Universitat Autònoma de Barcelona

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Drivers of presence, phenotypic responses and health concerns

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Tesi doctoral

Programa de doctorat en Biodiversitat

Departament de Biologia Animal, Biologia Vegetal i Ecologia

Facultat de Biociències

Universitat Autònoma de Barcelona

2019

SEFaS

Wildlife Ecology & Health

This research was partially funded by the contracts 13/051, 15/0174, 16/0243-00-CP/01 and 16/0243-00-PR/01 between Universitat Autònoma de Barcelona and Ajuntament de Barcelona.

Raquel Castillo Contreras was supported by a pre-doctoral grant by Agència de Gestió d'Ajuts Universitaris i de Recerca (Generalitat de Catalunya) and the European Social Fund, file numbers 2016FI_B 00425, 2017FI_B1 00040 and 2018FI_B2_00030.

Els doctors Jorge Ramón López Olvera, Professor Titular d'Universitat, i Santiago Lavín González, Catedràtic d'Universitat, del Departament de Medicina i Cirurgia Animals de la Universitat Autònoma de Barcelona, i Gregorio Mentaberre García, Professor Associat del Departament de Ciència Animal de la Universitat de Lleida,

Informen:

Que la memòria titulada "Urban wild boar. Drivers of presence, phenotypic responses and health concerns", presentada per Raquel Castillo Contreras per a la obtenció del grau de Doctora en Biodiversitat per la Universitat Autònoma de Barcelona, s'ha realitzat sota la nostra direcció i, un cop considerada satisfactòriament finalitzada, autoritzem la seva presentació per tal que sigui avaluada per la comissió corresponent.

I perquè així consti als efectes oportuns, signem el present informe a Bellaterra, a 19 de setembre de 2019.

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ACKNOWLEDGEMENTS, AGRADECIMIENTOS, AGRAÏMENTS

Y llegó el día. En primer lugar, me gustaría dar las gracias a mis directores Santiago, Jorge y Gregorio. A Santiago, porque decidió apostar por mí y no rendirse hasta que conseguimos una beca predoctoral, y a Jorge y Gregorio, porque han seguido mi camino y crecimiento científico de cerca, trabajando mano a mano en esta tesis y en otros muchos proyectos, ayudándome a llegar a donde estoy ahora. Sin vosotros tres, esta tesis no sería posible, y os agradezco de corazón que me hayáis brindado esta oportunidad y me hayáis acogido en el SEFaS con los brazos abiertos.

En segundo lugar, también quiero darle las gracias a Emmanuel por sus consejos sobre estadística, por su peculiar visión de las cosas, y por abrirnos las puertas del SEFaS a los biólogos; y a Roser, por su buen corazón y por siempre transmitir su gran sabiduría de una manera muy sencilla y cercana.

En tercer lloc, vull donar les gràcies a Marta Cerdà per la seva direcció i suport des del CReSA, que han fet possible el quart estudi d'aquesta tesi. I a la Tere, per tota la seva ajuda lluitant contra uns campylos que no volien créixer.

I en quart lloc, vull agrair a les colles de caçadors de Collserola, sobretot a Pere Martín, Fernando Moret, Joan Carles Montagut, Jesús Esquerrà i Jorge Sánchez, la seva col·laboració durant els mostrejos a les batudes.

Quiero dar gracias infinitas a Johan, a Arián y a –la más joven del SEFaS– Marta. Habéis sido sin duda una parte indispensable en el desarrollo de mi tesis, desde las batidas y sala de necropsias, hasta la estadística y la escritura. Johan, por tu serenidad (que no impasibilidad) en momentos clave y tu saber hacer, desde Valli di Lanzo hasta los cubículos, pasando por Sabadell sud. Arián, por tu buena compañía y muchísima ayuda durante casi toda mi tesis: en las batidas, el 333, la sala de necropsias y el despacho. Depositar el mismo día quizá tiene sus inconvenientes (como compartir director, hola Jorge), pero también sus ventajas; nos hemos animado mutuamente, nos hemos entendido a la perfección, y nos hemos echado unas buenas risas. Marta, perquè ets de les millors persones que conec, perquè et preocupes per tothom (sobretot animalons indefensos) i perquè t'enfades súper ràpid (cosa que m'agrada molt de tu). Encara et queda un llarg camí al SEFaS; aprofita'l, sigues forta, i molts ànims. Una abraçada al Jimbo i al Cotonet.

Carles, apareciste en el SEFaS cual chaleco salvavidas. Estaré eternamente agradecida, y sólo quiero decirte pronto serás doctor y podrás conseguir todo lo que deseas. Y un abrazo para Karma, que nos alegra los días en el despacho. Carlos, te has convertido en el chico de los modelos y las reuniones infinitas en Barcelona. Gracias por tu apoyo; tú también estás cerca de

terminar, así que mucho ánimo, te espera un futuro brillante. Y aquí voy a hacer una mención especial a Lucía, aunque sea ex-sefita, porque se lo merece por toda su ayuda a muchos niveles. Mucho ánimo también con tu tesis, no sabes cuánto me alegro de lo que estás consiguiendo, y esperamos (Johan y yo) visitarte pronto. Lo prometemos (de nuevo).

Xavi, Andreu y Diana, los doctorandos del SEFaS cuando yo me incorporé. El SEFaS no es lo mismo sin vosotros, ya os lo digo. Xavi, siempre dispuesto a hablar (y dar buenos consejos) sobre ciencia. Eres un modelo a seguir, y los cotilleos también eran divertidos, para qué negarlo. Andreu, no em contestes els correus, però no passa res. Moltes gràcies per la teva companyia, per aportar un altre punt de vista, per ser especial. I a tos dos, moltes gràcies per la vostra ajuda als mostrejos, tant a Barcelona, com a necros i a Collserola. Diana, ets una dona excepcional, model d'esforç i perseverança. Has deixat marca al SEFaS, i me n'alegro molt de saber que has començat una nova etapa i sou feliços amb el petit Jan.

Sefitos y ex-sefitos: gracias por vuestra colaboración, de una manera u otra, en esta tesis. Habéis sido clave en las fases de muestro y sin vuestra ayuda esto no habría sido posible. También destacáis por el apoyo moral y compañía dentro y fuera del despacho, y quiero hacer especial mención a Sara Chaves, Irene, Andrea, Little Italy -Federica, Mattia, Eugenia-, Wendy, y los "recién" llegados Genís y Clàudia. Y no querría olvidarme de Olivia, de Pollo, ni de la pizarra.

Gracias a Óscar por tu ayuda en el CReSA, con garrapatas, con caracoles, con lo que sea, y por darme la increíble oportunidad de ir a Uganda; junto con Ignasi hicisteis que fuera una experiencia inolvidable.

Richard, I want to thank you for all your help and guidance during my stay at Salford. I had a great time with you and I learned a lot about ticks and tick-borne pathogens. Jess, you also helped me a lot in the lab, and you also made me realize what was really important at that time. Plus, I tried the best pie ever with you and never got bitten by a tick.

Sílvia, Turon, Anna, Bigu i Ferran, us vull agrair que sempre us heu interessat pels senglars. Vam celebrar la meva primera beca, i la meva segona beca, i no m'ho crec però sembla que ja acabo.

Finalmente, quiero agradecer a mi familia que me hayan dado la oportunidad de estudiar que ellos no tuvieron, y que me hayan apoyado moral y económicamente durante toda mi vida, en especial mis padres, mi hermana y mis abuelas y abuelos.

Y termino con Marcel, que no es fácil. Gracias, gracias, gracias por todo tu apoyo, a tantos niveles, durante todos estos años. Eres la razón de mi cordura.

Y gracias a Nino.

