogens were detected in wild animals and in their ticks (51.3% of the total number of pathogens). Among them, the most reported were: *Rickettsia* spp. genus responsible of many diseases as the Spotted fever and the TIBOLA found in 20.8% of the Middle East publication (Abdad et al., 2018), the Crimean-Congo Hemorrhagic Fever virus mainly transmitted by *Hyalomma* ticks reported in 16.7% of the Middle East publication, *R. massiliae* a SFG bacterium reported in 12.5% of the publication (Figure 19.C), *R. aeschlimannii* and *C. burnetii* both reported in 8.3% of the area publications. Unlike Western Europe, the scientific focus seems to be not only on hunt mammals even if the red deer was highly researched, other wild animals host involved in tick live cycle and dispersion were focus as the bird and tortoise (Battisti et al., 2020).

### Balkans

In the Balkans, a total of 13 publications were found which represent 8.8% of the total publications (Figure 14.A). In these publications, eight animal hosts were found infested which equal 47.1% of the animal host found in the Mediterranean Rim. From them, the five main animal reported were: Red deer, Rodent, Wild boar, Roe deer and bird respectively reported in 26.7%, 26.7%, 13.3%, 13.3% and 6.7% of the area publications (Figure 19.B). From those wild animal hosts, a total of 16 tick species from six genera were reported (51.6.7% of the total tick species found). The main genera reported was *Hyalomma* spp. followed by *Rhipicephalus* spp., *Haemaphysalis* spp., *Ixodes* spp., *Argas* spp. and *Ornithodoros* spp.. A total of 16 pathogens were found in wild animals and their ticks in the Balkans, which represents 21.1% of the total pathogens found. The five main pathogens found were: *A. phagocytophilum* found in 20% of the publications, the genus *Babesia* found in 20% of the publications, the Tick-Borne Encephalitis Virus in 7.5% of the publications, followed by the genera *Leptospira* and *Rickettsia* both reported in 13.3% of the area publications (Figure 19.C). As the Western Europe, the scientific focus was on hunt animals as red deer and wild boar and micro mammals

#### *North-Africa*

The Mediterranean Rim with the fewest number of publication found is North Africa with 10 publications found which represents 6.8% of the publications found in the Mediterranean Rim (Figure 14.A). A total of six animal hosts were included in those studies which represents about 35.3% of the total host found. The main reported wild animal hosts in North Africa were: Tortoise and the Hedgehog both reported in 30% of the paper in North Africa. Birds, Rodent and Wild boar reported in 20% of the paper each (Figure 19.B). Out of these six hosts, 18 tick species from the eight tick genera were reported positives to TBPs (about 58% of the tick species reported). From them, the main reported species was also *Hyalomma* spp. followed by *Rhipicephalus* spp., *Ixodes* spp., *Haemaphysalis* spp., *Argas* spp., *Amblyomma* spp., *Dermacentor* spp. and *Ornithodoros* spp.. Eleven pathogens were reported from these ticks and animal hosts. This represent 14.5% of the total pathogens found. Among them the most found in North Africa were: *R. aeschlimannii* a SFG rickettsia found in 30% of the publications in North Africa, *Borrelia* spp., CCHF virus, *R. massiliae* and *R. slovaca* all found in 20% of the publications each (Figure 19.C). North Africa has the fewest number of publications in the Mediterranean with a focus on host of immature stage ticks as birds and hedgehog. A focus on host of *Hyalomma* tick like the tortoise is also observed.

#### *Focus on insular tick-borne pathogens in wild animals and their ticks.*

The Mediterranean islands are an important place for animal migrations, human activities and pathogen circulation due to their geographical situation. A total 19 publications were done on islands which represent 12.8% of the publications taken into account in this review. These 19 publications were included into the previous dataset. Out of these 19 publication, most came from both some of the largest islands of the Mediterranean Rim: Sardinia, Corsica, Cyprus and small Greek Island. We decided to split the island according to their geographic location between the western and the eastern parts of the Mediterranean Sea, in the western islands including Asinara, Capris, Columbretes, Corsica, Isla Grossa, Majorque, Medes, Pianosa, Ponza, Sardinia, Sicily, Ustica, Ventotene and Zalone (16 of the publications) and eastern islands including Antikythira and Cyprus (4 of the publications) (Table 6.). One publication was reported in both western and eastern publications.

**Table 6.** Tick and tick-borne pathogens from wild animals in Mediterranean islands

Country	Island	Surface area	Western basin /Eastern basin	Pathogen ( <sup>^</sup> : positive animals, <sup>T</sup> : positive ticks)	Positives ticks	Positives ticks host	Positive pathogen host	References
Cyprus	Cyprus	9251 km <sup>2</sup>	Eastern	<i>Anaplasma</i> spp. <sup>A,T</sup> , <i>A. ovis</i> <sup>A,T</sup> , <i>C. burnetii</i> <sup>A,T</sup> , <i>Rickettsia</i> spp. <sup>A,T</sup> , <i>R. aeschlimannii</i> <sup>T</sup> , <i>R. hoogstraalii</i> <sup>T</sup> , <i>R. massiliae</i> <sup>T</sup> , <i>R. sibirica mongolotimonae</i> <sup>T</sup> , <i>Candidatus R. barbariae</i> <sup>T</sup>	<i>H. punctata</i> , <i>H. sulcata</i> , <i>Hy. excavatum</i> , <i>Hy. marginatum</i> , <i>Hy. rufipes</i> , <i>I. gibbosus</i> , <i>I. ventalloi</i> , <i>Rh. bursa</i> , <i>Rh. sanguineus</i> s.l.	Birds, Mouflon, Red fox	Birds, Mouflon	(Chochlakis et al., 2012; Ioannou et al., 2011, 2009)
Greece	Antikythira	22 km <sup>2</sup>	Eastern	<i>Rickettsia</i> spp. <sup>T</sup> , <i>R. aeschlimannii</i> <sup>T</sup> , <i>R. africae</i> <sup>T</sup>	<i>Amblyomma</i> sp., <i>Haemaphysalis</i> sp., <i>Hy. marginatum</i> , <i>Hy. rufipes</i> , <i>Ixodes</i> sp., <i>I. frontalis</i> , <i>Rhipicephalus</i> sp.	Birds	Data not found	(Wallménius et al., 2014)
France	Corsica	8722 km <sup>2</sup>	Western	<i>Leptospira borpetersenii</i> <sup>A</sup> , <i>L. interrogans</i> <sup>A</sup> , <i>R. aechlimannii</i> <sup>T</sup> , <i>R. massiliae</i> <sup>T</sup> , <i>R. monacensis</i> <sup>T</sup> , <i>R. slovacca</i> <sup>T</sup> , <i>Candidatus R. barbariae</i> <sup>T</sup>	<i>D. marginatus</i> , <i>Hy. aegyptium</i> , <i>Hy. marginatum</i> , <i>Hy. rufipes</i> , <i>I. ricinus</i> , <i>Rh. bursa</i>	Birds, Tortoise and Wild boar	Rodent	(V. Cicculi et al., 2019b; Izquierdo-Rodríguez et al., 2020; Matsumoto et al., 2004)
Italy	Asinara	51.90 km <sup>2</sup>	Western	CCHF <sup>T</sup>	<i>Haemaphysalis</i> spp., <i>H. punctata</i> , <i>Hyalomma</i> sp., <i>I. ricinus</i>	Birds	Data not found	(De Liberato et al., 2018)
	Capri	10.40 km <sup>2</sup>	Western	<i>A. phagocytophilum</i> <sup>T</sup> , CCHF <sup>T</sup> , <i>Rickettsia</i> spp. <sup>T</sup> , <i>R. aechlimannii</i> <sup>T</sup> , <i>R. africae</i> <sup>T</sup>	<i>Amblyomma</i> sp., <i>Haemaphysalis</i> sp., <i>H. punctata</i> , <i>Hyalomma</i> sp., <i>Hy. marginatum</i> , <i>Hy. rufipes</i> , <i>Ixodes</i> sp., <i>I. frontalis</i> , <i>I. ricinus</i> , <i>Rhipicephalus</i> sp.	Birds	Data not found	(Hoffman et al., 2020; Wallménius et al., 2014)
	Pianosa	10.25 km <sup>2</sup>	Western	<i>Bo. spielmanni</i> <sup>T</sup> , <i>Bo. valaisiana</i> <sup>T</sup> , CCHF <sup>T</sup> , <i>R. aeschlimannii</i> <sup>T</sup> , <i>R. helvetica</i> <sup>T</sup>	<i>Haemaphysalis</i> spp., <i>H. punctata</i> , <i>Hyalomma</i> spp., <i>Hy. dentritum</i> , <i>Hy. marginatum</i> , <i>I. accuminatus</i> , <i>I. ricinus</i> , <i>I. ventalloi</i>	Birds	Data not found	(De Liberato et al., 2018; Tomassone et al., 2013)
	Ponza	7. 300 km <sup>2</sup>	Western	CCHF <sup>T</sup> , <i>R. aeschlimannii</i> <sup>T</sup> , <i>R. africae</i> <sup>T</sup> , <i>R. raoultii</i> <sup>T</sup>	<i>A. marmoreum</i> , <i>Haemaphysalis</i> spp., <i>H. punctata</i> , <i>Hyalomma</i> spp., <i>Hy. marginatum</i> , <i>I. frontalis</i> , <i>I. ricinus</i> , <i>I. ventalloi</i>	Birds	Data not found	(Battisti et al., 2020; De Liberato et al., 2018)
	Sardinia	24090 km <sup>2</sup>	Western	<i>A. ovis</i> <sup>T</sup> , <i>A. phagocytophilum</i> <sup>T</sup> , <i>B. bigemina</i> <sup>T</sup> , <i>Bartonella</i> spp. <sup>T</sup> , <i>C. burnetii</i> <sup>T</sup> , <i>Ch. abortus</i> <sup>T</sup> , <i>Ch. psittaci</i> <sup>T</sup> , <i>E. canis</i> <sup>T</sup> , <i>Rickettsia</i> spp. <sup>T</sup> , <i>R. aeschlimannii</i> <sup>T</sup> , <i>R. conorii israelensis</i> <sup>T</sup> , <i>R. helvetica</i> <sup>T</sup> , <i>R. hoogstraalii</i> <sup>T</sup> , <i>R. massiliae</i> <sup>T</sup> , <i>R. slovacca</i> <sup>T</sup> , <i>Candidatus R. barbariae</i> <sup>T</sup> , <i>T. buffeli/sergneti/orientalis</i> <sup>T</sup> , <i>T. equi</i> <sup>T</sup> , <i>T. ovis</i> <sup>T</sup>	<i>D. marginatus</i> , <i>H. punctata</i> , <i>H. sulcata</i> , <i>Hy. marginatum</i> , <i>I. festai</i> , <i>Rh. annulatus</i> , <i>Rh. bursa</i> , <i>Rh. pusillus</i> , <i>Rh sanguineus</i> s.l.	Birds, Hedgehog, Martes, Mouflon, Red deer, Red fox, Wild boar	Data not found	(Chisu et al., 2019, 2018b, 2018a, 2017, 2014; Masala et al., 2012; Satta et al., 2011)
	Sicily	25711 km <sup>2</sup>	Western	<i>A. marginale</i> <sup>A</sup> , <i>A. phagocytophilum</i> <sup>A</sup> , <i>A. ovis</i> <sup>A</sup>	Data not found	Data not found	Porcupine, Rodent	(Torina et al., 2008b)
	Ustica	8.240 km <sup>2</sup>	Western	CCHF <sup>T</sup>	<i>Haemaphysalis</i> spp., <i>H. punctata</i> , <i>Hyalomma</i> spp., <i>I. ricinus</i>	Birds	Data not found	(De Liberato et al., 2018)
	Ventotene	1.540 km <sup>2</sup>	Western	CCHF <sup>T</sup>	<i>Hy. rufipes</i>	Birds	Data not found	(Mancuso et al., 2019)
Zannone	0.9 km <sup>2</sup>	Western	CCHF <sup>T</sup>	<i>Haemaphysalis</i> spp., <i>H. punctata</i> , <i>Hyalomma</i> spp., <i>I. ricinus</i>	Birds	Data not found	(De Liberato et al., 2018)	
Spain	Columbretes	0.19 km <sup>2</sup>	Western	Maedan virus <sup>A,T</sup>	<i>O. maritimus</i>	Birds	Birds	(Arnal et al., 2014)
Spain	Isla Grosa	17.5 km <sup>2</sup>	Western	Maedan virus <sup>A,T</sup>	<i>O. maritimus</i>	Birds	Birds	(Arnal et al., 2014)
Spain	Mallorca	3640 km <sup>2</sup>	Western	Maedan virus <sup>A,T</sup>	<i>O. maritimus</i>	Birds	Birds	(Arnal et al., 2014)
Spain	Medes	0.215 km <sup>2</sup>	Western	Maedan virus <sup>A,T</sup>	<i>O. maritimus</i>	Birds	Birds	(Arnal et al., 2014)

A total of eleven wild animal hosts were reported carrier of tick positives to TBPs or directly positives to TBPs: Bird, Hare, Hedgehog, Fox, Mouflon, Porcupine, Red deer, Rodent, Tortoise, Wild boar and Wild carnivore (Table 6.). However, the most part of the data were reported from publications about TBPs in positive ticks collected on those wild animals (89.5% of the island publications). In the western islands ten of the eleven wild animal hosts were reported: Bird, Hedgehog, Mouflon, Porcupine, Red deer, Red fox, Rodent, Tortoise, Wild Boar and Wild carnivores. From those, the most part of the data was about the birds in 62.5% of the western publications. It was closely followed by the wild boar in 50% of the publications and mouflon in 31.3% of the publications. The two last main wild animal hosts reported were the red deer and wild carnivores both reported in 18.8% of the western publications (Figure 19.D). Meanwhile in eastern islands, fewest wild animal hosts were reported (4): Birds and mouflon both reported in 50% of the eastern publications and Hare and Red fox both reported in 25% of the eastern publications (Figure 19.D). That difference between western and eastern islands could be linked to the difference of data reported between these two areas.

From the wild animal hosts, the seven genera out of the eight reported in the whole data were reported in the islands data: *Amblyomma* spp., *Dermacentor* spp., *Haemaphysalis* spp., *Hyalomma* spp., *Ixodes* spp., *Ornithodoros* spp. and *Rhipicephalus* spp. (Table 6.). The seven genera were reported in the western islands data. The main positives tick genera reported was *Hyalomma* spp. in 64.3% followed by the genera *Rhipicephalus* and *Ixodes* respectively reported in 57.1% and 50% of the publications in ticks. This probably due to the repartition area of this genus and its spreading toward the North border of the Mediterranean Rim (Capek et al., 2014; Estrada-Peña et al., 2015). By this way, it may provoke the extend of TBPs circulation, and particularly in the islands which can have a role of sentinels (Wallménius et al., 2014). It could be performed by birds which are the main wild animal host of immature stage *Hyalomma* in the islands. The two last main genera were the genera *Dermacentor* and *Haemaphysalis* both reported in 35.7% of the positives tick publications in the western islands (Figure 19.D). As for the wild animal hosts, fewest positives tick genera were reported in the eastern islands data (5): *Haemaphysalis*, *Hyalomma* and the *Rhipicephalus* found in 100% of the positive tick publications in the eastern islands followed by *Ixodes* spp. and *Amblyomma* spp. respectively reported in 66.7% and 33.3% of the eastern publications in positives tick genera (Figure 19.D). The schema of the main positive tick genera reported in the islands data followed the same as the whole data excepted in the Wester Europe.

From both those wild animal hosts and positives tick genera, 36.8% of the total TBPs were reported (Table 6.). Even if a low percentage of the data were reported in the islands, an important percentage of the TBPs were reported which shown the importance of TBPs in the Mediterranean islands. In the western islands, a total of 11 genera were reported: *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Borrelia* spp., CCHF, *Chlamydia* spp., *Coxiella* spp., *Ehrlichia* spp., Maebian virus *Rickettsia* spp. and *Theileria* spp.. From those genera, the main reported was *Rickettsia* spp. in 68.8% the publications in the western islands followed by the virus CCHF and the genera *Anaplasma*, *Ehrlichia* and *Coxiella* reported respectively in 12.5%, 18.8%, 12.5% and 6.3% of the western publications (Figure 19.D). In the eastern islands, fewest TBPs were reported (3): *Rickettsia* spp., *Anaplasma* spp. and *Coxiella* spp. respectively reported in 100%, 50% and 50% of the eastern islands publications (Figure 19.D).

## 9. Conclusion

A total of 148 publications were reported about TBPs in wild animal and their ticks. From them, 66.9% were reported in the Western Europe countries, 16.2% from the Middle East, 10.1% in the Balkans and 6.8% in North–Africa in our research. This shows a most important focus of the scientific research on TBPs from wild animals in the Western Europe compare to the other areas in our data. From these publications, 85 pathogens were reported: nine genera of bacteria, eight genera of parasites and four genera of viruses which shown the high diversity

of TBPs. Despite the diversity of TBPs, the main genera reported were common TBPs bacteria: *Rickettsia* spp. and *Anaplasma* spp. These TBPs were reported from eight tick genera (six hard tick genera and two soft tick genera). The main positive genus reported was *Ixodes* spp. detected positive for more than 60% of the TBPs. However, it was the main positive genus reported only in Western Europe. The importance of this genus in the whole data could be due to the most important rate of publications reported in Western Europe (66.9%). In the other area and islands, the main positive tick genus was *Hyalomma* spp.. The amount of data found about these two tick genera could be explained by the amount of data reported on their wild animal hosts as the wild ungulates and birds. Among the 17 wild animal hosts reported, the main were composed of wild ungulate as red deer and wild boar host of adults' tick stages, and micro-vertebrates as birds and rodents host of immature tick stage. Also the amount of the data about birds in islands (63.2% of islands publications), could be due to the possible importance of migration path in ticks and TBPs dissemination in islands. This shows the importance of the wild animals in the circulation of tick-borne pathogens. The main lack of the survey of tick-borne pathogens in wild animals and their ticks reported was in the island with only 12.8% of the total publications, even if 36.8% of the pathogens and nearly 64.7% of the wild animal's host and tick species were found.

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10. Supplementary data

**Table S3.** Positives engorged ticks for TBPs collected on wild animal and their distribution in the Mediterranean Rim.

Tick genus	Number of tick species and Tick species (number of TBPs detected)	Stage	Number of countries with data and Countries	Number of tick host species with data and Host species	Number of pathogenic genera/ species and pathogens detected in ticks ( <sup>b</sup> bacteria, <sup>p</sup> parasite, <sup>v</sup> virus)
<i>Ixodes</i>	8 <i>I. acuminatus</i> (5) <i>I. festai</i> (2) <i>I. frontalis</i> (4) <i>I. hexagonus</i> (2) <i>I. ricinus</i> (32) <i>I. simplex</i> (1) <i>I. ventraloi</i> (5) <i>I. vespertilionis</i> (3)	Adults nymphs and larva	7 Cyprus, France, Greece, Italy, Slovenia, Spain and Turkey	23 Badgers, Bats, Birds, Chamois, Fallow deer, Genet, Hare, Hedgehog, Jackals, Lizard, Lynx, Martens, Mongoose, Mouflons, polecat, Red deer, Red fox, Rodent, Roe deer, Weasel, Wild boar and Wolf	39 <i>Anaplasma</i> spp. <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>B. acomodis</i> <sup>b</sup> , <i>B. bacilliformis</i> <sup>b</sup> , <i>B. bovis</i> <sup>b</sup> , <i>B. chomelli</i> <sup>b</sup> , <i>B. tamiiae</i> <sup>b</sup> , <i>B. tribocorum</i> <sup>b</sup> , <i>B. vinsonii berkoffi</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>Ba. capreoli</i> <sup>p</sup> , <i>Ba. microti</i> <sup>p</sup> , <i>Ba. ovis</i> <sup>p</sup> , <i>Ba. rodaini</i> <sup>p</sup> , <i>Ba. venatorum</i> <sup>p</sup> , <i>Bo. afzelii</i> <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Bo. burgdoferi</i> s.s. <sup>b</sup> , <i>Bo. garinii</i> <sup>b</sup> , <i>Bo. lusitaniae</i> <sup>b</sup> , <i>Bo. spielmani</i> <sup>b</sup> , <i>Bo. valaisiana</i> <sup>b</sup> , <i>Bo. turdi</i> <sup>b</sup> , CCHF <sup>v</sup> , <i>C. burnetii</i> <sup>b</sup> , <i>Candidatus</i> Noehrlichia mikurensis <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. africae</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. hoogstraalii</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. sibirica</i> <sup>b</sup> , <i>R. slovacca</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> . and <i>T. annae</i> <sup>p</sup>
<i>Rhipicephalus</i>	6 <i>Rh. (B). annulatus</i> (1) <i>Rh. bursa</i> (14) <i>Rh. (B). kohlsi</i> (1) <i>Rh. ovis</i> (1) <i>Rh. pusillus</i> (2) <i>Rh. sanguineus</i> s.l. (18)	Adults nymphs and larva	7 Cyprus, Greece, Israel, Italy, Palestine, Spain and Turkey	15 Birds, Fallow deer, Genet, Hare, Hedgehog, Jackals, Lynx, Marten, Mongoose, Mouflon, Red deer, Red fox, Roe deer, Wild boar and Wolf	22 <i>Anaplasma</i> spp. <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>Ba. rodaini</i> <sup>p</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Ch. abortus</i> <sup>b</sup> , <i>C. burnetii</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. slovacca</i> <sup>b</sup> , <i>Candidatus</i> <i>R. barbariae</i> <sup>b</sup> , <i>R. sibirica mongolitimonae</i> <sup>b</sup> , <i>Candidatus</i> <i>R. goldwasserii</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup> and <i>T. sergenti</i> <sup>p</sup>
<i>Haemaphysalis</i>	5 <i>H. adleri</i> (1) <i>H. erinacei</i> (1) <i>H. parva</i> (3) <i>H. punctata</i> (11) <i>H. sulcata</i> (5)	Adults nymphs and larva	7 Cyprus, Greece, Israel, Italy, Slovenia, Spain and Turkey	13 Birds, Fallow deer, Golden Jackals, Hare, Hedgehog, Lizards, Martens, Mouflon, Red deer, Red fox, Rodents, Roe deer and Wild boar	21 <i>Anaplasma</i> spp. <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>B. bacilliformis</i> <sup>b</sup> , <i>B. bovis</i> <sup>b</sup> , <i>B. chomelli</i> <sup>b</sup> , <i>Ba. crassa</i> <sup>p</sup> , <i>Ba. rossii</i> <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Bo. garinii</i> <sup>b</sup> , <i>Ch. abortus</i> <sup>b</sup> , <i>C. burnetii</i> <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. hoogstraalii</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. slovacca</i> <sup>b</sup> and <i>Candidatus</i> <i>R. goldwasserii</i> <sup>b</sup> ,
<i>Hyalomma</i>	6 <i>Hy. aegyptium</i> (4), <i>Hy. anatolicum excavatum</i> (1) <i>Hy. detritum</i> (1) <i>Hy. lusitanicum</i> (1) <i>Hy. marginatum</i> (13) <i>Hy. rufipes</i> (3)	Adults nymphs and larva	10 Algeria, Cyprus, Greece, France, Israel, Italy, Morocco, Palestine, Spain and Turkey	12 Birds, Fallow deer, Hare, Hedgehog, Lynx, Mouflon, Red deer, Red fox, Rodent, Roe deer, Turtles and Wild boar	16 <i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>Ba. occultans</i> <sup>p</sup> , CCHF <sup>v</sup> , <i>C. burnetii</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. africae</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , Tamdy orthonairovirus <sup>v</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> and <i>T. sergenti</i> <sup>p</sup>
<i>Dermacentor</i>	1 <i>D. marginatus</i> (12)	Adults nymphs and larva	5 Algeria, France, Italy, Slovenia and Spain	10 Chamois, Hare, Fallow deer, Jackals, Martens, Red deer, Red fox, Rodent, Roe deer and Wild boar	12 <i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>B. bovis</i> <sup>p</sup> , <i>B. chomelli</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>E. canis</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. slovacca</i> <sup>b</sup> and <i>Theileria</i> spp. <sup>p</sup>
<i>Amblyomma</i>	1 <i>Amblyomma</i> sp. (1) <i>Am. marmoreum</i> (2)	Adults nymphs and larva	3 Greece, Israel and Italy	1 Birds	2 <i>Ehrlichia</i> spp. <sup>b</sup> and <i>R. aeschlimannii</i> <sup>b</sup>
<i>Ornithodoros</i>	3 <i>O. erritacus</i> (3) <i>O. maritimus</i> (1) <i>O. normandi</i> (1)	Adults	3 Morocco, Spain and Tunisia	2 Bats and Rodent	5 <i>Bo. crocidurae</i> <sup>b</sup> , <i>Bo. hispanica</i> <sup>b</sup> , <i>Bo. merionesi</i> <sup>b</sup> , <i>Candidatus</i> <i>R. Africa septentrionalis</i> <sup>b</sup> and Maeban/Maeban-like virus <sup>v</sup>
<i>Argas</i>	2 <i>A. transgariiepimus</i> (1) <i>A. vespertilionis</i> (2)	Adults	1 Italy	1 Bats	2 <i>Bartonella</i> spp. <sup>b</sup> and <i>Rickettsia</i> spp. <sup>b</sup>
Unknown	X X	X	3 Greece, Palestine and Turkey	3 Birds, Lizard and Rodents	3 <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> and <i>R. africae</i> <sup>b</sup>

**Table S4. TBP detected wild animal and their ticks in the Mediterranean Rim.**

Animal names	Pathogens found in ticks ( <sup>b</sup> bacteria, <sup>p</sup> parasite, <sup>v</sup> virus)	Pathogens found in animals ( <sup>b</sup> bacteria, <sup>p</sup> parasite, <sup>v</sup> virus)
<b>Wild boar</b>	<i>Anaplasma marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. occultans</i> <sup>p</sup> , <i>B. rodaini</i> <sup>p</sup> , <i>B. rossi</i> <sup>p</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimanni</i> <sup>b</sup> , <i>R. africae</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. slovaca</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Babesia bigemina</i> <sup>p</sup> , <i>Borrelia burgdoferi</i> s.l. <sup>b</sup> , Louping ill, <i>Rickettsia slovaca</i> <sup>b</sup> , <i>Theileria</i> spp., Tick-Borne Encephalitis Virus <sup>v</sup>
<b>Red deer</b>	<i>Anaplasma marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Bartonella acomodis</i> <sup>b</sup> , <i>Ba. baciliformis</i> <sup>b</sup> , <i>Ba. bovis</i> <sup>b</sup> , <i>Ba. chomelii</i> <sup>b</sup> , <i>Ba. tribocorum</i> <sup>b</sup> , <i>Ba. vinsonii berkoffii</i> <sup>b</sup> , <i>Borrelia. burgdoferi</i> s.l. <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , Crimean-Congo Haemorrhagic Fever virus <sup>v</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. slovaca</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup>	<i>Anaplasma capra</i> <sup>b</sup> , <i>Babesia bigemina</i> <sup>p</sup> , <i>B. capreoli</i> <sup>p</sup> , <i>B. divergens</i> <sup>p</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup>
<b>Bird</b>	<i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia capreoli</i> <sup>p</sup> , <i>B. venatorum</i> <sup>p</sup> , <i>Borrelia afzelii</i> <sup>b</sup> , <i>Bo. garinii</i> <sup>b</sup> , <i>Bo. lusitaniae</i> <sup>b</sup> , <i>Bo. spielmanni</i> <sup>b</sup> , <i>Bo. valisiana</i> <sup>b</sup> , <i>Bo. turdi</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , Crimean-Congo Haemorrhagic Fever virus <sup>v</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , Maeban virus <sup>v</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimanni</i> <sup>b</sup> , <i>R. africae</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. sibirica</i> <sup>b</sup> , <i>Theileria buffeli</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. sergentis</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Borrelia burgdoferi</i> s.l. <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup>
<b>Rodents</b>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Babesia microti</i> <sup>p</sup> , <i>B. venatorum</i> <sup>p</sup> , <i>Borrelia bavariensis</i> <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Bo. burgdoferi</i> s.s. <sup>b</sup> , <i>Bo. crucidurae</i> <sup>b</sup> , <i>Bo. garinii</i> <sup>b</sup> , <i>Bo. hispanica</i> <sup>b</sup> , <i>Bo. lusitaniae</i> <sup>b</sup> , <i>Bo. merionesi</i> <sup>b</sup> , <i>Bo. valaisiana</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Neoehrlichia mikurensis</i> <sup>b</sup> , <i>Rickettsia helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. slovaca</i> <sup>b</sup> , <i>Candidatus R. africa septentrionalis</i> <sup>b</sup> , <i>Tamdy orthinaivirus</i> <sup>v</sup> , <i>Theileria annae</i> <sup>p</sup>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Ba. elizabethae</i> <sup>b</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Bo. afzelii</i> <sup>b</sup> , <i>Bo. bavariensis</i> <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Bo. burgdoferi</i> s.s. <sup>b</sup> , <i>Bo. garinii</i> <sup>b</sup> , <i>Bo. lusitaniae</i> <sup>b</sup> , <i>Bo. merionesi</i> <sup>b</sup> , <i>Bo. valaisiana</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Neoehrlichia mikurensis</i> <sup>b</sup> , <i>Leptospira</i> spp. <sup>b</sup> , <i>L. borpeterseni</i> <sup>b</sup> , <i>L. interrogans</i> <sup>b</sup> , <i>L. kishneri</i> <sup>b</sup> , <i>L. santaroni</i> <sup>b</sup> , <i>Rickettsia slovaca</i> <sup>b</sup> , Tick-Borne Encephalitis Virus <sup>v</sup>
<b>Wild carnivores</b>	<i>Anaplasma ovis</i> <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia helvetica</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>Candidatus R. barbariae</i> <sup>b</sup> , <i>Candidatus R. goldwasserii</i> <sup>b</sup>	<i>Babesia</i> spp. <sup>p</sup> , <i>B. canis</i> <sup>p</sup> , <i>Cytauxzonn</i> spp. <sup>p</sup> , <i>Ehrlichia canis</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>Theileria annulata</i> <sup>p</sup> , <i>T. capreoli</i> <sup>p</sup>
<b>Red fox</b>	<i>Anaplasma ovis</i> <sup>b</sup> , <i>Babesia microti</i> <sup>p</sup> , <i>Bartonella</i> spp. <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Ehrlichia canis</i> <sup>b</sup> , <i>Rickettsia helvetica</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>Candidatus R. barbariae</i> <sup>b</sup> , <i>Theileria annae</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. sergenti</i> <sup>p</sup>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Babesia annae</i> <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. vulpes</i> <sup>p</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia canis</i> <sup>b</sup> , <i>Hepatozoon canis</i> <sup>p</sup> , <i>Theileria annae</i> <sup>p</sup>
<b>Turtle/tortoise</b>	<i>Borrelia</i> spp. <sup>b</sup> , Crimean-Congo Haemorrhagic Fever virus <sup>v</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimanni</i> <sup>b</sup> , <i>R. africae</i> <sup>b</sup>	Crimean-Congo Haemorrhagic Fever virus <sup>v</sup> , <i>Hemolivia mauritanica</i> <sup>p</sup>
<b>Fallow deer</b>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Bartonella bacilliformis</i> <sup>b</sup> , <i>Ba. bovis</i> <sup>b</sup> , <i>Borrelia burgdoferi</i> s.l. <sup>b</sup> , <i>Rickettsia helvetica</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>R. sibirica mongolitimonae</i> <sup>b</sup> , <i>R. slovaca</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup>	<i>Anaplasma phagocytophilum</i> <sup>b</sup>
<b>Roe deer</b>	<i>Babesia</i> spp. <sup>p</sup> , <i>B. microti</i> <sup>p</sup> , <i>B. ovis</i> <sup>p</sup> , <i>B. rodaini</i> <sup>p</sup> , <i>Bartonella chomelii</i> <sup>b</sup> , <i>Borrelia burgdoferi</i> s.l. <sup>b</sup> , <i>Bo. burgdoferi</i> s.s. <sup>b</sup> , <i>Bo. lusitaniae</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimanni</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Borrelia burgdoferi</i> s.l. <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>B. bigemina</i> <sup>p</sup> , <i>B. capreoli</i> <sup>p</sup> , <i>B. venatorum</i> <sup>p</sup> , <i>Cercopithifilaria rugosicauda</i> <sup>p</sup> , Louping ill <sup>v</sup> , <i>Theileria</i> spp. <sup>p</sup> , Tick-Borne Encephalitis Virus <sup>v</sup>
<b>Hedgehog</b>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup>	
<b>Mouflon</b>	<i>Anaplasma ovis</i> <sup>b</sup> , <i>Chlamydia abortus</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Ehrlichia canis</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. hoogstraali</i> <sup>b</sup> , <i>Candidatus R. barbariae</i> <sup>b</sup> , <i>Theileria buffeli</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup> , <i>T. sergentis</i> <sup>p</sup>	<i>Anaplasma ovis</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup>
<b>Lizard</b>	<i>Borrelia afzelii</i> <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup> , <i>Bo. garinii</i> <sup>b</sup> , <i>Bo. lusitaniae</i> <sup>b</sup> , <i>Bo. valaisiana</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. hoogstraali</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup>	<i>Hepatozoon</i> spp. <sup>p</sup> , <i>H. kisrae</i> <sup>p</sup>
<b>Chamois</b>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>Rickettsia helvetica</i> <sup>b</sup>	<i>Babesia capreoli</i> <sup>p</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. ovis</i> <sup>p</sup>
<b>Hare</b>	<i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>Candidatus R. barbariae</i> <sup>b</sup>	<i>Babesia</i> spp. <sup>p</sup> , <i>Theileria</i> spp. <sup>p</sup>
<b>Bat</b>	<i>Bartonella</i> spp. <sup>b</sup> , <i>Ba. tamiae</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup>	
<b>Porcupine</b>		<i>Anaplasma phagocytophilum</i> <sup>b</sup>
<b>Swamp deer</b>		<i>Anaplasma capra</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup>

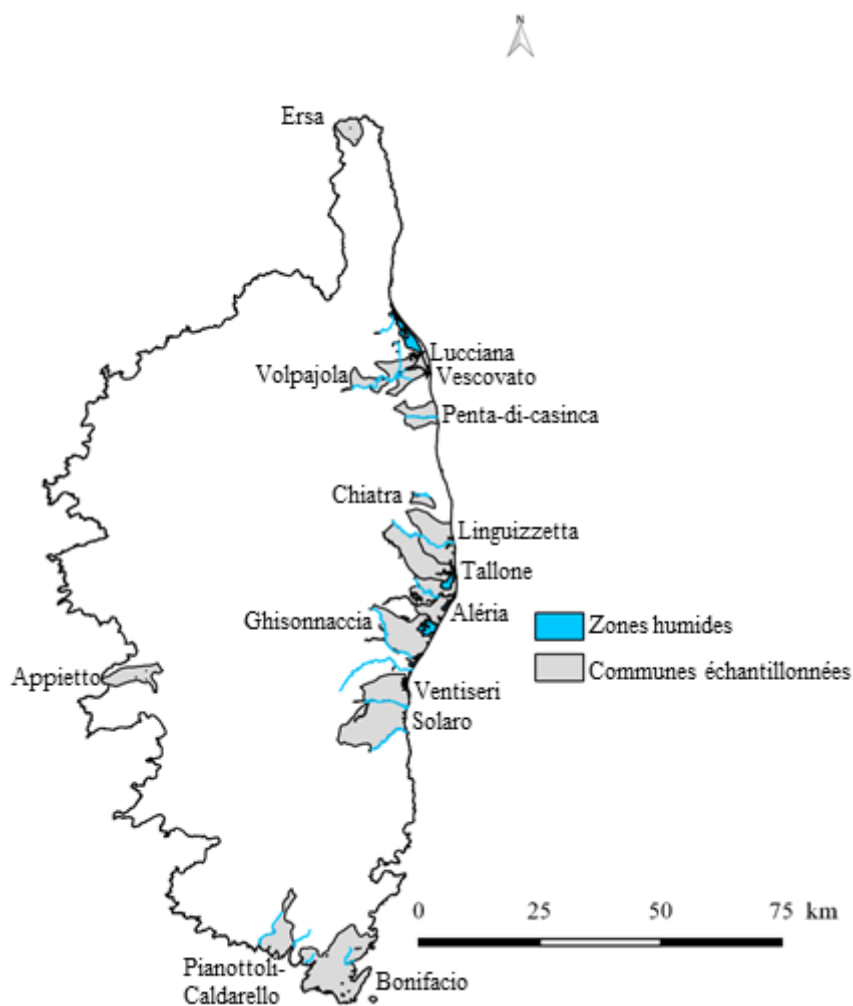
**Chapitre 3 : Agents pathogènes et ectoparasites de populations de sangliers en zones humides**

## 1. Résumé de la publication « Molecular Detection of Zoonotic and Non-Zoonotic Pathogens from Wild Boars and Their Ticks in the Corsican Wetlands »

Cet article a fait l'objet d'une publication dans la revue Pathogens : Baptiste Defaye, Sara Moutailler, Christian Pietri, Clémence Galon, Sébastien Grech-Angelini, Vanina Pasqualini, Yann Quilichini (2021). Molecular Detection of Zoonotic and Non-Zoonotic Pathogens from Wild Boars and Their Ticks in the Corsican Wetlands. Pathogens, 10(12), 1643. <https://doi.org/10.3390/pathogens10121643>

### Résumé

Le but de cette étude a été la détermination des agents pathogènes présents au sein de différentes populations d'un mammifère sauvage emblématique de Corse : le sanglier. Lors de notre étude, nous avons collecté des échantillons de tissus et des tiques sur des sangliers dans les zones humides corses. Nous avons axé notre travail de détection sur 34 espèces et 11 genres de bactéries, de virus et de parasites correspondant aux principaux agents pathogènes transmis par les tiques ou aux pathogènes non transmis par les tiques ayant un impact considérable sur la santé animale et humaine incluant : le virus de la peste porcine africaine, *Anaplasma* spp., *Anaplasma marginale*, *Anaplasma phagocytophilum*, virus d'Aujeszky, *Babesia bigemina*, *Babesia bovis*, *Babesia caballi*, *Babesia divergens*, *Babesia microti*, *Babesia ovis*, *Babesia vogeli*, *Babesia venatorum*, *Bartonella* spp. et *Bartonella henselae*, *Bartonella quintana*, *Borrelia afzelii*, *Borrelia burgdorferi* spp., *Borrelia burgdorferi sensu stricto*, *Borrelia lusitaniae*, *Borrelia miyamotoi*, *Borrelia spielmanii*, *Chlamydia psittaci*, *Coxiella burnetii*, *Ehrlichia* spp., *Ehrlichia ruminatum*, *Haemoproteus* spp, *Hepatozoon* spp., *Leishmania* spp., *Leishmania infantum*, *Leptospira* spp., *Leucocytozoon* spp, *Neoehrlichia mikurensis*, *Plasmodium* spp., *Rickettsia* spp., *R. aeschlimannii*, *R. conorii*, *R. helvetica*, *R. massiliae*, *R. monacensis*, *R. slovacica*, *Theileria annulata*, *Th. equi*, *T. gondii* et *Trypanosoma* spp. Nous avons utilisé la technique de la PCR en temps réel microfluidique. Les échantillons ont été récupérés dans différentes communes près de zones humides grâce à la participation de la Fédération de chasse de Haute-Corse et les Lieutenants de Louveterie Corse lors de battues de chasse et de battues administratives (Figure 20).



**Figure 20.** Carte des communes échantillonnées lors des campagnes de prélèvement sur sangliers.

Au total, 158 sangliers chassés ont été échantillonnés (523 échantillons). Sur ces échantillons, 113 étaient des tiques : 96,4 % étaient des *Dermacentor marginatus*, et le reste des *Hyalomma marginatum*, *Hyalomma scupense* et *Rhipicephalus sanguineus* s.l.. Dans ces différentes espèces de tiques, deux genres bactériens ont été détectés *Anaplasma* spp. et *Rickettsia* spp.. Deux espèces ont été identifiées dans le genre *Rickettsia* : *R. slovacica* qui a été l'espèce la plus détectée (70.8 % des tiques collectées) et principalement dans les tiques de l'espèce *D. marginatus* ; *R. aeschlimannii* qui a été détecté uniquement dans 5.3 % des tiques collectés, dans 100 % des *Hy. scupense*, *Rh. sanguineus* s.l. et dans 50 % des *Hy. marginatum*. Le genre *Anaplasma* a été uniquement détecté dans une tique *D. marginatus*. Parmi les échantillons de sangliers, seuls trois échantillons de sang se sont révélés positifs à *Babesia* spp, ce qui est la première détection de ce genre parasite dans les populations de sangliers en Corse.

Ces résultats dans les zones humides corses correspondent à ce qui a été retrouvé dans le pourtour méditerranéen où cinq genres de tiques ont été trouvés : *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* et *Rhipicephalus*. Comme dans notre étude, le genre prédominant est *Dermacentor* chez le sanglier suivi par des espèces appartenant aux genres *Hyalomma* et *Haemaphysalis*. Ce sont des genres avec un tropisme principal pour les ongulés sauvages et domestiques. Les résultats obtenus dans notre étude semblent indiquer que les principales espèces de tiques trouvées sur les sangliers en Corse seraient liées à la localisation géographique. *D. marginatus* est aussi bien à l'Ouest qu'à l'Est, les espèces *Hy. marginatum* et

*Hy. scupense* à l'Ouest et *H. parva* et *Hy. detritum* à l'Est. Contrairement à la précédente recherche faite par Grech-Angelini et al., le genre *Ixodes* n'a pas été retrouvé lors de nos échantillonnages (Grech-Angelini et al., 2016). Cela pourrait être lié au fait que ce genre a majoritairement été trouvé en altitude alors que nos principales zones échantillonnées ont été des zones humides littorales. De plus, notre cycle de collecte Automne-Hiver correspond à la période d'activité des tiques *D. marginatus* ce qui en plus du tropisme et la répartition géographique de cette espèce pourrait expliquer le pourcentage dominant de cette espèce dans notre étude.

Au cours de notre étude, les tiques collectées ont été positives à deux genres et à deux espèces : *Anaplasma* spp., *Rickettsia* spp., *R. aeschlimannii* et *R. slovaca*. Le genre *Anaplasma* spp. est un genre de bactérie intracellulaire principalement transmis par des tiques du genre *Ixodes* ciblant à la fois les ruminants, mais également l'homme et peuvent provoquer des maladies telles que l'anaplasmose granulocytaire humaine (HGA). Dans le pourtour méditerranéen, les principales espèces trouvées dans les tiques de sangliers sont *A. phagocytophilum* responsable du HGA et *A. marginale* responsable d'anaplasmose bovine. Sur le territoire corse, seulement la première a été retrouvée dans les tiques de sanglier cependant d'autres espèces tel qu'*A. marginale* et *A. ovis* ont été retrouvées dans des tiques d'autres ongulés sauvages et domestiques. Ces espèces ne sont pas les seules présentes en Corse, d'autres espèces telles qu'*A. bovis*, *A. omtjenn* ainsi qu'*A. marginale* ont été retrouvées dans des échantillons de sang d'ongulés domestiques. Le genre *Rickettsia* est un genre de bactérie intracellulaire obligatoire divisé en trois groupes : le groupe de la fièvre boutonneuse (SFG), le groupe Typhus et le groupe transitionnel. Le premier groupe (SFG) est uniquement transmis par les tiques et composé du nombre le plus important d'espèces qui sont majoritairement zoonotiques. Les deux espèces détectées dans notre étude appartiennent à ce groupe. *Rickettsia slovaca* a été fréquemment trouvé dans le pourtour méditerranéen et majoritairement dans des tiques du genre *Dermacentor* (*D. marginatus* et *D. reticulatus*) qui semblent être intimement impliquées dans le cycle de cette bactérie (Brouqui et al., 2007; Selmi et al., 2017; Spitalská et al., 2011). Elle a également été retrouvée dans des échantillons d'organes de sanglier en Algérie (Zeroual et al., 2018). En Corse, elle a été retrouvée dans différentes espèces de tique, mais également dans des chats. Dans notre étude, on démontre l'importance des populations de *D. marginatus* infectées par *R. slovaca* dans les zones humides qui pourraient représenter une menace pour les populations animales et humaines environnantes. La deuxième espèce retrouvée est *R. aeschlimannii* qui appartient également au SFG comme *R. slovaca*, mais semble plus liée aux tiques du genre *Hyalomma* dans le pourtour méditerranéen provenant de différents hôtes y compris de l'avifaune migratrice dans les îles méditerranéennes (Beati et al., 1997; Raoult et al., 2002). En Corse, elle a également été retrouvée dans des tiques des genres *Ixodes* et *Rhipicephalus* (Fernández-Soto, 2003). Dans notre étude, elle a été détectée avec un fort pourcentage dans des *Hyalomma* et *Rhipicephalus* mais toutefois elle n'a pas été détectée dans les échantillons de sangliers. Cela permet de conclure à la présence de ce pathogène dans les zones humides corses. Néanmoins, le faible pourcentage de *R. aeschlimannii* détecté et l'absence de la bactérie dans les sangliers ne permettent pas d'affirmer le rôle des populations de sangliers dans la circulation de cette bactérie. Dans les échantillons de sangliers, le genre *Babesia* a été le seul retrouvé dans trois échantillons de sang. Ce genre est composé de protozoaire intracellulaire infectant principalement les animaux domestiques et d'élevages (Beuglet and Moreau, 2015). Dans le pourtour méditerranéen, ce genre a été peu trouvé dans les sangliers sauf en Italie avec une prévalence de 4.67 % (Zanet et al., 2014). En Corse, l'espèce *B. bigemina* a été détectée dans des tiques collectées sur des ongulés domestiques et *B. ovis* chez le mouflon (Grech-Angelini et al., 2020). La détection de *Babesia* spp. pourrait être la première détection de ce genre de parasite dans les populations de sangliers en Corse. Néanmoins, le séquençage n'ayant pas été concluant, une confirmation est toujours nécessaire.

Ces résultats confirment l'importance du sanglier comme hôte de tiques porteuses de maladies telles que la rickettsiose près des zones humides et des sites de loisirs. Nos résultats

montrent également que le sanglier est un porteur potentiel de la babésiose en Corse, un agent pathogène détecté pour la première fois chez les sangliers de l'île.

# Molecular Detection of Zoonotic and Non-Zoonotic Pathogens from Wild Boars and Their Ticks in the Corsican Wetlands

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**Abstract:** Corsica is the main French island in the Mediterranean Sea and has high levels of human and animal population movement. Among the local animal species, the wild boar is highly prevalent in the Corsican landscape and in the island's traditions. Wild boars are the most commonly hunted animals on this island, and can be responsible for the transmission and circulation of pathogens and their vectors. In this study, wild boar samples and ticks were collected in 17 municipalities near wetlands on the Corsican coast. A total of 158 hunted wild boars were sampled (523 samples). Of these samples, 113 were ticks: 96.4% were *Dermacentor marginatus*, and the remainder were *Hyalomma marginatum*, *Hyalomma scupense* and *Rhipicephalus sanguineus* s.l. Of the wild boar samples, only three blood samples were found to be positive for *Babesia* spp. Of the tick samples, 90 were found to be positive for tick-borne pathogens (rickettsial species). These results confirm the importance of the wild boar as a host for ticks carrying diseases such as rickettsiosis near wetlands and recreational sites. Our findings also show that the wild boar is a potential carrier of babesiosis in Corsica, a pathogen detected for the first time in wild boars on the island.

**Keywords:** Anaplasma; Babesia; Rickettsia; Corsica; tick-borne pathogens; ticks; wild boars; wetlands

## 2. Introduction

The wild boar (*Sus scrofa*) is one of the most widely distributed ungulates in the world, present on all continents except Antarctica (Massei and Genov, 2004). The distribution of this species has increased over the years through increased farming or reintroduction or introduction for hunting, and due to vigorous reproduction and the animals' adaptable feeding habits (Courchamp et al., 2003; Goulding, 2001; Massei et al., 1996). Wild boars can represent a major hazard via direct or indirect interaction with many different species, including domestic pigs, livestock (Barasona et al., 2014; Barth et al., 2018; Cowie et al., 2016), and humans. These interactions can increase the risk of transmission of pathogens (Kazimírová et al., 2018; Kriz et al., 2014; Naranjo et al., 2008; Silaghi et al., 2014). Currently in the Mediterranean region, the wild boar is known to act as a reservoir for many disease-causing agents through interactions with many different populations and in multiple landscapes, including in Corsica (western Mediterranean) (Charrier et al., 2018; Naranjo et al., 2008).



Wild boars live close to water, especially wetlands, mainly because of their diet (Vanschoenwinkel et al., 2008). Wetlands are areas that are key to human activities and where there are high levels of interactions with animals. Wetlands represent one of the main watering and resting areas for both animals and humans (Bacon, 1987; Hammer, 1989). These habitats are important for public health given their role in vector proliferation and pathogen transmission (Jourdain et al., 2007; Rabou et al., 2015; Rey et al., 2012). Risks are mostly related to the animal species found in the wetland of interest. Depending on the ecosystem, the diversity and the prevalence of ectoparasite communities and pathogens can change (Dudek, 2014).

Worldwide, the wild boar is host to many types of pathogens, such as food-borne, tick-borne, and mosquito-borne pathogens (de la Fuente and Gortazar, 2012; Fredriksson-Ahomaa, 2018; Kriz et al., 2014). Across Europe, *S. scrofa* can carry a large spectrum of tick-borne pathogens, including *Anaplasma* spp., *Rickettsia* spp., *Ehrlichia* spp., *Theileria* spp., *Borrelia* spp., *Babesia* spp., and tick-borne encephalitis virus (de la Fuente et al., 2004; de la Fuente and Gortazar, 2012; Kazimírová et al., 2018; Kriz et al., 2014; Silaghi et al., 2014). It has been confirmed in Corsica that *S. scrofa* is an important host of many diseases and parasites (Charrier et al., 2018; Foata et al., 2006; Grech-Angelini et al., 2018). On this island, wild boar hunting is a very long-standing tradition that is still very popular, with over 26,000 wild boars hunted in 2017 (“Oncfs - Grands ongulés - Tableaux de chasse départementaux,” n.d.). One of the main routes of pathogen transmission from wild boars to humans is via food, especially in Corsica, where wild boar meat is widely consumed. Pathogens such as the hepatitis E virus are highly prevalent in the Corsican population (> 60% of blood donors) and in wild boars. The population may also be exposed to *Trichinella britovi* and *Echinococcus canadensis* (Charrier et al., 2018; Pavio et al., 2016; Richomme et al., 2010b; Umhang et al., 2014). Another route of transmission is direct or indirect interactions that cover different kinds of pathogens: viruses such as the Aujeszky’s disease virus, bacteria such as *Mycobacterium bovis*, and parasites such as *Toxoplasma gondii* have been found in wild boars in Corsica (Charrier et al., 2018; Richomme et al., 2010a, 2009). Transmission may also occur through vectors, especially ticks. The five tick species found on wild boars in Corsica are known to be vectors of different kinds of disease (Grech-Angelini et al., 2016). To date, *Rickettsia* from the spotted fever group (*Rickettsia slovaca* and *Rickettsia aeschlimannii*), *Rickettsia raoultii*, *Rickettsia conorii* (the agent of Mediterranean spotted fever), *Anaplasma* spp. and *Bartonella* spp. are the only tick-borne pathogens recorded from ticks collected from wild boars in Corsica (V. Cicculi et al., 2019b; Grech-Angelini et al., 2020; Selmi et al., 2017). The wild boar may play an important role in the transmission of these pathogens (Hrazdilová et al., 2021; Kriz et al., 2014). The species serves as a maintenance host and contributes to distribution by spreading ticks as part of its numerous interactions with other species (Barasona et al., 2014; Kriz et al., 2014). Even though wild boars can play a major role in pathogen transmission and ectoparasite spreading, few studies have focused on the extensive detection of pathogens in both animals and vectors. Detecting pathogens simultaneously in animals and in the ectoparasites they carry makes it possible to observe animal-borne and ectoparasite-borne pathogens in the same area.

Among potential vectors, ticks (Acarida) rank first for veterinary vector-borne pathogens and second for human disease, just after mosquitoes. Ticks are involved in the transmission, circulation and maintenance of pathogens between different groups of animals worldwide (Nicholson et al., 2019; Parola and Raoult, 2001). Tick-borne pathogens are maintained in a stable, natural cycle involving their incidental host and reservoir (such as animals) and accidental host (such as humans) by blood-feeding by vectors (de la Fuente et al., 2008). In this way, ticks can transmit a range of bacteria, viruses, and parasites. These tick-borne pathogens are known to be both pathogens of veterinary importance and zoonotic pathogens harmful to humans (Sonenshine, 1991). Many different animals are involved as reservoirs. In European countries, there are a diversity of important reservoir groups, such as birds, rodents, domestic and wild ungulates, such as the wild boar (Eldridge and Edman, 2003). In four surveys of tick fauna, 10 species were found in Corsica from rodent pets and domestic and wild ungulates: *Amblyomma variegatum* (sporadically), *Ixodes ricinus*, *Rhipicephalus*

*bursa*, *Rhipicephalus sanguineus* s.l., *Rhipicephalus annulatus*, *Hyalomma marginatum*, *Haemaphysalis sulcata*, *Haemaphysalis punctata*, *Hyalomma scupense* and *Dermacentor marginatus* (Cicculi et al., 2019b, 2019a; Cicculi et al., 2019; Grech-Angelini et al., 2016; Pérez-Eid, 2007). Five species have been found in wild boars. Out of the five species, *D. marginatus* was the most common, constituting >80% of the collected ticks. The other species are *Rh. bursa*, *Rh. sanguineus* s.l., *Hy. marginatum* and *I. ricinus*.

The aims of this study were to (i) determine the main tick species of wild boars in Corsican wetlands, and (ii) obtain an overview of the potential presence of a wide range of zoonotic and non-zoonotic pathogens in wild boars and their ticks in the Corsican wetlands.

For this purpose, we collected tissue samples and ticks from wild boar from the Corsican wetlands, and we focused the screening on the detection of 34 species and 11 genera of bacteria, viruses, and parasites expected to be present and belonging to the main tick-borne pathogens or non-tick-borne pathogens with an considerable impact on animal and human health, including: African swine fever virus, *Anaplasma* spp., *Anaplasma marginale*, *Anaplasma phagocytophilum*, Aujeszky's virus, *Babesia bigemina*, *Babesia bovis*, *Babesia caballi*, *Babesia divergens*, *Babesia microti*, *Babesia ovis*, *Babesia vogeli*, *Babesia venatorum*, *Bartonella* spp., *Bartonella henselae*, *Bartonella quintana*, *Borrelia afzelii*, *Borrelia burgdorferi* spp., *Borrelia burgdorferi sensu stricto*, *Borrelia lusitaniae*, *Borrelia miyamotoi*, *Borrelia spielmanii*, *Chlamydia psittaci*, *Coxiella burnetii*, *Ehrlichia* spp., *Ehrlichia ruminatum*, *Haemoproteus* spp., *Hepatozoon* spp., *Leishmania* spp., *Leishmania infantum*, *Leptospira* spp., *Leucocytozoon* spp., *Neoehrlichia mikurensis*, *Plasmodium* spp., *Rickettsia* spp., *R. aeschlimannii*, *R. conorii*, *Rickettsia helvetica*, *Rickettsia massiliae*, *Rickettsia monacensis*, *R. slovaca*, *Theileria annulata*, *Theileria equi*, *T. gondii* and *Trypanosoma* spp by means of microfluidic real-time PCR.

### 3. Results

#### *Tick Identification*

Of the 158 wild boar samples from 17 municipalities, a total of 113 ticks were collected from 32 wild boars from nine areas close to coastal wetlands (Table 7.). Overall, 96.4% of them were *D. marginatus*; the remainder belonged to three species: *Hy. marginatum*, *Hy. scupense* and *Rh. sanguineus* s.l.. They were all adults, with 53.6% being males and 46.4% being fully and partially engorged females.

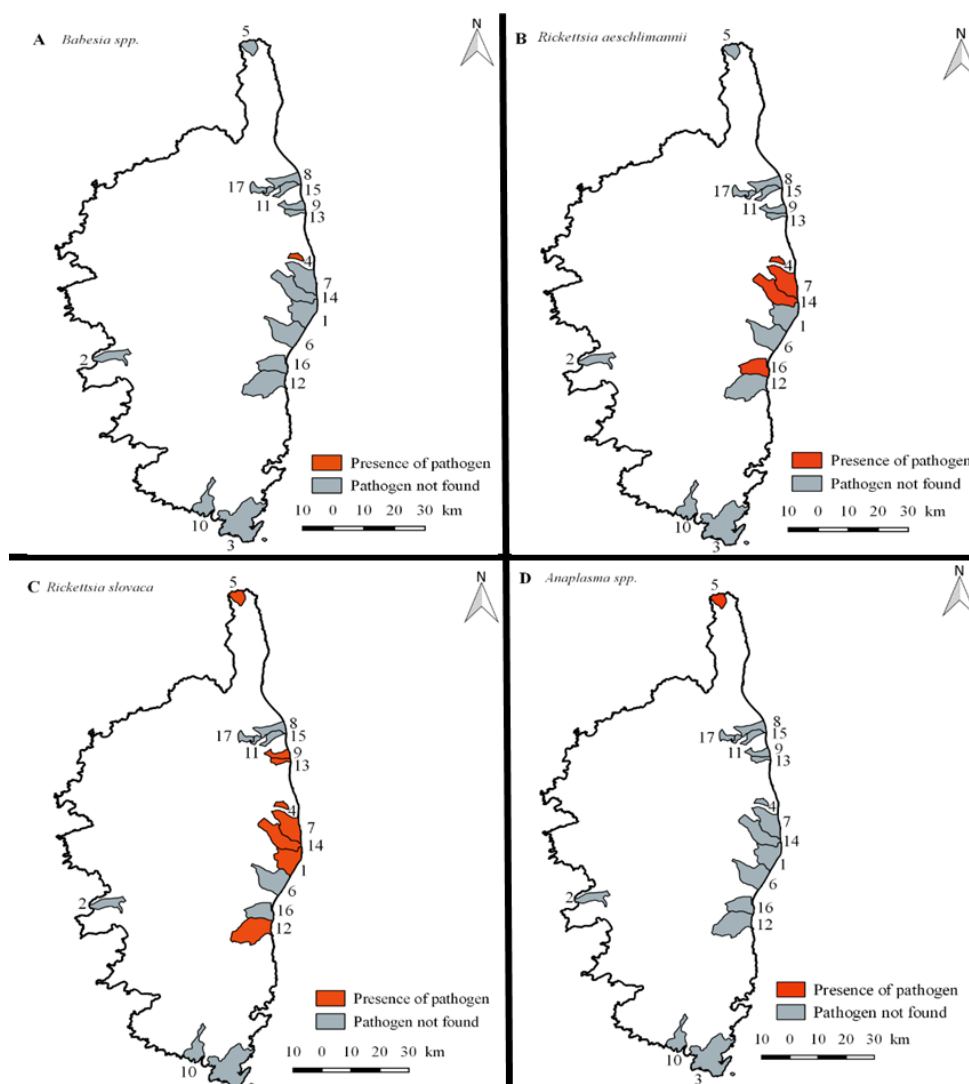
#### *Detection of Tick-Borne Pathogen DNA in Wild Boar Samples*

Only three wild boar samples were found to be positive. These were three blood samples from three juvenile males from Chiatria municipality which were positive for *Babesia* spp. (Table 7. and Figure 21.A). Unfortunately, the sequencing did not enable us to identify the *Babesia* species involved. This was the only pathogen found in wild boar blood samples, and no pathogens were found in the other tissue samples (spleen and liver).

**Table 7.** Tick-borne pathogen distribution in wild boar and their ticks in the Corsican wetlands.

Municipalities (No)	Sampled Wild Boar/Positive	<i>D. marginatus</i> Total/Positive (A/B/R/Ra/Rs)	<i>Hy. marginatum</i> Total/Positive (A/B/R/Ra/Rs)	<i>Hy. scupense</i> Total/Positive (A/B/R/Ra/Rs)	<i>Rh. sanguineus</i> s.l. Total/Positive (A/B/R/Ra/Rs)	Total Ticks Total/Positive (A/B/R/Ra/Rs)
-						
Aléria (1)	73/0	47/39 (Rs)	-	-	-	47/39 (Rs)
Appietto (2)	2/0	-	-	-	-	-
Bonifacio (3)	3/0	-	-	-	-	-
Chiatra (4)	11/3 (B)	24/1 (R), 24 (Rs)	2/1 (Ra)	-	1/1 (Ra)	27/1 (R), 2 (Ra), 24 (Rs)
Ersa (5)	3/0	5/1 (A), 4 (Rs)	-	-	-	5/1 (A), 4 (Rs)
Ghisonaccia (6)	10/0	-	-	-	-	-
Linguizetta (7)	11/0	4/1 (Rs)	-	1/1 (Ra)	-	5/1 (Rs), 1(Ra)
Luciana (8)	1/0	-	-	-	-	-
Penta di casinca (9)	2/0	8/4 (Rs)	-	-	-	8/4 (Rs)
Pianottoli-Caldarello (10)	1/0	-	-	-	-	-
Prunelli-di-Casaconi (11)	2/0	-	-	-	-	-
Solaro (12)	5/0	7/1 (Ra), 1 (Rs)	-	-	-	7/1 (Ra), 1 (Rs)
Taglio-Isolaccio (13)	2/0	5/1 (R), 3 (Rs)	-	-	-	5/1 (R), 3 (Rs)
Tallone (14)	20/0	8/1 (R), 2 (Ra), 4 (Rs)	-	-	-	8/1 (R), 2 (Ra), 4 (Rs)
Vescovato (15)	3/0	-	-	-	-	-
Ventiseri (16)	7/0	1/1	-	-	-	1
Volpajola (17)	2/0	-	-	-	-	-
Total/Positive	158/3 (B)	109/1 (A), 3 (R), 3 (Ra), 80 (Rs)	2/1 (Ra)	1/1 (Ra)	1/1 (Ra)	113/ 1 (A), 3 (R), 6 (Ra), 80 (Rs)

A: *Anaplasma* spp., B: *Babesia* spp., R: *Rickettsia* spp., Ra: *R. aeschlimanii*, Rs: *R. slovaca*.



**Figure 21.** Map of Corsica showing the locations of positive samples from wild boars and ticks (1. Aléria, 2. Appietto, 3. Bonifacio, 4. Chiatra, 5. Ersa, 6. Ghisonaccia, 7. Linguizetta, 8. Luciana, 9. Penta-di-casinca, 10. Pianottoli-Caldarelo, 11. Prunelli-di-Casaconi, 12. Solaro, 13. Taglio-Isolaccio, 14. Tallone, 15. Vescovato, 16. Ventiseri, 17. Volpajola).

#### *Detection of Tick-Borne Pathogen DNA in Ticks*

Of the 113 ticks collected from wild boars, 90 were positive (79.6%) and two genera were identified. The main genus was *Rickettsia* spp., with two species. Of the 89 tick samples positive for rickettsiae, three were *Rickettsia* spp. and two species were confirmed by sequencing: *R. slovaca* and *R. aeschlimannii*.

Six ticks were positive for *R. aeschlimannii*: 2.8% of the *D. marginatus*, 50% of the *H. marginatum*, and 100% of the *Hy. scupense* and *Rh. sanguineus* s.l. (Table 7 and Figure 21.B). Of the six positive samples, two were males collected on juvenile wild boars, and the other four were one female and three males collected on adult wild boars. These six samples were found to be related to the ompB sequence of *R. aeschlimannii* detected in *H. marginatum* collected from the environment in the Crimean Peninsula (GenBank: KU961544.1), with 88%–98% identity to the sequence for one *D. marginatus* and more than 99% for the other five.

A total of 80 ticks were positive for *R. slovaca*: 73.4% of the *D. marginatus* collected (Table 7 and Figure 21.C). About half of the ticks were male (52.5%), and the other half female

(47.5%). Most ticks were collected from adults rather than juveniles (98.8% and 1.2%) and female rather than male wild boars (68.8% and 31.8%). *R. slovaca* sequences found were related to the *R. slovaca* ompB sequence from a *Dermacentor* spp. nymph collected on a Royle's mountain vole (GenBank: MN581993.1), with an identity between 96% and 100%, and from an *R. slovaca* citrate synthase sequence from a *D. marginatus* tick from *S. scrofa* in the south of France (GenBank: AY129301.1), with an identity between 99% and 100%.

The last tick was detected as being positive for *Anaplasma* spp. It was a male *D. marginatus* collected from a male wild boar in Ersa municipality (Table 7 and Figure 21.D). Unfortunately, the sequencing of *Anaplasma* spp. in this sample was unsuccessful.

#### 4. Discussion

In this paper, we report two wild boar hunting season surveys (2018–2019 and 2019–2020) in several coastal or near-coastal administrative municipalities of Corsica with the aim of identifying major non-vector pathogens, tick-borne pathogens, and their vectors.

##### *Ticks on Wild Boar Population in Corsican Wetland*

Across the Mediterranean Rim, a high diversity of Ixodidae ticks is found on wild boars. Their distribution can be divided into two areas: the western side, with the highest diversity, and the eastern side, where ticks were found only in Israel and Turkey. A total of five genera were found on the western side: *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*, with a total of nine different species; and four genera on the eastern side: *Dermacentor*, *Haemaphysalis*, *Hyalomma*, and *Rhipicephalus*, with a total of seven different species (Table 8.).

Of these tick species, five are the main ticks found in various studies. The term main specie defines the species found with the highest number of tick individuals on the wild boar in each study. The most important is *D. marginatus*, which is the main tick found on wild boars in more than half of the publications in the Mediterranean Rim. This can be explained by the tropism of the adult stage mainly for wild ungulates, especially wild boars (Pérez-Eid, 2007). On the western side, the other main species are *Hy. marginatum* and *Hy. lusitanicum*, which mainly target livestock but can occur on wild ungulates (Estrada-Peña, 2004). In the Eastern part, the two other main species are *H. parva* and *Hy. detritum*, which have previously been found mainly in domestic ruminants but can also target wild ungulates (Estrada-Peña, 2004; Raad et al., 2020).

In Corsica, ticks from four genera have previously been found on wild boars: *Dermacentor*, *Ixodes*, *Hyalomma* and *Rhipicephalus*. Tick belonging to the genus *Rhipicephalus* and the species *Hy. marginatum* were the main ones found on domestic ungulates and pets. On the other hand, *I. ricinus* was more commonly found on deer and pets (V. Cicculli et al., 2019b; Grech-Angelini et al., 2016). Our results are consistent with those of studies performed elsewhere in the Mediterranean Rim. This can be explained by the distribution of *D. marginatus*, which is mainly found in the west Mediterranean Rim (Figure 22.), due to its tropism for the wild boar, and its activity peak in autumn and winter (Estrada-Peña, 2004). The three other species found (*Hy. marginatum*, *Hy. scupense*, which also infest ungulates with a tropism for domestic animals, and *Rh. sanguineus* s.l., which infest both dog and ungulates in the Mediterranean Rim) have an activity peak in spring (Pérez-Eid, 2007). However, the tropism for the wild boar, the activity peak of *D. marginatus* and the seasonal collection of ticks (autumn and winter) can explain the difference in proportions between the *D. marginatus* found and other tick species, such as *Hyalomma* spp., which are more present in spring and summer. Seasonal surveys of ticks on wild boars from wetlands could be useful to determine the circulation of tick-borne pathogens linked to wild boars, and the threat to animals and humans.

**Table 8.** Tick-borne pathogens detected in the wild boar (*Sus scrofa*) and its ticks in the Mediterranean Rim.

Area (Island)	Main tick species	Other tick species	Pathogens found in ticks (bacterium <sup>b</sup> , parasite <sup>p</sup> and virus <sup>v</sup> )	Pathogens found in wild boars (bacterium <sup>b</sup> , parasite <sup>p</sup> and virus <sup>v</sup> )	References
Algeria	<i>D. marginatus</i>	<i>H. punctata</i> , <i>Rh. sanguineus</i>	<i>R. massiliae</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup>	Not found	(Leulmi et al., 2016)
Algeria			Not screened	<i>R. slovacica</i> <sup>b</sup>	(Zeroual et al., 2018)
France (Corsica)	<i>D. marginatus</i>	<i>Hy. marginatum</i> , <i>H. scupense</i> , <i>I. ricinus</i>	<i>A. phagocytophilum</i> <sup>b</sup> , <i>R. spp.</i> <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup>	<i>Babesia</i> spp. <sup>b</sup>	Present study
France (Corsica)	<i>D. marginatus</i>	<i>Hy. marginatum</i> , <i>I. ricinus</i> , <i>Rh. bursa</i>	<i>R. aeschlimannii</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup>	Not screened	(V. Cicculi et al., 2019b)
France (Corsica)	<i>D. marginatus</i>	<i>Hy. marginatum</i> , <i>I. ricinus</i> , <i>Rh. bursa</i> , <i>Rh. sanguineus</i>	<i>A. phagocytophilum</i> <sup>b</sup> , <i>Ba. henselae</i> <sup>b</sup> , <i>R. aeschlimannii</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup>	Not screened	(Grech-Angelini et al., 2020)
France (Corsica)	<i>Hy. marginatum</i>		<i>R. aeschlimannii</i> <sup>b</sup>	Not screened	(Matsumoto et al., 2004)
France			Not screened	Tick-borne encephalitis virus <sup>v</sup> , Louping ill <sup>v</sup>	(Bournez et al., 2019)
France			Not screened	<i>A. phagocytophilum</i> <sup>b</sup>	(Dugat et al., 2016)
France	<i>D. marginatus</i>		<i>Rickettsia</i> spp. <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup>	Not search	(Sanogo et al., 2003)
Israel	<i>H. parva</i>	<i>H. aleri</i> , <i>Hyalomma</i> spp., <i>Rh. turanicus</i>	<i>R. massiliae</i> <sup>b</sup>	Not screened	(Keysary et al., 2011)
Israel	<i>Hy. detritum</i>		<i>R. africae</i> <sup>b</sup>	Not screened	(Waner et al., 2014)
Italy			Not screened	<i>A. phagocytophilum</i> <sup>b</sup> , <i>Bo. burgdoferi</i> s.l. <sup>b</sup>	(V. Ebani et al., 2017)
Italy	<i>D. marginatus</i>		<i>R. raoultii</i> <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup>	Not screened	(Raele et al., 2018)
Italy (Sardinia)	<i>D. marginatus</i>		<i>R. slovacica</i> <sup>b</sup>	Not screened	(Chisu et al., 2014)
Italy (Sardinia)	<i>D. marginatus</i>		<i>R. spp.</i> <sup>b</sup>	Not screened	(Chisu et al., 2018a)
Italy	<i>D. marginatus</i>		<i>R. slovacica</i> <sup>b</sup>	Not screened	(Pascucci et al., 2019)
Italy	<i>D. marginatus</i>		<i>E. canis</i> <sup>b</sup>	Not screened	(Satta et al., 2011)
Italy			Not screened	<i>B. bigemina</i> <sup>p</sup> , <i>Theileria</i> spp. <sup>p</sup>	(Zanet et al., 2014)
Slovenia			Not screened	<i>A. phagocytophilum</i> <sup>b</sup>	(Smrdel et al., 2009)
Slovenia			Not screened	<i>A. phagocytophilum</i> <sup>b</sup>	(Zele et al., 2012)
Spain	<i>Hy. marginatum</i>	<i>D. marginatus</i> , <i>Rh. bursa</i>	<i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , <i>Piroplasmid</i> <sup>p</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. slovacica</i> <sup>b</sup>	Not screened	(de la Fuente et al., 2004)
Spain	<i>D. marginatus</i>	<i>D. reticulatus</i> , <i>Rh. bursa</i>	Not found	<i>C. burnetii</i> <sup>b</sup>	(Astobiza et al., 2011)

Spain	<i>D. marginatus</i>		<i>R.slovaca</i> <sup>b</sup>	Not screened	(Oteo et al., 2006)
Spain	<i>D. marginatus</i>		<i>R. slovaca</i> <sup>b</sup>	Not screened	(Ortuño et al., 2006)
Spain	<i>Hy. lusitanicum</i>	<i>D. marginatus</i>	<i>R. raoultii</i> <sup>b</sup> , <i>R. slovaca</i> <sup>b</sup>	Not screened	(Márquez, 2008)
Turkey	<i>D. marginatus</i>	<i>H. parva</i> , <i>Hy. excavatum</i> , <i>Hy. marginatum</i> , <i>Rh. turanicus</i>	<i>B. crassa</i> <sup>p</sup> , <i>B. occultans</i> <sup>p</sup> , <i>B. rossi</i> <sup>p</sup>	Not found	(Orkun and Karaer, 2017)

### *Pathogens Detected in Wild Boar and Their Ticks in the Corsican Wetlands*

Another objective of our study was to overview the potential presence of a wide range of pathogens in wild boars and their ticks in the Corsican wetlands. Concerning wild boars in the Mediterranean Rim, most pathogens were found on ticks. We found only one article focusing on Algeria, and another on Turkey (Leulmi et al., 2016; Orkun and Karaer, 2017), in which the pathogens were searched for in both ticks and wild boars. Screening for pathogens in both ticks and hosts enables us to research tick-borne pathogens in ticks and non-vector pathogens in wild boars from the Corsican wetlands.

#### *Babesia* spp.

In this study, only the *Babesia* genus was found in three wild boar blood samples. All of the remaining tissue and blood samples were negative. This genus is composed of an intracellular protozoan parasite from the piroplasmid group. It targets erythrocyte cells and is transmitted by hard ticks. It mainly infects livestock and pets, but can also be found in wild ungulates and rodents (Beuglet and Moreau, 2015; Zanet et al., 2014). Parasites of the *Babesia* genus can be responsible for moderate symptoms such as myalgia, headache, anorexia, as well as severe symptoms such as pulmonary edema, organ dysfunction syndrome and coma (Vannier et al., 2015). The zoonotic species are mainly transmitted by ticks from the *Ixodes* genus and are primarily *B. divergens*, *B. duncani*, *B. microti*, and *B. venatorum*. Their primary reservoir is often rodents, which contaminate immature tick stages, and the pathogens can be transmitted to ungulates during the adult stage (Vannier et al., 2015). In the Mediterranean Rim, one species of the *Babesia* genus (*B. bigemina*) has already been detected in wild boar in Italy, with a prevalence of 4.67% (Zanet et al., 2014), and no *Babesia* was found in ticks. In Corsica, *B. bigemina* was found in *I. ricinus* collected from cattle and goats; *B. ovis* was found only in *I. ricinus* collected from cattle for domestic animals, and from mouflons for wild ungulates (Grech-Angelini et al., 2020). Our findings are the first possible report of *Babesia* spp. DNA in wild boars from Corsica; however, the sequences could not be obtained, so the presence of *Babesia* spp. in wild boar still requires confirmation.

#### *Rickettsia* spp.

The *Rickettsia* genus was the most detected in the tick samples; however, *Rickettsia* DNA was not detected in the tissue samples of wild boars. This genus is composed of three groups: the spotted fever group (SFG), the typhus group and the transitional group. The SFG is the only group transmitted exclusively by ticks, which have both the role of vector and reservoir thanks to their transstadial and transovarial transmission of the pathogens. It is composed of many zoonotic agents with diverse pathogenicity levels, but most of these species can be a threat for human health, with symptoms ranging from a cutaneous rash to lymphadenopathy, for example (Blanton, 2019; Davoust et al., 2010). In Corsica, even if the exact impact of *Rickettsia* species is still unknown, diverse species were already found in animals (*R. aeschlimannii*, *R. africae*, *R. felis*, *R. helvetica*, *R. massiliae* and *R. slovaca* (V. Cicculli et al., 2019b; V Cicculli et al., 2019; Grech-Angelini et al., 2020; Matsumoto et al., 2004)), and a

previous sero-epidemiological study showed the exposition of 4.8% of the people to these pathogens in “Corse du Sud”(Raoult et al., 1985).