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LIST OF ABBREVIATIONS

AFS: African swine fever

ASSuT: Ampicillin, streptomycin, tetracycline and sulphametoxazole

AIC: Akaike's information criterion

BC: Before Christ

bp: base pairs

CC: Clonal complex

C_t value: Cycle threshold value

DEBONEL: *Dermacentor*-borne necrosis erythema and lymphadenopathy

ERIC-PCR: Enterobacterial repetitive intergenic consensus PCR

*Fla*A-RFLP: Restriction fragment length polymorphism of the *fla*A gene

GAM(s): Generalised additive model(s)

GLM(s): Generalised linear model(s)

HEV: Hepatitis E virus

LM(s): Linear model(s)

MAB: Metropolitan Area of Barcelona

MIC: Minimum inhibitory concentration

MLST: Multilocus sequence typing

MDR: Multidrug resistant

PFGE: Pulse-field gel electrophoresis

PCR: Polymerase chain reaction

PCV2: Porcine circovirus type 2

RLB: Reverse line blot hybridization assay

RT-PCR: Real Time PCR

S.: serovar (from *Salmonella*)

SFG: Spotted fever group

ST(s): Sequence type(s)

s.l.: *sensu lato*

s.s.: *sensu stricto*

TBP(s): Tick-borne pathogen(s)

T6SS: Type 6 secretion system

TIBOLA: Tick-borne lymphadenopathy

UAB: Universitat Autònoma de Barcelona

1. SUMMARY

There are wildlife species able to exploit the resources offered by urban environments. Wild boars can explore urban and peri-urban areas, but their presence in these areas is a nuisance and poses a risk for public health and safety. Interactions between wild boars and people are expected to continue rising in Europe owing to increasing wild boar and human population trends, particularly in urban settings. This thesis determines the drivers of wild boar presence in the urban area of Barcelona, addresses the phenotypic changes shown by urban individuals with respect to non-urban ones, and identifies wild boar related public health concerns in an urban environment.

Wild boar presence in the city of Barcelona was positively correlated to proximity to streams in the bordering Collserola natural space, higher landscape fragmentation and the presence of both urban green areas and stray cat colonies. The presence was also more frequent in spring and summer, which could be related to births leading to a higher group size and increased energetic needs, juvenile and yearling dispersal and lower availability of food resources in the warm seasons in Mediterranean regions. Moreover, urban wild boars used more anthropogenic food resources, showed higher body mass and grew faster than non-urban ones. Urban female wild boars started reproducing earlier than non-urban ones, probably as a result of achieving the required body mass earlier. However, urban wild boars died at a younger age than non-urban wild boars, indicating a possible cost of exploring the urban area for the wild boar in Barcelona.

This thesis also describes the presence of zoonotic tick-borne and foodborne pathogens carried by ticks parasitizing wild boars and wild boars, respectively. Wild boars in the Metropolitan Area of Barcelona (MAB) carried ticks belonging to species *Hyalomma lusitanicum*, *Dermacentor marginatus*, *Rhipicephalus sanguineus* sensu lato and, anecdotally, *Rhipicephalus bursa*. Screening of tick pools revealed the presence of three emerging zoonotic *Rickettsia* species (*R. massiliae*, *R. slovaca* and *R. raoultii*), whereas wild boar spleen samples yielded negative results. Therefore, despite wild boars do not seem to act as reservoirs of *Rickettsia* spp. in the MAB, they could be favouring tick dispersion and promoting *Rickettsia* spp. circulation among ticks by sustaining abundant tick populations and facilitating the transmission via co-feeding.

Wild boars in the MAB also carried *Campylobacter* spp., *C. lanienae* being more prevalent than *C. coli*, and different serovars of *Salmonella enterica* subsp. *enterica.* There was a high genetic diversity among *Campylobacter* isolates, some of which showed a high virulence potential.

None of the *Campylobacter* isolates were susceptible to all the antimicrobials tested, and nearly 60% of *C. coli* isolates and one *Salmonella* isolate were multiresistant, the latter being a monophasic *S.* Typhimurium clone of public health concern in Europe. These results provide further evidence on the role of wild boars as reservoirs of zoonotic thermophilic *Campylobacter* species, and show that they can carry and spread these foodborne zoonotic bacteria into urban and peri-urban areas in the MAB.

Results from this thesis have management and public health implications, and several management measures derived from these results are currently being applied and scientifically evaluated in Barcelona. This thesis contributes to improve the incipient knowledge of wild boars in urban and peri-urban areas from an ecological, epidemiological and applied management approach.

1.1. RESUM

Els recursos disponibles al medi urbà poden ser aprofitats per diverses espècies de fauna salvatge, entre elles el porc senglar (*Sus scrofa*), que porta temps explorant àrees urbanes i peri-urbanes. Això causa problemes i riscs per a la seguretat i la salut de les persones. Les interaccions entre persones i porcs senglars van en augment, especialment a les àrees urbanitzades d'Europa, paral·lelament a les tendències creixents de les seves poblacions. Aquesta tesi identifica els factors que determinen la presència de porcs senglars a l'àrea urbana de Barcelona, descriu els canvis fenotípics dels porcs senglars urbans respecte als no urbans, i posa de manifest l'existència de riscs sanitaris derivats de dita presència a un àrea urbana.

La presència de porcs senglars a la ciutat de Barcelona va ser més freqüent en punts pròxims a les rieres que baixen del massís de Collserola cap a la ciutat, en paisatges urbans fragmentats i amb major presència de zones verdes i colònies de gats de carrer. Així mateix, també va ser més abundant durant la primavera i l'estiu, possiblement degut a la major grandària dels grups familiars en aquestes èpoques i als seus majors requeriments energètics després dels parts a la primavera, a la dispersió de juvenils i subadults, i a la menor disponibilitat d'aliment d'origen natural durant l'època de calor a les regions mediterrànies. A més, els porcs senglars urbans van consumir més aliment d'origen antropogènic, van tenir un creixement més accelerat, assolint una major massa corporal que els no urbans. Les femelles d'origen urbà també començaren a reproduir-se abans que les d'origen no urbà, probablement pel fet que arribaren abans al pes mínim requerit per a la reproducció. No obstant això, l'esperança de vida es va veure minvada en els porcs senglars urbans, assenyalant una possible conseqüència negativa derivada d'explorar la ciutat.

Aquesta tesi també descriu la presència de patògens zoonòsics en el porc senglar i en les seves paparres. Els porcs senglars de l'Àrea Metropolitana de Barcelona (AMB) es trobaren parasitats per paparres de les espècies *Hyalomma lusitanicum*, *Dermacentor marginatus*, *Rhipicephalus sanguineus* sensu lato i, de forma anecdòtica, *Rhipicephalus bursa*. Alhora, les paparres analitzades foren portadores de tres espècies zoonòsiques i emergents de *Rickettsia* (*R. massiliae*, *R. slovaca* i *R. raoultii*), tot i que les mostres de melsa de porc senglar en foren negatives. Per tant, malgrat que els porcs senglars no semblen actuar com a reservoris de *Rickettsia* spp. a l'AMB, sí que podrien afavorir la dispersió de paparres i desenvolupar un paper en la circulació de *Rickettsia* spp. de manera indirecta en afavorir el cicle, l'abundància i la dispersió de les paparres. A més, podrien facilitar la transmissió de *Rickettsia* spp. entre les paparres en alimentar-se simultàniament del mateix hoste.

Tanmateix, els porcs senglars de l'AMB també van ser portadors de dues espècies de *Campylobacter*, essent *C. lanienae* més prevalent que *C. coli*, i diversos serotips de *Salmonella enterica* subsp. *enterica.* Els aïllats de *Campylobacter* mostraren una gran diversitat genètica, i alguns a més van exhibir un gran potencial de virulència. Tots els aïllats de *Campylobacter* van ser resistents com a mínim a un dels antimicrobians analitzats, i prop del 60% dels aïllats de *C. coli* i un aïllat de *Salmonella* van mostrar multirresistència. Aquest aïllat de *Salmonella* és la variant monofàsica del serotip Thyphimurium, amb un perfil de resistència que s'ha relacionat amb problemes de salut pública a Europa. Aquests resultats confirmen la importància del porc senglar com a reservori d'espècies de *Campylobacter* zoonòsiques i termòfiles, i posen de manifest que els porcs senglars poden ser portadors i potencials propagadors d'aquests bacteris a zones urbanes i peri-urbanes.