In our study, the main *Rickettsia* species detected was *R. slovaca*, found in 73.4% of collected *D. marginatus*. *R. slovaca* is a tick-borne rickettsia species from the spotted fever group, mainly transmitted by *D. marginatus* (Reháček, 1984). This rickettsia species is mainly known for causing tick-borne lymphadenopathy in humans (de Sousa et al., 2013). It was first isolated in a *D. marginatus* tick in Slovakia in 1968. In Europe, *R. slovaca* has already been detected in several countries in Eastern Europe, including Slovakia, Romania, and Bulgaria, as well as in southern Europe, including France, Spain, Portugal and Italy, mainly with the involvement of *D. marginatus* and *D. reticulatus* (Brouqui et al., 2007; de Sousa et al., 2013; Selmi et al., 2009; Spitalská et al., 2012). In the Mediterranean Rim, *R. slovaca* is one of the most frequent tick-borne pathogens found in wild boar ticks, mainly in the north-west of the Mediterranean Rim and in Algeria. It is mainly found in *D. marginatus*, and the only detection in wild boar tissues was from the North of Algeria, with a prevalence of 5.4% in wild boar spleens (Zeroual et al., 2018). Concerning Mediterranean islands, *R. slovaca* has already been found in Sardinia from *D. marginatus*, *Rh. bursa* and *H. marginatum* collected from wild boars, mouflons and foxes, and in *D. marginatus* collected from humans in Sicily. In Corsica, *R. slovaca* has already been detected from *D. marginatus*, *I. ricinus* and *Rh. bursa* from wild boars, and *D. marginatus* from cats (Blanda et al., 2017; Chisu et al., 2017; V. Cicculli et al., 2019b; Grech-Angelini et al., 2020; Masala et al., 2012). The prevalence of *R. slovaca* (70.8%) found in our study was quite similar to that previously found in Corsica (66%) (V. Cicculli et al., 2019b; Grech-Angelini et al., 2020). In this study, we show the importance of *D. marginatus* ticks in *R. slovaca*'s possible presence near wetland areas in Corsica, where humans and animals could be at risk from this pathogen.



**Figure 22.** Distribution of Rickettsiae species found in ticks collected from wild boars (*Sus scrofa*) in the Mediterranean Rim (data from the literature).

*Rickettsia aeschlimannii* belongs to the spotted fever group of rickettsiae, similarly to *R. slovaca*, and was first found in *Hy. marginatum* from Morocco. This disease was detected in a man who traveled to Morocco in 2000 (Beati et al., 1997; Raoult et al., 2002). Given its circulation via *Hy. marginatum*, *R. aeschlimannii* has a different distribution pattern compared to *R. slovaca*, following the distribution of *H. marginatum*, mainly in Africa. It has also been found in southern Europe and in the Mediterranean Rim. *R. aeschlimannii* has been detected in species other than *H. marginatum*, such as *Rhipicephalus* and *Ixodes* ticks (Fernández-Soto, 2003). As with *R. slovaca*, *R. aeschlimannii* was mainly found in ticks. Regarding the



Mediterranean islands, *R. aeschlimannii* seems to follow migratory birds that carry ticks from the *Hyalomma* genus, such as *Hy. marginatum* and *Hy. rufipes*, carrying *R. aeschlimannii* in Greece and the Italian islands (Battisti et al., 2020; Wallménus et al., 2014). In wild boars, unlike *R. slovaca*, *R. aeschlimannii* is only found on the north-west border of the Mediterranean Rim. In Corsica, *R. aeschlimannii* was also detected in *Rhipicephalus*, *Hyalomma*, and *Ixodes* ticks collected from a wide range of domestic ungulates and wild boars (V. Cicculli et al., 2019b; Grech-Angelini et al., 2020). In wetlands, *R. aeschlimannii* was detected in wild boars with a lower prevalence, but at a high prevalence in the ticks of the genus *Hyalomma* and *Rhipicephalus* (50–100%), which confirms the importance of these tick genera in *R. aeschlimannii*'s potential circulation in Corsica. On the other hand, since the pathogen was not found in the wild boar, this animal species seems not to be involved in *R. aeschlimannii*'s circulation in this area.

#### *Anaplasma* spp.

The other genus found in ticks was *Anaplasma* spp., which belongs to the *Anaplasmataceae*, and is known to be a pathogen targeting ruminants. The members of this genus are obligatory intracellular bacteria which can be responsible of febrile illness and can cause diseases such as human granulocytic anaplasmosis. They are mainly transmitted by hard ticks of the *Ixodes* genus (Madison-Antenucci et al., 2020). The confirmation of the transstadial and transovarial transmission of *Anaplasma* bacteria seems to also show the reservoir behavior of the ticks (Baldrige et al., 2009; Taank et al., 2020). This genus has a worldwide distribution (Guzman et al., 2021; Kocan et al., 2015; Nguyen et al., 2020). From ticks collected from wild boars of the Mediterranean Rim, *A. marginale* and *A. phagocytophilum* have been detected in Slovenia and Spain. This genus was also one of the most frequently detected in wild boar tissue samples, with the detection of *A. phagocytophilum* in France, Italy, and Slovenia. In the Mediterranean Rim, unlike *Rickettsia* spp., *Anaplasma* spp. was found to a moderate extent in ticks and wild boars. In Corsican ticks, *A. phagocytophilum* has already been found in ticks collected from birds, livestock, and wild boars, while *A. marginale* was detected in ticks from cattle and mouflons and *A. ovis* was detected in ticks from goats (Cabezas-Cruz et al., 2019; Cicculli et al., 2020; Grech-Angelini et al., 2020). From animals in Corsica, *A. bovis*, *A. marginale*, and *A. omtjenn* were found in cattle's blood samples and *A. ovis* was found in goats' blood samples (Cabezas-Cruz et al., 2019; Dahmani et al., 2017b; Grech-Angelini et al., 2020). The observed prevalence of *Anaplasma* spp. (0.9%) was lower than the prevalence previously found in wild boars from Corsica (2%) (Grech-Angelini et al., 2020). In our study, we confirmed the presence of *Anaplasma* spp. near the Corsican wetlands.

The results of this study confirm the importance of pathogen research in both animals and their associated tick species, in order to draw conclusions described above about the potential risks to animal and human health in the Corsican wetland. In our study, as no pathogen DNA was found in common between the wild boar and their ticks, no statement can be made regarding the transmission of these pathogens between both species. However, the methodology used was based on PCR detection and did not allow us to confirm the presence and the circulation of these pathogens. Nevertheless, these results are a first step in the characterization of the potential threat regarding those pathogens in hotspots of human and animal activity in Corsica. Obviously, serological surveys and pathogen isolations are needed to draw conclusions regarding the presence and potential circulation of these pathogens in the wild boar population of the Corsican wetlands. Future studies are needed to assess the extent of major non-vector pathogens, tick-borne pathogens and their vectors in wetland compared to other land types in the wild boar population of Corsica.

## 5. Materials and Methods

### *Animals and sampling*

Corsica is the third largest island in the Mediterranean Sea (8722 km<sup>2</sup>). It is located 15 km north of the closest Italian territory (Sardinia) and 160 km from the coast of mainland France. It is characterized by a mild Mediterranean climate and has a huge spectrum of landscapes, such as wetlands (mainly on the coast), forests, and mountains (Monte Cintu peaks at 2706 meters). Corsica is divided into two administrative departments, Haute-Corse and Corse du Sud, with a population of 322,000, which is multiplied by 10 during the tourist season, with approximately 3 million tourists, located primarily around the coastal wetlands. From autumn 2018 to winter 2020, a total of 523 samples were collected from 158 hunter-harvested wild boars in 17 municipalities close to wetlands. In all, 100 blood samples, 158 liver samples, 152 spleen samples, and 113 fully and partially engorged ticks were collected by hunters just after wild boar death during the hunting season, in compliance with current regulations. All samples were stored at -80°C until further study. Every animal was morphometrically characterized by the hunter. Age was determined on the basis of tooth eruption patterns. The animals were classified as juveniles if they were under 12 months of age, and as adults above 12 months of age. Wetlands were divided into 4 categories: lagoon, river/river mouth, artificial lake, and temporary pool.

### *DNA Extraction and PCR Pre-Amplification*

Ticks were washed in ethanol and distilled water and then morphologically determined to the species level using standard keys [37,38]. Prior to extraction, tissues and ticks were crushed in microtubes filled with six metal beads using a fisher brand Bead Mill 24 (ThermoFisher, Waltham, MA, USA) at 5500 rpm for 20s. DNA from ticks and tissues was extracted using a Nucleospin tissue kit (Macherey-Nagel, Düren, Germany). For the blood samples, a Nucleospin Quickpure Blood kit (Macherey-Nagel, Düren, Germany) was used, according to the manufacturer's instructions. All the ticks were analyzed individually.

To improve the detection of pathogen DNA, total DNA was pre-amplified using a PreAmp Master Mix, according to the manufacturer's instructions (Fluidigm, South San Francisco, CA, USA). Prior to experiments, primers targeting all pathogens were pooled in equal volumes (200 nM each). The experiment was performed with 1 µL of PreAmp Master Mix, 1.25 µL of pooled primers mix, 1.5 µL distilled water, and 1.25 µL DNA for 5 µL final volume. The pre-amplifications were performed with one cycle at 95 °C for 2 mins, 14 cycles at 95 °C for 15 s and 60 °C for 4 mins. At the end of pre-amplification, samples were diluted to 1:10. Pre-amplified DNA was stored at -20°C until further use.

### *Assay Design*

Vector-borne pathogens and their targeted genes were *Anaplasma* spp. (16S), *A. marginale* (Msp1b), *A. phagocytophilum* (Msp2), *Borrelia* spp. (23S), *Bo. burgdorferi* s.s. (rpoB), *Bo. afzelii* (flagellin), *Bo. miyamotoi* (glpQ), *Bo. lusitaniae* (rpoB), *Bo. spielmanii* (flagellin), *Rickettsia* spp. (gltA), *R. slovacica* (23S-5S ITS), *R. helvetica* (23S-5S ITS), *R. aeschlimannii* (23S-5S ITS), *R. massiliae* (23S-5S ITS), *R. conorii* (23S-5S ITS), *C. burnetii* (idc, IS1111), *N. mikurensis* (ARN16S), *Bartonella* spp. (ssrA), *Ba. henselae* (pap31), *Ba. quintana* (bqtR), *Ehrlichia* spp. (16S), *E. ruminatum* (dsb), *Hepatozoon* spp. (18S), *T. gondii*, *B. microti* (CCTeta), *B. bigemina* (ARN 18S), *B. vogeli* (hsp70), *B. caballi* (rap1), *B. bovis* (CCTeta), *B. ovis* (ARN18S), *B. divergens* (hsp70), *T. equi* (Ema1), *T. annulata* (ARN 18S), *Leishmania* spp. (hsp70), *L. infantum* (ITS), and African swine fever virus (Vp72). The primers and probes for these pathogens selected from the literature were designed by Grech-Angelini et al., Gondard et al. and Michelet et al. (Gondard et al., 2018; Grech-Angelini et al., 2020; Michelet et al., 2014). The primer and probe sets created for this study are listed in Table 9.

**Table 9.** List of pathogens, targets and primer/probe sets created.

Pathogen	Name	Sequence	Length (nucleotide)	Genes	Genes references
Aujeszký's virus	Aujv_gp50_F	CTTTATCGAGTACGCCGACTG	225	gp50	Y14834.1
	Av_gp50_R	AACGGGCACTCTTGCCCC			
	Av_gp50_P	CAGATCTTTGGGGCGTGCCGGC			
<i>Ch. Psitacii</i>	Chl_psi_16S-23S_F	ACGCCGTGAATACGTTCCC	214	16S-23S rRNA	U68450.1
	Chl_psi_16S-23S_R	AGTCAAACCGTCCTAAGACAG			
	Chl_psi_16S-23S_P	CCTTGACACACCCGCCGTCACATC			
<i>Haemoproteus</i> spp.	Hae_cytB_F	ATATGCATGCTACTGGTGCTAC	240	cytochrome B	AF465579.1
	Hae_cytB_R	CAAATCCATGAAACAAGTCCAGG			
	Hae_cytB_P	CGGTTGCACCCAGAAACTCATTGAC			
<i>Leptospira</i> spp.	Lep_Lipl32_F	CTCTATGTTTTGGATTCTCTGCC	158	LipL32	MK54189.1.1
	Lep_Lipl32_R	CCAAGTATCAAACCAATGTGGC			
	Lep_Lipl32_P	ATTGATTTTTCTTCTGGGGTAGCCGCTTTG			
<i>Leucocytozoon</i> spp.	Leu_cytB_F	GGGTTATGTCTTACCATGGGG	177	cytochrome B	KF717066.1
	Leu_cytB_R	AATTGCTAGTGCTACGAATGGG			
	Leu_cytB_P	AAATGAGTTTTTGGGGAGCAACCGTTATTAC			
<i>Plasmodium</i> spp.	Pla_ssrRNA_F	ATATAGAACTGCGAACGGCTC	339	ssrRNA	MK65062.0.1
	Pla_ssrRNA_R	TTTCTCAGGCTCCCTCTCC			
	Pla_ssrRNA_P	CTCTAATTCGCCGTTACCCGTCATAGC			
<i>R. monacensis</i>	Ric_mon_F	CTCGGTGCCGGTACTTTAAAC	192	ompB	KU96154.3.1
	Ric_mon_R	GAGCACCGCCAATAAGAGC			
	Ric_mon_P	AGTGCCGATGCAAATACTCCGGTGAC			
<i>Trypanosoma</i> spp.	Try_18SRNA_F	GTAATTCAGCTCCAAAAGCG	178	18SRNA	EU596263.1
	Try_18SRNA_R	TCAGGAAGGAACCACTCCC			
	Try_18SRNA_P	ACCTCAAGGGCATGGGTCACCAATCC			

#### DNA Amplification and Microfluidic Real-Time PCR

Prior to the microfluidic real-time PCR, DNA from each sample for all targeted pathogens was amplified by simple PCR for better detection. The primers used were the same as those used for the microfluidic real-time PCR, except for *Escherichia coli*, which was not amplified.

The target vector-borne pathogens were detected using a BioMark™ real-time PCR system (Fluidigm, South San Francisco, CA, USA) for high-throughput microfluidic real-time PCR amplification using 48.48 dynamic arrays (Fluidigm). Fluidigm chips can be used to perform 2304 real-time PCR reactions thanks to 48 PCR mixes and 48 samples placed into individual wells, prior to transfer into individual chambers for the reaction. The thermal cycling conditions were 50 °C for 2 mins, 95 °C for 10 mins, and 40 cycles at 95 °C for 15 s and 60 °C for 1 min. One negative water control, one inhibitory molecule control (*Escherichia coli* EDL933 strain), and one DNA extraction control (animal targeting primers) were added to each chip. Specific primers and probes of *E. coli* were used.

#### Confirmation of Pathogen Detection

Confirmation of pathogen detection was performed by nested PCR or real-time PCR. As for the assay design, most of the primers were selected from the literature if they fit into the confirmation design. The positive sequences after gel migration from the nested PCR products were sent to sequencing at Eurofins MWG Operon (Germany) and assembled using BioEdit software (Ibis Biosciences, version 7.2.5, Carlsbad, CA, USA). An online BLAST (NCBI; National Center for Biotechnology Information) was used to identify the sequenced organisms. All the target genes and primer sequences from the literature were described in the studies by Gondard et al. and Michelet et al. 95,96. The primer/probe sets used for confirmation of detection in this study are listed in Table 10.

**Table 10.** List of pathogens, targets and primer/probe sets used for confirmation.

Pathogen	Name	Sequence	Length (nucleotide)	Genes	Genes references
Aujeszky's virus	Aujv_gp50_F2	AACATCCTCACCGACTTCATG	158	<i>gp50</i>	Y14834.1
	Av_gp50_R2	CTGGTAGAACGGCGTCAGG			
	Av_gp50_P2	AATCGCATCACGTCCACGCCCC			
<i>Ch. Psitacci</i>	Chl_psi_16S-23S_F2	AGTAATCTTCGGCGAGCTGG	177	<i>16S-23S rRNA</i>	U68450.1
	Chl_psi_16S-23S_R2	CGTACTTAGGGAATCTCTTT G			
	Chl_psi_16S-23S_P2	TATAAAGCTATGACCCGGAGGTCTCCG			
<i>Haemoproteus</i> spp.	Hae_cytB_F2	CCTTGGGGTCAAATGAGTTTC	231	<i>cytochrome</i>	BAF465579.1
	Hae_cytB_R2	AAGCCGTATCATATCCTAAAGG			
	Hae_cytB_P2	CCTGGACTTGTTCATGGATTGTGGAGG			
<i>Leucocytozoon</i> spp.	Leu_cytB_F2	GAGTTTCTGGGGAGCTACTG	197	<i>cytochrome</i>	BKU842391.1
	Leu_cytB_R2	GGATTAGTGCTACCTTGAATATG			
	Leu_cytB_P2	TGAATAAATACAATTGCTAGTGCTACGAATGG			
<i>Plasmodium</i> spp.	Pla_ssrRNA_F2	TCGAGTTTCTGACCTATCAGC	264	<i>ssrRNA</i>	MK650620.1
	Pla_ssrRNA_R2	AGACTTGCCCTCCAATTGTTA C			
	Pla_ssrRNA_P2	TGGCCTTGCATTGTTATTCTTGTCACTACC			
<i>Trypanosoma</i> spp.	Try_18SRNA_F2	CAACACGGGGAACTTTACCAG	141	<i>18SRNA</i>	EU596263.1
	Try_18SRNA_R2	ATCCTACTGGGCAGCTTGG			
	Try_18SRNA_P2	CAGGGTGAGGATTGACAGATTGAGTGTTTC			

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**Institutional Review Board Statement:** Ethical review and approval were waived for this study. The wild boars were killed by hunters for hunting reason during the legal hunting season and not specifically for this study. The study complies with the current French laws and there is in accordance with the conservation guidelines. Therefore, approval of the ethics committee was not required.

**Informed Consent Statement:** Not applicable.

#### Data Availability Statement:

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

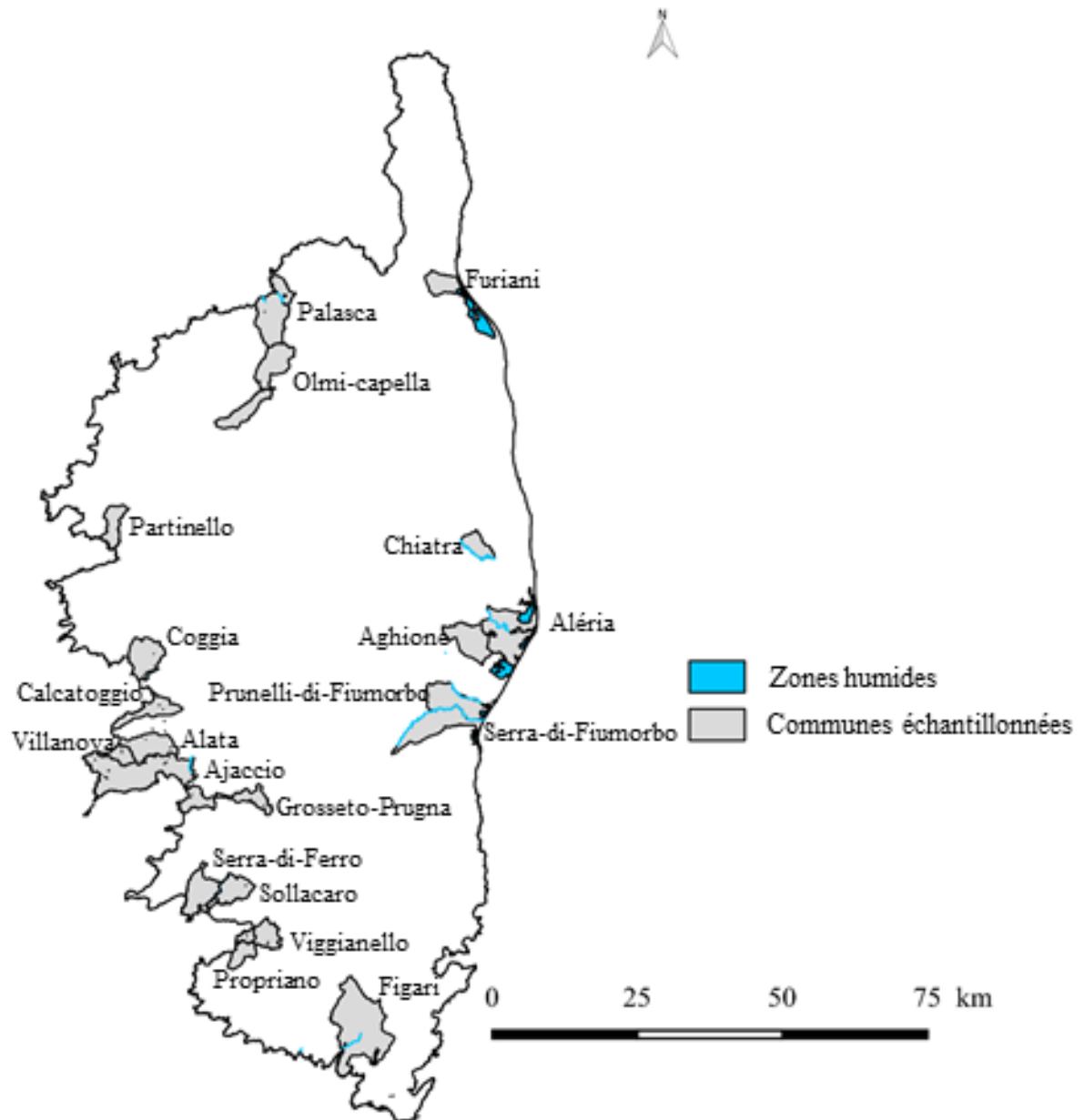
**Chapitre 4 : Agents pathogènes et ectoparasites des élevages bovins et ovins de zones humides**

## 1. Résumé de la publication « Detecting zoonotic and non-zoonotic pathogens in livestock and their ticks in Corsican wetlands »

Cet article a été accepté avec révision mineure dans la revue *Veterinary Medicine and Science* : Baptiste Defaye, Sara Moutailler, Sébastien Grech-Angelini, Clémence Galon, Sandrine Ferrandi, Vanina Pasqualini, Yann Quilichini (soumis). Detecting zoonotic and non-zoonotic pathogens in livestock and their ticks in Corsican wetlands.

### Résumé en Français

L'objectif de ce travail a été la détection d'agents pathogènes au sein d'élevages bovins et ovins et de leurs tiques à proximité de zones humides. Dans cette optique, nous avons collecté aussi bien des échantillons de sang de bovins et d'ovins que des tiques dans les zones humides corses. Nous avons axé le dépistage sur la détection de 34 espèces et de 11 genres de bactéries, de virus et de parasites correspondant aux principaux agents pathogènes transmis par les tiques ou aux pathogènes non transmis par les tiques ayant un impact considérable sur la santé animale et humaine incluant : le virus de la peste porcine africaine, *Anaplasma* spp., *Anaplasma marginale*, *Anaplasma phagocytophilum*, virus d'Aujeszky, *Babesia bigemina*, *Babesia bovis*, *Babesia caballi*, *Babesia divergens*, *Babesia microti*, *Babesia ovis*, *Babesia vogeli*, *Babesia venatorum*, *Bartonella* spp. et *Bartonella henselae*, *Bartonella quintana*, *Borrelia afzelii*, *Borrelia burgdorferi* spp., *Borrelia burgdorferi sensu stricto*, *Borrelia lusitaniae*, *Borrelia miyamotoi*, *Borrelia spielmanii*, *Chlamydia psittaci*, *Coxiella burnetii*, *Ehrlichia* spp., *Ehrlichia ruminatum*, *Haemoproteus* spp., *Hepatozoon* spp., *Leishmania* spp., *Leishmania infantum*, *Leptospira* spp., *Leucocytozoon* spp., *Neoehrlichia mikurensis*, *Plasmodium* spp., *Rickettsia* spp., *R. aeschlimannii*, *R. conorii*, *R. helvetica*, *R. massiliae*, *R. monacensis*, *R. slovaca*, *Theileria annulata*, *Th. equi*, *T. gondii* et *Trypanosoma* spp. Nous avons utilisé la technique de la PCR en temps réel microfluidique. Les échantillons ont été récupérés dans différentes communes proches de zones humides grâce à la participation des vétérinaires du Groupements Technique Vétérinaire Corse lors de campagnes de prophylaxie (Figure 23).



**Figure 23.** Carte des communes échantillonnées lors des campagnes de prélèvement dans les élevages bovins et ovins.

362 échantillons de sang ont été récupérés (288 de bovins et 74 d'ovins). De ces échantillons, 79,9 % et 78,4 % des échantillons de bovins et ovins respectivement ont été détectés positifs à au moins un pathogène.

Le genre *Anaplasma* spp., qui a été retrouvé le plus souvent, est un genre de bactérie intracellulaire principalement transmis par des tiques du genre *Ixodes* ciblant à la fois les ruminants comme avec *A. marginale*, mais également l'homme où elles peuvent provoquer des maladies telles que l'anaplasmose granulocytaire humaine (HGA) avec *A. phagocytophilum* (Kocan et al., 2015). Dans les échantillons de sang d'ovins nous avons trouvé deux agents pathogènes : *A. ovis* et *A. capra* qui provoquent tous les deux des anaplasmoses chez les petits ruminants (Peng et al., 2021; Yasini et al., 2012). Actuellement en Corse, *A. ovis* a déjà été détecté dans des élevages de caprins et d'ovins, tandis que *A. capra* ne l'a pas encore été en Corse (Dahmani et al., 2017b). Dans notre étude, nous avons détecté pour la première fois *A. capra* en Corse. Chez les bovins, uniquement *A. marginale* a été détecté, mais dans plus de 60% des bovins échantillonnés. En Corse, cette bactérie avait déjà été identifiée dans des tiques collectées sur bovins et dans des échantillons de bovins (Dahmani et al., 2017b). Nos résultats montrent que comme dans le reste de la Corse, le genre *Anaplasma* est très présent dans les élevages de zones humides notamment chez les bovins.

Le deuxième genre détecté est *Trypanosoma* spp., dans 58,3 % des échantillons de sang de bovins. Dans le pourtour Méditerranéen, il a été trouvé en Espagne (Villa et al., 2008). A notre connaissance, il s'agit de la première détection d'ADN de *Trypanosoma* spp. chez des bovins en Corse. Malheureusement, la séquence d'ADN n'ayant pu être obtenue, l'isolement du parasite ou une enquête sérologique sont à réaliser.

Le dernier genre retrouvé est le genre *Babesia* avec une prévalence de 12,2 %. Au niveau des îles méditerranéennes, seules deux espèces ont été détectées chez les bovins : *B. bigemina* et *B. bovis*, toutes deux responsables de la babésiose bovine avec une prévalence de 26,3 % et 20 % en Sicile (Torina et al., 2007a). Dans notre étude, nous n'avons pu détecter les agents pathogènes qu'au niveau du genre, le séquençage a échoué et la détermination de l'espèce reste à faire.

Au sein des 444 tiques collectées sur bovins provenant de 18 communes, 38,7 % d'entre elles étaient *Rh. bursa*, le reste était composé de *Rh. sanguineus* s.l. (22,8 %), *Rhipicephalus* spp. (17,1 %), *Hy. marginatum* (8,6 %), *Hy. scupense* (7,2 %), *D. marginatus* (0,7 %) et *Rh. (Bo.) annulatus* (0,2 %). 4,7 % des tiques n'ont pas pu être déterminées et ont été désignées comme tiques indéterminées (Tableau 3). 55,6 % étaient des nymphes et 44,4 % des adultes, parmi ces adultes 57,4 % étaient des femelles partiellement ou totalement engorgées et 42,6 % étaient des mâles. Aucune tique n'a été trouvée sur les moutons.

Dans notre étude, nous nous sommes concentrés sur les zones humides et pendant les mois de janvier-février, les principales espèces de tiques trouvées appartiennent au genre *Rhipicephalus* : *Rh. (Bo.) annulatus*, *Rh. bursa* et *Rh. sanguineus* s.l. Elles représentent respectivement 0,2 %, 38,7 % et 22,7 % des tiques collectées. Ce genre était suivi par le genre *Hyalomma* avec deux espèces : *Hy. marginatum* et *Hy. scupense* représentaient respectivement 8,6% et 7,2% des tiques collectées sur le bétail. La dernière espèce trouvée est *Dermacentor marginatus* (0,7 %). Les espèces de tiques trouvées dans notre étude correspondent de manière générale à la collecte effectuée par Grech-Angelini et al., en 2016. Cependant, nous n'avons pas collecté d'*Haemaphysalis* et d'*Ixodes*, ce qui peut être expliqué par la saisonnalité de l'échantillonnage et l'altitude. La présence de certaines espèces comme *Hy. scupense* en janvier-février et l'absence d'autres comme *Hae. punctata* et *I. ricinus* pourraient être liées à la saisonnalité. De même, le ciblage plus précis des zones humides dans notre étude pourrait expliquer la différence entre notre étude et l'étude précédemment réalisée par Grech-Angelini et al. 2016.



Trois genres d'agents pathogènes ont été détectés : *Anaplasma* spp. dans 0,4 % des tiques, *Babesia* spp. dans 0,4 % des tiques et le genre *Rickettsia* dans 5,2 % des tiques au niveau du genre uniquement et 19,8 % à *R. aeschlimannii*. Uniquement une tique a été détectée porteuse de deux agents pathogènes : *Babesia* spp. et *R. aeschlimannii*. Le genre *Rickettsia* a été détecté dans 25 % des tiques collectées. En Corse, même si l'impact exact des espèces de *Rickettsia* est encore inconnu, diverses espèces ont déjà été trouvées chez les animaux (*R. aeschlimannii*, *R. africae*, *R. felis*, *R. helvetica*, *R. massiliae* et *R. slovaca*) (Cicculli et al., 2019a, 2019b; Cicculli et al., 2019; Grech-Angelini et al., 2020; Matsumoto et al., 2004) et une précédente étude séro-épidémiologique a montré une exposition de 4,8 % de la population à ces pathogènes en Corse du Sud (Raoult et al., 1985). La *Rickettsia* détectée appartient aux rickettsies du groupe de la fièvre tachetée (SFG). Chez les tiques positives, 79,3 % étaient positives à *R. aeschlimannii*, le reste n'a pas pu être déterminé au niveau de l'espèce. *Rickettsia aeschlimannii* a été trouvé pour la première fois chez *Hy. marginatum* au Maroc. La maladie chez l'homme a été documentée chez un homme ayant voyagé au Maroc en 2000 (Beati et al., 1997; Raoult et al., 2002). En Corse, *R. aeschlimannii* a été précédemment rapporté avec une prévalence de 23 % dans les tiques collectées sur les bovins et de 58 % dans les tiques collectées sur les ovins par Grech-Angelini et al., 2020. Il a également été détecté dans *Hy. marginatum* collecté sur des bovins par Cicculli et al., 2019. Dans notre étude, nous avons confirmé la présence de *R. aeschlimannii* dans les tiques collectées sur des bovins avec une prévalence proche de celles trouvées précédemment (19,8 %) mais dans une grande diversité d'espèces de tiques. Il semble que l'ADN de *R. aeschlimannii* soit présent dans les tiques issues de l'élevage bovin quelle que soit la zone d'échantillonnage ainsi que dans les élevages d'ovins bien que nous ne l'ayons pas détecté en zones humides. Néanmoins, *R. aeschlimannii* n'a pas pu être trouvé dans le sang des animaux.

Le second genre est *Anaplasma* spp. dans 0,4 % des tiques collectées. Sur les îles méditerranéennes, *A. phagocytophilum* et *A. marginale* étaient les deux espèces détectées en Sicile et en Corse (de la Fuente et al., 2004; Grech-Angelini et al., 2020). *A. marginale* a également été détectée en Sardaigne (Chisu et al., 2018a). Il était présent dans 2 % des *Rh. sanguineus* s.l. collectés, ce qui est plus bas que la prévalence précédemment trouvée par Grech-Angelini et al. (22 %), cependant il a été détecté de manière plus importante dans les tiques *I. ricinus* (Grech-Angelini et al., 2020). Dans notre étude, les tiques positives ont été collectées dans la municipalité de Viggianello, où *A. marginale* a également été trouvé dans des échantillons de sang de bovins.

Le dernier genre est le genre *Babesia* retrouvé dans 3 % des *Rh. sanguineus* s.l. collectés et dans 2,6 % des *Hy. marginatum*. C'est dans la commune de Figari que *Babesia* spp. a été trouvé également dans des échantillons de sang de bovins, ce qui montre que *Babesia* spp. est probablement présent à la fois dans les tiques et dans la population bovine de cette commune. Nos résultats ont montré pour la première fois la présence de *Babesia* spp. dans l'élevage bovin en Corse. La présence de l'ADN de *B. bigemina* dans les tiques du bétail corse avait déjà été mise en évidence chez les tiques *Rh. bursa* (Grech-Angelini et al., 2020). L'espèce pourrait être *B. bigemina* ou *B. bovis* qui sont très présentes sur le pourtour méditerranéen. Cependant, l'isolement des agents pathogènes et la détermination des espèces sont encore nécessaires.

En conclusion, les résultats de cette étude confirment l'importance de la recherche de pathogènes à la fois chez les animaux et chez leurs tiques. Dans notre étude, nous avons confirmé la présence d'ADN de pathogènes chez les bovins corses comme *A. marginale* et dans leurs tiques comme *R. aeschlimannii*, *Anaplasma* spp. et *Babesia* spp. D'autre part, nous avons détecté pour la première fois en Corse l'ADN de *Babesia* spp. et de *Trypanosoma* spp. chez des bovins corses. L'ADN de deux genres bactériens a été trouvé à la fois chez les animaux et les tiques dans les mêmes municipalités : *Babesia* spp. à Figari et *Anaplasma* spp. à Viggianello. Ces résultats montrent une possible circulation de ces bactéries dans ces communes. Cependant, la méthodologie utilisée était basée sur la détection par PCR et ne nous a pas permis de

confirmer la présence et la circulation de ces pathogènes. Ces résultats constituent une première étape dans la caractérisation de la menace potentielle que représentent ces pathogènes dans les activités d'élevage à proximité des zones humides corses. Il est évident que des enquêtes sérologiques et des isolations d'agents pathogènes sont nécessaires pour tirer des conclusions sur la présence et la circulation potentielle de ces agents.

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

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## Abstract

Corsica is a large French island in the Mediterranean Sea with high human and animal migration rates, especially near wetlands where these migrations are particularly frequent. Among the livestock populations, cattle and sheep are widely present all across the entire Mediterranean region. Trade can be responsible for the circulation of numerous pathogens and their vectors, thereby representing a health and economic threat for the livestock industry. In our study, blood samples and ticks were collected from cattle and sheep in 20 municipalities near Corsican wetlands to screen for the presence of various types of pathogens. The samples were processed using a high-throughput screening technique based on real-time microfluidic PCR: 45 pathogens were screened in 47 samples simultaneously. A total of 372 cattle and 74 sheep were sampled, and 444 ticks were collected from cattle. Out of the eight tick species detected, the main one was *Rhipicephalus bursa* (38.7% of the ticks collected). From cattle blood samples, one species and two genera were found: *Anaplasma marginale*, *Trypanosoma* sp. and *Babesia* sp. in respectively 61.5%, 58.3% and 12.2% of the cattle blood samples. From sheep blood samples, 74.3% were positive for *Anaplasma* sp, 2.7% for *Anaplasma ovis* and 1.4% for *Anaplasma capra*. This is the first report of *A. ovis* DNA in blood samples from sheep in Corsica. Out of 444 the tick samples, 114 were positive: 77.2% for *Rickettsia aeschlimannii*, 20.2% for *Rickettsia* sp., 3.5% for *Babesia* sp. and 1.8% for *Anaplasma* sp. Among them, 2.7% were co-infected with *R. aeschlimannii* and *Babesia* sp. Our results confirm the extent of possible circulation of different pathogens near Corsican wetlands, not only in ticks collected from livestock but also directly in cattle and sheep, with two (*Trypanosoma* sp. and *Babesia* sp.) being detected for the first time in cattle, one for the first time in sheep (*A. ovis*) and one for the first time in Corsica (*A. capra*)

**Keywords:** *Anaplasma*, *Babesia*, *Rickettsia*, *Trypanosoma*, Cattle, Sheep, Ticks, Tick-borne pathogens, High-throughput screening technique, Wetlands

## 2. Introduction

Livestock rearing appeared early in the Mediterranean region with cattle, goat, pig and sheep (Zeder, 2008). Today, in the early 21<sup>st</sup> century, grazing pastures represent half the agricultural pasturelands and more than 130 million ha (Pardini, 2004). Livestock are highly affected by all types of pathogens (e.g. bacteria, parasites, viruses) which can cause important economic losses. For instance, €1.660 billion was spent in 2013 in Europe just for animal diseases control including viral diseases like the avian influenza and African swine fever, bacterial diseases such as bovine tuberculosis and brucellosis, and parasite diseases such as toxoplasmosis in pig and sheep, or cryptosporidiosis in cattle (Costa and Akdeniz, 2019; Knap and Doeschl-Wilson, 2020; Mévélec et al., 2020; Thomson et al., 2017). In the Mediterranean

Basin, sheep are the main livestock species, especially in North Africa and Eastern Mediterranean countries, followed by cattle and goat (Montgomery, 2014). Combined with their role in foodborne diseases and their interaction with humans, livestock can interact with wild animals, all of which may increase the potential risk of spread or creation of a disease reservoir in the animal population by these interactions, such as for bovine tuberculosis (Cowie et al., 2016). One of the main areas of interaction are feeding areas, which can be human-managed pastures or natural wetlands (Cowie et al., 2016). In 2019, the main livestock species raised in Corsica was sheep, with a total over 100,000 heads, followed by cattle, pig and goat (Chambre d'agriculture Corse, 2021).

Livestock are frequently present in wetlands and their interactions, particularly with wild ungulates (such as wild boar) can occur (Robertson, 1997). Wetlands are key areas for human activities, biodiversity and historically providing one of the main watering and resting areas for both livestock and humans; and highly impact the breeding and road construction (Bacon, 1987; Fornell-Muñoz and Guerrero, 2019; Hammer, 1989). They represent 2-3% of the land area in the Mediterranean basin nevertheless, they regroup more than 30% of the vertebrate biodiversity. However, even if wetlands represent a major importance in the biodiversity and animals and human wellbeing these various activities are disturbance factors of the wetlands ecology such as the water use due to human and agricultural activities, the impact of agriculture chemicals and the livestock grazing (Middleton, 2016; Taylor et al., 2021). Unsurprisingly, these habitats are important for public health given their role in vector proliferation and pathogen transmission (Jourdain et al., 2007; Rabou et al., 2015; Rey et al., 2012). Furthermore, depending on the ecosystem, the diversity and the prevalence of ectoparasite communities and pathogens can change (Dudek, 2014).

Ticks (Acarida) are the leading vectors of veterinary pathogens and the second, after mosquitos, of human pathogens (Nicholson et al., 2019; Parola and Raoult, 2001). Furthermore, ticks are responsible of the transmission, circulation and maintenance of pathogens between different groups of animals all over the world. They allow for the maintenance and transmission of tick-borne pathogens in a natural cycle that involves feeding on the blood of their intermediate and final animal hosts and accidental human hosts (de la Fuente et al., 2008). Ticks can transmit a wide spectrum of bacteria, viruses and parasites. These tick-borne pathogens are known to be both pathogens of veterinary importance and zoonotic pathogens harmful for humans (Sonenshine, 2014). Many different animals can be reservoirs. Worldwide, one of the largest groups of reservoirs is probably ungulates (wild or domestic) as the livestock (Eldridge and Edman, 2003). The livestock are highly affect by various tick-borne pathogens (TBPs) as a target and like host of tick carrying pathogens of sanitary importance such as the bacteria responsible of the Lyme disease and the CCHF virus (Dernat Sylvain et al., 2021). In the Mediterranean Basin, more precisely, the livestock are among the most studied and reported animals host of pathogens and ectoparasites. They also belong the main targets of various ticks' genera as *Rhipicephalus* and *Hyalomma*. By these ticks' genera infestations, the livestock can be targets or reservoir of several pathogens as the causative agents of piroplasmosis, Lyme disease and Q fever (Defaye et al., 2022a; Estrada-Peña, 2004). This potential role of target and reservoir is particularly important in Corsica where livestock represents more than 270000 animals for a human population of 322,000 and coupled with both a lack of knowledge of the farmer about the ticks and TBPs; and under estimation of their risk (Chambre d'agriculture Corse, 2021; Dernat Sylvain et al., 2021). Recently, in two surveys of ticks, nine species were found in Corsica: *Ixodes ricinus*, *Rhipicephalus bursa*, *Rh. sanguineus* s.l., *Rh. (Bo.) annulatus*, *Hyalomma marginatum*, *Hy. scupense*, *Haemaphysalis sulcata*, *Hae. punctata* and *Dermacentor marginatus* (Grech-Angelini et al., 2016). Eight species were found on Corsican cattle. Out of the eight species, *Rh. bursa* was the most commonly found on cattle (>50% of the ticks collected on cattle), with only *Hae. sulcata* not being sampled at all on cattle. In sheep, three species were identified: *Rh. bursa* (91% of the collected ticks), followed by *H. marginatum* and *Rh. sanguineus* s.l. (Grech-Angelini et al., 2016).

The aims of this study were (i) to determine the main tick species of cattle and sheep in Corsican wetland areas and (ii) to investigate the potential occurrence of zoonotic and non-

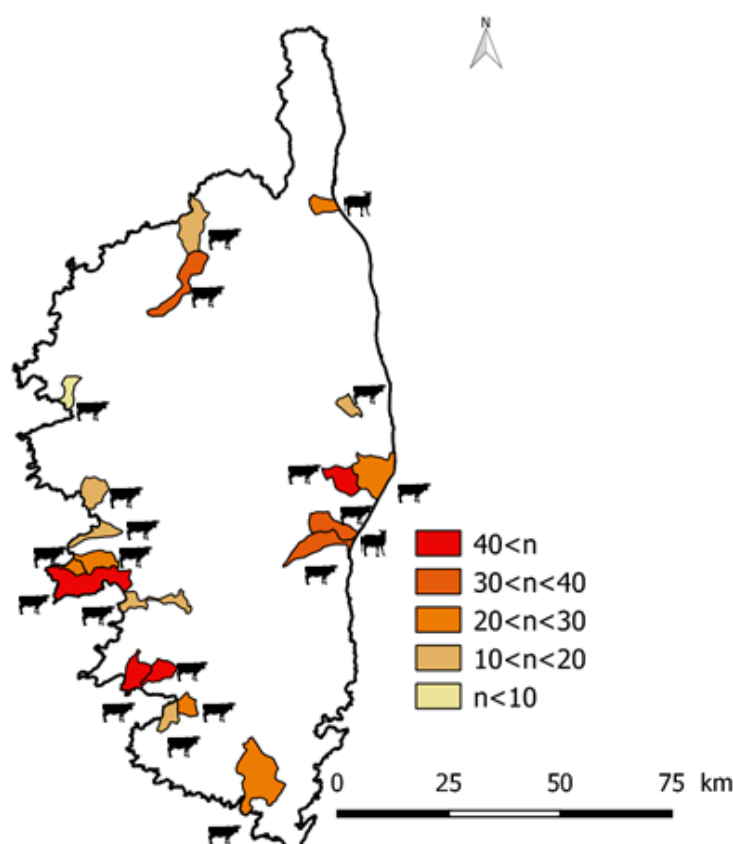
zoonotic pathogens in ticks and hosts (livestock) from Corsican wetlands. To do so, we focused our screening efforts on the detection of 34 species and 11 genera of bacteria, viruses and parasites with a high impact on animal and human health suspected to be present in Corsica.

### 3. Methods

#### *Sites and animal samples*

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

A total of 372 cattle and 74 sheep were sampled across Corsica on respectively 22 and 3 farms during the winter and spring seasons in 2019 (February to June) and winter in 2020 (October 2019 to February 2020). For cattle, 288 blood samples and 444 ticks were collected in 18 municipalities, and for sheep, 74 blood samples were collected in 3 municipalities (Figure 24). Samples were collected only from animals older than 6 months of age. Every blood sample was collected in an EDTA tube by veterinarians as part of national surveillance program for animal diseases. All samples were stored at  $-80^{\circ}\text{C}$  within a few hours after sampling until further analysis.



**Figure 24.** Map of Corsica showing the municipalities samples ( $n$ = number of animal sampled by municipalities).

*DNA extraction and PCR pre-amplification*

Ticks were washed in ethanol and distilled water and then morphologically determined to the species level using species keys (Estrada-Peña, 2004; Pérez-Eid, 2007). Prior to extraction, ticks were crushed in microtubes filled with six metal beads by using a Fisherbrand™ bead mill 24 homogenizer (Thermo Fisher, USA) at 5500 rpm for 20 s. Tick DNA was extracted using a NucleoSpin™ Tissue kit (Macherey-Nagel, Germany). For blood samples, a Nucleospin Quickpure Blood kit (Macherey-Nagel, Germany) was used according to the manufacturer's instructions. All ticks from the same animal were pooled by species, stage and sex (minimum number of ticks = 1; maximum number of ticks = 8).

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

*Assay design*

Pathogens and their targeted genes were *Anaplasma* spp. (16S rRNA), *A. marginale* (msp1b), *A. phagocytophilum* (msp2), *Borrelia* spp. (23S rRNA), *Bo. burgdorferi* s.s. (rpoB), *Bo. afzelii* (flagellin), *Bo. miyamotoi* (glpQ), *Bo. lusitaniae* (rpoB), *Bo. spielmanii* (flagellin), *Rickettsia* spp. (gltA), *R. slovaca* (23S-5S ITS), *R. helvetica* (23S-5S ITS), *R. aeschlimanii* (23S-5S ITS), *R. massiliae* (23S-5S ITS), *R. conorii* (23S-5S ITS), *C. burnetii* (idc, IS1111), *N. mikurensis* (16S rRNA), *Bartonella* spp. (ssrA), *Ba. henselae* (pap31), *Ba. quintana* (bqtR), *Ehrlichia* spp. (16S rRNA), *E. ruminatum* (dsb), *Hepatozoon* spp. (18S rRNA), *To. gondii*, *B. microti* (CCTeta), *B. bigemina* (18S rRNA), *B. vogeli* (hsp70), *B. caballi* (rap1), *B. bovis* (CCTeta), *B. ovis* (18S rRNA), *B. divergens* (hsp70), *T. equi* (ema1), *T. annulata* (18S rRNA), *Leishmania* spp. (hsp70), *L. infantum* (ITS) and *African swine fever virus* (Vp72). Primers and probes were designed by Gondard et al. (2018), Grech-Angelini et al. (2019) and Michelet et al. (2014). The set of primers and probes created especially for this study is listed in the Table 11.

**Table 11.** List of pathogens, targets and primers/probes created for microfluidic real-time PCR screening.

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

### DNA amplification and microfluidic real-time PCR

The detection of the vector-borne pathogen targets was performed on pre-amplified DNA by using the BioMark™ real-time PCR system (Fluidigm, California, USA) for high-throughput microfluidic real-time PCR amplification using the 48.48 Dynamic Array™ (Fluidigm). Fluidigm chips can carry out 2304 real time PCR reactions simultaneously with 48 PCR mixes on 48 samples placed in individual wells prior to transfer to individual chambers for the reaction. The thermal cycling conditions were 50°C for 2 min, 95°C for 10 min and 40 cycles at 95°C for 15 s and 60°C for 1 min. One negative water control, one inhibitory molecule control (*E. coli* EDL933 strain) and one DNA extraction control (animal species target) were added to each array. *E. coli*-specific primers and probes were used.

### Confirmation of pathogen detection

The confirmation of the pathogen detection was performed using nested PCR or real-time PCR targeting a gene other than the one used in the microfluidic experiment. The targets and primers were selected from the literature when available or newly designed for this study. The positive sequences after gel migration of the nested PCR product were sent to Eurofins MWG Operon (Cologne, Germany) for sequencing and assembled using BioEdit software (Ibis Biosciences, Carlsbad, CA, USA). An online BLAST (National Center for Biotechnology Information) was used to identify the sequenced organisms. All targeted genes and primer sequences from the literature are described in Gondard et al. (2018) and Michelet et al. (2014). The set of primers/probes created for this publication is listed in Table 12.

**Table 12.** List of pathogens, targets and primers/probes used for confirmation by real-time PCR.

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

## 4. Results

### *Tick collection*

A total of 444 ticks were collected on 178 cattle in 18 municipalities close to wetlands (Table 13). Six tick species were identified: *Rhipicephalus bursa* (38.7%), *Rh. sanguineus* s.l. (22.8%), *Rhipicephalus* sp. (17.1%), *Hy. marginatum* (8.6%), *Hy. scupense* (7.2%), *D. marginatus* (0.7%) and *Rh. (Bo.) annulatus* (0.2%). Finally, 4.7% of the ticks could not be determined and were designated as undetermined ticks (Table 13). Regarding their life-cycle stage, 55.6% were nymphs and 44.4% were adults; of these adults 57.4% were partially or fully engorged females and 42.6% were males. No ticks were found on sheep.

### *Pathogens detected in ticks*

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

One pool was positive for *Anaplasma* sp. It was composed of *Rh. sanguineus* s.l. ticks collected on a farm in Viggianello (Table 13, Figure 25A). However, sequencing was unsuccessful for *Anaplasma* sp.

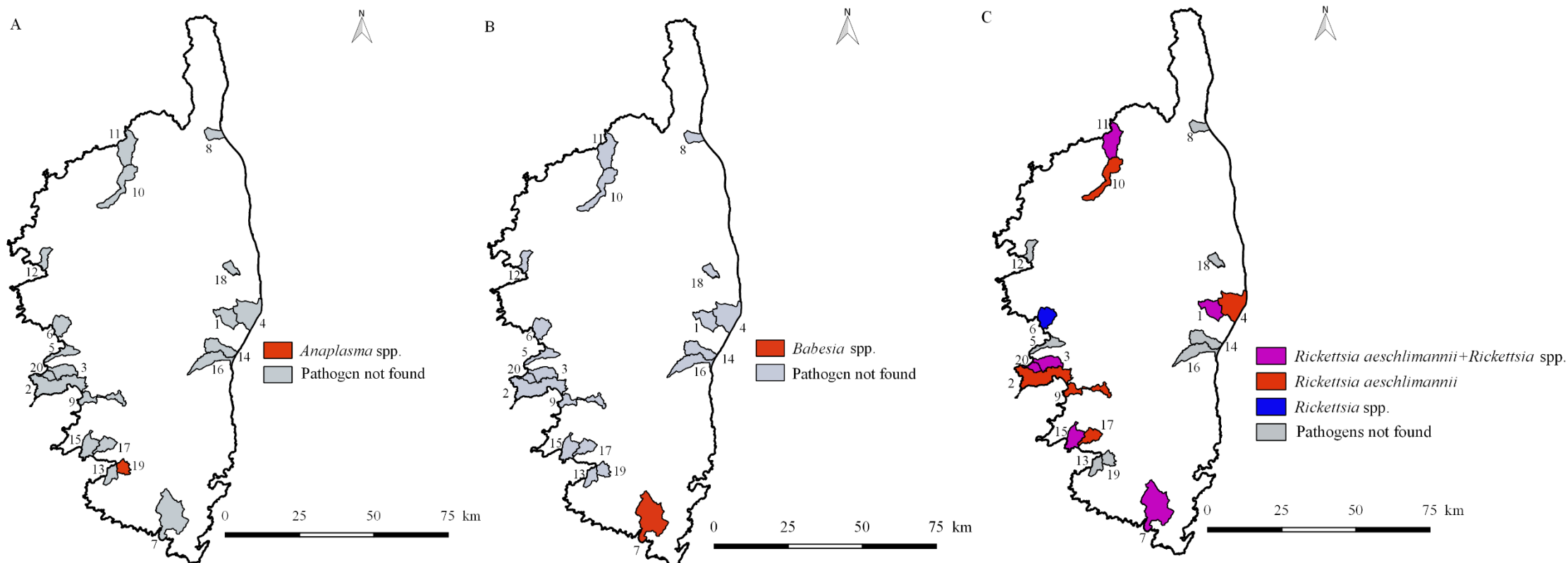
Two pools were positive for *Babesia* sp. They were one pool of *Rh. sanguineus* s.l. and one of *Hy. marginatum* both collected on a farm in Figari (Table 13, Figure 25B). Unfortunately, the sequencing of the *Babesia* sp. positive samples was unsuccessful.

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

The most frequently found pathogen was *Rickettsia* sp., detected in 93 pools; 15 of the pools detected positives to *Rickettsia* sp. could not be determined to the specific level. These



bacteria were detected in *Rh. sanguineus* s.l. (46.6%), *Rh. bursa* (33.3%), but only 6.7% for *Rhipicephalus* sp., *Hy. scupense* and undetermined ticks. The remaining 78 pools were positive for *R. aeschlimannii*. This pathogen was mainly found in *Rh. bursa* (41%), with the remaining positive tick species being *Hy. marginatum* (29.5%), *H. scupense* (14.1%), *Rh. sanguineus* s.l. (10.2%), *Rhipicephalus* sp. (3.8%) and unknown (1.4%) (Table 13, Figure 25.C). These samples were found to be related to the ompb gene of a *R. aeschlimannii* sequence detected in a *Hy. marginatum* individual in Russia (Genbank: KU961544.1) with an identity between 96% and 100%.



**Figure 25.** Map of Corsica showing the locations of tick samples from cattle positive for *Rickettsia aeschlimannii* and *Rickettsia* spp. (A), *Anaplasma* spp. (B) and/or *Babesia* spp. (C) (1. Aghione, 2. Ajaccio, 3. Alata, 4. Aleria, 5. Calcatoggio, 6. Coggia, 7. Figari, 8. Furiani, 9. Grosseto-Prugna, 10. Olmi-Capella, 11. Palasca, 12. Partinello, 13. Propriano, 14. Prunelli-Di-Fiumurbo, 15. Serra-Di-Ferro, 16. Serra-Di-Fiumorbo, 17. Sollocaro, 18. Tox, 19. Viggianello, 20. Villanova)

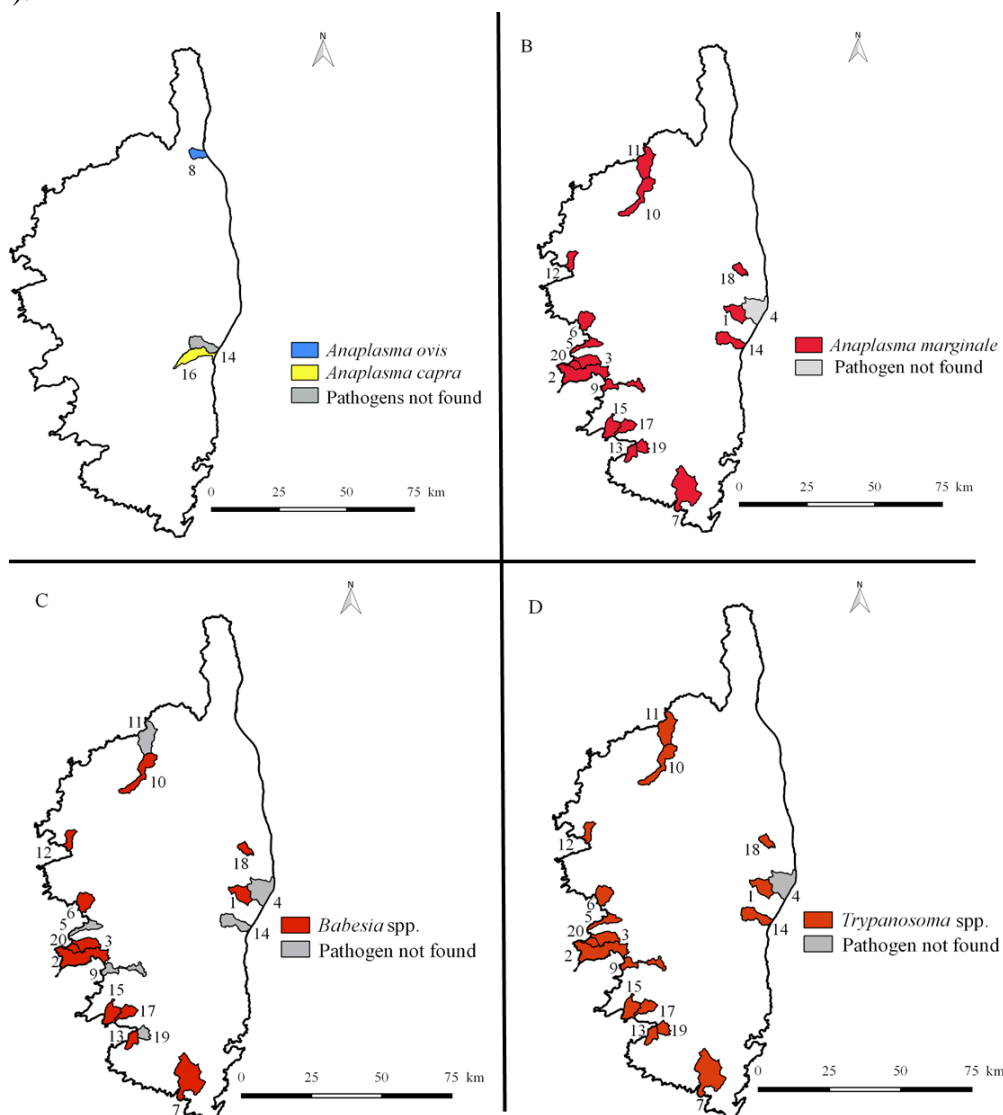
**Table 13.** Tick-borne pathogens and tick distribution on livestock in Corsican wetlands. (abbreviations: A: *Anaplasma* spp., Ac: *A. capra*, Am: *A. marginale*, Ao: *A. ovis*, B: *Babesia* spp., R: *Rickettsia* spp., Ra: *R. aeschlimannii*, T: *Trypanosoma* spp.)

Municipality (n°)	No. sampled sheep/no. positives (A/Ao/Ac)	No. sampled cattle/no. positives (Am/B/T)	<i>D. marginatus</i> (A/B/R/Ra)	<i>Hy. Marginatum</i> (A/B/R/Ra)	<i>Hy. Scupense</i> (A/B/R/Ra)	<i>Rhipicephalus</i> spp. (A/B/R/Ra)	<i>Rh. Annulatus</i> (A/B/R/Ra)	<i>Rh. Bursa</i> (A/B/R/Ra)	<i>Rh. sanguineus</i> s.l. (A/B/R/Ra)	Undetermined tick species (A/B/R/Ra)	Total ticks from cattle (A/B/R/Ra)
Aghione (1)	-	28/9 (Am), 1 (B), 15 (T)	-	2/1 (Ra)	1/1 (R)	2/2 (Ra)	-	2/2 (Ra)	1/1 (R)	9/0	17/2 (R), 5 (Ra)
Ajaccio (2)	-	38/14 (Am), 1 (B), 22 (T)	-	-	-	16/0	1/0	26/3 (Ra)	14/0	-	57/3 (Ra)
Alata (3)	-	6/6 (Am), 3 (B), 2 (T)	1/0	1/1 (Ra)	1/0	1/0	-	1/0	4/4 (R)	-	9/4 (R), 1 (Ra)
Aleria (4)	-	22/0	-	3/3 (Ra)	3/0	2/0	-	22/ 3 (Ra)	2/ 2 (Ra)	2/0	34/8 (Ra)
Calcatoggio (5)	-	10/9 (Am), 4 (T)	-	1/0	-	-	-	-	-	1/0	2/0
Coggia (6)	-	10/10 (Am), 4 (B), 9 (T)	-	1/0	8/0	-	-	-	-	2/1 (R)	11/1 (R)
Figari (7)	-	14/14 (Am), 2 (B), 11 (T)	1/0	4/1 (B) , 2 (Ra)	-	6/0	-	9/2 (R) , 1 (Ra)	12/3 (B), 3 (R), 6 (Ra)	1/0	33/4 (B), 5 (R), 9 (Ra)
Furiani (8)	25/22 (A), 2 (Ao)	-	-	-	-	-	-	-	-	-	-
Grosseto-Prugna (9)	-	11/1 (Am), 6 (T)	-	6/3 (Ra)	3/3 (Ra)	-	-	1/0	-	-	10/6 (Ra)
Olmi-Capella (10)	-	19/19 (Am), 4 (B), 13 (T)	-	4/3 (Ra)	3/1 (Ra)	12/1 (Ra)	-	2/1 (Ra)	3/0	2/1 (Ra)	26/7 (Ra)
Palasca (11)	-	12/10 (Am), 10 (T)	-	12/9 (Ra)	8/3 (Ra)	-	-	54/3 (R), 26 (Ra)	-	-	74/3 (R), 38 (Ra)
Partinello (12)	-	8/5 (Am), 4 (B), 7 (T)	-	-	-	-	-	-	-	-	-
Propriano (13)	-	10/7 (Am), 3 (B), 4 (T)	-	-	-	1/0	-	8/0	3/0	2/0	14/0
Prunelli-Di-Fiumorbo (14)	25/0	8/6 (Am), 4 (T)	-	-	-	-	-	-	-	-	-
Serra-Di-Ferro (15)	-	34/29 (Am), 5 (B), 20 (T)	-	-	-	2/2 (Ra)	-	25/2 (R)	14/3 (R)	-	41/5 (R), 2 (Ra)
Serra-Di-Fiumorbo (16)	34/33 (A), 1 (Ac)	-	-	-	-	-	-	-	-	-	-
Sollacaro (17)	-	28/18 (Am), 4 (B), 20 (T)	1/0	-	-	17/0	-	15/1 (Ra)	28/ 1 (Ra)	1/0	62/2 (Ra)
Tox (18)	-	9/9 (Am), 2 (B), 9 (T)	-	-	-	-	-	1/0	-	-	1/0
Viggianello (19)	-	15/7 (Am), 3 (T)	-	1/0	-	16/0	-	-	8/2 (A)	1/0	26/2 (A)
Villanova (20)	-	16/4 (Am), 2 (B), 9 (T)	-	3/1 (Ra)	5/4 (Ra)	1/1 (R)	-	6/0	12/2 (R), 2 (Ra)	-	27/3 (R), 7 (Ra)
<b>Total sampled/positives</b>	74/55 (A),1 (Ac), 2 (Ao)	288/177 (Am), 35 (B), 168 (T)	3/0	38/1 (B), 23 (Ra)	32/1 (R), 11 (Ra)	76/1 (R), 5 (Ra)	1/0	172/7 (R), 37 (Ra)	101/2 (A), 3 (B), 13 (R), 11 (Ra)	21/1 (R), 1 (Ra)	444/2 (A), 4 (B), 23 (R), 88 (Ra)

The most frequently found pathogen was *Rickettsia* sp., detected in 93 pools; 15 of the pools detected positives to *Rickettsia* sp. could not be determined to the specific level. These bacteria were detected in *Rh. sanguineus* s.l. (46.6%), *Rh. bursa* (33.3%), but only 6.7% for *Rhipicephalus* sp., *Hy. scupense* and undetermined ticks. The remaining 78 pools were positive for *R. aeschlimannii*. This pathogen was mainly found in *Rh. bursa* (41%), with the remaining positive tick species being *Hy. marginatum* (29.5%), *H. scupense* (14.1%), *Rh. sanguineus* s.l. (10.2%), *Rhipicephalus* sp. (3.8%) and unknown (1.4%) (Table 13, Figure 25.C). These samples were found to be related to the ompB gene of a *R. aeschlimannii* sequence detected in a *Hy. marginatum* individual in Russia (Genbank: KU961544.1) with an identity between 96% and 100%.

*Pathogens detected in blood samples*

From the 362 blood samples (288 cattle and 74 sheep), 79.9% of cattle and 78.4% of sheep were positive for at least one pathogen (Table 13). Bacteria from the genus *Anaplasma* were found in both livestock species (Table 13). In sheep blood samples, two *Anaplasma* species were found: *A. capra* in Serra-Di-Fiumorbo and *A. ovis* in Furiani (Table 13, Figure 26.A).



**Figure 26.** Map of Corsica showing location of sheep blood samples positive for *Anaplasma capra* and *Anaplasma ovis* (A), and cattle blood samples positive for *Anaplasma marginale* (B), *Babesia* spp. (C) and/or *Trypanosoma* spp. (1. Aghione, 2. Ajaccio, 3. Alata, 4. Aleria, 5. Calcatoggio, 6. Coggia, 7. Figari, 8. Furiani, 9. Grosseto-Prugna, 10. Olmi-Capella, 11. Palasca, 12. Partinello, 13. Propiano, 14. Prunelli-Di-Fiumurbo, 15. Serra-Di-Ferro, 16. Serra-Di-Fiumorbo, 17. Sollocaro, 18. Tox, 19. Viggianello, 20. Villanova).

The *A. capra* sequence reported was found to be related to one 16S rRNA sequence from a blood sample collected from a Korean water deer in South Korea (GenBank: LC432126.1) with an identity of 98.69%. The *A. ovis* sequence reported was found to be related to a 16S rRNA sequence found in sheep blood in China (GenBank: KX579073.1) with an identity of 99.71%.

The *Anaplasma* species detected in cattle was *A. marginale* (Table 13, Figure 26.B) in 61.4% of the cattle blood samples. These samples were related to a 16S rRNA sequence detected in cattle blood samples from Iran (GenBank: MK016525.1) and from Cuba (GenBank: MK804764.1) with an identity between 99-100% for both.

The parasite genus *Babesia* sp. was found in 35 cattle blood samples in 12 municipalities (Table 13, Figure 26.C). The sequencing of *Babesia* sp. was unsuccessful and did not allow the identification of the species.

Out of the 288 cattle blood samples, 168 were positive for *Trypanosoma* sp. in 19 of the 20 sampled municipalities (Table 3, Figure 26D). Unfortunately, the sequencing of *Trypanosoma* sp. was unsuccessful in these samples and the species could not be identified.

From the 230 positive cattle blood samples, 42.7% were positive for one pathogen, 46% for two pathogens and 11.3% for three pathogens (Table 14).

**Table 14.** Number of cattle blood samples found positive for *Anaplasma marginale*, *Babesia* sp. and/or *Trypanosoma* sp. DNA.

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

## 5. Discussion

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

The main objective of our study was to screen cattle and sheep blood samples and their ticks for tick-borne pathogens and non-vectorized pathogens (but with a high risk of presence in wetlands) involved in animal and human health threats. In the literature, many pathogens have been found all around the Mediterranean Basin in ticks collected on cattle and sheep or in cattle and sheep blood (Table S5).

### *Tick species collected on cattle*

In the Mediterranean region, ticks are present in 14 countries and are highly diverse. About 27 species from six genera carrying tick-borne pathogens were collected on cattle and sheep (Table S5). These genera are widely distributed in the four areas of the Mediterranean Basin: the Balkans, the Maghreb or southern border, the Middle East and Western Europe. In all four areas, cattle and sheep are host to four of the six genera: *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*. *Dermacentor* is found in the Balkans, the Middle East and Western Europe on cattle and sheep. Finally, the last genus, *Amblyomma* has only been collected sporadically in Western Europe on cattle. In our study, four of these six genera were found on cattle: *Dermacentor*, *Hyalomma*, *Ixodes* and *Rhipicephalus* (Table S5).

Among these genera, different species are frequently found on cattle or sheep. In the continental areas of the Balkans, the Maghreb, the Middle East and Western Europe, a total of 10 main tick species have been found: the term main species defines the species found with the highest number of tick individuals on the cattle or sheep per study.

Among the genera found in our study, in the *Dermacentor* genus, we identified one species in our study, *D. marginatus*, which has also been found on cattle in Italy and Turkey, and on sheep in Greece, Italy, Spain and Turkey, and can be responsible for the circulation of pathogens such as rickettsiae from the spotted fever group (Buczek et al., 2020). The second one is the *Hyalomma* genus, in which we found two species: *Hy. marginatum* and *Hy. scupense*. *Hyalomma marginatum*, also reported on cattle in Algeria, Spain, France, Greece, Italy and Turkey, and on sheep from Algeria, Cyprus, Greece, Italy and Turkey, is responsible for the transmission of *Babesia* spp., *R. aeschlimannii* and the CCHF virus (Table S5). *Hyalomma scupense* has been found on cattle in Corsica and sheep in Turkey, and it mainly transmits *Theileria* parasites, but can also transmit the CCHF virus (Gharbi and Aziz Darghouth, 2014). The third tick genus and probably the most frequently encountered tick genus on cattle and sheep was *Rhipicephalus*, with: *Rh.(Bo). annulatus*, a species known to have a tropism for cattle and responsible for the transmission of *Babesia*. It has been found on cattle in Algeria, Egypt, France, Italy and Lebanon and on sheep in Lebanon and Tunisia (Table S5). *Rhipicephalus bursa*, which has a tropism for domestic ungulates and transmits pathogens/parasites from the *Anaplasma*, *Babesia* and *Theileria* genera, has been found on cattle in nearly every Mediterranean country and on sheep in Algeria, France, Greece, Italy, Lebanon and Tunisia (Table S5). *Rhipicephalus sanguineus* s.l., which targets both dogs and domestic ungulates, has been found in nearly every Mediterranean country on both cattle and sheep (Table S5).

To our knowledge, nine tick species have been found on cattle and sheep in Corsica: *Amblyomma variegatum*, responsible for the transmission of *Rickettsia* spp. and *Ehrlichia ruminatum*, which is an African livestock tick and has been sporadically collected on cattle in Corsica (V Cicculi et al., 2019; Yonow, 1995); *D. marginatus*, *Hae. punctata*, *Hy. scupense*, *Hy. marginatum*, *I. ricinus*, *Rh.(Bo.) annulatus*, *Rh. Bursa* and *Rh. sanguineus* s.l. were previously found in exhaustive survey (Grech-Angelini et al., 2016). However, this survey covered the entire island and all seasons. In our study, we focused on wetlands during January-February, the main tick species found belong to the *Rhipicephalus* genus: *Rh. annulatus*, *Rh bursa* and *Rh sanguineus* s.l., representing respectively 0.2%, 38.7% and 22.7% of the ticks

collected. Prevalence of *Rh. annulatus* was same as previously found and *Rh. bursa* was previously described as the main tick species found on cattle in Corsica (more than 50% of the ticks collected) (Grech-Angelini et al., 2016). However, *Rh. sanguineus* s.l. was represented by only 2.3% of the specimens collected in this previous survey (Grech-Angelini et al., 2016). This genus was followed by the *Hyalomma* genus with two species: *Hy. marginatum* and *Hy. scupense* represented respectively 8.6% and 7.2% of the ticks collected on cattle, which is lower than the *Hy. marginatum* collected (21.5%) and nearly equal to the *Hy. scupense* collected in a previous study (8.7%) (Grech-Angelini et al., 2016). The last species found was *D. marginatus* found in a low proportion (0.7%), which is similar to the one found in the previous study (Grech-Angelini et al., 2016). The tick species found in our study are similar to those reported from an exhaustive survey carried out by Grech-Angelini et al.,. However, we collected no *Haemaphysalis* and *Ixodes*, which can be attributed to season and altitude. Indeed, most of the *Haemaphysalis* ticks found by Grech-Angelini et al. (2016) were collected in November-December between 400 and 600 m altitude and *Ixodes* ticks in September-October at above 600 m of altitude, whereas we mainly collected our ticks in January-February in wetlands close to sea level. The seasonality of some species such as *Hy. scupense* in January-February and the absence of others such as *Hae. punctata* and *I. ricinus* during these months – which was observed in Grech-Angelini et al. may explain the presence of the former species and the absence of the latter two during our sampling period (Grech-Angelini et al., 2016). In addition, specific targeting of the wetlands in our study may also explain the difference between our study and the 2016 survey.

#### *Detected pathogens*

##### *Anaplasma sp.*

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

*Anaplasma ovis* is responsible for anaplasmosis in small ruminants which can lead to severe anemia, lower milk production and abortion (Yasini et al., 2012). It is mainly transmitted by *Rhipicephalus* ticks (Cabezas-Cruz et al., 2019; Friedhoff, 1997). In the Mediterranean region, *A. ovis* has already been detected in Algeria, France, Italy and Turkey, in both cattle and sheep, and in sheep in Tunisia. In ticks, it has been detected in Algeria, France, Italy and Morocco (Table S5). *Anaplasma ovis* seems to be mainly distributed in the western part of the Mediterranean Basin and is relatively common in cattle and sheep (Table S5). On Mediterranean islands, it has been detected in Sicily in cattle and sheep; and in Corsica where it has been found in goat flocks with a prevalence of 52.0% (Cabezas-Cruz et al., 2019). In our study, the presence of *A. ovis* DNA was detected in Corsican sheep blood (2.7%) for the first time, but at a lower prevalence (2.7%) than reported in goats (Cabezas-Cruz et al., 2019). The second *Anaplasma* species detected in blood samples was *A. capra*, an emerging zoonotic pathogen targeting small ruminants and humans. *Anaplasma capra* is known to be mainly asymptomatic, but sometimes it can cause fever, myalgia, thrombocytopenia and lymphadenopathy (Li et al., 2015; Peng et al., 2018). Actually, it was mainly reported in Asia (Amer et al., 2019; Peng et al., 2021). However, in the Mediterranean region, it has already been detected in deer in France (Jouglin et al., 2019). The detection of *A. capra* in sheep in our study is the first report of *A. capra* DNA in sheep in the Mediterranean area and in Corsica (Table S5).

The *Anaplasma* species reported in cattle blood samples was *Anaplasma marginale*. *A. marginale* is a bacterium responsible for bovine anaplasmosis occurring across nearly the entire continent of Europe, transmitted by *Rhipicephalus* ticks (Ferrolho et al., 2016; Khodadadi et al., 2021; Martins et al., 2020) and mainly targeting cattle and sheep (Atif, 2015). This bacterial species can cause symptoms such as fever, anemia, temporal infertility and anorexia, for

example (Kocan et al., 2015). *Anaplasma marginale* has been detected in cattle in six Mediterranean countries: Algeria, Egypt, France, Italy, Tunisia and Turkey (Açici et al., 2016; Al-Hosary et al., 2020; Belkahia et al., 2017; Boucheikhchoukh et al., 2018; Ceci et al., 2014; Dahmani et al., 2017c). In Italy, *A. marginale* has also been detected in sheep (Torina et al., 2007a). In ticks, *A. marginale* has been detected in Egypt, France, Italy and Morocco (Table S5). *Anaplasma marginale* seems to be present in both ticks and domestic animals, especially in western Mediterranean countries. On the Mediterranean islands, *Anaplasma* species has only been detected on French and Italian islands. *Anaplasma marginale* appears to be widespread in Sicily, where it has been detected in cattle, sheep and ticks. On other islands, this bacterial species has only been detected in Corsica in *Rh. bursa* ticks collected on cattle (Dahmani et al., 2017c) (Table S5). In our study, we detected *A. marginale* DNA in cattle at a prevalence of 61.4%, which is lower than the 100% detected in a previous study in cattle (Dahmani et al., 2017c). This difference could be explained by difference in the methodology as the number of farm and cattle blood samples which is higher in our study (288 blood samples and 22 farms) compare to Dahmani *et al.* which was a study on multiple animal (12 samples and one farm). Our result along with the previous discovery of *A. marginale* DNA in ticks collected on cattle indicate the high prevalence of *A. marginale* DNA in Corsican cattle (V. Cicculli et al., 2019a; Dahmani et al., 2017c; Grech-Angelini et al., 2020).