Els resultats d'aquesta tesi són rellevants tant per a la gestió del porc senglar a zones urbanes, com per a la protecció de la salut pública a l'AMB. Diverses mesures de gestió derivades dels resultats d'aquesta tesi ja s'estan aplicant i avaluant a la ciutat de Barcelona. Aquesta tesi contribueix de forma transcendent a la millora del coneixement sobre el porc senglar urbà amb una perspectiva ecològica, epidemiològica i de gestió.

1.2. RESUMEN

Ciertas especies de fauna salvaje pueden explotar los recursos disponibles en ambientes urbanos. Los jabalíes (*Sus scrofa*) llevan años adentrándose en zonas urbanas y peri-urbanas, con los riesgos que esto supone para la seguridad y la salud de las personas. La interacción entre jabalíes y personas sigue una tendencia ascendente, en concordancia con el aumento de sus poblaciones, especialmente en zonas urbanizadas de Europa. Esta tesis identifica los factores determinantes de la presencia del jabalí en zonas urbanas de Barcelona, describe los cambios fenotípicos mostrados por jabalíes urbanos respecto a los no urbanos, y pone de manifiesto la existencia de riesgos sanitarios asociados a la presencia de jabalíes en una zona urbana.

La presencia de jabalíes en la ciudad de Barcelona fue más frecuente en puntos cercanos a torrenteras que bajan del macizo de Collserola hacia la ciudad, en zonas urbanas con alta fragmentación del paisaje y con mayor presencia de zonas verdes y de colonias de gatos callejeros. La presencia de jabalíes también fue más abundante en primavera y verano, posiblemente debido al mayor tamaño de los grupos familiares y a sus mayores necesidades energéticas tras los nacimientos en primavera, a la dispersión de juveniles y subadultos y a la menor disponibilidad de alimento de origen natural que se produce en la estación cálida en ambientes mediterráneos. Además, los jabalíes urbanos consumieron más comida de origen antropogénico, crecieron más rápido y alcanzaron una mayor masa corporal que los jabalíes no urbanos. De igual manera, las hembras de origen urbano también se reprodujeron antes que las de origen no urbano, probablemente debido a que alcanzaron antes el peso necesario. Por otro lado, la esperanza de vida fue menor en jabalíes urbanos que en no urbanos, lo que indica que explorar la ciudad tuvo un coste asociado.

Esta tesis también describe la presencia de patógenos zoonósicos en jabalíes y en sus garrapatas. Los jabalíes del Área Metropolitana de Barcelona (AMB) estaban parasitados por garrapatas de las especies *Hyalomma lusitanicum*, *Dermacentor marginatus*, *Rhipicephalus sanguineus* sensu lato y, de forma anecdótica, *Rhipicephalus bursa*. Las garrapatas, a su vez, portaban tres especies de *Rickettsia* (*R. massiliae*, *R. slovaca* y *R. raoultii*), las tres zoonósicas y emergentes, si bien las muestras de bazo de jabalí fueron todas negativas. Por lo tanto, pese a que los jabalíes no parecen actuar como reservorios de *Rickettsia* spp. en el AMB, sí que podrían estar favoreciendo la dispersión de garrapatas y contribuyendo de manera indirecta a la circulación de *Rickettsia* spp. al favorecer con su abundancia el ciclo, la abundancia y la

dispersión de las garrapatas. También podrían estar facilitando la transmisión de *Rickettsia* spp. entre garrapatas al alimentarse simultáneamente del mismo hospedador.

Los jabalíes del AMB también portaban dos especies de *Campylobacter*, siendo más prevalente *C. lanienae* que *C. coli*, así como varios serotipos de *Salmonella enterica* subsp. *enterica*. Los aislados de *Campylobacter* mostraron una gran diversidad genética, y algunos también presentaron un gran potencial de virulencia. Todos los aislados de *Campylobacter* fueron resistentes como mínimo a uno de los antimicrobianos analizados. Cerca del 60% de los aislados de *C. coli* y un aislado de *Salmonella* fueron multirresistentes. Este aislado de *Salmonella* es la variante monofásica del serotipo Typhimurium, cuyo perfil de resistencia se ha relacionado con problemas de salud pública en Europa. Estos resultados confirman la importancia del jabalí como reservorio de especies de *Campylobacter* zoonósicas y termófilas, y muestran que los jabalíes del AMB pueden ser portadores y propagar estas bacterias a zonas urbanas y peri-urbanas.

Los resultados de esta tesis tienen relevancia para la gestión del jabalí en zonas urbanas y la protección de la salud pública en el AMB. De hecho, varias medidas de gestión que se derivan de los resultados de esta tesis ya se están aplicando y evaluando en Barcelona. Esta tesis contribuye, por tanto, a la mejora del conocimiento sobre el jabalí urbano con un enfoque ecológico, epidemiológico y de gestión.

2. INTRODUCTION

2.1. Ungulates: trends, relevance and conflict

In recent decades, ungulate populations are recovering from large declines suffered between the 19th and 20th centuries, mostly thanks to changes in land use and cover (reforestation, reduction of free-ranging livestock), species management (reintroductions, changes in hunting regimes), and legal protection, in addition to an increase in respectful human attitudes towards wildlife (Deinet et al., 2013; Linnell & Zachos, 2011; Martínez-Abraín, Jiménez, & Oro, 2019).

Ungulates are a natural renewable resource; more than 5.2 million are hunted every year in Europe, producing 120,000 tonnes of meat and a potential hunting revenue of several hundred million euros (Apollonio, Andersen, & Putman, 2010). Ungulates also have aesthetic and cultural value, as they are symbolic species with local histories and traditions associated (Kenward & Putman, 2011). Moreover, grazing and browsing from wild ungulates are a normal part of balanced ecological dynamics of woodlands, and ungulates modulate ecosystem processes such as nutrient cycles, net primary production and abiotic disturbance (e.g. fire regimes) (Hobbs, 1996; Putman, 1996).

However, ungulates have also become a nuisance due to damages to agricultural crops, forestry, traffic accidents, and because they can act as reservoirs of diseases shared with livestock and people (Barrios-Garcia & Ballari, 2012; Bruinderink & Hazebroek, 1996; Côté, Rooney, Tremblay, Dussault, & Waller, 2004; Putman, 1996). Furthermore, they can exert negative effects on the growth and regeneration of plant species, altering the relative abundance and composition of plant communities (Putman, 1996; Rooney & Waller, 2003).

Therefore, there is currently a need for ungulate population management, and the approach used should consider ungulates as part of the ecosystem as well as being adaptive, that is, subjected to changes based on scientific evidence (Apollonio et al., 2017).

2.2. Wild boar

2.2.1. Taxonomy and distribution

The Eurasian wild boar (*Sus scrofa* Linnaeus, 1758) is an artiodactyl mammal from the Suidae family, within the paraphyletic group of ungulates (Groves & Grubb, 2011). According to previous taxonomy classifications, there were 16 different subspecies recognised within the Eurasian wild boar species (Groves, 2007; Groves & Grubb, 1993). More recently, most of these subspecies were elevated to the species level, and currently there is a minimum of eight species and several subspecies within the *S. scrofa* group (Groves & Grubb, 2011). This new

classification, however, needs to be complemented with genetic, genomic, morphometric and reproductive analyses (Keuling et al., 2017). The species *S. scrofa* is the one present in most of Europe (including Spain), the Middle East and North Africa.