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

#### *Babesia* sp.

The second genus found in cattle blood samples was *Babesia*, at a prevalence of 12.2%. This genus is an intracellular protozoan parasite that belongs to the piroplasmid taxon, along with the genus *Theileria*. They mainly infect livestock, pets and rodents in which they can cause piroplasmosis (Beuglet and Moreau, 2015). In humans, *Babesia* parasites can cause moderate symptoms such as myalgia, headache, anorexia as well as severe symptoms with pulmonary edema, organ dysfunction syndrome and coma, (Vannier et al., 2015). Their primary reservoir is often rodents, which contaminate immature tick stages and the pathogens can be transmitted to domestic ungulates during the adult tick stage (Vannier et al., 2015). The zoonotic species are principally transmitted via *Ixodes* ticks, vector to mainly *B. divergens*, *B. duncani*, *B. microti* and *B. venatorum*. The *Babesia* genus is common in the Mediterranean region where it has been detected in cattle and sheep blood samples in seven countries: Algeria, Egypt, France, Italy, Morocco, Spain and Turkey, with a predominance of *B. bigemina*, the parasite responsible for bovine piroplasmosis (Table S5). On Mediterranean islands, only two species have been detected in cattle: *B. bigemina* and *B. bovis*, both responsible for bovine babesiosis at a prevalence of 26.3% and 20% respectively in Sicily. From ticks collected on cattle and sheep, this genus has been detected in Algeria, France, Italy, Palestine and Turkey with a predominance of *B. bigemina* and *B. ovis* (Table S5). In our study, *Babesia* sp. were identified in 3% of the *Rh. sanguineus* s.l. and in 2.6% of the *Hy. marginatum* ticks collected. In Figari, *Babesia* sp. was also found in one cattle blood sample, which indicates that *Babesia* sp. is possibly present in ticks and in cattle in this municipality. Our results demonstrate the presence of *Babesia* sp. in cattle for the first time in Corsica. The presence of *B. bigemina* DNA in ticks on Corsican cattle had already been shown in *Rh. bursa* ticks (Grech-Angelini et al., 2020). The



species may be *B. bigemina* or *B. bovis*, which are highly present in the Mediterranean region. However, in our study, we could only identify *Babesia* to the genus level due to unsuccessful sequencing and species determination is still needed.

#### *Rickettsia aeschlimannii*

The genus *Rickettsia* was only found in ticks, and it was the most frequently detected pathogen genus. It was detected in 25% of the collected ticks. It is composed of many zoonotic agents with diverse pathogenicity, but most of these species can be a threat for human health with symptoms ranging from cutaneous rashes to lymphadenopathy, for example (Blanton, 2019; Davoust et al., 2010). In Corsica, even if the exact impact of *Rickettsia* species is still unknown, diverse species have already been found in animals (*R. aeschlimannii*, *R. africae*, *R. felis*, *R. helvetica*, *R. massiliae* and *R. slovaca*) (V. Cicculli et al., 2019; V. Cicculli et al., 2019b; Grech-Angelini et al., 2020; Matsumoto et al., 2004) and a previous sero-epidemiological study showed exposure of 4.8% of the people to these pathogens in Corse-du-Sud (Raoult et al., 1985). The rickettsiae detected belong to the spotted fever group (SFG) rickettsiae. SFG is transmitted exclusively by ticks, which play the roles of both vector and reservoir owing to the trans-stadial and transovarial transmission of these pathogens (Blanton, 2019). Out of the positive ticks, 79.3% were *R. aeschlimannii*, the rest could not be determined to the species level. *Rickettsia aeschlimannii* was first found in *Hy. marginatum* from Morocco. The disease in humans was documented in a man who had traveled to Morocco in 2000 (Beati et al., 1997; Raoult et al., 2002). Given its circulation in *Hy. marginatum*, *R. aeschlimannii* is distributed throughout Africa. In the Mediterranean region, *R. aeschlimannii* is widely distributed in ticks. It has been found in Algeria, Egypt, France, Greece, Lebanon, Spain and Turkey (Table S5). On Mediterranean islands, it has been found in Cyprus with a prevalence of 12% from *Hy. marginatum* collected on goats and sheep (Chochlakis et al., 2012), and on the Greek island of Cephalonia in *Hy. anaticum excavatum* collected on sheep (Psaroulaki et al., 2006)(Table S5). In Corsica, *R. aeschlimannii* has previously been reported with a prevalence of 23% in ticks collected on cattle and 58% in ticks collected on sheep (Grech-Angelini et al., 2020). It was also detected in *Hy. marginatum* collected on cattle in 2019 (V. Cicculli et al., 2019b). In our study, we confirmed the presence of *R. aeschlimannii* in ticks collected from cattle at a prevalence similar to that previously found (19.8%), but in a wide diversity of tick species (Table S5). It seems that *R. aeschlimannii* DNA is present in ticks on cattle regardless of the sampling area. Nevertheless, *R. aeschlimannii* was not detected in cattle or sheep blood.

#### *Trypanosoma sp.*

*Trypanosoma sp.* was detected in 58.3% of the cattle blood samples. The *Trypanosoma* genus is mainly known as the pathogen of sleeping sickness in humans, caused by *Tr. brucei*, and Chagas disease, caused by *Tr. cruzi*, in Africa and South America, respectively. It is an ubiquitous genus, infecting a wide range of vertebrates and are mainly transmitted by biting flies, bugs and occasionally ticks (Kaufer et al., 2017). In domestic animals (cattle, horses, sheep, carnivores, etc.), these pathogens are transmitted mechanically by a wide range of biting midges. This protozoan parasite can be transmitted horizontally and vertically (Desquesnes et al., 2013). In the Mediterranean region, two species have been found: *Tr. evansi* responsible for “surra” a trypanosomiasis with high morbidity and mortality rates in animals (Erekat et al., 2020). According to the infected animal host, it can be lethal (horses), cause acute disease (dogs) and variable symptoms including lethal symptoms (cattle). However, although it is morphologically similar to *Tr. brucei*, *Tr. evansi* is rarely detected in humans and is still not considered as zoonotic (Desquesnes et al., 2013). It has been found in Algeria, Egypt, Palestine and Tunisia (Figure 27). *Trypanosoma theileri* is a non-pathogenic hemoparasite except for cattle in which it can cause anemia, abortion, fever and weight loss (Villa et al., 2008). It can be transmitted by tabanid flies and *Hy. anaticum anaticum* ticks (Lee et al., 2013). It has been found in Italy and in Spain (Figure 26). To our knowledge, this is the first report of *Trypanosoma sp.* DNA in cattle in Corsica. Unfortunately, the DNA sequence was not readable, so the sequence failed which could be due to divers factors as the quality or a degradation of

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



**Figure 26.** Distribution of *Trypanosoma* spp. in cattle in the Mediterranean region (data from the literature) and in our study (red square).

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

#### *Potential role of the livestock in the pathogens circulation*

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

needed to draw conclusions regarding the actual presence and potential circulation of these pathogens in the livestock population in Corsican wetlands.

## 6. Conclusion

In our study, we confirmed the presence of pathogen DNA in Corsican cattle, including *A. marginale* and in their ticks, such as *R. aeschlimannii*, *Anaplasma* sp. and *Babesia* sp. In addition, we detected the DNA of *Babesia* sp. and *Trypanosoma* sp. in cattle blood for the first time in Corsica with potential co-infection. The DNA of two different pathogen genera were found in both animals and ticks in the same municipalities: *Babesia* sp. in Figari and *Anaplasma* sp. in Viggianello which suggests the circulation of these pathogens in these municipalities. We also detected for the first time the presence of *Anaplasma capra* and *A. ovis* DNA in sheep flocks in Corsica. However, our methodology was based on PCR and it was not possible to confirm the actual presence and circulation of these pathogens. Obviously, serological surveys and pathogen isolations are needed to draw conclusions regarding the actual presence and potential circulation of these pathogens in the livestock population in Corsican wetlands. Nevertheless, our results are a first step in the characterization of potential threats regarding these pathogens and farming activities near Corsican wetlands.

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

**Competing interests:** The authors declare that they have no competing interests.

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

7. Supplementary data

**Table S5.** Distribution of tick-borne pathogens in ticks and in cattle and sheep blood samples in the Mediterranean region

Area (Island)	Main tick species (host: cattle <sup>c</sup> , sheep <sup>s</sup> )	Other tick species (host: cattle <sup>c</sup> , sheep <sup>s</sup> )	Pathogens found in ticks (bacteria <sup>b</sup> , parasite <sup>p</sup> and virus <sup>v</sup> )	Pathogens found in cattle (bacteria <sup>b</sup> , parasite <sup>p</sup> and virus <sup>v</sup> )	Pathogens found in sheep (bacteria <sup>b</sup> , parasite <sup>p</sup> and virus <sup>v</sup> )	References
Albania			Not screened	CCHF virus <sup>v</sup>	CCHF virus <sup>v</sup>	(Lugaj et al., 2014a, 2014c, 2014b; Schuster et al., 2016)
Algeria	<i>Rhipicephalus annulatus</i> <sup>c</sup> , <i>Rh. bursa</i> <sup>s</sup>	<i>Haemaphysalis punctata</i> <sup>c,s</sup> , <i>Hae. sulcata</i> <sup>s</sup> , <i>Hyalomma detritum</i> <sup>c,s</sup> , <i>Hy. marginatum</i> <sup>c,s</sup> , <i>Ixodes ricinus</i> <sup>c</sup> , <i>Rhipicephalus bursa</i> <sup>c</sup> , <i>Rh. sanguineus</i> s.l. <sup>c,s</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>Babesia ovis</i> <sup>p</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Candidatus</i> , <i>Ehrlichia urmitei</i> <sup>b</sup> , <i>Rickettsia aeschlimanii</i> <sup>b</sup> , <i>R. helvetica</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. monacensis</i> <sup>b</sup> , <i>R. raoultii</i> <sup>b</sup> , <i>Theileria annulata</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. ovis</i> <sup>p</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>Babesia bigemina</i> <sup>p</sup> , <i>B. bovis</i> <sup>p</sup> , <i>Borrelia</i> spp. <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. annulata</i> <sup>p</sup> , <i>T. buffeli</i> <sup>p</sup> , <i>T. equi</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>Borrelia theileria</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Theileria ovis</i> <sup>p</sup> , <i>T. annulata</i> <sup>p</sup>	(Aouadi et al., 2017; Bitam et al., 2006; Boucheikhchoukh et al., 2018; Dahmani et al., 2015; Dib et al., 2018, 2009; Sadeddine et al., 2020; Ziam et al., 2020, 2015; Ziam and Benaouf, 2004)
Cyprus	<i>Hyalomma excavatum</i> <sup>c</sup> , <i>Rhipicephalus bursa</i> <sup>c</sup> , <i>Rhipicephalus sanguineus</i> s.l. <sup>s</sup>	<i>Hyalomma</i> spp. <sup>s</sup> , <i>Hy. excavatum</i> <sup>s</sup> , <i>Hy. marginatum</i> <sup>s</sup> , <i>Hy. rufipes</i> <sup>c</sup> , <i>Rhipicephalus bursa</i> <sup>c</sup>	<i>Coxiella burnetii</i> <sup>b</sup> , <i>Rickettsia</i> spp. <sup>b</sup> , <i>R. aeschlimanii</i> <sup>b</sup> , <i>R. sibirica mongolotimonae</i> <sup>b</sup> ,	Not screened	Not screened	(Chochlakis et al., 2012; Spyridaki et al., 2002)
Egypt	<i>Rhipicephalus annulatus</i> <sup>c</sup> , <i>Hyalomma</i> spp. <sup>s</sup>	<i>Amblyomma</i> spp. <sup>c</sup> , <i>A. hebraeum</i> <sup>c</sup> , <i>Hyalomma</i> spp. <sup>c</sup> , <i>Hy. anatolicum</i> <sup>c</sup> , <i>Hy. excavatum</i> <sup>c</sup> , <i>Hy. dromedarii</i> <sup>c</sup> , <i>Hy. impeltatum</i> <sup>c</sup> , <i>Hy. rufipes</i> <sup>c</sup> , <i>Hy. truncatum</i> <sup>c</sup> , <i>Rhipicephalus</i> spp. <sup>c</sup> , <i>Rh. appendiculatus</i> <sup>c</sup> , <i>Rh. microplus</i> <sup>c</sup> , <i>Rh. sanguineus</i> s.l. <sup>c</sup>	<i>Anaplasma marginale</i> <sup>b</sup> , Crimean Congo Haemorrhagic Fever virus <sup>v</sup> , <i>Ehrlichia</i> spp. <sup>b</sup> , Lumpy skin disease virus <sup>v</sup> , <i>Rickettsia aeschlimanii</i> <sup>b</sup>	<i>Anaplasma marginale</i> <sup>b</sup> , <i>A. platys</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>b</sup> , <i>B. bigemina</i> <sup>b</sup> , <i>B. bovis</i> <sup>b</sup> , <i>B. occulta</i> <sup>b</sup> , Crimean Congo Haemorrhagic Fever virus <sup>v</sup> , <i>Leptospira</i> spp. <sup>b</sup> , <i>Theileria annulata</i> <sup>p</sup> , <i>T. orientalis</i> <sup>p</sup>	CCHF virus <sup>v</sup> , <i>Babesia bovis</i> <sup>p</sup> , <i>B. bigemina</i> <sup>b</sup> , <i>Coxiella burnetii</i> <sup>b</sup> , <i>Leptospira</i> spp. <sup>b</sup>	(Abdel-Shafy et al., 2012; Al-Hosary et al., 2020, 2018, 2018; Chisholm et al., 2012; El-Ashker et al., 2015; Elsify et al., 2015; Horton et al., 2014; Klemmer et al., 2018; Loftis et al., 2006; Mohamed et al., 2008; Tuppurainen et al., 2015)
France	<i>Rhipicephalus bursa</i> <sup>s</sup>		<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>Babesia divergens</i> <sup>p</sup> , <i>Ehrlichia</i> spp. <sup>b</sup>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Babesia divergens</i> <sup>p</sup> , <i>B. major</i> <sup>p</sup> , <i>Theileria buffeli</i> <sup>p</sup>	<i>Anaplasma phagocytophilum</i> <sup>b</sup> , <i>Theileria</i> spp. <sup>p</sup> , <i>T. ovis</i> <sup>p</sup>	(Agoulon et al., 2012; Chastagner et al., 2014; Criado-Fornelio et al., 2009; Dahmani et al., 2017a; Devos and Geysen, 2004; Dugat et al., 2014)
France (Corsica)	<i>Rhipicephalus bursa</i> <sup>c,s</sup>	<i>Amblyomma variegatum</i> <sup>c</sup> , <i>Haemaphysalis punctata</i> <sup>c</sup> , <i>Hyalomma marginatum</i> <sup>c</sup> , <i>Hy. scupense</i> <sup>c</sup> , <i>Ixodes ricinus</i> <sup>c</sup> , <i>Rhipicephalus sanguineus</i> s.l. <sup>c</sup> , <i>Rh. (Bo.) annulatus</i> <sup>c</sup>	<i>Anaplasma</i> spp. <sup>b</sup> , <i>A. marginale</i> <sup>b</sup> , <i>A. phagocytophilum</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>E. canis</i> <sup>b</sup> , <i>E. minasensis</i> <sup>b</sup> , <i>Rickettsia aeschlimanii</i> <sup>b</sup> , <i>R. africae</i> <sup>b</sup> , <i>Candidatus</i> , <i>R. barbariae</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup>	<i>Anaplasma marginale</i> <sup>b</sup> , <i>Babesia</i> spp. <sup>p</sup> , <i>Trypanosoma</i> spp. <sup>p</sup>	<i>Anaplasma capra</i> <sup>b</sup> , <i>A. ovis</i> <sup>b</sup> , <i>Candidatus</i> , <i>A. corsicanum</i> <sup>b</sup> , <i>Candidatus</i> , <i>A. mediterraneum</i> <sup>b</sup>	(V Cicculi et al., 2019; V. Cicculi et al., 2019b, 2019c; Dahmani et al., 2017b; Grech-Angelini et al., 2020; Matsumoto et al., 2004), present study
Greece	<i>Hae. sulcata</i> <sup>s</sup> , <i>Rhipicephalus sanguineus</i> s.l. <sup>c</sup>	<i>Dermacentor marginatus</i> <sup>s</sup> , <i>Haemaphysalis</i> spp. <sup>s</sup> , <i>Hae. parva</i> <sup>s</sup> , <i>Hae. punctata</i> <sup>s</sup> , <i>Hae. sulcata</i> <sup>s</sup> , <i>Ixodes gibbosus</i> <sup>s</sup> , <i>Rhipicephalus bursa</i> <sup>c,s</sup> , <i>Rh. sanguineus</i> s.l. <sup>s</sup>	Crimean Congo Haemorrhagic Fever virus <sup>v</sup> , <i>Rickettsia conorii</i> <sup>b</sup> , <i>R. massiliae</i> <sup>b</sup> , <i>R. rhipicephali</i> <sup>b</sup>		<i>Coxiella burnetii</i> <sup>b</sup>	(Chochlakis et al., 2018; Fernández de Mera et al., 2017; Filioussis et al., 2017; Pape et

						al., 2009; Psaroulaki et al., 2003)
<b>Greece (Cephalonia)</b>	<i>Hy. marginatum<sup>f</sup>, Rhipicephalus sanguineus s.l.<sup>s</sup></i>	<i>D. marginatus<sup>s</sup>, Haemaphysalis punctata<sup>s</sup>, Hyalomma excavatum<sup>c,s</sup>, Hy. marginatum<sup>c,s</sup>, Ixodes gibbosus<sup>c,s</sup>, Rhipicephalus sanguineus s.l.<sup>c</sup></i>	<i>Coxiella burnetii<sup>b</sup>, Rickettsia aeschlimannii<sup>b</sup>, R. massiliae<sup>b</sup></i>			(Psaroulaki et al., 2006)
<b>Greece (Crete)</b>	<i>Rhipicephalus bursa<sup>c</sup></i>	<i>Rhipicephalus sanguineus s.l.<sup>c</sup></i>	<i>Bartonella vinsonii subsp. arupensis<sup>b</sup></i>	<i>Bartonella vinsonii subsp. arupensis<sup>b</sup></i>		(Chochlakis et al., 2020)
<b>Italy</b>	<i>Rhipicephalus annulatus<sup>c</sup>, I. ricinus<sup>s</sup>,</i>	<i>Dermacentor marginatus<sup>c,s</sup>, Haemaphysalis punctata<sup>c</sup>, Hyalomma marginatum<sup>c</sup>, Rhipicephalus bursa<sup>c,s</sup>, Rh. sanguineus s.l.<sup>c,s</sup></i>	<i>Anaplasma phagocytophilum<sup>b</sup>, Babesia bigemina<sup>p</sup>, B. bovis<sup>p</sup>, B. caballi<sup>p</sup>, B. divergens<sup>p</sup>, B. ovis<sup>p</sup>, Bartonella spp.<sup>b</sup>, Borrelia burgdoferi s.l.<sup>b</sup>, Coxiella burnetii<sup>b</sup>, Ehrlichia spp.<sup>b</sup>, Francisella spp.<sup>b</sup>, Rickettsia spp.<sup>b</sup>, R. felis<sup>b</sup>, Theileria annae<sup>p</sup>, T. buffeli/sergenti/orientalis<sup>p</sup>, T. equi<sup>p</sup></i>	<i>Anaplasma bovis<sup>b</sup>, A. centrale<sup>b</sup>, A. marginale<sup>b</sup>, A. phagocytophilum<sup>b</sup>, Babesia bigemina<sup>p</sup>, B. bovis<sup>p</sup>, B. divergens<sup>b</sup>, Coxiella burnetii<sup>b</sup>, Theileria buffeli<sup>p</sup></i>	<i>Anaplasma phagocytophilum<sup>b</sup>,</i>	(Baráková et al., 2018; Cassini et al., 2012; Ceci et al., 2014; Cringoli et al., 2002; Di Domenico et al., 2014; Ebani et al., 2008; Mancini et al., 2019; Raele et al., 2018; Toma et al., 2017)
<b>Italy (Sardinia)</b>	<i>Rhipicephalus bursa<sup>c,s</sup></i>	<i>Dermacentor marginatus<sup>c,s</sup>, Haemaphysalis punctata<sup>c,s</sup>, Hae. sulcata<sup>a</sup>, Hyalomma marginatum<sup>c</sup>, Rhipicephalus annulatus<sup>c</sup>, Rh. sanguineus s.l.<sup>c,s</sup></i>	<i>Anaplasma spp.<sup>b</sup>, A. phagocytophilum<sup>b</sup>, Bartonella spp.<sup>b</sup>, Chlamydia abortus<sup>b</sup>, Chlamydomphila psitacci<sup>b</sup>, Coxiella burnetii<sup>b</sup>, Rickettsia spp.<sup>b</sup>, R. hoogstrali<sup>b</sup>, R. massiliae<sup>b</sup>, Theileria buffeli/sergenti/orientalis<sup>p</sup></i>		<i>Coxiella burnetii<sup>b</sup></i>	(Chisu et al., 2019, 2018a, 2018b, 2017; Masala et al., 2004; Mura et al., 2008; Satta et al., 2011)
<b>Italy (Sicily)</b>	<i>Rhipicephalus sanguineus s.l.<sup>c,s</sup></i>	<i>D. marginatus<sup>c,s</sup>, Haemaphysalis punctata<sup>c,s</sup>, Hyalomma marginatum<sup>c,s</sup>, Hy. lusitanicum<sup>c</sup>, Ixodes ricinus<sup>s</sup>, Rhipicephalus bursa<sup>c</sup></i>	<i>Anaplasma marginale<sup>b</sup>, A. phagocytophilum<sup>b</sup>, Rickettsia africae<sup>b</sup>, R. conorii<sup>b</sup>, R. slovaca<sup>b</sup></i>	<i>Anaplasma marginale<sup>b</sup>, A. ovis<sup>b</sup>, A. phagocytophilum<sup>b</sup>, Babesia bigemina<sup>p</sup>, B. bovis<sup>p</sup>, Coxiella burnetii<sup>b</sup>, Theileria annulata<sup>p</sup></i>	<i>Anaplasma spp.<sup>b</sup>, A. marginale<sup>b</sup>, A. ovis<sup>b</sup>, A. phagocytophilum<sup>b</sup>, Babesia spp.<sup>p</sup>, Coxiella burnetii<sup>b</sup>, Theileria annulata<sup>p</sup></i>	(Beninati et al., 2005; De La Fuente et al., 2005; Gargano et al., 2021; Torina et al., 2008b, 2007a)
<b>Lebanon</b>	<i>Rhipicephalus annulatus<sup>c,s</sup></i>	<i>Hyalomma anatolicum<sup>c,s</sup>, Rh. bursa<sup>c,s</sup>, Rhipicephalus sanguineus s.l.<sup>c,s</sup></i>	<i>Rickettsia spp.<sup>b</sup>, R. aeschlimannii<sup>b</sup>, Candidatus R. barbariae<sup>b</sup>, R. massiliae<sup>b</sup></i>			(Fernández de Mera et al., 2018)
<b>Morocco</b>	<i>Rhipicephalus sanguineus s.l.<sup>c</sup></i>	<i>Ixodes ricinus<sup>c</sup>, Rhipicephalus bursa<sup>c</sup></i>	<i>Anaplasma centrale<sup>b</sup>, A. marginale<sup>b</sup>, A. ovis<sup>b</sup></i>		<i>Anaplasma spp.<sup>b</sup>, Babesia spp.<sup>p</sup>, Theileria spp.<sup>p</sup></i>	(Lbacha et al., 2017; Seng et al., 2009)
<b>Montenegro</b>					<i>Coxiella burnetii<sup>b</sup></i>	(Lausevic, 2001)
<b>Palestine</b>	<i>Rhipicephalus sanguineus s.l.<sup>s</sup></i>	<i>Haemaphysalis adleri<sup>s</sup>, Hae. parva<sup>s</sup>, Rhipicephalus spp.<sup>s</sup>, Rh. bursa<sup>s</sup></i>	<i>Anaplasma spp.<sup>b</sup>, A. platys<sup>b</sup>, Babesia ovis<sup>p</sup>, Bartonella chomelii<sup>b</sup>, Ba. rochalimae<sup>b</sup>, Ba. koehlerae<sup>b</sup>, E. canis<sup>b</sup>, H. canis<sup>p</sup>, Theileria ovis<sup>s</sup></i>		<i>Anaplasma spp.<sup>b</sup></i>	(Azmi et al., 2016; Erekat et al., 2016b; Zaid et al., 2019)
<b>Slovenia</b>	<i>Ixodes ricinus<sup>c</sup></i>	<i>Haemaphysalis punctata<sup>c</sup></i>	<i>Coxiella burnetii<sup>b</sup></i>	<i>Coxiella burnetii<sup>b</sup></i>	<i>Coxiella burnetii<sup>b</sup></i>	(Knap et al., 2019)
<b>Spain</b>	<i>Hyalomma marginatum<sup>f</sup>, Rhipicephalus sanguineus s.l.<sup>s</sup></i>	<i>Dermacentor marginatus<sup>s</sup>, Hyalomma lusitanicum<sup>c</sup>, Hy. marginatum<sup>s</sup>, Ixodes ricinus<sup>s</sup>, Rhipicephalus spp.<sup>c</sup></i>	<i>Anaplasma phagocytophilum<sup>b</sup>, CCHF virus<sup>v</sup>, Noerlichia mikurensis<sup>b</sup>, Rickettsia spp.<sup>b</sup>, R. aeschlimannii<sup>b</sup>, R. massiliae<sup>s</sup>, R. raoultii<sup>s</sup>, R. slovaca<sup>b</sup></i>	<i>Coxiella burnetii<sup>b</sup>, R. slovaca<sup>b</sup>, Theileria annulata<sup>p</sup>, T. buffeli<sup>p</sup>, T. sergenti<sup>p</sup></i>	<i>Babesia ovis<sup>p</sup>, B. motasi<sup>p</sup>, Coxiella burnetii<sup>b</sup>, Rickettsia slovaca<sup>b</sup>, Theileria spp.<sup>p</sup>, T. ovis<sup>p</sup></i>	(Astobiza et al., 2011; Giménez Pardo et al., 2009; Nagore et al., 2004; Negro et al., 2019; Ortuño et al., 2012; Palomar et al., 2014, 2017b; Ruiz-Fons et al., 2008a)
<b>Tunisia</b>	<i>Rhipicephalus sanguineus s.l.<sup>s</sup></i>	<i>Rhipicephalus annulatus<sup>s</sup>, Rh. bursa<sup>s</sup></i>	<i>Anaplasma bovis<sup>b</sup>, R. conorii<sup>b</sup></i>	<i>Anaplasma spp.<sup>b</sup>, A. bovis<sup>b</sup>, A. centrale<sup>b</sup>, A. marginale<sup>b</sup>, A. phagocytophilum<sup>b</sup>, Theileria annulata<sup>p</sup></i>	<i>Anaplasma bovis<sup>b</sup>, A. ovis<sup>b</sup>, A. phagocytophilum<sup>b</sup>, Coxiella burnetii<sup>b</sup></i>	(Barkallah et al., 2018; Belkahia et al., 2019, 2017, 2014; Ben Said et al., 2017, 2015; Khrouf et al., 2014; Sallemi et al., 2017)

<b>Turkey</b>	<i>Hyalomma anatolicum<sup>s</sup></i> , <i>Rhipicephalus bursa<sup>c</sup></i>	<i>Dermacentor niveus<sup>b</sup></i> , <i>D. marginatus<sup>c,s</sup></i> , <i>Haemaphysalis concinna<sup>s</sup></i> , <i>Hae. parva<sup>c,s</sup></i> , <i>Hae. punctata<sup>c,s</sup></i> , <i>Hae. sulcata<sup>c,s</sup></i> , <i>Hyalomma</i> <i>spp.<sup>c</sup></i> , <i>Hy. aegyptium<sup>s</sup></i> , <i>Hy. detritum<sup>c,s</sup></i> , <i>Hy.</i> <i>excavatum<sup>c,s</sup></i> , <i>Hy. marginatum<sup>c,s</sup></i> , <i>Hy.</i> <i>scupense<sup>c</sup></i> , <i>Ixodes</i> <i>I. hexagonus<sup>c</sup></i> , <i>I. ricinus<sup>c,s</sup></i> , <i>Ornithodoros lahorensis<sup>c</sup></i> , <i>Rhipicephalus</i> <i>bursa<sup>c</sup></i> , <i>Rh. sanguineus</i> s.l. <sup>c,s</sup> ,	<i>Anaplasma spp.<sup>b</sup></i> , <i>A. centrale<sup>b</sup></i> , <i>A.</i> <i>phagocytophilum<sup>b</sup></i> <i>Babesia spp.<sup>p</sup></i> <i>B.</i> <i>bigemina<sup>p</sup></i> , <i>B. microti<sup>p</sup></i> , <i>B. ovis<sup>p</sup></i> , Crimean Congo Haemorrhagic Fever virus <sup>v</sup> , Phlebovirus <sup>v</sup> , <i>Rickettsia spp.<sup>b</sup></i> , <i>R. aeschlimanii<sup>b</sup></i> , <i>R. africae<sup>b</sup></i> , <i>R.</i> <i>raoultii<sup>b</sup></i> , <i>R. slovaca<sup>b</sup></i> <i>Theileria spp.<sup>p</sup></i> , <i>T. annulata<sup>p</sup></i> , <i>T. ovis<sup>p</sup></i> , <i>T.</i> <i>buffeli/sergenti/orientalis<sup>p</sup></i>	<i>Anaplasma spp.<sup>b</sup></i> , <i>A. centrale<sup>p</sup></i> , <i>A. marginale<sup>b</sup></i> , <i>A. ovis<sup>b</sup></i> , <i>A.</i> <i>phagocytophilum<sup>b</sup></i> , <i>Babesia</i> <i>spp.<sup>p</sup></i> , <i>B. bigemina<sup>p</sup></i> , <i>B. bovis<sup>p</sup></i> , <i>B. major<sup>p</sup></i> , <i>Ehrlichia spp.<sup>b</sup></i> , <i>Theileria spp.<sup>p</sup></i> , <i>T. annulata<sup>p</sup></i> , <i>T. buffeli/sergenti/orientalis<sup>p</sup></i>	<i>Anaplasma ovis<sup>b</sup></i> , <i>A.</i> <i>phagocytophilum<sup>b</sup></i> , <i>Babesia</i> <i>spp.<sup>p</sup></i> , <i>B. crassa<sup>p</sup></i> , <i>B. ovis<sup>b</sup></i> , <i>B. motasi<sup>p</sup></i> , <i>Theileria spp.<sup>p</sup></i> , <i>T. lestoquardii<sup>p</sup></i> , <i>T.</i> <i>luwenshuni<sup>p</sup></i> , <i>T. ovis<sup>p</sup></i> , <i>T.</i> <i>uilenberg<sup>p</sup></i>	(Açici et al., 2016; Aktas et al., 2012, 2011, 2006, 2006, 2004, 2004, 2002; Albayrak et al., 2010; Altay et al., 2014, 2012; K. Altay et al., 2008; Kursat Altay et al., 2008; Aydin et al., 2015; Aydin et al., 2019, 2019; Bilgic et al., 2017; Brinkmann et al., 2019; Dinçer et al., 2017; Dumanli et al., 2005; Ekici et al., 2012; Emanet et al., 2019; Gokce et al., 2008; Inci et al., 2016; Negredo et al., 2019; Orkun et al., 2014; Oter et al., 2016; Sayin et al., 2004; Tonbak et al., 2006)
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**Chapitre 5 : Agents pathogènes et ectoparasites de l'avifaune en zones humides de Corse**

## 1. Résumé de la publication « Detection of pathogens and ticks on sedentary and migratory birds in two Corsican wetlands (France, Mediterranean area) »

Cet article a fait l'objet d'une soumission dans la revue Parasites : Baptiste Defaye, Sara Moutailler, Benjamin Vollot, Clémence Gallon, Rayane Amaral Moraes, Antoine-Simon Leoncini, Amalia Rataud, Gilles Le Guillou, Vanina Pasqualini, Yann Quilichini (soumis). Detection of pathogens and ticks on sedentary and migratory birds in two Corsican wetlands (France, Mediterranean area).

### Résumé en Français

Dans cette étude, nous nous sommes focalisés sur les agents pathogènes au sein de l'avifaune circulant dans les zones humides corses. Pour cela, nous avons collecté des échantillons de sang et des ectoparasites sur l'avifaune dans les zones humides corses, et nous avons axé le dépistage sur la détection de 34 espèces et de 11 genres de bactéries, de virus et de parasites appartenant aux principaux pathogènes transmis par les tiques ou aux pathogènes non transmis par les tiques mais ayant un impact considérable sur la santé animale et humaine incluant : le virus de la peste porcine africaine, *Anaplasma* spp., *Anaplasma marginale*, *Anaplasma phagocytophilum*, virus d'Aujeszky, *Babesia bigemina*, *Babesia bovis*, *Babesia caballi*, *Babesia divergens*, *Babesia microti*, *Babesia ovis*, *Babesia vogeli*, *Babesia venatorum*, *Bartonella* spp. et *Bartonella henselae*, *Bartonella quintana*, *Borrelia afzelii*, *Borrelia burgdorferi* spp., *Borrelia burgdorferi sensu stricto*, *Borrelia lusitaniae*, *Borrelia miyamotoi*, *Borrelia spielmanii*, *Chlamydia psittaci*, *Coxiella burnetii*, *Ehrlichia* spp., *Ehrlichia ruminatum*, *Haemoproteus* spp, *Hepatozoon* spp., *Leishmania* spp., *Leishmania infantum*, *Leptospira* spp., *Leucocytozoon* spp, *Neoehrlichia mikurensis*, *Plasmodium* spp., *Rickettsia* spp., *R. aeschlimannii*, *R. conorii*, *R. helvetica*, *R. massiliae*, *R. monacensis*, *R. slovacica*, *Theileria annulata*, *Th. equi*, *T. gondii* et *Trypanosoma* spp. au moyen de la PCR en temps réel microfluidique. En complément, une détection du West Nile virus et du virus Ustu a été effectuée dans les échantillons de sang de l'avifaune. Les échantillons ont été récupérés grâce à la participation de Benjamin Vollot, bagueur assermenté du Centre de Recherche sur la Biologie des Populations d'Oiseaux (C.R.B.P.O.).

Trente-sept mouches plates de l'espèce *Ornithomya biloba* ont été collectées sur 2,5 % des oiseaux capturés. De ces mouches plates, une seule était positive à *Trypanosoma* spp., malheureusement le séquençage n'a pas été concluant. La famille des *Hippoboscidae* appartient à la superfamille des *Hippoboscoidea*, qui regroupe de nombreuses familles de mouches suceuses de sang. Cette famille a une distribution mondiale et peut être trouvée dans les mammifères et dans les oiseaux (Rahola et al., 2011). En Europe, différents genres ont été trouvés comme le genre *Liptotena* sur les cervidés, *Melophagus* spp. sur les ruminants domestiques et le genre *Ornithomya* sur les oiseaux, principalement sur les passereaux (Oboňa et al., 2019). Le genre *Ornithomya* est impliqué dans la transmission de parasites des genres *Haemoproteus* et *Trypanosoma* à leur hôte aviaire (Karadjian et al., 2013; Mungomba et al., 1989). Dans notre étude, nous avons détecté l'ADN de *Trypanosoma* spp. dans 2,7 % des mouches collectées, ce qui est une première en Corse. Cependant, la séquence n'a pas pu être confirmée. Néanmoins, elle permet d'avancer, soit l'hypothèse du passage de mouches plates potentiellement infectées par des pathogènes en Corse par le biais des oiseaux migrateurs, soit l'hypothèse de l'infestation des oiseaux migrateurs par des mouches plates en Corse.

Quarante-quatre tiques ont également été collectées sur 24 oiseaux (1,7% de la capture d'oiseaux), uniquement sur différentes espèces d'oiseaux sédentaires ou sédentaires/migratrices: 36,3 % sur *Erithacus rubecula*, 22,7 % sur *Saxicola rubicola*, 18,2 % sur *Cettia cetti*, 6,9 % sur *Turdus merula* et 4,5 % sur *Acrocephalus arundinaceus* et *Prunella modularis*, 2,3 % sur *Luscinia Megarhyncos*, *Parus major* et *Sylvia atricapilla*. Toutes les tiques collectées appartenaient aux genres *Ixodes* : *Ixodes* spp. (8,5 %), *I. accuminatus/ventalloi* (2,9 %), *I.*



*arboricola/lividus* (14,3 %), *I. frontalis* (5,7 %) et *I. ricinus* (68,6 %). 97,1 % des tiques ont été collectées au niveau de la lagune de Biguglia et 2,9 % au niveau de l'étang de Gradugine. Les tiques dures (*Ixodidae*) sont des vecteurs de plusieurs agents pathogènes comme les espèces *Rickettsia*, *Borrelia* et la fièvre hémorragique de Crimée-Congo (CCHF) (Estrada-Peña et al., 2012b; Rataud et al., 2021; Wallménius et al., 2014). Dans la zone européenne et méditerranéenne, la plupart des tiques présentes sur les espèces aviaires sont trouvées à des stades immatures pour le genre *Hyalomma* et à tous les stades pour le genre *Ixodes*. Sporadiquement, des stades immatures des genres *Haemaphysalis* et *Rhipicephalus* peuvent également être retrouvés. A l'heure actuelle, aucune information n'est disponible sur les espèces aviaires infestées par les tiques en Corse (Capligina et al., 2014; Wallménius et al., 2014). Dans notre étude, nous avons trouvé exclusivement des tiques du genre *Ixodes*. Ces tiques ont été fréquemment trouvées sur des espèces de passereaux (Baráková et al., 2018; Battisti et al., 2020).

Dans ces tiques, trois espèces de bactéries ont été détectées : *A. phagocytophilum*, *E. chaffeensis* et *R. helvetica*. Au sein des 1377 oiseaux capturés, 762 échantillons de sang ont été effectués. Deux agents pathogènes ont été trouvés. Le virus West Nile a été détecté dans deux échantillons de sang provenant de mésange charbonnière à l'étang de Gradugine lors de la migration automnale. *E. chaffeensis* a été détecté dans un *E. rubecula* sur lequel des tiques également positives à *E. chaffeensis* ont été collectées au sein de la lagune de Biguglia.

*A. phagocytophilum* a été la seule espèce du genre *Anaplasma* à avoir été trouvée, dans le pool de nymphes d'*I. ricinus* collectées à partir de *S. atricapilla*. Cet agent pathogène avait déjà été retrouvé dans les tiques *I. frontalis* et *I. ricinus* de quatre espèces d'oiseaux capturées dans notre étude : *E. rubecula*, *L. megarhynchos*, *S. atricapilla* et *T. merula* (Palomar et al., 2015). Il a également été trouvé dans la rate d'un *T. merula* des Pays-Bas. La présence d'*A. phagocytophilum* dans le nord de l'Amérique est très documentée (Jahfari et al., 2014; Johnston et al., 2013; Ogden et al., 2008). Plus récemment, un variant différent a également été trouvé dans une île grecque dans une tique du genre *Ixodes* infestant une pie-grièche écorcheur (*Lanius senator senator*) (Hoffman et al., 2020). En Corse, *A. phagocytophilum* a été trouvé principalement chez *I. ricinus*, mais également chez *D. marginatus*, *Ha. punctata*, *H. marginatum*, *Hy scupense*, *Rh. bursa* et *Rh. sanguineus* s.l. collectés sur des mammifères domestiques et sauvages (Cicculli et al., 2020; Grech-Angelini et al., 2020). A notre connaissance, il s'agit de la première détection de cet agent pathogène dans des tiques collectées sur oiseaux en Corse.

Au sein du genre *Ehrlichia*, nous avons détecté une espèce : *E. chaffeensis*. Elle a été détectée à partir d'un pool de larves d'*I. ricinus* collectées sur *E. rubecula* ainsi que dans le sang de l'oiseau. Chez les oiseaux, un *Anaplasma* étroitement lié à *Eh. chaffeensis*, a été trouvé dans des échantillons de tissus et de sang d'une grive musicienne (*Turdus philomelo*) en Hongrie (Hornok et al., 2014). Il a également été trouvé dans un foie de faisan commun en Chine (Yang et al., 2015). Même si le genre *Ehrlichia* a déjà été observé dans des tiques d'oiseaux (Špitalská et al., 2006; Toma et al., 2014), il y a peu d'informations sur la détection d'*E. chaffeensis* dans des tiques d'oiseaux en Europe et dans la zone méditerranéenne. Nos résultats constituent la première détection d'*Eh. chaffeensis* dans des tiques d'oiseaux sur le pourtour méditerranéen.

Concernant le genre *Rickettsia*, six pools ont été trouvés positifs et sur les six, trois étaient positifs à *R. helvetica* à partir de trois espèces différentes d'*Ixodes*. En Hongrie, des recherches avancent un statut de réservoir potentiel des oiseaux pour *R. helvetica* avec la détection de la bactérie dans 51,4 % des tiques des espèces *I. ricinus* et *Haemaphysalis* collectées sur des passereaux et également avec 4,7 % des oiseaux trouvés positifs à la bactérie (Hornok et al., 2014). Dans la zone méditerranéenne, il a été trouvé en Espagne avec une prévalence de 6 % dans des tiques *I. ricinus* collectées (Baráková et al., 2018). D'après la littérature, *R. helvetica* n'a été trouvée en Corse que chez des tiques *I. ricinus* collectées sur bovins (Grech-Angelini et al., 2020). Dans notre recherche, nous avons détecté pour la première

fois la présence de *R. helvetica* avec une prévalence de 11,1 % chez les oiseaux infestés de tiques.

Le dernier agent pathogène détecté a été le West Nile virus dans deux *P. major*. Ce virus est un flavivirus transmis par les moustiques et infectant principalement les oiseaux et les moustiques qui sont impliqués dans le cycle de vie du virus. Il peut également être trouvé chez l'homme et le cheval qui sont considérés comme des hôtes accidentels du virus. Dans les îles méditerranéennes, il a déjà été trouvé à Chypre chez des oiseaux ainsi que dans le sang de donneurs humains (Pallari et al., 2021), en Sardaigne dans un cas humain (Magurano et al., 2012) et en Crète dans du sang de poulet (Fotakis et al., 2022). En Corse, il a été trouvé avec une prévalence de 9,4 % chez des chevaux et 8,4 % chez des chiens (Maquart et al., 2017). Il s'agit de la première détection de l'ARN du WNV dans la population d'oiseaux en Corse.

Nos résultats démontrent l'importance de l'avifaune dans la surveillance des agents pathogènes et de leurs vecteurs arthropodes. Des analyses supplémentaires sur *Eh. chaffeensis* et West Nile virus sont encore nécessaires dans les populations d'oiseaux et leurs tiques pour déterminer la situation réelle de ces pathogènes dans les zones humides corses.

## Detection of pathogens and ticks on sedentary and migratory birds in two Corsican wetlands (France, Mediterranean area)

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### Abstract

Birds are one of the most species-diverse vertebrate groups and are susceptible to numerous hematophagous ectoparasites. Migratory birds likely contribute to the circulation of these ectoparasites and their associated pathogens. One of the many migration paths crosses the Mediterranean islands including Corsica and its wetlands, which are migration stopovers. Corsica, the main French island in the Mediterranean Sea, has high levels of human and animal population movement. Its numerous wetlands include the Biguglia Lagoon, which is the largest wetlands of the island. In our study, we collected blood samples and hematophagous ectoparasites in migratory and sedentary bird populations in two coastal lagoons: Biguglia and Gradugine. A total of 1,377 birds were captured during banding session over 3 years, from which 762 blood samples, 37 louse flies and 44 ticks were collected. All the louse flies were identified as *Ornithomya biloba* and all the ticks were from the *Ixodes* genus: *Ixodes* sp. (8.5%), *I. accuminatus/ventalloi* (2.9%), *I. arboricola/lividus* (14.3%), *I. frontalis* (5.7%) and *I. ricinus* (68.6%). From these samples, five pathogens including bacteria and viruses were detected: *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis* and *Rickettsia helvetica* in ticks, *Trypanosoma* sp. in louse flies. *E. chaffeensis* and the West Nile virus were both detected for in bird blood samples in Corsica. This is the first report of these tick, louse fly and pathogen species isolated on the bird population in Corsican. Our finding highlights the importance of bird populations in the presence of arthropod-borne pathogens in Corsican wetlands. Furthermore, we provide evidence that birds and their ticks are potentially involved in the circulation of *E. chaffeensis*, which was found in both bird and tick samples from Corsica.

**Keywords:** Arthropod-borne pathogens, ticks, louse flies, migratory birds, sedentary birds, wetlands

## 2. Introduction

Birds are one of the largest vertebrate groups, with approximately 10,000 species, of which approximately 5,000 belong to the order Passeriformes (Schmidt et al., 2015). Passerines can be sedentary — a lifestyle more susceptible to be infected by ectoparasites — or migratory — making them potential dispersal agents of ectoparasites and their pathogens along the migration routes (Battisti et al., 2020; Klaus et al., 2016). The Mediterranean area spans the main migration areas for autumn migration (postnuptial) from Northern Europe to North or sub-Saharan Africa, and spring migration (prenuptial) from Africa to Europe. The migration route is mainly defined by two migration paths: one across France, Spain and Gibraltar, and one across Turkey and Israel (Bruderer & Liechti 1999; Mellone 2020). However, some migration also covers the Mediterranean islands (Bruderer & Liechti 1999; Maggini et al., 2020; Vagliano, 1985) such as Corsica.

Migratory birds are frequently present in wetlands, which serve as water spots during migration stopovers, where they may interact with sedentary birds (Jourdain et al., 2007). Wetlands are areas of high levels of interaction between animals occur and are also strategic places for human activities: they represent one of the main watering and resting areas for both animals and humans (Bacon 1987; Hammer 1989). These habitats are important for public health given their role in vector proliferation and pathogen transmission (Jourdain et al., 2007; Rabou et al., 2015; Rey et al., 2012). The diversity and the prevalence of ectoparasite communities and pathogens can vary according to the ecosystem, leading to different health-related threats (Dudek, 2014).

Via their migrations routes, birds can play a role in the circulation of numerous pathogens, becoming infected, reservoir or acting as transport infected vectors. Avian influenza is the perfect example of a large-scale virus infection affecting many birds, including domestic or poultry species. Avian influenza is responsible for numerous outbreaks around the world every year (Levison, 2015; Pantin-Jackwood and Swayne, 2009). Birds can also transmit bacteria responsible for public or animal health and economic issues such as *Chlamydia psittaci*, the causative agent of psittacosis (Levison 2015) or numerous tick-borne pathogens (Hildebrandt et al., 2010), vector-borne viruses, e.g. West Nile virus (WNV) and Usutu virus (USUV), or parasites such as *Trypanosoma* spp. and *Hepatozoon* spp. (O'Donoghue 2017). West Nile Virus and USUV are emerging zoonotic pathogens transmitted by mosquitoes that can infect birds, humans and a large panel of other animal species (Levison, 2015; Maquart et al., 2017; Vilibic-Cavlek et al., 2020). As transport vectors, migratory birds can be involved in the circulation of vectors, such as ticks, which can carry pathogens, e.g. *Borrelia burgdorferi sensus lato*, the causative agent of Lyme borreliosis (Benskin et al., 2009).

Among the arthropods responsible for the circulation of pathogens, ticks and louse flies can be carried by birds over long distances. Ticks (Acarida) are the leading vector-borne pathogens in animals and second in humans, just after mosquitoes (de la Fuente et al., 2008). They are involved in pathogen transmission and circulation and serve as pathogen reservoirs in various types of animals all over the world: by feeding on their host's blood, ticks harbor and transmit tick-borne pathogens (TBPs) in a natural cycle involving their intermediate and final animal hosts and accidental human hosts (de la Fuente et al., 2008). As such, ticks can transmit a wide spectrum of bacteria, viruses and parasites. TBPs are known to be both pathogens of veterinary importance and zoonotic pathogens harmful for humans (Sonenshine, 1991). Through the dissemination of different tick species, migratory birds can facilitate the spread of a large panel of TBPs (Hasle 2013). In two tick surveys, 10 species have been recorded in Corsica (Mediterranean island): *Amblyomma variegatum* (sporadically), *Ixodes ricinus*, *Rhipicephalus bursa*, *Rh. Sanguineus* s.l., *Rh. Annulatus*, *Hyalomma marginatum*, *H. scupense*, *Haemaphysalis sulcata*, *Ha. punctata* and *Dermacentor marginatus* (V Cicculli et al., 2019; Grech-Angelini et al., 2016), but no research has been conducted on tick infestations of birds in Corsica. Louse flies (Hippoboscoidae) are biting flies that infest a large diversity of mammals

and birds, and occasionally humans. They are divided into two families: family Hippoboscidae targeting birds and mammals and family Nycteribiidae targeting bats (Reeves & Lloyd, 2019). Even though they have a lower health impact on humans than ticks and mosquitoes, they can have an impact on animals through the blood lost during feeding and the transmission of pathogens such as *Rickettsia* spp., *Bartonella* spp. and *Trypanosoma* spp. In wild ungulates (Foley et al., 2016; Halos et al., 2004; Reeves & Lloyd 2019; Werszko et al., 2020). In birds, louse flies are responsible for the transmission of parasites such as *Haemoproteus* spp. involved in bird mortality and morbidity (Žiegytė et al., 2020). Until this study, the only louse flies identified in Corsica were collected on bats and belonged to the family Nycteribiidae.

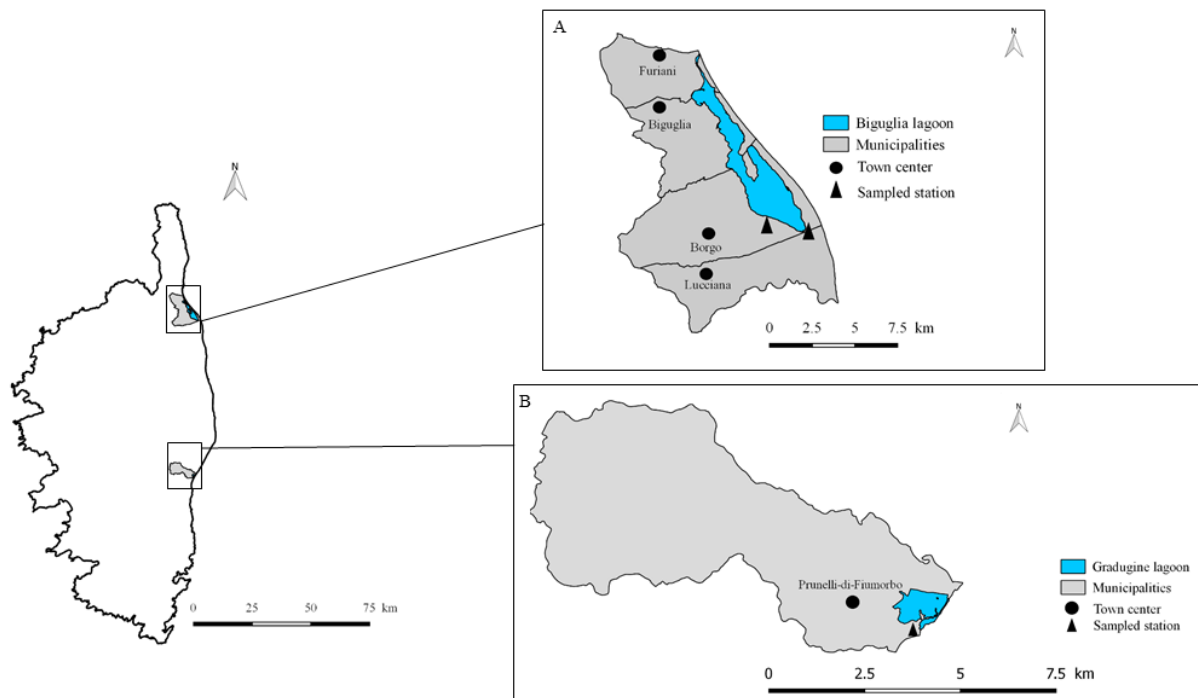
The aims of this study were (i) to identify and determine the ectoparasite species in birds sampled from Corsican wetlands and to (ii) to investigate the potential occurrence of zoonotic and non-zoonotic pathogens in birds and in their ectoparasites in Corsican wetlands. To do so, we restricted our screening to the detection of 34 species and 11 genera of bacteria, viruses and parasites suspected to be present in Corsica that have an important impact on animal and human health and belong to the main TBPs (i.e. African swine fever virus, *Anaplasma* spp., *A. marginale*, *A. phagocytophilum*, *Babesia bigemina*, *B. bovis*, *B. caballi*, *B. divergens*, *B. microti*, *B. ovis*, *B. vogeli*, *B. venatorum*, *Bartonella* spp., *Ba. henselae*, *Ba. quintana*, *Bo. afzelii*, *Bo. burgdorferi* spp., *Bo. burgdorferi* sensu stricto, *Bo. lusitaniae*, *Bo. miyamotoi*, *Bo. spielmanii*, *Coxiella burnetii*, *Hepatozoon* spp., *Ehrlichia* spp., *E. ruminantium*, *Neoehrlichia mikurensis*, *Rickettsia* spp., *R. aeschlimannii*, *R. conorii*, *R. helvetica*, *R. massiliae*, *R. monacensis*, *R. slovacica*, *Theileria annulata* and *T. equi*) or non-TBPs such as Aujeszky's disease virus, *Chlamydia psittaci*, *Haemoproteus* spp., *Leishmania* spp., *L. infantum*, *Leptospira* spp., *Leucocytozoon* spp., *Plasmodium* spp., *Toxoplasma gondii* and *Trypanosoma* spp.

### 3. Methods

#### *Sites and animal sampling*

Corsica is the largest French island in the Mediterranean Sea. It is located 15 km from the closest Italian coast (Sardinia Island) and 160 km to the nearest French coast. It is characterized by mild Mediterranean climate and a wide spectrum of landscapes, including as wetlands (mainly along the coastline), forests and mountains (up to 2,706 m). Corsica is divided into two administrative units (called *départements*: *Haute-Corse* and *Corse du Sud*), with a population 340,000 inhabitants, which increases nearly 10-fold during the tourist season (summer), especially in the coastal wetlands. These wetlands can be classified into five categories: lagoons, rivers and river mouths, artificial lakes, altitude lakes and temporary pools. Eastern Corsica is characterized by a particularly high number of coastal wetlands. In this study, all samples were collected in coastal wetlands.

A total of 1,377 birds were captured during regular banding programs using Japanese nets (2.5 m x 12m) in two main migration hotspot wetlands on the eastern coast of Corsica, namely the Biguglia and Gradugine lagoons, during autumn migrations in 2019 and 2020; and during spring migration in 2021 (Figure 27.). From these birds, 762 blood samples, 37 louse flies and 35 ticks were collected. All blood samples and ectoparasites were collected by a certified bird bander from the CRBPO (*Centre de Recherches sur la Biologie des Populations d'Oiseaux du Muséum National d'Histoire Naturelle*) in order to minimized as possible the animal pain, trapping and handling procedures were approved by the appropriate authorities (authorization no. PP 1062). All blood samples were stored at -80°C and the ectoparasites were stored in 70% ethanol at -20°C within a few hours after sampling.



**Figure 27.** Map of Corsica with the sampled wetland locations (A: Biguglia lagoon; B: Gradugine lagoon).

#### *DNA extraction and PCR pre-amplification*

The ectoparasites were washed in ethanol and distilled water and then morphologically determined to the species level using species identification keys (Estrada-Peña, 2004; Pérez-Eid, 2007) for ticks or sent for determination to the entomologist in charge of the PUIPO project for louse flies. Prior to extraction, ectoparasites were crushed in microtubes filled with six metal beads using a Fisherbrand Bead Mill 24 homogenizer (Thermo Fisher Scientific, USA) at 5,500 rpm for 20 s. DNA from ticks was extracted using a Nucleospin Tissue kit (Macherey-Nagel, Germany). The DNA and RNA in the blood samples were extracted using a FastPrep Ribolyzer (Thermo Fisher Scientific, USA) in BSL3 facilities. All ticks were pooled by species, stage and sex collected from the same bird (minimum number of ticks = 1, maximum number of ticks = 11). The louse flies were crushed and analyzed individually.

In order to improve the detection of DNA pathogens, total DNA was preamplified by using the PreAmp Master Mix according to the manufacturer's instructions (Fluidigm, California, USA). Prior to the experiments, primers targeting all pathogens were pooled combining equal volumes of primers (200 nM final each). The experiment was performed with 1 µl PreAmp Master Mix, 1.25 µl pooled primers mix, 1.5 µl distilled water and 1.25 µl DNA for a 5 µl of final volume. The preamplification cycling was as follows: one cycle at 95°C for 2 min, 14 cycles at 95 °C for 15 s and 60 °C for 4 min. At the end, the samples were diluted 1:10 as performed in Michelet et al., 2014. Preamplified DNA and RNA samples were stored at -20 °C until further use.

#### *Assay design*

Pathogens and their targeted genes were *Anaplasma* spp. (16S), *A. marginale* (Msp1b), *A. phagocytophilum* (Msp2), *Borrelia* spp. (23S), *Bo. burgdorferi* s.s. (rpoB), *Bo. afzelii* (flagellin), *Bo. miyamotoi* (glpQ), *Bo. lusitaniae* (rpoB), *Bo. spielmanii* (flagellin), Chlamydiaceae (23S) (Ehrlich et al., 2006), *Rickettsia* spp. (gltA), *R. slovaca* (23S-5S ITS), *R. helvetica* (23S-5S ITS), *R. aeschlimanii* (23S-5S ITS), *R. massiliae* (23S-5S ITS), *R. conorii* (23S-5S ITS), *C. burnetii* (idc, IS1111), *N. mikurensis* (ARN16S), *Bartonella* spp. (ssrA), *Ba. henselae* (pap31), *Ba. quintana* (bqtR), *Ehrlichia* spp. (16S), *E. ruminantium* (dsb), *Hepatozoon*

spp. (18S), *To. gondii*, *B. microti* (CCTeta), *B. bigemina* (ARN 18S), *B. vogeli* (hsp70), *B. caballi* (rap1), *B. bovis* (CCTeta), *B. ovis* (ARN18S), *B. divergens* (hsp70), *T. equi* (Ema1), *T. annulata* (ARN 18S), *Leishmania* spp. (hsp70), *L. infantum* (ITS) and *African swine fever virus* (Vp72). The primers and probes selected for these pathogens were designed by Gondard et al. (2018), Grech-Angelini et al. (2020) and Michelet et al. (2014) excepted for Chlamydiaceae. The primer/probe sets created in this study are listed in the Table 15.

**Table 15.** List of pathogens, targets and primer/probe sets created for microfluidic real-time PCR screening.

Pathogens	Name	Sequence	Length (nucleotide)	Gene	Gene references
Aujeszký's disease virus	Aujv_gp50_F	CTTTATCGAGTACGCCGACTG	225	gp50	Y14834.1
	Av_gp50_R	AACGGGCACTCTTGCCCC			
	Av_gp50_P	CAGATCTTTGGGCGCTGCCGGC			
<i>Haemoproteus</i> spp.	Hae_cytB_F	ATATGCATGCTACTGGTGCTAC	240	cytochrome B	AF465579.1
	Hae_cytB_R	CAAATCCATGAAACAAGTCCAGG			
	Hae_cytB_P	CGGTTGCACCCAGAAACTCATTGAC			
<i>Leptospira</i> spp.	Lep_Lipl32_F	CTCTATGTTTGGATTCTGACC	158	LipL32	MK541891.1
	Lep_Lipl32_R	CCAAGTATCAAACCAATGTGGC			
	Lep_Lipl32_P	ATTGATTTTTCTTCTGGGGTAGCCGCTTTG			
<i>Leucocytozoon</i> spp.	Leu_cytB_F	GGGTTATGTCTTACCATGGGG	177	cytochrome B	KF717066.1
	Leu_cytB_R	AATTGCTAGTGCTACGAATGGG			
	Leu_cytB_P	AAATGAGTTTTTGGGGAGCAACCGTTATTAC			
<i>Plasmodium</i> spp.	Pla_ssrRNA_F	ATATAGAAACTGCGAACGGCTC	339	ssrRNA	MK650620.1
	Pla_ssrRNA_R	TTTCTCAGGCTCCCTCTCC			
	Pla_ssrRNA_P	CTCTAATTCCCCGTTACCCGTCATAGC			
<i>Rickettsia monacensis</i>	Ric_mon_F	CTCGGTGCCGGTACTTTAAAC	192	ompB	KU961543.1
	Ric_mon_R	GAGCACCGCCAATAAGAGC			
	Ric_mon_P	AGTGCCGATGCAAATACTCCGGTGAC			
<i>Trypanosoma</i> spp.	Try_18SRNA_F	GTAATTCCAGCTCCAAAAGCG	178	18SRNA	EU596263.1
	Try_18SRNA_R	TCAGGAAGGAACCACTCCC			
	Try_18SRNA_P	ACCTCAAGGGCATGGGTCACCAATCC			

#### DNA amplification, microfluidic real-time PCR and RT-PCR

The detection of the targets vector-borne pathogens was performed on preamplified DNA using the BioMark™ real-time PCR system (Fluidigm, California, USA) for high-throughput microfluidic real-time PCR amplification using 48.48 dynamic arrays (Fluidigm). Fluidigm chips can handle 2,304 real-time PCR reactions, i.e. from 48 PCR mixes for 48 samples placed into individual wells prior to transfer into individual chambers for the reaction. The thermal cycling conditions were 50°C for 2 min, 95°C for 10 min and 40 cycles at 95°C for 15 s and 60°C for 1 min. One negative water control, one inhibitory molecule control (*E. coli* EDL933 strain) and one DNA extraction control (bird-specific primers) were added to each chip. Specific primers and probe of *E. coli* were used. All RNA extracts underwent real-time RT-PCR following the protocol described previously (Linke et al., 2007) for the detection of WNV and USUV.

*Confirmation of detected pathogens*

Pathogen detection was confirmed using nested PCR or real-time PCR targeting a gene other than that used for the microfluidic experiment. Primer/probe sets were selected from the literature when available or newly designed in this study. The positive sequences after gel migration from the nested PCR product were sent for sequencing at Eurofins MWG Operon (Cologne, Germany) and assembled using BioEdit software (Ibis Biosciences, Carlsbad). An online BLAST (National Center for Biotechnology Information) was used to identify the sequenced organisms. All the target genes and primer sequences from the literature are described in Ehricht et al., (2006) Gondard et al., (2018), Michelet et al., (2014). The primer/probe sets created for this publication are listed in the Table 16.

**Table 16.** List of pathogens, targets and primer/probe sets used for confirmation using real-time PCR.

Pathogens	Name	Sequence	Length (nucleotide)	Genes	Genes references
Aujeszky's disease virus	Aujv_gp50_F2	AACATCCTCACCGACTTCATG	158	gp50	Y14834.1
	Av_gp50_R2	CTGGTAGAACGGCGTCAGG			
	Av_gp50_P2	AATCGCATCACGTCCACGCCCC			
<i>Haemoproteus</i> spp.	Hae_cytB_F2	CCTTGGGGTCAAATGAGTTTC	231	cytochrome B	AF465579.1
	Hae_cytB_R2	AAGCCGTATCATATCCTAAAGG			
	Hae_cytB_P2	CCTGGACTTGTTTCATGGATTTGTGGAGG			
<i>Leptospira</i> spp.	Lep_Lipl32_F2	CTCTATGTTTGGATTCCTGCC	158	LipL32	MK541891.1
	Lep_Lipl32_R2	CCAAGTATCAAACCAATGTGGC			
	Lep_Lipl32_P2	ATTGATTTTTCTCTGGGGTAGCCGCTTTG			
<i>Leucocytozoon</i> spp.	Leu_cytB_F2	GAGTTTCTGGGGAGCTACTG	197	cytochrome B	KU842391.1
	Leu_cytB_R2	GGATTAGTGCTACCTTGAATATG			
	Leu_cytB_P2	TGAATAAATACAATTGCTAGTGCTACGAATGG			
<i>Plasmodium</i> spp.	Pla_ssrRNA_F2	TCGAGTTTCTGACCTATCAGC	264	ssrRNA	MK650620.1
	Pla_ssrRNA_R2	AGACTTGCCCTCCAATTGTTAC			
	Pla_ssrRNA_P2	TGGCCTTGCAATTGTTATTCTTGTCACTACC			
<i>Trypanosoma</i> spp.	Try_18SRNA_F2	CAACACGGGGAACTTACCAG	141	18SRNA	EU596263.1
	Try_18SRNA_R2	ATCCTACTGGGCAGCTTGG			
	Try_18SRNA_P2	CAGGGTGAGGATTGACAGATTGAGTGTTTC			

**4. Results**

In our research the birds captured and sampled had different behavior which had already been defined in previous studies (Table 17) (Thibault and Bonaccorsi, 1999). In purpose of a better legibility of the birds' behavior, we decided in our study to regroup them in three behavior categories: Sedentary, Migratory and Sedentary/Migratory. They respectively regroup former breeder, introduced breed and resident breeder for sedentary birds; accidental visitor, breeding visitor, occasional breeder, passage migrant and winter visitor for migratory birds; birds which have behaviors from these two groups will be defined as Sedentary/Migratory birds. However, for the sake of accuracy, the terms define by Thibault and Bonaccorsi will be kept in the Table 17.

*Ectoparasite carriers of pathogens in wild birds*

From the ectoparasites sampled in wild birds, 37 louse flies were collected on 35 birds representing 2.5% of the bird capture with infestation intensity of 1.06 (ticks per infested birds) (Table 18). All were collected on the migratory bird species *Hirundo rustica* at a rate of about 10.6% of the captured individuals. All louse flies collected were adult *Ornithomya biloba* (Table 17 and 18) and 94.3% of them were collected near the Gradugine Lagoon and 5.7% in the Biguglia Lagoon. Of these, 67.6% were female and 32.4% were male; only one was positive for *Trypanosoma* spp., for which the sequencing was unfortunately not successful. All other louse flies were negative for all the other targeted pathogens (Table 17 and 18).



The other ectoparasites sampled on wild birds were 44 ticks, sampled from 24 birds (1.7% of the birds captured). They were collected on bird species with various behavior (Table 3): 36.3% from *Erithacus rubecula* (Sedentary/Migratory), 22.7% from *Saxicola rubicola* (Sedentary/Migratory), 18.2% from *Cettia cetti* (Sedentary), 6.9% from *Turdus merula* (Sedentary/Migratory), 4.5% from *Acrocephalus arundinaceus* (Migratory), 4.5% from *Prunella modularis* (Migratory), 2.3% for *Luscinia megarhynchos* (Migratory), 2.3% from *Parus major* (Sedentary) and 2.3% from *Sylvia atricapilla* (Sedentary/Migratory) (Table 17 and 18). All collected ticks were from the *Ixodes* genus: *Ixodes* spp. (8.5%), *I. accuminatus/ventaloi* (2.9%), *I. arboricola/lividus* (14.3%), *I. frontalis* (5.7%) and *I. ricinus* (68.6%), with 97.1% of the ticks sampled in the Biguglia Lagoon wetlands and 2.9% in the Gradugine Lagoon. A total of 27 different pools were constituted. Among the pools, one pool of nymphs collected on *S. atricapilla* in the Biguglia Lagoon was positive for *A. phagocytophilum*. Another one pool of larvae collected on *E. rubecula* from the Biguglia Lagoon was positive for *Ehrlichia* spp. which appears to be closely related to *E. chaffeensis* sample from goat's blood in China (GenBank: KX505292.1). Three other pools (one of larvae, one of nymphs and one of adults) were positive for *R. helvetica* collected from one *C. cetti* and two *E. rubecula* in the Biguglia Lagoon. One pool of larvae from *L. megarhynchos*, one pool of 10 engorged nymphs from *Sax. rubicola* and another one pool of engorged nymphs from *T. merula* were positive for *Rickettsia* spp. (Table 17). Unfortunately, the sequencing of *A. phagocytophilum*, *R. helvetica* and *Rickettsia* spp. was not successful.

#### *Pathogens detected in blood samples*

From bird blood samples, two pathogens were detected. WNV was found in two adult *Parus major* (Sedentary/Migratory) from the Gradugine Lagoon during autumn migration in 2019 (Table 17). Unfortunately, sequencing was unsuccessful. Regarding TBPs, one *Erithacus rubecula* (Sedentary/Migratory) from the Biguglia Lagoon was positive for *Ehrlichia chaffeensis* which appears to be closely related to a sample from goat's blood in China (GenBank: KX505292.1). The same passerine individual harbored tick larvae positive for *E. chaffeensis* (see above, Table 18).

**Table 17.** Samples and positive samples collected on birds in two Corsican lagoons.

Bird species	Behavior <sup>†</sup>	Lagoon	Blood samples	<i>Ixodes</i> spp. pools/positive pools			<i>I. accumanitus/ventalloi</i> pools/positive pools			<i>I. arboricola/lividus</i> pools/positive pools			<i>I. frontalis</i> pools/positive pools			<i>I. ricinus</i> pools/positive pools			<i>O. biloba</i> individuals/positive individuals
				Adults	Nymphs	Larvae	Adults	Nymphs	Larvae	Adults	Nymphs	Larvae	Adults	Nymphs	Larvae	Adults	Nymphs	Larvae	
<b>Sedentary</b>																			
<i>Accipiter nisus</i>	RB	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aegithalos caudatus</i>	RB	Biguglia, Gradugine	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chloris chloris</i>	RB	Gradugine	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Curruca melanocephala</i>	RB	Biguglia, Gradugine	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cyanistes caeruleus</i>	RB	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Garrulus glandarius</i>	RB	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Parus major</i>	RB	Biguglia, Gradugine	80/2 <sup>WNV</sup> ‡	-	-	-	-	-	-	1/0	-	-	-	-	-	-	-	-	-
<i>Sturnus unicolor</i>	RB	Biguglia	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Migratory</b>																			
<i>Acrocephalus arundinaceus</i>	MB PM	Biguglia, Gradugine	18	-	-	-	-	-	-	-	-	1/0	-	-	-	-	1/0	-	-
<i>Acrocephalus schoenobaenus</i>	PM	Biguglia, Gradugine	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acrocephalus scirpaceus</i>	MB PM	Biguglia, Gradugine	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Anthus spinoletta</i>	MB PM WV	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Anthus trivialis</i>	PM	Gradugine	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Curruca cantillans</i>	MB PM	Biguglia, Gradugine	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Curruca communis</i>	PM OB	Biguglia, Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Emberiza schoeniclus</i>	PM WV	Biguglia, Gradugine	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<i>Hippolais icterina</i>	PM	Biguglia,	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Gradugine																	
<i>Hirundo rustica</i>	PM MB	Biguglia,	177	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37/1 <sup>T‡</sup>
		Gradugine																	
<i>Hirundo rustica</i> X	hybrid	Biguglia	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Delichon urbicum</i>																			
<i>Luscinia megarhynchos</i>	MB PM	Biguglia,	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/1 <sup>R‡</sup>	-
		Gradugine																	
<i>Luscinia svecica</i>	PM WV	Biguglia	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Motacilla flava</i>	PM OB	Biguglia,	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Gradugine																	
<i>Phoenicurus phoenicurus</i>	PM	Biguglia,	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Gradugine																	
<i>Phylloscopus collybita</i>	PM WV OB	Biguglia,	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Gradugine																	
<i>Phylloscopus sibilatrix</i>	PM	Biguglia	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Phylloscopus trochilus</i>	PM	Biguglia,	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Gradugine																	
<i>Prunella modularis</i>	WV PM	Biguglia,	1	-	-	-	-	-	-	-	-	-	-	1/0	-	-	1/0	-	-
		Gradugine																	
<i>Remiz pendulinus</i>	PM WV	Gradugine	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Riparia riparia</i>	PM	Gradugine	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Saxicola rubetra</i>	PM OB	Biguglia	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Sylvia borin</i>	PM	Biguglia,	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Gradugine																	
<b>Sedentary/Migratory</b>																			
<i>Alcedo atthis</i>	PM WV RB	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Buteo buteo</i>	RB PM	Gradugine	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Carduelis carduelis</i>	RB PM	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cettia cetti</i>	RB PM WV	Biguglia,	95	-	-	-	1/1 <sup>Rh‡</sup>	-	-	-	-	-	-	-	-	-	3/0	-	-
		Gradugine																	
<i>Circus aeruginosus</i>	PM RB WV	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cisticola juncidis</i>	RB PM	Gradugine	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<i>Erithacus rubecula</i>	RB PM WV	Biguglia, Gradugine	68/1 <sup>Ec</sup>	-	1/0	1/0	-	-	-	-	-	1/0	-	-	1/1 <sup>Rh</sup> ‡	1/0	4/1 <sup>Rh</sup> ‡	3/1 <sup>Ec</sup> ‡	-
<i>Falco tinnunculus</i>	RB PM WV	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Fringilla coelebs</i>	RB PM WV	Biguglia, Gradugine	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Jynx torquilla</i>	RB PM	Biguglia	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Otus scops</i>	RB PM	Biguglia, Gradugine	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Passer italiae</i>	RB PM	Gradugine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Saxicola rubicola</i>	PM WV RB	Gradugine	1	-	-	-	-	-	-	1/1 <sup>R</sup> ‡	-	-	-	-	-	-	-	-	-
<i>Sylvia atricapilla</i>	RB PM WV	Biguglia, Gradugine	148	-	-	-	-	-	-	-	-	-	-	-	-	-	1/1 <sup>Ap</sup> ‡	-	-
<i>Turdus merula</i>	RB PM WV	Biguglia, Gradugine	17	-	-	1/0	-	-	-	-	-	-	-	-	-	-	2/1 <sup>R</sup> ‡	-	-
<b>Total</b>			<b>762/2<sup>WNV</sup></b> <b>‡, 1<sup>Ec</sup>‡</b>	<b>-</b>	<b>1/0</b>	<b>2/0</b>	<b>1/1<sup>Rh</sup>‡</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>2/1<sup>R</sup>‡</b>	<b>2/0</b>	<b>-</b>	<b>1/0</b>	<b>1/0</b>	<b>1/0</b>	<b>11/1<sup>Ap</sup>‡</b>	<b>4/ 1<sup>Ec</sup>‡</b>	<b>37/1<sup>T</sup>‡</b>
																	<b>1<sup>R</sup>‡</b>	<b>1<sup>Rh</sup>‡</b>	<b>1<sup>R</sup>‡</b>

‡Av: Accidental visitor, FB: Formal breeder, IB: introduce breeder, MB: Breeding visitor, OB: Occasional breeder, PM: Passage migrant, RB: Resident breeder, WV: Winter visitor

‡Ap: *Anaplasma phagocytophilum*, Ec: *Ehrlichia chaffeensis*, R: *Rickettsia* spp., Rh: *Rickettsia helvetica*, T: *Trypanosoma* spp. and WNV: West Nile virus

**Table 18.** Ectoparasites (ticks and louse flies) infesting passerine birds.

Bird species	Tick species															Louse fly species	Number of ectoparasites (Number of infested birds)	Mean infestation intensity
	<i>Ixodes</i> spp.			<i>Ixodes accuminatus/ventalloi</i>			<i>Ixodes arboricola/lividus</i>			<i>Ixodes frontalis</i>			<i>Ixodes ricinus</i>					
	Adults	Nymphs	Larvae	Adults	Nymphs	Larvae	Adults	Nymphs	Larvae	Adults	Nymphs	Larvae	Adults	Nymphs	Larvae	Adults		
<i>Acrocephalus arundinaceus</i>	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	2 (2)	1.0
<i>Cettia cetti</i>	-	-	-	1	-	-	-	-	-	-	-	-	-	7	-	-	8(3)	2.66
<i>Erithacus rubecula</i>	-	1	1	-	-	-	-	-	2	-	-	1	1	7	3	-	16(11)	1.45
<i>Hirundo rustica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	37(35)	1.06
<i>Luscinia megarhynchos</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1 (1)	1.0
<i>Parus major</i>	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1 (1)	1.0
<i>Prunella modularis</i>	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	2 (2)	1.0
<i>Saxicola rubicola</i>	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	10 (1)	10
<i>Sylvia atricapilla</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1 (1)	1
<i>Turdus merula</i>	-	-	1	-	-	-	-	-	-	-	-	-	-	2	-	-	3 (2)	1.5

## 5. Discussion

### *Bird ectoparasites and their pathogens*

#### *Louse flies*

The Hippoboscidae family belongs to the superfamily Hippoboscoidea, which groups numerous bloodsucking fly families. This family has a worldwide distribution and can be found in both mammals and birds (Rahola et al., 2011). In Europe, different genera have been detected, such as *Liptotena* on cervids, *Melophagus* on domestic ruminants and *Ornithomya* on birds, mainly on passerines (Oboňa et al., 2019). Two *Ornithomya* species (*O. avicularia* and *O. chloropus*) have a wide distribution and, for example, have been reported in bird nests in Norway (Eeva et al., 2015). Both species have also been found in the Czech Republic along with a third species *O. biloba*, on birds (Sychra et al., 2008). Although this genus is mainly a nest parasite, it is also observed during summer and autumn migration (Eeva et al., 2015; Sychra et al., 2008). In our study, we only found one species: *O. biloba* collected on *Hirundo rustica* in autumn 2020. This species is mainly present on Hirundinidae passerines, such as *H. rustica* (Maa, 1969; Oboňa et al., 2019). This species has already been reported in France during a survey of Hippoboscidae carried out by the PUPIPO project on different hosts; it was the second-most frequent louse fly species and was found nearly exclusively on *H. rustica*. However, this survey miss the information on the situation on bird population in Corsica, only louse flies from *Nycteribiidae* bats were collected in the PUPIPO project. This study is thus the first report of bird louse flies in Corsica.