Wild boars, together with their domesticated forms, feral pigs and hybrids among them, are one of the most widely distributed mammals over the world. Native to Eurasia, wild boars and their domesticated forms have been introduced by humans throughout the globe, with some introductions dating back to the fifth millennium BC, and they are currently present in all the continents but Antarctica (Keuling et al., 2017; Long, 2003).

2.2.2. Habitat and diet

Wild boars live in habitats with dense vegetation, such as forests and scrublands, which provide food and shelter (Abaigar, Del Barrio, & Vericad, 1994; Meriggi & Sacchi, 2001), but they also can inhabit agricultural areas (Herrero, García-serrano, & García-gonzález, 2008; Virgós, 2002). Factors such as hunting disturbance and seasonal changes in food and shelter availability affect their habitat use (Boitani, Mattei, Nonis, & Corsi, 1994; Santos, Mexia-de-Almeida, & Petrucci-Fonseca, 2004; Spitz & Janeau, 1995), highlighting the plasticity of this species. Furthermore, in recent years they have been increasingly present in urban and peri-urban areas (Licoppe et al., 2013).

Wild boars are omnivorous and opportunistic, and their diet depends on energy requirements, region, season and food availability (Ballari & Barrios-García, 2014). Plant matter is the main component of their diet and includes mast, roots, green plant matter and agricultural crops (Schley & Roper, 2003). High-energy seeds such as acorns and pine seeds are preferred (Massei, Genov, & Staines, 1996), and agricultural crops including maize, rice, wheat, sorghum and potatoes, among others, are part of the wild boar diet when available (Herrero, García-Serrano, Couto, Ortuño, & García-González, 2006; Schley & Roper, 2003). Animal matter constitutes a small proportion of the wild boar diet, which they obtain through scavenging or predation (Barrios-Garcia & Ballari, 2012; Giménez-Anaya, Herrero, Rosell, Couto, & García-Serrano, 2008), and it includes invertebrates (earthworms, insects and snails, among others), small mammals, fish, amphibians, reptiles and birds (Schley & Roper, 2003).

2.2.3. Activity, movement and social organisation

In general, wild boars are nocturnal (Brivio et al., 2017), although some populations also show activity during daylight, mainly when undisturbed by human activities (Keuling, Stier, & Roth,

2008; Podgórski et al., 2013). Searching for food is the main driver of wild boar movements (Morelle et al., 2015), and they show high intraspecific variability in home range size, with described annual home ranges from 100 to over 6,000 ha (Maillard & Fournier, 1995; Podgórski et al., 2013).

Wild boars form matriarchal groups composed of one to several adult females and their offspring from the current and previous years, while adult males are usually solitary but also join female groups during the rut season (Dardaillon, 1988; Kaminski, Brandt, Baubet, & Baudoin, 2005). Regarding dispersion, most wild boars remain within or close to the natal range (Keuling, Lauterbach, Stier, & Roth, 2010; Podgórski, Scandura, & Jedrzejewska, 2014).

2.2.4. Reproduction

Wild boar is a multiparous species, having from 3.6 to 7.6 piglets per litter (Fonseca, da Silva, Alves, Vingada, & Soares, 2011), although lower values have been reported under drought conditions (Fernández-Llario & Carranza, 2000). The life-history strategy of wild boar, towards the fast end of the fast-slow continuum, is different from what would be expected from a mammal of its size; wild boar exhibits high fecundity despite its relatively large size (Bieber & Ruf, 2005; Focardi, Gaillard, Ronchi, & Rossi, 2008; Oli, 2004). Litter size and prevalence of pregnancies depends on female age and body mass (Massei et al., 1996; Šprem, Piria, Prđun, Novosel, & Treer, 2016), food availability, and climate (Bieber & Ruf, 2005; Fernández-Llario & Carranza, 2000; Frauendorf, Gethöffer, Siebert, & Keuling, 2016; Santos et al., 2006). Gestation duration is approximately 115 days (Henry, 1968b) and pregnancy within the first year of life is possible if females reach a body mass of 30-35 kg (Fernández-Llario & Mateos-Quesada, 1998; Sabrina, Jean-Michel, Carole, Serge, & Eric, 2009). In Europe, there is a peak of farrowing from March through May (Fonseca et al., 2011; Gethöffer, Sodeikat, & Pohlmeyer, 2007), but under good environmental conditions births can happen outside this period (Fernández-Llario & Mateos-Quesada, 1998).

2.2.5. Increasing abundance, distribution and conflicts

Estimating wild boar population numbers is difficult due to their aggregated distribution, nocturnal activity and habitat preferences (Náhlik et al., 2017). Nonetheless, wild boar populations have increased and expanded across Europe in the last decades, as shown by increasing hunting bags, vehicle collisions and damage to crops (Massei et al., 2015; Sáez-Royuela & Tellería, 1986). Probably more than three million wild boars were harvested in 2012

in all Europe, while this number was less than 900,000 individuals twenty years before that (Massei et al., 2015).

The geographic and demographic expansion of wild boar could be explained, as already argued more than 30 years ago (Sáez-Royuela & Tellería, 1986), by a combination of socio-economic changes including the depopulation of rural areas, changes in agricultural practices, lack of predators, limited hunting, additional food, reintroductions, and mild winters. Nowadays, authors still agree on most of these causes, but they highlight the importance of 1) supplementary food (agricultural crops, food provided by hunters, food available in human-dominated areas) since it favours wild boar survival and reproduction (Geisser & Reyer, 2005); 2) reforestation after the abandonment of traditional activities, which increases food and shelter (Meriggi & Sacchi, 2001); and 3) insufficient hunting, as recreational hunting is not enough to control the wild boar population growth (Massei et al., 2015).

Regarding the lack of predators, it seems to affect the growth and density of wild boar populations, but only when the wild boar density is low and/or the habitat is not optimal (Kanzaki, Perzanowski, & Nowosad, 1998). Likewise, global increasing temperatures can alleviate the negative effect of cold winters on wild boar survival and reproduction, but mainly at high latitudes (Melis, Szafrańska, Jędrzejewska, & Bartoń, 2006; Vetter, Ruf, Bieber, & Arnold, 2015). Despite hybridisation between wild boars and domestic pigs has occurred in some regions and could improve the reproductive success of female wild boar (Fulgione et al., 2016), it seems to be a minor source of genetic variation for wild boar populations (Scandura, Iacolina, & Apollonio, 2011).

The increase in wild boar abundance and distribution has generated conflicts with humans due to crop damages (Schley, Dufrêne, Krier, & Frantz, 2008), road traffic accidents (Lagos, Picos, & Valero, 2012) and increased risk of disease transmission to people and livestock (Meng, Lindsay, & Sriranganathan, 2009). Furthermore, negative impacts on plant and animal communities due to rooting, predation and habitat destruction have been reported (Barrios-Garcia & Ballari, 2012; Massei & Genov, 2004).

2.2.6. Management

Wild boar management depends on the legal framework and the magnitude of the wild boar-human conflicts to deal with (Náhlik et al., 2017). In Europe, hunting is the main mortality factor of the species (Keuling et al., 2013), although there is a mismatch between the number of hunted wild boar and the number of hunters (Massei et al., 2015). Hunters are aware of the

problems, solutions and factors contributing to wild boar population increase, but most of them do not feel responsible for their management (Keuling, Strauß, & Siebert, 2016).

Hunting periods vary among countries, and hunting techniques include drive-hunts (with beaters and dogs), stalk hunting and lookout hunting (with or without bait) (Náhlik et al., 2017). Several studies suggest that the most effective ways to stop further population increases are the reduction of supplementary feeding and the selective hunting of juveniles, yearlings and adult females, depending on the environmental conditions (Bieber & Ruf, 2005; González-Crespo et al., 2018). Other management methods, mainly where hunting is not allowed, include trapping and euthanasia, fertility control, fencing, repellents, diversionary feeding and translocations (Massei, Roy, & Bunting, 2011).