The Hippoboscidae family may be responsible for the circulation of various pathogens; however, to date, there is little information on them (Bezerra-Santos and Otranto, 2020). Nevertheless, one study has suggested that cervid louse flies *Liptotena cervi* may be involved in the mechanical transmission of *A. phagocytophilum* (Buss et al., 2016). Similarly, in birds, the genus *Ornithomya* is involved in the transmission of *Haemoproteus* and *Trypanosoma* parasites to their avian host (Karadjian et al., 2013; Mungomba et al., 1989). In our study, we detected *Trypanosoma* spp. DNA in 2.7% of the louse flies collected, which is the first record of carriage for Corsica. Although the species could not be identified, our study demonstrates the occurrence of infected louse flies in Corsica — which are either vectored by migratory birds or migratory birds are infested by infected Corsican louse flies. However, the absence of louse flies on sedentary birds cannot exclude the possibility that louse flies are transmitted between migratory birds and sedentary birds. Further research is needed on louse flies to better determine their role in the circulation of pathogens and their impact on bird and human health in Corsica.

#### *Ticks*

Hard ticks (Ixodidae) are vectors of several pathogens, such as *Rickettsia* spp., *Borrelia* spp. and Crimean-Congo Hemorrhagic Fever virus (CCHFV) (Estrada-Peña et al., 2012a; Rataud et al., 2021; Wallménius et al., 2014). In Europe and the Mediterranean, most ticks found on avian species are immature stages from the genus *Hyalomma*, *Ixodes* ticks of all stages and, sporadically, immature stages from the genera *Haemaphysalis* and *Rhipicephalus* (Capligina et al., 2014; Wallménius et al., 2014). However, to date, no information is available on the ticks infesting avian species in Corsica. In our study, we found ticks exclusively from the *Ixodes* genus: *Ixodes* spp., *I. accuminatus/ventalloi*, *I. arboricola/lividus*, *I. frontalis* and *I. ricinus*. These ticks are frequently found on passerine species among other tick genera, such as *Hyalomma* (Baráková et al., 2018; Battisti et al., 2020; Capligina et al., 2014). All ticks in our study were sampled on sedentary birds. The *Erithacus rubecula* represented the highest proportion of infested birds (36.3%), on which a total of three different *Ixodes* species were found: *I. arboricola/lividus*, *I. frontalis* and *I. ricinus*. This latter species was the most frequently detected tick species, accounting for 68.8% of the ticks population on *E. rubecula* and 54.5% of the total of ticks collected on other wild birds. Whether migratory or sedentary (as shown in the Corsican wetlands in this study), *E. rubecula* appears to be an important host for immature tick stages, particularly for *Ixodes* ticks. In various countries, *E. rubecula* is the

main *Ixodes* tick host; for example, Denmark shows an even higher infestation intensity (between 1.93 and 3 ticks per bird) than in our study (1.45 ticks per bird) (Klitgaard et al., 2019). Similarly, other countries show heavy bird tick infestations, such as Latvia, with a mean *Ixodes* tick infestation intensity of 2.4 ticks per bird, or Italy, with a prevalence of 4.5% on *E. rubecula* (Battisti et al., 2020; Capligina et al., 2014). However, in these cited countries, only migratory *E. rubecula* individual tick-infested were considered migratory, in contrast to Corsica, where *E. rubecula* was considered both sedentary and migratory birds (Klitgaard et al., 2019). This bird species is also the main host of the immature stages of *I. frontalis* ticks in Turkey (Keskin and Erciyas-Yavuz, 2016). However, *E. rubecula* is not the only passerine infested by *Ixodes* ticks, which target a wide spectrum of passerines, both sedentary and migratory (Estrada-Peña et al., 2005; Klitgaard et al., 2019; Palomar et al., 2017a; Wallménus et al., 2014). On the other hand, the bird species with the highest infestation rate was *Cettia cetti* (2.66 ticks per bird), which is a ground-feeding sedentary bird in Corsica. This high infestation intensity may be linked with the ground-feeding behavior of *C. cetti* which makes it a more susceptible target of ticks (Hasle 2013). Our results demonstrate the potential importance of the sedentary birds in the stabilization of the *Ixodes* tick population in Corsican wetlands, but also it suggests possible tick circulation between the sedentary and migratory birds of the same species that co-occur in Corsican wetlands (Klitgaard et al., 2019; Ogden et al., 2008). Determining the mechanism of tick circulation requires a comparison of tick infestation during migration and non-migration.

A total of three pathogen genera were found in bird ticks: *Anaplasma* spp., *Ehrlichia* spp. and *Rickettsia* spp. One *Anaplasma* species was found: *A. phagocytophilum* in an *I. ricinus* nymph pool collected from *Sylvia atricapilla*. *A. phagocytophilum* is a worldwide intracellular bacterium mainly transmitted by *Ixodes* ticks, leading to tick-borne fever in a wide range of hosts and to human granulocytic anaplasmosis in humans (Stuenkel et al., 2013). In birds, this pathogen has already been reported in *I. frontalis* and *I. ricinus* ticks sampled from four bird species captured in our study: *E. rubecula*, *Luscinia megarhynchos*, *S. atricapilla* and *Turdus merula* (Palomar et al. 2015). It has also been detected in the spleen from *T. merula* from the Netherlands; furthermore, the presence of *A. phagocytophilum* in North America is often documented in birds and their ticks (Jahfari et al., 2014; Johnston et al., 2013; Ogden et al., 2008). More recently, a different variant was reported from a Greek island in an *Ixodes* tick infesting a woodchat shrike (*Lanius senator senator*) (Hoffman et al., 2020). In Corsica, *A. phagocytophilum* has been mainly reported in *I. ricinus*, but it has also been detected in the tick species *Dermacentor marginatus*, *Haemaphysalis punctata*, *Hyalomma marginatum*, *Hy. scupense*, *Rhipicephalus bursa* and *Rh. sanguineus* s.l. collected on domestic and wild mammals (Cicculli et al., 2020; Grech-Angelini et al., 2020). To our knowledge, this is the first detection of *A. phagocytophilum* in ticks collected from sedentary birds in Corsica. However, due to the low prevalence of *A. phagocytophilum* plus the possibility of trans ovarian transmission in ticks, it is not possible to determine precisely whether sedentary birds play a reservoir or dispersal role with regard to this pathogen in Corsica (Hauck et al., 2020). Nevertheless, our study clearly indicates the potential presence of this pathogen in tick-infested sedentary birds in Corsica, although confirmation of this presence requires isolating the pathogen directly from birds.

The *Ehrlichia* genus is closely related to *Anaplasma*; we detected one species: *E. chaffeensis*. It was detected from a pool of *I. ricinus* larvae collected on *E. rubecula*. *Ehrlichia chaffeensis* is a tick-borne intracellular bacterium causing human monocytotropic ehrlichiosis, but is usually found in *Amblyomma americanum* ticks and mainly present in the United States (Madison-Antenucci et al., 2020; Paddock and Childs, 2003). Although the *Ehrlichia* genus has already been observed in bird ticks (Špitalská et al., 2006; Toma et al., 2014), this is the first detection of *E. chaffeensis* in bird ticks in the Mediterranean Rim and in Corsica where it has not previously been reported in any animal population. The predominance of *Ixodes* ticks as hosts of *E. chaffeensis* in this study supports the previous suggestion that birds are potential reservoir hosts for this bacterial species. However, the isolation of the pathogens and serological

analysis of bird sera are required for confirmation. Further analyses on *E. chaffeensis* are still needed in bird tick population to determine the actual status of this pathogen in the Corsican wetlands.

Six tick pools were found positive for *Rickettsia* spp. Of these six pools, three were positive for *R. helvetica*. Each of the three pools were from different *Ixodes* species. *Rickettsia helvetica* is a tick-borne rickettsia belonging to the spotted fever group (SFG) associated with symptoms such as fever, myalgia and meningitis. It has a nearly worldwide distribution and is mainly transmitted by *Ixodes* ticks (Påhlson et al., 2021; Parola et al., 2013). In birds, it has been detected in 70% of the immature ticks from *I. ricinus* collected on passerines in Poland, 21.6% of *I. frontalis* and *I. ricinus* ticks from birds in the Netherlands and in 5.6% of *I. ricinus* ticks collected on migratory birds in Sweden (Biernat et al., 2016; Elfving et al., 2010; Heylen et al., 2017). In Hungary, research suggests that birds are a potential reservoir for *R. helvetica*, with the detection of this bacterial species in 51.4% of the *I. ricinus* and *Haemaphysalis* ticks collected on passerines and also in 4.7% of the birds (Hornok et al., 2014). In the Mediterranean area, it has been found in Spain with a prevalence of 6% of *I. ricinus* ticks (Baráková et al., 2018). In our research, we detected the presence of *R. helvetica* for the first time with a prevalence of 11.1% in tick-infested birds. However, in Corsica, *R. helvetica* has already been reported in *I. ricinus* ticks collected on cattle (Grech-Angelini et al., 2020).

#### *Pathogens found in birds' blood*

Another objective of the research was to screen for pathogens in bird blood samples. We found two pathogens: one virus, WNV, and one bacterium *E. chaffeensis*.

In birds, an Anaplasmacetae closely related to *E. chaffeensis* has been reported in tissue and blood samples from a song thrush (*Turdus philomelo*) in Hungary (Hornok et al., 2020a). It was also found in the liver of a common pheasant (*Phasianus colchicus*) in China (Yang et al., 2015). In Corsica, *E. chaffeensis* has not previously been detected in animal samples. Furthermore, the *E. rubecula* individual found positive was also infested with infected *I. ricinus* larvae (*E. chaffeensis*-positive larvae). Therefore, *E. chaffeensis* may circulate in sedentary and migratory birds in the Biguglia Lagoon in Corsica. This is the first Corsican record of this bacterium in birds and their ticks. Further analyses are still needed to confirm if this is just a sporadic infection or if the infection of more ticks collected on sedentary birds suggests a more frequent presence of this pathogen in sedentary bird population in Corsican wetlands. Furthermore, confirmation of the presence of this pathogen requires the isolation of the pathogen itself.

The second pathogen identified here was the West Nile Virus found in two *Parus major* individuals in the Gradugine Lagoon. This virus is maintaining in a bird-mosquito-bird transmission cycle. It can also infect mammals as humans and equines and induce diseases ranging from mild illness to severe neurological disorder. (Rossi, Ross, and Evans 2010). In the Mediterranean area, equine and humans outbreaks are reported every years across the Mediterranean Rim, including in Algeria, France, Greece, Italy, Israel, Spain and Tunisia (Bakonyi & Haussig 2020; Zeller et Schuffenecker 2004). On Mediterranean islands, it has already been found in Cyprus with a prevalence of 1.3% in birds, 4.5% in human blood donors (Pallari et al., 2021), a human case in Sardinia (Magurano et al., 2012), 1.7% in chicken blood in Crete (Fotakis et al., 2022) and, in Corsica, with a prevalence of 9.4% in horses and 8.4% in dogs (Maquart et al., 2017). In our research, we found WNV RNA in two sedentary *P. major* adults with a prevalence of 0.3% in the wild bird blood samples. This is the first record of WNV RNA in the Corsican bird population. Our results, complements previous record of WNV-positives horses and dogs, appear to confirm the presence of the virus in Corsica (Beck et al., 2020). Further analyses of WNV are required to determine its actual impact on the bird and mosquito populations involved in its transmission in Corsica. For both bird pathogens, comparative analysis between the migratory and sedentary birds can help to determine the possible circulation of those pathogens between sedentary and migratory birds.



In conclusion, our results show the importance of the research about pathogens from birds and their ectoparasites. It is allowed the monitoring of known pathogens as the West Nile virus in Corsica, and also in the monitoring and discovery of new pathogens in the Corsican islands as *Erhlichia chaffeensis* and *Rickettsia helvetica*.

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**Discussion**

L'objectif de la thèse visait à identifier les ectoparasites et les agents pathogènes au sein de trois groupes d'animaux et de leurs ectoparasites, échantillonnés au niveau de différentes zones humides de Corse. Ce travail était divisé en plusieurs parties :

- Un premier bilan sur les agents pathogènes détectés dans les animaux domestiques et sauvages, ainsi que leurs tiques, dans le bassin méditerranéen entre 2000 et début 2021 à travers deux revues bibliographiques.
- L'identification des tiques présentes dans des populations de sangliers vivants à proximité de zones humides ainsi que la détection d'agents pathogènes au sein de ces populations et leurs tiques.  
La détermination des espèces de tiques collectées dans des élevages bovins et ovins à proximité de zones humides ainsi que la détection d'un panel d'agents pathogènes au sein de ces élevages et leurs tiques.  
L'identification des ectoparasites collectés sur l'avifaune sédentaire et migratrice à proximité de deux zones humides corses ainsi que la détection d'agents pathogènes au sein de ces populations et de leurs ectoparasites.

Dans ce chapitre, nous orienterons la discussion de nos résultats autour des ectoparasites et des agents pathogènes identifiés au sein des trois groupes d'animaux étudiés.

### 1. Ectoparasites récoltés au sein des différents groupes d'animaux

#### 1.1. Les tiques

Lors de nos campagnes de prélèvement, 601 tiques ont été collectées sur sangliers, animaux d'élevage et avifaune. Ces différentes tiques appartiennent à quatre genres différents : *Dermacentor* spp. (*D. marginatus*), *Hyalomma* spp. (*Hy. marginatum* et *Hy. scupense*), *Ixodes* spp. (*I. acuminatus/ventaloi*, *I. arboricola/lividus*, *I. frontalis* et *I. ricinus*) et *Rhipicephalus* spp. (*Rh (Bo). annulatus*, *Rh. bursa* et *Rh sanguineus* s.l.) (Figure 28). Suivant les groupes d'animaux, les espèces de tiques collectées varient, ce qui peut être dû à la fois à la saisonnalité mais également au tropisme des tiques.

##### Le genre *Dermacentor*

C'est le genre de tique principalement retrouvé sur les sangliers dans notre étude. Ce genre est connu pour cibler les ongulés sauvages et domestiques lors des stades adultes et les micromammifères lors des stades immatures (Pérez-Eid, 2007). Dans le bassin méditerranéen, deux espèces sont prédominantes : *D. reticulatus* et *D. marginatus* (Estrada-Peña, 2004). Cette deuxième espèce est l'unique que nous ayons retrouvé, comme ça a été le cas dans d'autres études en Corse (V. Cicculi et al., 2019b; Grech-Angelini et al., 2016). Cette espèce est vectrice de nombreuses rickettsies notamment *R. slovaca* en partie responsable de la fièvre boutonneuse Méditerranéenne (V. Cicculi et al., 2019b; Zeroual et al., 2018). Elle présente un fort tropisme pour les sangliers et représente 96,4 % des tiques collectées lors de notre étude (Figure 28). Les fortes abondances de cette espèce sont également liées à la période d'échantillonnage hivernale qui correspond à la principale période de présence des stades adultes sur leurs hôtes (Estrada-Peña, 2004). Cette espèce a également été retrouvée au sein des animaux d'élevage dans la commune de Figari où elle représente 0,7 % du total des tiques collectées sur bovins. Ce faible

pourcentage peut être ici expliqué par la prédominance d'autres espèces de tiques chez les populations de bovins tels que les genres *Hyalomma* et *Rhipicephalus*. Cette prédominance de ces deux genres pourrait être dû à leur tropisme plus prononcé pour les bovins que le genre *Dermacentor*.

#### Le genre *Ixodes*

Le genre *Ixodes* est un genre ubiquiste ciblant une large variété du vivant avec un tropisme pour les grands mammifères lors du stade adulte et les micro mammifères et l'avifaune lors des stades immatures (Estrada-Peña, 2004). Il est principalement représenté par l'espèce *I. ricinus* vectrice de nombreux agents pathogènes tels que les agents causatifs de la maladie de Lyme et de piroplasmose (Estrada-Peña, 2004). Malgré son tropisme ubiquiste, ce genre a uniquement été collecté dans notre étude sur l'avifaune aux stades adulte et immature mais présente une importante diversité spécifique : *Ixodes* spp. (8,5 %), *I. accuminatus/ventalloi* (2,9 %), *I. arboricola/lividus* (14,3 %), *I. frontalis* (5,7 %) et *I. ricinus* (68,6 %) (Figure 28). Dans le cas de *I. accuminatus/ventalloi* et *I. arboricola/lividus*, la détermination n'a pu être affinée au niveau spécifique. Cette forte présence du genre *Ixodes* chez l'avifaune peut être expliquée par le tropisme des stades immatures du genre pour ce groupe animal, comme cela a déjà été observé dans le pourtour méditerranéen. Ce genre a été détecté dans plusieurs pays tels que la France, l'Italie, l'Espagne et la Grèce (Battisti et al., 2020; Falchi et al., 2012; Hoffman et al., 2020; Palomar et al., 2015, 2012; Pascucci et al., 2019; Rataud et al., 2022; Wallménus et al., 2014). Au sein de ces pays une large variété d'oiseaux hôtes ont été retrouvés porteurs de ce genre comme en France avec 36 espèces hôtes, sept en Espagne, cinq en Italie et deux en Grèce. Cela montre l'importance de ce genre de tique comme ectoparasite de l'avifaune en Méditerranée et témoigne de l'intérêt de poursuivre la recherche de ce taxon dans l'avifaune insulaire. Cela est d'autant plus important que les espèces d'*Ixodes* retrouvées dans notre étude ont également été identifiées chez l'avifaune de ces quatre pays comme positives à des agents pathogènes pas encore détectés en Corse (e.g. *Borrelia valaisiana* en Espagne)(Palomar et al., 2012). Actuellement ce genre *Ixodes* a été retrouvé uniquement au stade adulte sur des bovins en Corse à des altitudes supérieures à 600m (Grech-Angelini et al., 2016) et à des saisons différentes de nos périodes d'échantillonnages. Cela pourrait expliquer pourquoi nous n'avons pas retrouvé d'*Ixodes* adultes sur les bovins pris en compte dans notre étude.

#### Le genre *Hyalomma*

Ce genre cible principalement les ongulés domestiques et sauvages lors de leurs stades adultes. Comme préalablement observé en Corse (Grech-Angelini et al., 2016), deux espèces de *Hyalomma* ont été collectées : *Hy. marginatum*, *Hy. Scupense* représentant respectivement 1,8 % et 0,9 % des tiques collectées sur sangliers ; et 8,5 % et 7,2 % des tiques collectées sur animaux d'élevage (Figure 28). Parmi ces deux espèces, la plus fréquente est *Hy. marginatum*, retrouvées dans 11 communes sur 20 échantillonnées, et connue pour être vectrice du Virus hémorragique Crimée-Congo (CCHF) ainsi que de nombreuses rickettsies. *Hy. Scupense*, qui a un tropisme similaire à *Hy. Marginatum*, a été retrouvée dans huit des 20 communes échantillonnées. Chez les bovins ce genre est le deuxième genre le plus retrouvé. Cela pourrait être dû au tropisme principal du stade adulte pour les grands ongulés (Estrada-Peña, 2004). Pour autant de faibles proportions de tiques du genre *Hyalomma* ont été collectés, ce qui peut s'expliquer par la saisonnalité des échantillonnages sur bovin qui ne correspond pas à la période d'activité du stade adulte contrairement à d'autres genres (Estrada-Peña, 2004). Les stades immatures du genre *Hyalomma* et notamment de *H. marginatum* ont quant à eux un tropisme plus marqué pour les micromammifères et l'avifaune (Capek et al., 2014; Estrada-Peña, 2004)

mais n'ont pour autant pas été observés dans le cadre de notre étude au niveau de l'avifaune. Néanmoins, ce genre a été collecté sur l'avifaune au niveau de plusieurs pays du pourtour méditerranéen comme en Grèce et Italie où plus de 90% des tiques collectées étaient du genre *Hyalomma* (Wallménius et al., 2014). De plus, l'hypothèse de l'expansion de l'aire de répartition de *H. marginatum* vers le nord du pourtour méditerranéen, et potentiellement en Corse, par le biais de l'avifaune migratrice se vérifie de plus en plus (Estrada-Peña et al., 2021). L'avifaune pourrait être ainsi une voie d'entrée naturelle du genre *Hyalomma* sur le territoire insulaire et expliquerait la présence d'individus collectés récemment sur bovins (Cicculi et al., 2019a; Dahmani et al., 2017b; Grech-Angelini et al., 2016). Cette présence représente un risque sanitaire par rapport aux agents pathogènes transmis par ce genre de tique tel que le virus du CCHF, responsable de fièvres hémorragiques et qui est retrouvé dans différents pays du pourtour méditerranéen tel que l'Italie (Mancuso et al., 2019), et la Turquie (Kar et al., 2020) par exemple. Les zones présentant des cas positifs au CCHF dans des tiques du genre *Hyalomma* pourrait être des sources d'introduction de ce virus via l'avifaune migratrice. Le risque est d'autant plus élevé que ces zones se situent sur la même voie de migration aviaire que la Corse comme l'Italie. De plus de nombreux agents pathogènes peuvent être détectés sur d'autres espèces de tiques collectées sur l'avifaune. C'est le cas par exemple en Allemagne où 10% des tiques du genre *Ixodes* spp. collectées sur avifaune ont été détectées positives aux bactéries du groupe *Borrelia burgdoferi* (Klaus et al., 2016). L'impact de la migration aviaire est d'autant plus marquant que de nombreux agents pathogènes, tel que *Anaplasma marginale* ou encore *Borrelia burgdoferi sensus lato* pouvant circuler via l'avifaune, ont une transmission transstadiale de larve à nymphe et de nymphe à adulte (Hasle, 2013). Cela pourrait faciliter l'infestation d'hôtes par des stades adultes de tiques rendues positives lors de leur stade immature pendant ou avant la migration aviaire. Il y aurait donc un intérêt majeur à poursuivre les recherches de ce genre au sein de l'avifaune dans les zones humides de Corse ainsi que dans d'autres zones où des stades adultes ont été collectés.

### Le genre *Rhipicephalus*

Il s'agit d'un genre de tique dure ayant un tropisme principalement pour les ongulés domestiques et chiens pour les stades matures et également une partie des immatures (Estrada-Peña, 2004). Dans le bassin méditerranéen, il est principalement représenté par deux espèces : *Rh. bursa* qui représente 38,7 % des tiques collectées sur bovins dans notre étude, responsable de la transmission de nombreuses *Babesia* et *Anaplasma* fortement présentes dans le bassin méditerranéen comme en Espagne (García-Sanmartín et al., 2008) , et *Rh. sanguineus* s.l. représentant 22,7% des tiques collectées sur bovins et 0,9 % des tiques collectées sur sangliers, et responsable de la circulation de divers *Rickettsia* et piroplasmides déjà observés en Sardaigne notamment (Chisu et al., 2019, 2018a). La troisième espèce à avoir été collectée : *Rh. (Bo). boophilus*, représente 0,2% des tiques collectées sur bovins (Figure 28) et est impliquée dans la transmission de la babésiose bovine comme en Italie (Toma et al., 2017).

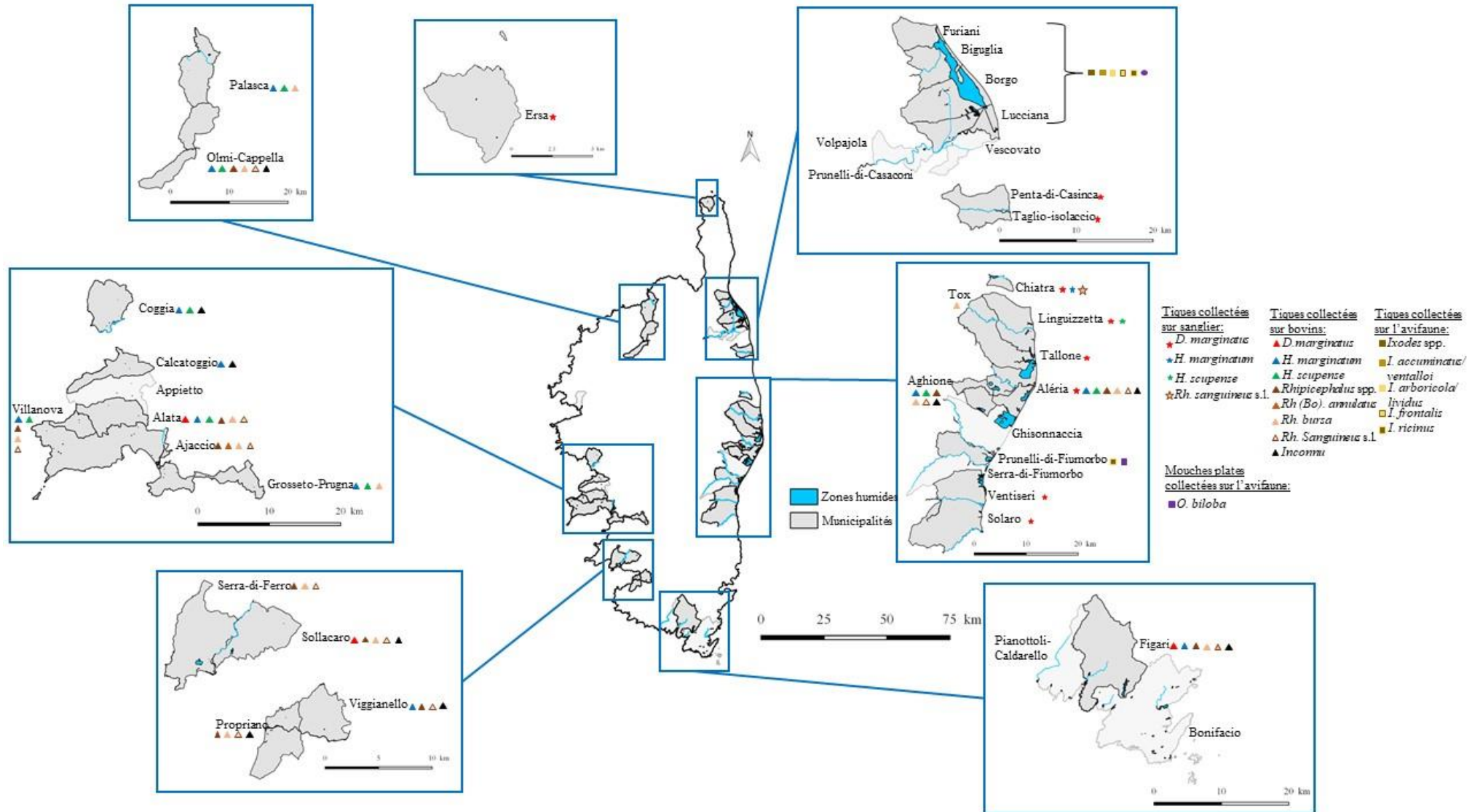
Le genre *Rhipicephalus* représente donc plus de 50 % des tiques collectées sur bovins. Cela pourrait être expliqué par le fort tropisme de ces trois espèces pour les animaux d'élevage comme les bovins. Ce genre a souvent été retrouvé sur bovins dans diverses études au niveau du pourtour méditerranéen où il a été identifié dans 13 pays du pourtour et s'est révélé positif à plus de 50 agents pathogènes (Defaye et al., 2022a). La deuxième explication de cette forte présence pourrait être liée à la saisonnalité de notre échantillonnage (Hiver - Printemps) favorable à leur collecte contrairement à d'autres genres ayant un tropisme similaire, tel que le genre *Hyalomma*, mais une saisonnalité différente (Printemps - Été) (Estrada-Peña, 2004). Ces

deux facteurs ont certainement rendu plus favorable le prélèvement des stades immatures et adultes de *Rhipicephalus* par rapport au genre *Hyalomma*.

## 1.2. Les mouches plates

Un autre groupe d'ectoparasites retrouvé lors de notre étude a été celui des mouches plates qui ont uniquement été collectées sur l'avifaune. Ce sont des arthropodes hématophages de la famille des *Hippoboscidae* composée de différents genres : les *Lipoptena* ciblant principalement les cervidés ; les *Melophagus* ciblant principalement les petits ruminants ; les *Hippobosca* ciblant les bovins et les équins ; les *Orthinomya* ciblant l'avifaune ; et les *Nycteribia* ciblant les chauves-souris (Reeves and Lloyd, 2019). Les mouches plates ont une morphologie adaptée à leurs hôtes, comme par exemple les mouches plates ciblant des grands mammifères qui ont une grande taille et dotées de grandes ailes leur permettant de changer d'individus plus rapidement contrairement aux *Orthinomya* qui vont être de petite taille, plates avec des griffes leur permettant de se déplacer sous les plumes et de rester accrochées lors de grandes migrations (Reeves and Lloyd, 2019). L'intégralité des mouches plates dans notre étude appartenait à l'espèce *Orthinomya biloba* (Figure 28). Elles ont toutes été collectées sur des hirondelles rustiques (*H. rustica*). Cela est le premier signalement de cette espèce de mouche plate en Corse. Cette espèce est trouvée principalement sur des Passeriformes de la famille des *Hirundinidae* comme *Hirundo rustica* (Maa, 1969; Oboňa et al., 2019) et a déjà été trouvée en France lors d'une enquête réalisée par le projet PUPIPO où elle était la deuxième espèce la plus importante recensée ("la\_mouche\_plate\_n\_6.pdf," n.d.). Bien que des agents pathogènes aient déjà été détectés dans d'autres espèces de mouches plates tels que *Anaplasma phagocytophilum* chez la mouche plate du cerf (*Liptotena cervi*), ou encore *Trypanosoma melophagium* chez des *Melophagus* collectées sur moutons (Halos et al., 2004; Martinković et al., 2012), l'impact sanitaire d'*Orthinomya biloba* est toujours inconnu et sa compétence vectorielle n'est pas encore confirmée. Néanmoins des agents pathogènes des genres *Plasmodium* et *Haemoproteus* ont déjà été détectés dans ces mouches plates en France (Karadjian et al., 2013), ou du genre *Trypanosoma* en République tchèque (Santolíkuvá et al., 2022).

## Discussion



**Figure 28.** Carte des espèces d'ectoparasites collectés dans les groupes d'animaux échantillonnés au niveau de différentes zones humides de Corse.

## 2. Agents pathogènes retrouvés dans les ectoparasites et les animaux des zones humides

Les études précédemment menées sur les agents pathogènes en Corse suivaient diverses approches. Certaines se focalisaient sur un agent pathogène au niveau des hôtes définitifs telles que la recherche d'*Echinococcus Canadensis* dans les élevages de porcs et les populations de sangliers (Umhang et al., 2014) ou encore *Anaplasma ovis* chez les chèvres (Cabezas-Cruz et al., 2019), tandis que d'autres recherchaient un large panel d'agents pathogènes uniquement dans les tiques collectées sur divers hôtes (Cicculli et al., 2019b, 2019a; Grech-Angelini et al., 2020). Dans le cadre de notre étude, nous avons voulu mettre en place une recherche basée sur la détection d'un large panel d'agents pathogènes à la fois dans les animaux mais également dans les ectoparasites collectés sur animaux au niveau des zones humides. Cela avait pour but d'obtenir à la fois des informations sur les agents pathogènes présents dans les animaux et les ectoparasites mais également d'observer s'il y a des potentielles interactions entre les animaux et leurs tiques (Figure 29) et/ou entre les différents groupes d'animaux.

### 2.1. Le genre *Anaplasma*

Le genre *Anaplasma* a été détecté dans les trois groupes d'animaux étudiés, directement chez les animaux ou dans leurs ectoparasites. De faibles prévalences ont été observées dans les populations de tiques (*D. marginatus*) de sangliers et au niveau des ectoparasites de l'avifaune où *A. phagocytophilum*, l'agent de l'anaplasmose granulocytaire humaine, a été détecté dans un pool de nymphes d'*I. ricinus* collectées chez *Sylvia atricapilla*. Mais ce genre a été majoritairement détecté au niveau des animaux d'élevage.

Dans les tiques de bovins, le genre *Anaplasma* a été détecté chez *Rh. sanguineus* s.l.. La faible prévalence du genre *Anaplasma* au sein des tiques collectées ne permet pas d'affirmer la circulation du genre par leur intermédiaire à l'exception de la commune de Viggianello où *Anaplasma marginale* a également été retrouvé dans des échantillons de sang de bovins. *A. marginale* est la bactérie responsable de l'anaplasmose bovine présente sur la quasi-totalité du continent européen, transmise par les tiques *Rhipicephalus* (Ferrolho et al., 2016; Khodadadi et al., 2021; Martins et al., 2020) et ciblant principalement les bovins et les ovins (Atif, 2015). Dans notre étude, nous avons détecté l'ADN d'*A. marginale* chez les bovins avec une prévalence de 61,4%, ce qui comparé à des études antérieures confirme la forte présence de cet agent pathogène dans les élevages bovins corses notamment de zones humides (Cicculli et al., 2019a; Dahmani et al., 2017b; Grech-Angelini et al., 2020).

Dans les élevages d'ovins, un genre et deux espèces ont été détectés : *Anaplasma* spp. (74,3% des échantillons), *Anaplasma capra* (1,3%) et *Anaplasma ovis* (2,7%). *A. ovis* est responsable de l'anaplasmose chez les petits ruminants qui peut entraîner une anémie sévère, une baisse de la production laitière et des avortements (Yasini et al., 2012). Elle est principalement transmise par les tiques *Rhipicephalus* (Cabezas-Cruz et al., 2019; Friedhoff, 1997). En Corse, elle a été trouvée dans des troupeaux de chèvres avec une prévalence de 52,0 % (Cabezas-Cruz et al., 2019). Dans notre étude, la présence de l'ADN d'*A. ovis* a été détectée dans le sang de moutons (2,7 %) pour la première fois, mais avec une prévalence plus faible que celle rapportée chez les chèvres (Cabezas-Cruz et al., 2019). La deuxième espèce d'*Anaplasma* détectée dans les échantillons de sang d'ovins était *A. capra*, un agent pathogène zoonotique émergent ciblant les petits ruminants et les humains. *A. capra* est connu pour être principalement asymptomatique, mais il peut parfois provoquer de la fièvre, des myalgies, une thrombocytopenie et des lymphadénopathies (Peng et al., 2018). Actuellement, on le trouve principalement en Asie (Amer et al., 2019; Peng et al., 2021). Dans la région méditerranéenne, il a déjà été détecté chez des cerfs en France (Jouglin et al., 2019). Notre étude semble être le premier rapport de présence d'ADN d'*A. capra* chez des moutons dans la zone méditerranéenne.