2.3. Diseases of wild boar

Wild boars can host many pathogens shared with livestock, companion animals and humans (Meng et al., 2009), and the increasing distribution and abundance of wild boar populations are increasing the risk for disease transmission (Ruiz-Fons, 2017). In rural areas, wild boars pose a threat primarily to the livestock industry, especially the pig industry, because they are reservoirs of pathogens that may have been eradicated in domestic pigs but still circulate in wild boar populations (Wu et al., 2011; Wyckoff, Henke, Campbell, Hewitt, & VerCauteren, 2009).

The Catalan wildlife health surveillance programme includes diseases mainly due to their relevance to the livestock industry, but also to human and wildlife health. The diseases under surveillance in wild boar are classical swine fever (CSF), African swine fever (ASF), swine vesicular disease, Aujeszky's disease, bovine tuberculosis, brucellosis and trichinellosis (Generalitat de Catalunya, 2016).

With natural habitats being replaced by human-altered ones, new scenarios appear where environmental changes linked to urbanisation can affect the transmission of zoonotic pathogens (Bradley & Altizer, 2007). Moreover, evidence suggests that the loss of biodiversity, as occurs in urbanised areas, frequently tends to increase disease transmission (Keesing et al., 2010). Thus, in an urban context, shared diseases other than the ones commonly included in wildlife health surveillance plans have more relevance because of the risk for people and pets (instead of livestock).

2.3.1. Parasitic diseases

Trichinellosis is a parasitic zoonosis caused by a nematode that affects mammals almost all over the world (Dupouy-Camet, 2000), and it is, perhaps, the most notorious wild boar-transmitted disease among hunters. There are several species within the genus *Trichinella*; *T. spiralis* and *T. britovi* are the most relevant for humans, and transmission occurs through the consumption of raw or undercooked meat containing larvae, primarily from pork (Gottstein, Pozio, & Nöckler, 2009). There have also been cases linked to wild boar products (Fichi et al., 2015). Since some *Trichinella* species resist freezing, the best way to prevent human infestation is by cooking the meat at a certain combination of temperature and time (Kotula, Murrell, Acosta-Stein, Lamb, & Douglass, 1983).

Another worldwide parasitic zoonosis is toxoplasmosis, caused by *Toxoplasma gondii*. This protozoan affects a wide variety of mammal and avian species and has been detected in different wild boar populations, also in Spain (Calero-Bernal et al., 2015; Fredriksson-Ahomaa, 2019). Most cases in adult humans are subclinical, but toxoplasmosis can be severe and cause death in foetuses, children and immunocompromised people. Humans can acquire *T. gondii* through the consumption of raw or undercooked meat containing cysts, or by drinking water contaminated with oocysts (Hill & Dubey, 2002). Domestic cats might be the major source of contamination, since they shed millions of oocysts (Dubey, 2001). Cooking is, as with *Trichinella* spp., a proper method to kill tissue cysts and prevent infestation (Dubey, Kotula, Sharar, Andrews, & Lindsay, 1990).

Ticks are blood-feeding ectoparasites that can cause paralyses, toxicoses, allergic reactions, and are among the most important vectors of viral, bacterial and protozoan pathogens to humans, livestock and pets (Estrada-Peña & Jongejan, 1999; Jongejan & Uilenberg, 2004; Parola & Raoult, 2001). One of the factors influencing the life cycle of ticks, and therefore the circulation of tick-borne pathogens (TBPs), is their host communities, since the abundance of reservoir hosts can greatly affect the prevalence of such pathogens (Estrada-Peña & de la Fuente, 2014). Ticks are receiving increasing attention in recent years because the spectrum of tick-borne diseases of domestic animals and humans has increased, as well as the list of potential TBPs (Dantas-Torres, Chomel, & Otranto, 2012).

2.3.2. Bacterial diseases

Rickettsiales infections (rickettsiosis, anaplasmosis and ehrlichiosis) have public health and veterinary relevance and are transmitted in most cases via tick bite. Subclinical infections have been detected in wildlife and several wild species are considered reservoirs, but whether these pathogens pose a threat to their health is still uncertain (Birtles, 2012). Many *Rickettsia* species cause disease to humans, with thousands of cases reported from 2000 to 2010 across Europe, where rickettsioses are regarded as emerging diseases (ECDC, 2013; Jones et al., 2008).

Campylobacter spp. are Gram-negative bacteria distributed worldwide among wild and domestic animals, and wild birds and mammals are regarded as reservoirs (Speck, 2012). Campylobacteriosis is the most commonly reported zoonosis in Europe since 2005, with over 246,000 confirmed human cases in 2017 (EFSA and ECDC, 2018). *Campylobacter* species isolated from wildlife include *C. coli*, *C. jejuni* and *C. lanienae*, among others, and while the two first species have been associated to disease or lesions in food-producing and companion animals, no clinical disease has been described in free-ranging wild animals (Schweitzer et al., 2011; Speck, 2012).

Salmonellosis is the most commonly reported zoonosis in Europe after campylobacteriosis, with nearly 92,000 confirmed human cases in 2017 (EFSA and ECDC, 2018). The genus *Salmonella* includes two species of Gram-negative bacteria: *S. enterica* and *S. bongori*. The former is divided into six subspecies, each of which includes different serotypes, and *S. enterica* subsp. *enterica* is mostly formed by warm-blooded animal pathogens (Gaffuri & Holmes, 2012). Human infections are mainly caused by food contaminated with *Salmonella* spp. and wildlife infections probably occur through ingestion of contaminated water or food. Many wildlife species carry *Salmonella* without signs of clinical disease, although clinical infections have been described in free-living animals, mainly birds but also mammals including wild boar (Gaffuri & Holmes, 2012). Together with *Campylobacter* spp., *Salmonella* spp. has been identified as one of the most prone bacteria to be transmitted from wild swine to humans in the near future (Ruiz-Fons, 2017).

Streptococcus suis is an emerging zoonotic agent (Goyette-Desjardins, Auger, Xu, Segura, & Gottschalk, 2014). Human cases of meningitis by *S. suis* have been associated to contact with wild boar carcasses (Halaby et al., 2000; Rosenkranz et al., 2003), and this is especially relevant since urban wild boars from Barcelona harbour *S. suis* strains with identical molecular profile as local human cases (Fernández-Aguilar et al., 2018).

Leptospira interrogans causes leptospirosis, which is a widespread emerging zoonosis affecting livestock, pets and humans. It can be acquired through urine-contaminated water or soil and most human infections are mild, although they can also be severe and lead to death (Levett, 2001). *Leptospira* spp. has also been identified as one of the most prone bacteria to be transmitted from wild swine to humans (Ruiz-Fons, 2017), and antibodies against *Leptospira* spp. have been detected in wild boars in the city of Berlin (Jansen et al., 2007).

Q fever is another widespread zoonotic disease caused by *Coxiella burnetii*. This disease is usually asymptomatic or causes mild clinical signs in humans, although it can sometimes lead to severe complications. Domestic animals are frequently the source of infection for humans, and over 40 species of ticks can acquire *C. burnetii*. However, the main route of *C. burnetii* transmission to humans is airborne (Maurin & Raoult, 1999). *Coxiella burnetii* circulates in wildlife, including wild boars in Spain, and they are a potential source of infection for humans (Astobiza et al., 2011). Moreover, different species of domestic animals, wild boar and humans share the same *C. burnetii* genotypes (Jado et al., 2012), and antibodies against *C. burnetii* have been found, again, in urban wild boars from Berlin (Henning, Angela, & Wittstatt, 2015).

Mycobacterium bovis, within the *Mycobacterium tuberculosis* complex, is the causative agent of bovine tuberculosis, a disease with huge socio-economic impact (Caminiti et al., 2016; O'Reilly & Daborn, 1995). Bovine tuberculosis affects cattle and other ruminants, as well as humans and wildlife, leading to persistent infection with granulomas in lymph nodes, lung and other internal organs when becoming systemic (Wedlock, Skinner, De Lisle, & Buddle, 2002). In continental Western Europe, Spain has a complex epidemiology involving different *Mycobacterium* species and hosts (Gortázar et al., 2012). Wild boar can become infected from cattle and then turn into a reservoir and the source of *M. bovis* for livestock, even in areas where the risk is supposed to be low (Mentaberre et al., 2014). *Mycbacterium bovis* also causes tuberculosis in humans, although the majority of infections are caused by *M. tuberculosis* (Wedlock et al., 2002). Wild boars can be a source of infection especially for hunters and veterinarians (Meng et al., 2009).

Another bacterial agent with relevance in the livestock industry is *Brucella suis*, which causes brucellosis and leads to abortions and infertility in pigs, wild boars and other animals, with the consequent economic losses, especially in developing countries (McDermott, Grace, & Zinsstag, 2013; Meng et al., 2009). Wild boars can be a reservoir of *B. suis* (Szulowski et al., 2015) and a risk of contagion to domestic pigs (Risco et al., 2014). *Brucella suis* also cause brucellosis in humans, who suffer from fever and reproductive problems, and can be infected through

handling wild boar carcasses (Eales, Norton, & Ketheesan, 2010; Franco-Paredes, Chastain, Taylor, Stocking, & Sellers, 2017).

2.3.3. Viral diseases

Hepatitis E virus (HEV) causes hepatitis E in humans, an endemic disease in tropical and subtropical regions that has been increasingly detected in developed countries (Emerson & Purcell, 2003; Wichmann et al., 2008). It occurs with increased severity in pregnant women and children, and its main way of transmission is faeco-oral, for instance through contaminated water (Panda, Thakral, & Rehman, 2007), although human cases have been linked to the consumption of raw or undercooked wild boar products (Kim et al., 2011; Matsuda, Okada, Takahashi, & Mishiro, 2003). Moreover, a recent study reported that 59% of wild boars from Barcelona were seropositive, and HEV RNA was detected in 20% of their faeces (Wang et al., 2019).

CSF is a major disease for the pig industry that causes economic losses (Terpstra & de Smit, 2000). It produces high mortality in young pigs and wild boars, although CSF outbreaks are generally self-limiting in wild boar populations after initial mortality (Ruiz-Fons, Segalés, & Gortázar, 2008). Wild boars can serve as reservoir hosts and as a source of infection for domestic pigs (Artois et al., 2002).

Aujeszky's disease affects suids, which are its natural hosts, although it can also affect other mammals. It is caused by the pseudorabies virus, and it is another relevant disease of domestic pigs worldwide (Müller et al., 2011). Aujeszky's disease is also widespread in wild boar populations, including Spain (Gortázar et al., 2002; Müller et al., 2011), and transmission between pigs and wild boars is possible, although the sylvatic and domestic epidemiological cycles seem to be independent (Charrier et al., 2018). Moreover, this disease usually causes death in dogs, and hunting dogs can become infected by eating infected wild boar tissues (Cay & Letellier, 2009; Leschnik et al., 2012).

ASF, which affects both pigs and wild boars, among other suids, causes massive losses in the pork industry due to mortality, eradication costs and trade restrictions (Costard, Mur, Lubroth, Sanchez-Vizcaino, & Pfeiffer, 2013; Halasa et al., 2016). In 2007, ASF was reintroduced into continental Europe (in Georgia) and it has since then expanded towards Western Europe (Gavier-Widén et al., 2015). The last infected country was Belgium, in late 2018 (Linden et al., 2019), when the closest affected countries were Poland and Hungary, thus increasing the risk of ASF being introduced to the neighbouring countries including France (Andraud, Halasa, Boklund,

& Rose, 2019). Wild boars can play an important role in the spread of ASF, but contact with infected domestic pigs or other sources seems necessary to maintain the virus circulation in wild boar populations (Mur et al., 2012).

Porcine circovirus type 2 (PCV2) is worldwide distributed in the domestic pig, and has been linked to a number of diseases. Among these, postweaning multisystemic wasting syndrome has the biggest impact on pig production (Segalés, Allan, & Domingo, 2005). PCV2 antibodies have been detected at high rate in different wild boar populations in Europe, including Spain, although infections are often subclinical (Vicente et al., 2004). Wild boar PCV2 viruses or close relatives have been found in domestic pigs, indicating that virus transmission between the two porcine species would be possible (Cságola, Kecskeméti, Kardos, Kiss, & Tuboly, 2006).

2.4. Wildlife in an urbanising world

Urbanisation affects land use and cover, hydrosystems, biogeochemical cycles, climate and biodiversity, which are five major types of global environmental change (Grimm et al., 2015). Focusing on biodiversity, urbanisation reduces species richness and alters species composition, causing physical and biological homogenization simultaneously (Chace & Walsh, 2006; McKinney, 2006). With the spread of urbanised areas, new opportunities arise for individuals or populations that are able to exploit new resources (DeStefano & DeGraaf, 2003), while many others disappear along with their natural habitat (McKinney, 2006).

Urban wildlife ecology has received more attention since the 20th century (Adams, 2005; DeStefano & DeGraaf, 2003). Generalist species do better in urban environments than habitat specialists, and most studies have focused on birds (Adams, 2005). Wildlife species can adopt different strategies, from thriving and even depending on urban areas to survive (urban exploiters) to just taking advantage of the resources this environment offers, but still depending on the natural habitat (urban adapters; Blair, 1996; Shochat, Warren, Faeth, McIntyre, & Hope, 2006).

Urban wildlife species can experience changes in behaviour, morphology or genetics as a response to urban selection pressures (Shochat et al., 2006). These changes may come from the ability of a species to alter the phenotype within its lifetime (phenotypic plasticity; DeWitt & Scheiner, 2004), and can result in shifts in genotypes (microevolution), which do not necessarily indicate a process of adaptation to the urban environment because this would confer a genetically based fitness advantage (Donihue & Lambert, 2015; McDonnell & Hahs, 2015).

Conversely, genetic differences between urban and non-urban populations might arise from founder events and random genetic drift, as is the case of red foxes (*Vulpes vulpes*) in the city of Zurich (Wandeler, Funk, Largiadèr, Gloor, & Breitenmoser, 2003).

From a human perspective, biodiversity in urban ecosystems can improve quality of life and education of human population (Savard, Clergeau, & Mennechez, 2000), but the increases in wild populations and their interactions with humans can lead to a change in the perception of these species from beneficial to pest (DeStefano & DeGraaf, 2003; Kansky & Knight, 2014). Human-wildlife conflicts arise when the needs or behaviour of wildlife negatively affect humans, or when human activities negatively affect the needs of wildlife (Madden, 2004). However, any conflict between people and wildlife can make a certain species be perceived by people as problematic, even without consistent evidence, because human perception is shaped by past interactions with a species (Angelici, 2016; Conejero et al., 2019; Kansky & Knight, 2014). Thus, in urban and peri-urban areas, wildlife management currently faces two opposite challenges: the habitat and biodiversity loss, and the problem of overabundant wildlife species (DeStefano & DeGraaf, 2003).