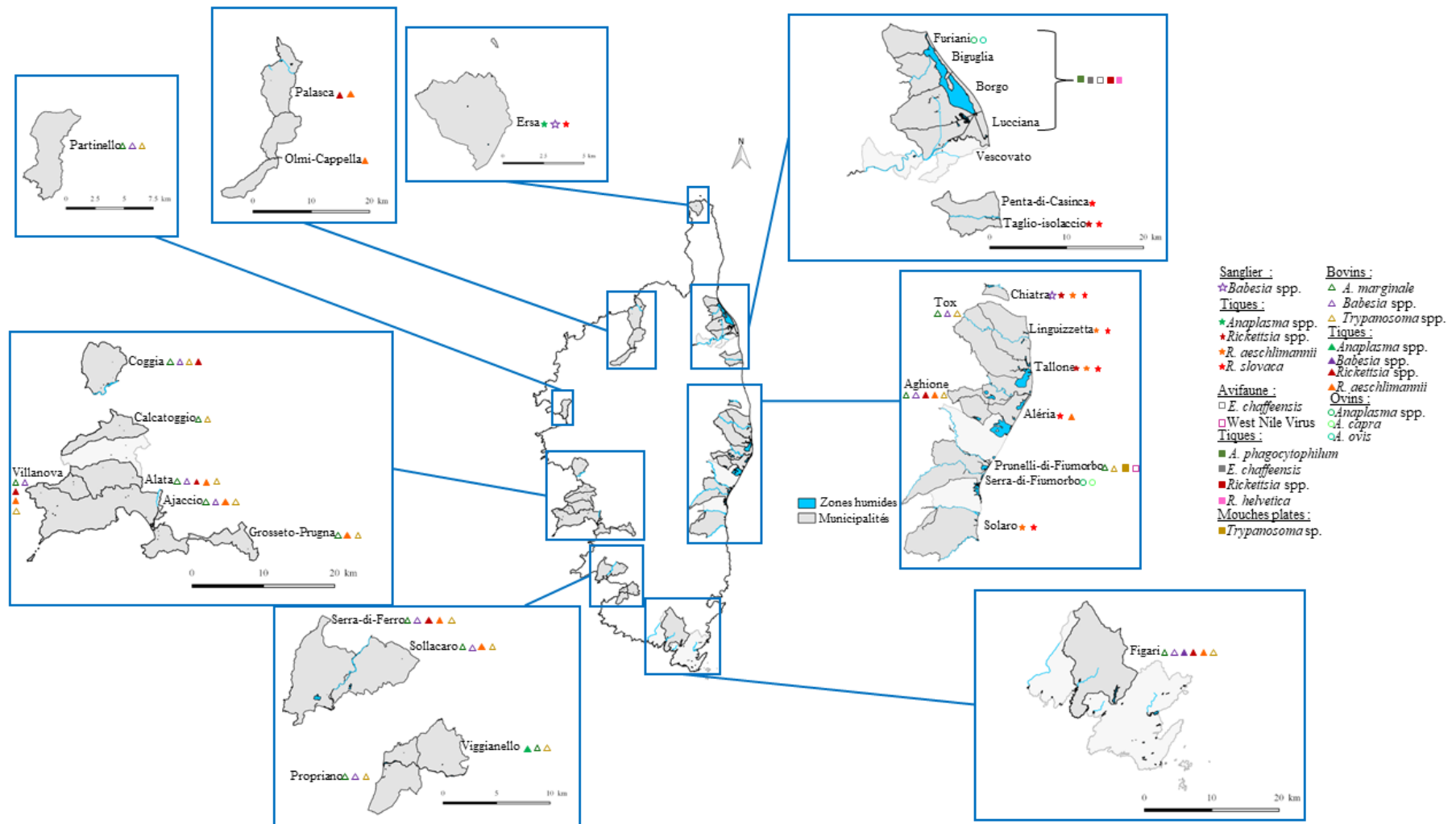


Figure 29. Carte des agents pathogènes détectés dans les animaux et espèces de tiques collectées dans les différents groupes échantillonnés dans les zones humides de Corse.

Malheureusement, aucune tique n'a pu être prélevée dans les élevages de moutons. Néanmoins, cela confirme l'importance de la prise en compte et du suivi du genre *Anaplasma* dans les élevages d'ovins et de bovins corses.

### 2.2. Le genre *Babesia*

Ce genre est composé de parasites protozoaires intracellulaires du groupe des piroplasmides. Il est transmis par des tiques dures notamment par le genre *Ixodes* spp.. Il infecte principalement les bovins, les animaux domestiques et les rongeurs chez lesquels il peut provoquer la piroplasmose (Beuglet and Moreau, 2015). Chez l'homme, les parasites du genre *Babesia* peuvent provoquer des symptômes modérés tels que des myalgies, des maux de tête, de l'anorexie ainsi que des symptômes sévères avec un œdème pulmonaire, un syndrome de dysfonctionnement des organes et un coma (Vannier et al., 2015). Bien que leur réservoir primaire soit souvent les rongeurs, ces parasites infectent également le bétail, les animaux domestiques et les ongulés sauvages. Dans notre étude il a uniquement été retrouvé dans des échantillons de sangliers de la commune de Chiatra, en Plaine Orientale, et dans des échantillons de sang de bovins, avec une prévalence de 12,2% à Figari, seule commune positive (Figure 31). Nos résultats font état du premier signalement de l'ADN de *Babesia* spp. dans les populations de sangliers et de bovins en Corse. Néanmoins la présence de ce genre en Corse a déjà été détecté dans des tiques. *Babesia bigemina* a été trouvé dans les tiques *I. ricinus* collectées sur des bovins et des chèvres (Grech-Angelini et al., 2020) et *Babesia ovis* a été trouvé dans les tiques *I. ricinus* collectées sur des bovins pour les animaux domestiques, et sur des mouflons pour les ongulés sauvages (Grech-Angelini et al., 2020). Dans notre étude, *Babesia* spp. a été identifié dans 3% des tiques *Rh. sanguineus* s.l. et dans 2,6% des tiques *Hy. marginatum* collectées dans la commune de Figari ainsi que dans des échantillons de sang. Ce qui suggère une circulation de ce genre au sein des élevages bovins et de leurs tiques dans cette commune. Cependant, les séquences n'ont pas pu être obtenues, donc la présence de *Babesia* spp. chez les sangliers et les bovins doit encore être confirmée ainsi que la détermination de l'espèce.

### 2.3. Le genre *Ehrlichia*

Le genre *Ehrlichia* est étroitement lié au genre *Anaplasma*. Dans notre étude, une espèce de ce genre a été identifiée : *Ehrlichia chaffeensis*. Elle a été détectée à partir d'un pool de larves d'*I. ricinus* collectées sur rouge-gorge *Eritacus rubecula*. *E. chaffeensis* est une bactérie intracellulaire transmise par les tiques provoquant l'ehrlichiose monocytotrope humaine. On la trouve généralement comme agent pathogène de la tique étoilée d'Amérique (*Amblyomma americanum*) et principalement aux États-Unis (Madison-Antenucci et al., 2020; Paddock and Childs, 2003). Même si le genre *Ehrlichia* a déjà été observé dans des tiques d'oiseaux dans le bassin Méditerranéen (Špitalská et al., 2006; Toma et al., 2014), notre résultat est la première détection d'*E. chaffeensis* dans des tiques d'oiseaux sur le pourtour méditerranéen. C'est également son premier signalement en Corse. La prédominance des tiques *Ixodes* dans notre étude pourrait confirmer son rôle de vecteur potentiel d'agents pathogènes chez les oiseaux. De plus, *E. rubecula* porteur des larves *I. ricinus* positives à *E. chaffeensis* s'est avéré être lui-même positif à cette bactérie. Ceci montre une possible circulation d'*E. chaffeensis* chez les oiseaux sédentaires de la lagune de Biguglia. Néanmoins actuellement, la circulation de cette bactérie a surtout été démontrée chez la tique *Amblyomma americanum* (Cohen et al., 2010), ce qui n'est pas encore le cas pour *Ixodes ricinus*. Des analyses complémentaires sont encore nécessaires pour confirmer s'il s'agit d'une infection sporadique ou si l'infection d'un plus grand nombre de

tiques collectées sur des oiseaux sédentaires suggère une présence importante de cet agent pathogène. L'isolement des agents pathogènes serait nécessaire.

#### 2.4. Le genre *Rickettsia*

Le genre *Rickettsia* a été détecté dans des échantillons de tiques des trois groupes d'animaux étudiés, cependant, l'ADN de *Rickettsia* spp. n'a pas été détecté dans les échantillons de tissus ou de sang des animaux. Ce genre est composé de trois groupes : le groupe de la fièvre tachetée (SFG), le groupe du typhus et le groupe transitoire. Le SFG est le seul groupe transmis quasi-exclusivement par les tiques, qui ont à la fois le rôle de vecteur et de réservoir grâce à leur transmission transstadiale et transovarienne des pathogènes (Robinson et al., 2019). Il est composé de nombreux agents pathogènes zoonotiques avec des niveaux de pathogénicité divers, mais la plupart de ces espèces peuvent constituer une menace pour la santé humaine, avec des symptômes allant d'une éruption cutanée à une lymphadénopathie. En Corse, même si l'impact exact des espèces de *Rickettsia* est encore inconnu, diverses espèces ont déjà été trouvées dans les tiques d'animaux (*R. aeschlimannii*, *R. africae*, *R. felis*, *R. helvetica*, *R. massiliae* et *R. slovacica*) (Cicculli et al., 2019a; Cicculli et al., 2019; Cicculli et al., 2019b; Grech-Angelini et al., 2020; Matsumoto et al., 2004), et une précédente étude séro-épidémiologique réalisée entre 1982 et 1987 en Corse du Sud a montré l'exposition de 355 cas, avec une moyenne d'âge de 49 ans (["bulletin\\_epidemiologique\\_hebdomadaire\\_34\\_1988.pdf," n.d.](#)).

L'espèce principale retrouvée chez les tiques de sangliers (70% de tiques positives) a été *Rickettsia slovacica*, appartenant au groupe de rickettsies provoquant la fièvre boutonneuse Méditerranéenne. Ce résultat conforte ceux déjà observés dans des études réalisées en Corse (Grech-Angelini et al., 2020). Néanmoins, le fait d'avoir détecté cette espèce uniquement dans les tiques et non dans les sangliers ne permet pas de déterminer le rôle du sanglier dans la circulation du pathogène, ni l'affinité de l'agent pathogène pour cet hôte. D'un autre côté le fait que la totalité des *R. slovacica* se retrouvent chez 73,4 % des *Dermacentor marginatus* collectés reflèterait plus la spécificité de *R. slovacica* pour cette espèce de tique. D'autant plus que cette rickettsie est souvent retrouvée dans des *Dermacentor marginatus* au sein du pourtour Méditerranéen que ce soit sur des tiques d'animaux domestiques comme en Espagne, Italie, France ou Turquie par exemple ; ou encore sur des tiques d'animaux sauvages dont les sangliers en Algérie, Espagne, France et Italie (Defaye et al., 2022a, 2022b). La potentielle affinité de *R. slovacica* pour le sanglier serait donc due à la spécificité de l'agent pathogène pour *D. marginatus*.

L'autre espèce de rickettsies détectée est *Rickettsia aeschlimannii* qui, contrairement à *R. slovacica*, a été détectée uniquement dans les tiques *Hyalomma marginatum* (50 %), *Hyalomma scupense* (100%) et *Rhipicephalus sanguineus* s.l. (100 %) collectées sur sangliers. Cette espèce est également la principale espèce détectée dans les tiques provenant d'élevages de bovins avec 79,3% des *Rickettsia* détectés, le reste n'ayant pas pu être déterminé au niveau spécifique. Elle a été détectée aussi bien dans les tiques du genre *Hyalomma* (38,6% positives) que dans celle du genre *Rhipicephalus* (60.2% positives) collectées sur bovins. En Corse, *R. aeschlimannii* a été signalée précédemment avec une prévalence de 23% dans des tiques collectées sur bovins et de 58% dans des tiques collectées sur moutons (Grech-Angelini et al., 2020). Elle a également été détectée chez *Hy. marginatum* collectés sur bovins en 2019 (Cicculli et al., 2019b). Il semble que l'ADN de *R. aeschlimannii* soit présent dans les tiques de bovins quelle que soit la zone d'échantillonnage en Corse. Cela suit également les résultats reportés dans le bassin Méditerranéen où cette bactérie a été retrouvée de nombreuses fois dans des tiques de bovins notamment du genre *Hyalomma* que ce soit en Europe (France et Italie), en Afrique ( Egypte et Tunisie) et en Israël (Defaye et al., 2022a). Nos résultats suivent donc les

observations faites sur *R. aeschmimannii* dans le pourtour Méditerranéen à savoir que cette rickettsie semble fortement liée aux tiques du genre *Hyalomma* collectées sur bovins.

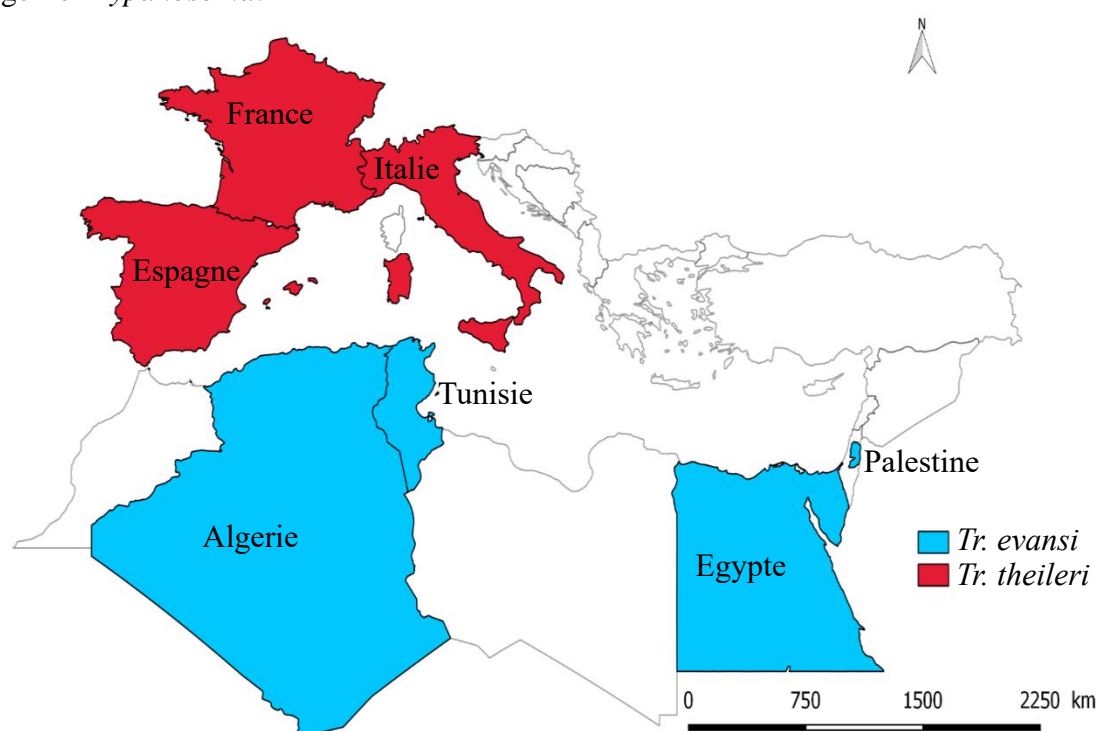
La dernière espèce de rickettsie détectée est *Rickettsia helvetica* dans des pools constitués d'espèces d'*Ixodes* différentes. *R. helvetica* est une rickettsie transmise par les tiques, appartenant au groupe de la fièvre tachetée (SFG), associée à des symptômes tels que la fièvre, la myalgie et la méningite. Elle a une distribution quasi mondiale et est principalement transmise par des tiques du genre *Ixodes* (Påhlson et al., 2021; Parola et al., 2013). Cette détection a été la première sur le territoire Corse. Néanmoins la présence de cette rickettsie a déjà été observée dans le bassin méditerranéen principalement dans des tiques du genre *Ixodes* que ce soit sur animaux domestiques en Algérie, Italie et Tunisie ou sur une large variété d'animaux sauvages (avifaune, micromammifères, mammifères carnivores et ongulés) en Espagne, France et Italie (Defaye et al., 2022a, 2022b). Comme pour les espèces de *Rickettsia* précédentes, il semblerait que *R. helvetica* ait une affinité pour un genre de tiques qui est ici le genre *Ixodes* spp.

Les résultats obtenus montrent l'importance du genre *Rickettsia* au sein des populations de tiques parasitant la faune de zones humides corses. Il serait d'autant plus intéressant d'étudier ce genre que les différentes espèces de *Rickettsia* semblent avoir des affinités pour différents genres de tiques. La surveillance des différents genres de tiques sur le territoire Corse permettrait une surveillance efficace des espèces de *Rickettsia* circulant en Corse. Néanmoins, le genre *Rickettsia* n'a pas été détecté au sein des animaux mais uniquement dans les tiques.

## 2.5 Le genre *Trypanosoma*

Le genre *Trypanosoma* est principalement connu comme l'agent pathogène de la maladie du sommeil humaine, causée par *Trypanosoma brucei*, et de la maladie de Chagas, causée par *Tr. cruzi*, respectivement en Afrique et en Amérique du Sud. C'est un genre ubiquitaire, qui infecte un large éventail de vertébrés et qui est principalement transmis par les mouches piqueuses, les punaises de lit et parfois les tiques (Kaufer et al., 2017). Chez les animaux domestiques (bovins, chevaux, moutons, carnivores, etc.), ces agents pathogènes sont transmis mécaniquement par un large éventail de moucheron piqueurs. Ce parasite protozoaire peut être transmis horizontalement et verticalement (Desquesnes et al., 2013). Dans la région méditerranéenne, deux espèces ont été trouvées. *Trypanosoma evansi* responsable de la " surra " qui est une trypanosomiase avec des taux de morbidité et de mortalité élevés chez les animaux (Erekat et al., 2020). Selon l'animal hôte infecté, elle peut être létale (chevaux), provoquer une maladie aiguë (chiens) et des symptômes variables dont des symptômes létaux (bovins). Cependant, *Tr. evansi* est rarement détecté chez l'homme et n'est toujours pas considéré comme zoonotique (Desquesnes et al., 2013). La deuxième espèce est *Trypanosoma theileri*, un hémoparasite non pathogène dont le potentiel pathogénique est encore peu connu chez les bovins (Villa et al., 2008) (Figure 30). Il peut être transmis par les mouches tabanides et les tiques *Hy. anatolicum anatolicum* (Lee et al., 2013). Ce genre a été identifié pour la première fois dans notre étude en Corse depuis 1989 où il avait été détecté dans des grenouilles vertes (Barta et al., 1989), et a été détecté dans 58,3% des échantillons de sang de bovins analysés. Cette prévalence couplée au fait qu'aucune mortalité ou morbidité ne soit observée au sein des élevages bovins échantillonnés tend à supposer la présence de *Tr. theileri* plutôt que *Tr. evansi*.

Au niveau de l'avifaune, le genre *Trypanosoma* a été détecté dans 2,7% des mouches plates *Ornithomya biloba* collectées sur des hirondelles rustiques (*Hirundo rustica*). A notre connaissance, il s'agit du premier signalement d'ADN de *Trypanosoma* sp. chez les ectoparasites d'oiseaux en Corse et en Méditerranée. Néanmoins, ce genre a déjà été détecté chez *Ornithomya biloba* collecté sur l'avifaune en République Tchèque où, comme dans le cadre de notre étude, l'espèce et la pathogénicité n'ont pas pu être déterminées (Santolíkova et al., 2022). La faible prévalence dans les mouches plates d'oiseaux ne permet pas de conclure quant à la circulation du genre via les oiseaux migrateurs et leurs ectoparasites. Que ce soit pour les bovins et l'avifaune, la séquence d'ADN n'a pas pu être obtenue. Par conséquent, l'isolement du parasite ou des enquêtes sérologiques seraient nécessaires pour confirmer la présence du genre *Trypanosoma*.

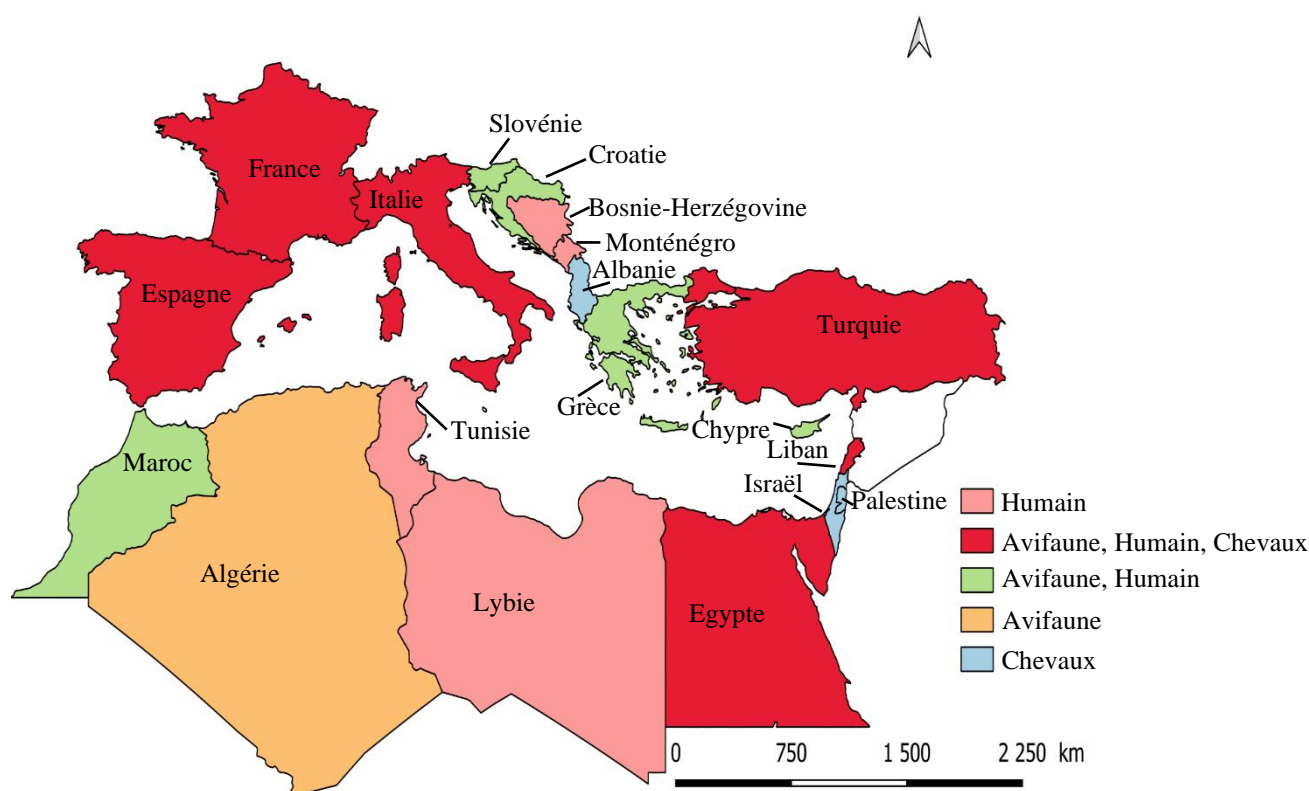


**Figure 30.** Carte des espèces de *Trypanosoma* spp. détectées sur des bovins dans le bassin méditerranéen.

## 2.6 Le West Nile virus

Le West Nile virus (WNV) a été détecté dans l'avifaune chez deux mésanges charbonnières (*Parus major*) au niveau de l'étang de Gradugine. Ce virus est un flavivirus transmis par les moustiques et infectant principalement les oiseaux qui sont tous deux impliqués dans le cycle de vie du virus. Il peut également être trouvé chez l'homme et le cheval qui sont des hôtes accidentels et pour lesquels le virus peut s'avérer mortel (Rossi et al., 2010). Il se manifeste principalement par une maladie fébrile moyenne avec fièvre, maux de tête et myalgie et un syndrome de Guillain-Barré (Beshai et al., 2020). En Corse, il a été trouvé avec une prévalence de 9,4% chez les chevaux et 8,4% chez les chiens (Maquart et al., 2017). Dans notre étude, nous avons trouvé l'ARN du WNV chez deux adultes sédentaires *P. major*, ce qui représente une prévalence de 0,3% des échantillons de sang totaux d'oiseaux sauvages. Il s'agit du premier enregistrement de l'ARN du WNV dans la population d'oiseaux en Corse. Nos résultats, ajoutés aux précédents signalements chez des chevaux et des chiens, pourraient confirmer la présence du virus dans l'île. De plus, ce virus est largement répandu dans le monde (Rossi et al., 2010)

et a une distribution globale en Méditerranée (Figure 31). Au sein de la zone méditerranéenne, le virus du West Nile a été retrouvé dans l'intégralité des pays (en dehors de la Syrie), aussi bien chez les humains, les équins ou l'avifaune. Au sein de ce dernier groupe, une large variété d'oiseaux a été détectée positive au virus comme en Italie (Angelini et al., 2010; Calzolari et al., 2012; Llopis et al., 2015; Monaco et al., 2010) et en Espagne avec un prévalence 42,6 % (Figuerola et al., 2007). Cela démontre l'importance du virus West Nile dans le bassin méditerranéen. Néanmoins, l'isolation du virus, l'utilisation de méthodes sérologiques, ainsi que la recherche du virus au sein d'autres groupes que les Passeriformes et dans des populations de moustiques permettraient de confirmer la circulation du virus sur le territoire corse.



**Figure 31.** Carte des hôtes positifs au West Nile Virus dans les pays du bassin méditerranéen.

**Conclusion et perspectives**

Les travaux réalisés dans le cadre de cette thèse présentent des résultats majeurs. Les premiers, portant sur la recherche bibliographique, ont permis de démontrer une augmentation de l'effort de recherches croissant sur les agents pathogènes transmis par les tiques chez les animaux sauvages et domestiques dans le bassin méditerranéen de 2000 à 2021. Cette augmentation semble corrélée à l'augmentation de l'utilisation de la biologie moléculaire comme moyen de détection. Néanmoins la majorité des études reportées sur les animaux domestiques et sauvages proviennent principalement des pays de l'Europe de l'Ouest, ce qui n'est pas représentatif de la totalité du pourtour méditerranéen. Des divergences dans l'effort d'échantillonnage sont également observées au sein des hôtes et les données proviennent principalement des animaux associés aux activités humaines tels que les animaux d'élevages et les animaux chassables. Cela a entraîné une collecte et une identification plus importante de leurs tiques et des agents pathogènes associés pas forcément représentative de la diversité globale. C'est le cas par exemple pour le genre *Rhipicephalus* sur les animaux d'élevages et des genres *Hyalomma* et *Ixodes* pour les animaux chassables et l'avifaune fréquemment identifiés et ainsi davantage pris en considération que d'autres. Un dernier résultat qui a pu être mis en évidence est l'importance des îles, où malgré le faible pourcentage de publications reportées, une large variété d'agents pathogènes, d'espèces de tiques et d'hôtes commune aux différentes régions du pourtour méditerranéen, a été retrouvée. Cela pourrait montrer l'importance que peuvent avoir les îles méditerranéennes en tant que « sentinelles » pour la surveillance de la circulation des tiques et de leurs agents pathogènes. Néanmoins, un plus grand nombre de données permettrait de confirmer ce rôle.

Au sein des trois groupes d'animaux étudiés, une large variété d'espèces d'ectoparasites a été collectée avec dix espèces de tiques : *Dermacentor marginatus*, *Hyalomma marginatum*, *Hyalomma rufipes*, *Ixodes accuminatus/ventralloii*, *Ixodes arboricola/lividus*, *Ixodes frontalis*, *Ixodes ricinus*, *Rhipicephalus bursa*, *Rhipicephalus sanguineus* s.l. et *Rhipicephalus (Bo) boophilus* ; et une mouche plate : *Orthinomya biloba*. Parmi cette diversité, une forte similitude peut être constatée entre les sangliers et les bovins où 4 espèces de tiques ont été retrouvées en commun. Tandis que l'avifaune ne partage aucune espèce d'ectoparasites en commun avec les deux autres groupes. Cela démontre une possible circulation des ectoparasites entre les bovins et sangliers, ce qui ne peut pas être démontré pour l'avifaune. Cependant, il est connu que l'avifaune regroupe des hôtes parasités par des stades immatures de tiques des genres *Ixodes* et *Hyalomma* déjà retrouvés sur des bovins et sangliers en Corse. En dehors du genre *Ixodes* identifié dans notre étude, des recherches sur la présence de *Hyalomma marginatum* et *Ixodes* spp. dans l'avifaune corse permettraient de déterminer leurs impacts dans l'introduction et la circulation d'agents pathogènes sur le territoire corse. Le deuxième résultat d'importance pour l'avifaune est lié à la première collecte de mouches plates réalisée en Corse et l'identification de l'espèce *Orthinomya biloba*. Elle pourrait représenter un risque d'entrée d'agents pathogènes sur le territoire bien que pour l'instant la compétence vectorielle de cette mouche plate ne soit pas encore connue. Actuellement, les populations de mouches plates sont très peu connues en Corse ainsi que le risque sanitaire qu'elles pourraient entraîner. Des campagnes de collectes sur des hôtes diversifiés de mouches plates au sein des différentes populations animales corses, ainsi que la recherche d'agents pathogènes associés permettraient d'approfondir cette question.

Par le moyen d'outils d'investigation épidémiologique à haut débit mis en place par le Laboratoire de Santé Animale ANSES de Maisons-Alfort, nous avons pu mener une recherche explorant la diversité des agents pathogènes au sein des trois groupes d'animaux pris en compte dans notre étude dans les zones humides de Corse : sangliers, animaux d'élevages et avifaune. Cette approche nous a permis de mettre en avant la présence de divers agents pathogènes au sein de ces différents groupes. C'est le cas pour le genre *Babesia* détecté pour la première fois au sein de populations de sangliers dans une commune de Corse. Ce genre a également été



retrouvé dans des bovins et des tiques collectés dans un même élevage. Une isolation et une identification spécifique de l'agent pathogène, ainsi que des recherches sur la présence du genre *Babesia* au sein des élevages bovins où il a été détecté à la fois dans les tiques et les animaux (commune de Figari), serait nécessaire pour confirmer sa présence, sa circulation et les risques en santé animale potentiels. C'est également le cas pour le genre *Trypanosoma* qui a été retrouvé dans plus de 50% des échantillons de sang de bovins collectés et dans des échantillons de mouches plates de l'avifaune. Des isolations et une détermination spécifique permettrait d'avoir une meilleure connaissance de ce genre parasitaire en Corse et d'en évaluer l'éventuel impact sanitaire. Un autre résultat marquant de notre étude est la détection du virus West Nile dans deux mésanges charbonnières qui est la première détection de ce virus dans son hôte naturel en Corse. Cela confirme la présence de ce virus sur le territoire insulaire après la détection d'anti-corp positif au virus chez des chevaux. Des recherches sur la présence de ce virus dans son vecteur et au sein d'autres populations animales au niveau des zones humides permettraient d'améliorer les connaissances sur sa circulation éventuelle en Corse.

La mise en place d'une d'étude comparative entre zones humides et zones non humides, avec la mise en œuvre de méthodologies identiques, permettrait d'avoir un aperçu plus précis des risques sanitaires inféodées aux zones humides. Néanmoins ces milieux particulièrement impactés par le changement climatique, soumis à de nombreuses perturbations et au centre de nombreuses activités humaines et animales leurs confèrent un caractère évolutif, nécessitant une surveillance accrue dans le domaine de la santé animale et humaine.

Il est également important de préciser que nos résultats confirment la présence de l'ADN de divers agents pathogènes et non la présence d'agents pathogènes vivants et infectieux. Le couplage de la détection d'agents pathogènes à large spectre grâce aux outils d'investigation épidémiologique à haut débit mis en place par le laboratoire de Santé Animale ANSES de Maisons-Alfort avec des méthodes sérologiques et microscopiques permettrait de confirmer la présence de ces agents pathogènes ainsi que leurs identifications. Cette approche pourrait également s'appliquer à une recherche dans d'autres groupes d'animaux (comme les micromammifères) et vecteurs hématophages afin d'avoir une vision plus étendue des agents pathogènes et des vecteurs présents dans les zones humides.

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**Résumé :**

La Corse est une île méditerranéenne française marquée par de fortes activités humaines et animales au sein de différents biotopes. Parmi ces biotopes, les plus marquants sont les zones humides. Au sein du territoire Corse, sept catégories de zones humides ont été dénombrées, allant de zones humides temporaires, telles les mares et les estuaires temporaires, à des zones permanentes de grande taille, telles les lagunes littorales. Ces zones sont des zones clef des activités humaines (agricoles, touristiques, récréatives), animales (agriculture, abreuvements, repos, migration) et favorables à la prolifération et à la circulation d'agents pathogènes et de leurs vecteurs. C'est dans ce contexte que nous avons mis en place cette étude, basée sur la recherche d'agents pathogènes dans différents groupes d'animaux et leurs vecteurs dans les zones humides de Corse. Elle s'est focalisée sur trois groupes d'animaux. Le premier est celui des mammifères sauvages, ayant pour modèle le sanglier. Le deuxième groupe est celui des animaux d'élevages Corse. Le troisième est celui de l'avifaune. Lors de notre étude, 601 tiques ont été collectées, réparties dans 4 genres différents : *Dermacentor* spp. (*D. marginatus*), *Hyalomma* spp. (*Hy. marginatum* et *Hy. scupense*), *Ixodes* spp. (*I. acuminatus/ventalloi*, *I. arboricola/lividus*, *I. frontalis* et *I. ricinus*) et *Rhipicephalus* spp. (*Rh (Bo). annulatus*, *Rh. bursa* et *Rh sanguineus* s.l.). Parmi ces genres, des préférences d'infestations ont été observées, comme cela a été le cas pour le genre *Dermacentor* pour le sanglier, le genre *Rhipicephalus* pour les bovins et le genre *Ixodes* pour l'avifaune. En complément des tiques collectées, 37 mouches plates de l'espèce *Ornithomya biloba* ont été collectées sur l'avifaune migratrice. Il s'agit de leur première identification sur le territoire Corse.

Par le biais d'un outil de PCR micro-fluidique en temps réel à haut débit (BioMark™ dynamic arrays, Fluidigm Corporation, USA), 34 espèces et 11 genres de bactéries, parasites et virus ont été recherchés dans les trois groupes d'animaux cibles. En complément, les virus Usutu et West Nile virus l'ont été dans les échantillons de l'avifaune. Six genres d'agents pathogènes ont été détectés dans les ectoparasites et dans les animaux : trois genres bactériens (*Anaplasma*, *Ehrlichia*, *Rickettsia*), deux genres de parasites (*Babesia*, *Trypanosoma*) et un genre viral (Flavivirus). Parmi ces différents genres, certaines espèces ont été identifiées pour la première fois en Corse comme c'est le cas pour : *Rickettsia helvetica* dans les tiques de l'avifaune, *Ehrlichia chaffeensis* dans les tiques et oiseaux échantillonnés et *Anaplasma capra* dans des élevages d'ovins. La présence de certains agents pathogènes a également été confirmée dans des zones humides telles que des bactéries des genres *Rickettsia* dans des tiques de sangliers et bovins, *Anaplasma* dans des élevages d'ovins et bovins, *Babesia* dans les tiques de sangliers et bovins ainsi que dans les populations de sangliers, *Trypanosoma* dans les populations de bovins et le West Nile virus dans l'avifaune.

**Mots-clés :** Agents pathogènes, Avifaune, Corse, Animaux d'élevage, Mouches plates, Sangliers, Tiques, Zones humides

**Abstract:**

Corsica is a French Mediterranean island marked by strong human and animal activities within different biotopes. Among these biotopes, the most outstanding are the wetlands. Within the Corsican territory, seven categories of wetlands have been counted, ranging from temporary wetlands such as ponds and temporary estuaries to large permanent areas such as coastal lagoons. These areas are key areas for human (agricultural, tourist, recreational) and animal (agriculture, watering, resting) activities. Due to their characteristics, these habitats are important in public health by their role in the proliferation and circulation of pathogens and their vectors. In the context of Corsica, the sanitary importance of wetlands is all the more important due to the insular context in the Mediterranean, which makes these areas points of resting for animal migrations such as avifauna. This favorable context for the research of pathogens in groups of animals and their vectors in Corsica wetlands was the target in our study. It focused on three groups of animals. The first one is the wild mammals where we focused on the wild boar. The second group is that of the animals of the Corsican breeding. The third and last group is the avifauna. During our study, a total of 601 ticks were collected. They are composed of 4 different genera: *Dermacentor* spp. (*D. marginatus*), *Hyalomma* spp. (*Hy. marginatum* and *Hy. scupense*), *Ixodes* spp. (*I. acuminatus/ventalloi*, *I. arboricola/lividus*, *I. frontalis*, and *I. ricinus*), and *Rhipicephalus* spp. (*Rh (Bo). annulatus*, *Rh. bursa*, and *Rh sanguineus* s.l.). Among these genera, infestation preferences were observed, as for the genus *Dermacentor* for wild boar, the genus *Rhipicephalus* for cattle and the genus *Ixodes* for avifauna. In addition to the ticks collected, 37 loose flies of the species *Ornithomya biloba* were collected from migratory avifauna. This is their first identification on the Corsican territory.

Using a high throughput real-time micro-fluidic PCR tool (BioMark™ dynamic arrays, Fluidigm Corporation, USA), a total of 34 species and 11 genera of bacteria, parasites and viruses were searched for in the three target animal groups. In addition, Usutu and West Nile viruses were tested in avian samples. A total of six pathogen genera were detected in ectoparasites and animals: three bacterial genera (*Anaplasma*, *Ehrlichia*, *Rickettsia*), two parasite genera (*Babesia*, *Trypanosoma*) and one viral genus (Flavivirus). Among these different genera, some species have been identified for the first time in Corsica as it is the case for: *Rickettsia helvetica* in avifauna ticks, *Ehrlichia chaffeensis* in sampled ticks and birds and *Anaplasma capra* in sheep farms. The presence of some pathogens was also confirmed in wetlands such as bacteria of the genera *Rickettsia* in wild boar and cattle ticks, *Anaplasma* in sheep and cattle farms, *Babesia* in wild boar and cattle ticks as well as in wild boar populations, *Trypanosoma* in cattle populations and West Nile virus in avifauna.

**Keywords:** Avifauna, Corsica, Livestock, Loose flies, Pathogens, Ticks, Wetlands, Wild boar