2.4.1. Urban wild boar

During the last years, increasing attention has been paid to the presence of wild boar in urban and peri-urban areas (Amendolia, Lombardini, Pierucci, & Meriggi, 2019; Cahill, Llimona, Cabañeros, & Calomardo, 2012), the derived problems (Fernández-Aguilar et al., 2018; Jansen et al., 2007; Kotulski & Koenig, 2008), and the relationship between wild boars and the urban environment (Cahill et al., 2012; Stillfried, Gras, Börner, et al., 2017). Easy access to food and/or water, absence of or insufficient hunting pressure outside the peri-urban area, expanding urbanisation into the wild boar habitat, and perhaps rivers and highways as routes of entry seem to favour wild boar entrance and/or establishment in these areas (Licoppe et al., 2013; Toger, Benenson, Wang, Czamanski, & Malkinson, 2018). Urban food sources include plant matter available in parks and gardens, rubbish and food left for stray cats (Cahill et al., 2012; Licoppe et al., 2013; Stillfried, Gras, Busch, et al., 2017).

Different European cities such as Berlin, Vienna, Cracow and Rome, among others, have wild boar as visitors (Kotulski & Koenig, 2008; Licoppe et al., 2013). In Spain, wild boar presence has been reported in several cities including Barcelona, Zaragoza, Pamplona and Las Rozas de Madrid (Cahill et al., 2012; Licoppe et al., 2013; López, López, Gavela, Bosch, & Ballesteros, 2010; Sanz, 2000). Wild boar presence in the streets causes public concern due to damages to

green spaces through rooting (Licoppe et al., 2013) (Figure 2.1), traffic accidents (Zuberogoitia et al., 2014) (Figure 2.2), risk of disease transmission to people and pets (Fernández-Aguilar et al., 2018), and risk of attacks (Silwal, Kolejka, & Sharma, 2016).

Figure 2.1. Urban green space damaged by wild boars through rooting.

Figure 2.2. Dangerous situation caused by wild boars in an urban area.

Wild boars can be seen as urban adapters (Stillfried, Fickel, et al., 2017; Stillfried, Gras, Busch, et al., 2017), which are those species relatively associated to the urban environment, i.e. that still use their natural habitat but can exploit urban resources (Blair, 1996; McKinney, 2006). Urban wild boars can adjust their spatiotemporal behaviour to avoid human disturbance (Podgórski et al., 2013), or develop a higher tolerance to human disturbance (Stillfried, Gras, Börner, et al., 2017). In addition, urban wild boar stomachs show a high proportion of food from anthropogenic origin (Hafeez, Abbas, Khan, & Rehman, 2011), possibly meaning that they change their foraging behaviour to exploit food resources in the city. Regarding morphology, adult female wild boars habituated to humans have higher body mass than non-habituated ones (Cahill et al., 2012).

The abovementioned changes would be non-evolutionary adaptive responses, which are adjustments of physiology, behaviour or morphology in response to urbanisation, not necessarily related to changes in gene frequency nor to benefits in their survival or reproduction in the new area (McDonnell & Hahs, 2015; Ricklefs, 1990). Since the wild boar is widespread and flexible in its responses to different situations (i.e. phenotypically plastic; (Gamelon et al., 2013; Podgórski et al., 2013), it is a good model to study how a medium to large mammal responds to urban environmental change.

2.4.1.1. Urban wild boar in Barcelona and Collserola

The wild boar population in Collserola has been increasing in recent years (González-Crespo et al., 2018), according with the aforementioned world trend, to reach a peak density of almost 15

wild boars per 100 ha in the hunting season 2012-2013 (Minuartia, 2018). The Serra de Collserola Natural Park, within the Collserola massif, is a protected natural area that receives three million visitors annually (Generalitat de Catalunya, 2010; Parc de Collserola, n.d.-C). Approximately 30% of its surface is covered by urban or peri-urban areas, and the Collserola wild boar population is probably partly isolated from other populations because it is surrounded by rivers, roads and other human infrastructures (Cahill & Llimona, 2004) (Figure 2.3).

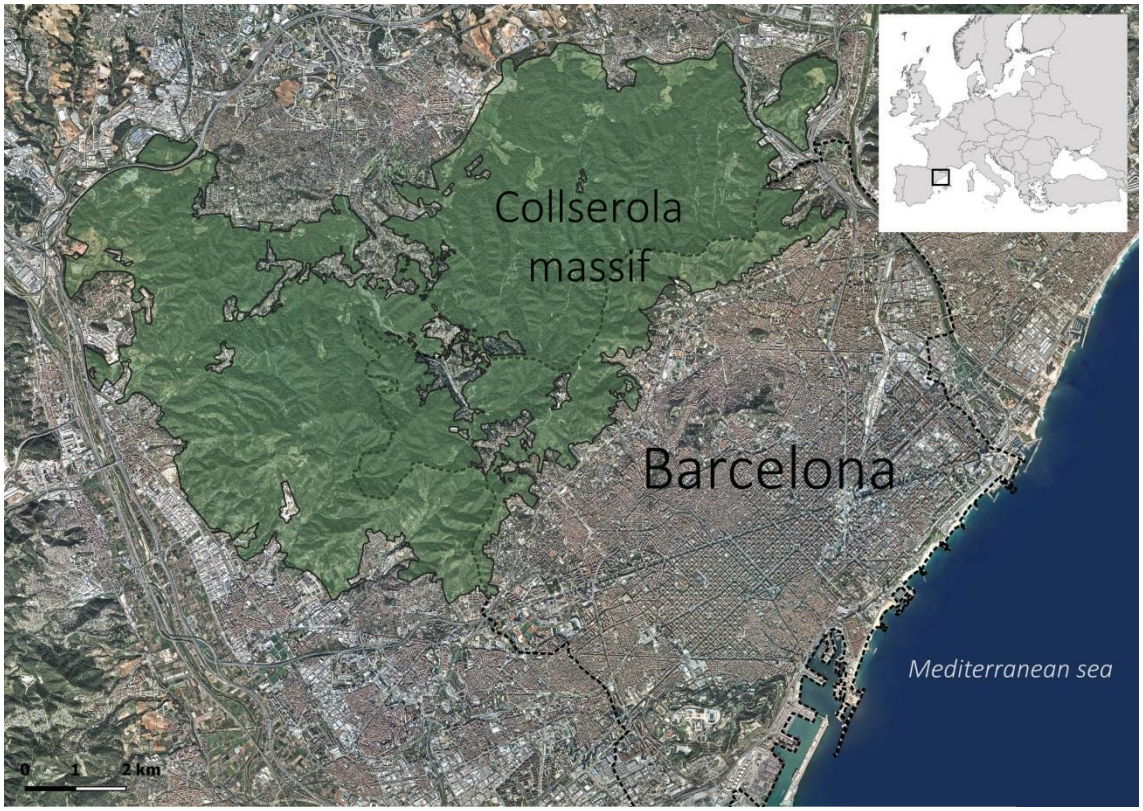

Figure 2.3. Collserola massif and the city of Barcelona. Orthophoto: Institut Cartogràfic i Geològic de Catalunya (ICGC).

In this metropolitan context, isolation caused by barriers, increased urbanisation (and the food resources therein) and the abovementioned adaptable nature of wild boars might explain why they approach urban and peri-areas during periods of food scarcity (Cahill, Llimona, & Gràcia, 2003; Llimona et al., 2007). Barcelona is the biggest municipality bordering Collserola, and it is home to 1,600,000 people (Institut d'Estadística de Catalunya, 2019). In recent years, the wild boar has been approaching the city (Figure 2.4), originating an increasing trend of conflicts from two incidents registered in 1998 (Llimona et al., 2007) to over 1,100 calls to the local emergency number in 2016 (Ajuntament de Barcelona, 2018).

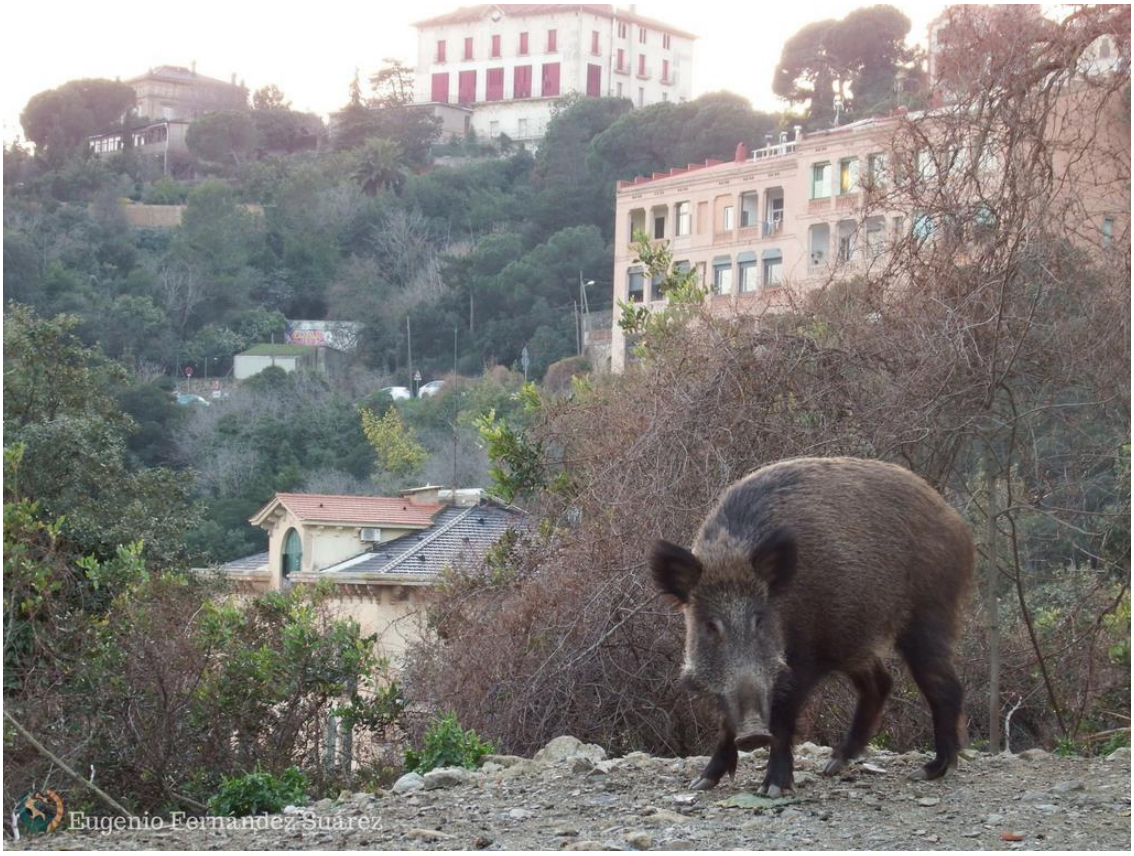

Figure 2.4. Wild boar in the Barcelona-Collserola interface. Credit: Eugenio Fernández Suárez.

This thesis determines the drivers of wild boar presence in the urban area of Barcelona (Study 1), addresses the phenotypic changes shown by wild boars captured in Barcelona with respect to wild boars hunted in Collserola (Study 2), and identifies TBPs and foodborne pathogens carried by ticks and wild boars in the Metropolitan Area of Barcelona (MAB) (Studies 3 and 4).

2.5. Hypotheses

The hypotheses of this thesis are:

1. Wild boar presence in Barcelona does not follow a random pattern, but is determined by temporal, spatial and environmental drivers (Study 1).

2. Adjusting to the urban environment must be related to behavioural, morphological, physiological, and life-history changes in wild boar (Study 2).

3. Wild boars in the Metropolitan Area of Barcelona (MAB) can carry parasites and zoonotic pathogens of public health concern (Studies 3 and 4).

2.6. Objectives

Consequently, the objectives of this thesis are:

1. To identify the factors driving the presence of wild boar in the urban area of Barcelona. (Study 1).

2. To measure the phenotypic changes shown by wild boars in response to urbanisation in Barcelona (Study 2).

3. To provide a scientific basis for decision-making on management measures in Barcelona (Studies 1 and 2).

4. To identify the tick species parasitizing wild boars in the Metropolitan Area of Barcelona (MAB) (Study 3).

5. To describe the tick-borne pathogens infecting ticks and their wild boar hosts in the MAB (Study 3).

6. To identify zoonotic *Campylobacter* spp. and *Salmonella* sp. in wild boar faeces from the MAB (Study 4).

7. To determine the genetic diversity, virulence potential and antimicrobial susceptibility of *Campylobacter* spp. and *Salmonella* sp. carried by wild boars in the MAB (Study 4).

3. STUDIES

3.1. Study 1

Urban wild boars prefer fragmented areas with food resources near natural corridors

Science of the Total Environment, 615 (2018): 282-282

3.1.1. Abstract

Wild boar populations are expanding throughout the world and intruding into peri-urban and urban areas. In the last years, wild boar has colonized several European cities, including our study area, the city of Barcelona. It is required to identify the main factors driving wild boar into urban areas prior to establish management measures.

We built Boosted Regression Trees using 3,148 wild boar presences registered in the urban area of Barcelona from 2010 to 2014 to identify the variables correlated with these presences. The variables analysed included proxies for distance to the source population, urban food resources, climate and urban habitat structure.

Wild boars enter the urban area from close natural habitat using corridors such as streams, preferably in fragmented urban environment, looking for food such as urban green areas or dry pet food from cat colonies. Wild boar presence is higher in spring possibly due to the births of piglets and the dispersion of yearlings during that season, and also when natural resources in the Mediterranean habitat fail to satisfy the nutritional requirements of the wild boar population during the summer season.

Management measures derived from this study are currently being applied in the city of Barcelona, including vegetation clearings in the wild boar entrance areas and an awareness campaign aimed at reducing the anthropogenic food availability for wild boars. The methodology used can be applied to other cities with wild boar or even other wildlife species issues. The comparison between the factors attracting wild boars into different urban areas would be helpful to understand the global phenomenon.

Keywords: Boosted Regression Trees, Mediterranean climate, native invader, wildlife management, *Sus scrofa*, urban area

3.1.2. Introduction

Wild boar populations are geographic and demographically increasing throughout the world as a result of socio-economic and ecological changes, such as natural forest regeneration, increased anthropogenic food resources, limited hunting and translocations (Massei et al., 2011; Sáez-Royuela & Tellería, 1986; Snow, Jarzyna, & VerCauteren, 2017). The movement ecology of wild boar, together with its high diet plasticity and high prolificacy, have also contributed to the worldwide spread of wild boar populations (Morelle et al., 2015). As a

consequence of these human-induced changes and natural features of the wild boar, the interaction between wild boars, humans and the environment have increased, namely crop damages, road traffic accidents, increased risk for shared diseases including zoonoses, altered food webs and damage to some plant and animal species (Massei et al., 2011).

This way, wild boar has been acting as a native invader, spreading within their historical range, reaching extreme abundances and producing severe effects on other species (Carey, Sanderson, Barnas, & Olden, 2012). The concept "native invader" was introduced by Simberloff (2011) to refer to a species that has become invasive in its own native distribution range, but has not been widely applied to wild boar yet. However, it is a clear example of a human-wildlife conflict, where management strategies applied try to mitigate the symptoms rather than address the causes, and then both social and political challenges arise (Carey et al., 2012).

The easy access to food and water, the absence or insufficient hunting pressure and the expanding urbanisation into the forested areas occupied by wild boar have similarly favoured its intrusion into peri-urban and urban areas. Rivers and roads act as corridors which facilitate wild boar occurrence in these areas (Licoppe et al., 2013). The risks and disturbances associated with the wild boar presence in urban environments include, apart from traffic accidents, damages to street furniture, parks and private gardens, and ransacking of rubbish bins and containers. They can also share diseases with pets and humans (Meng et al., 2009) and may occasionally attack people (Cahill et al., 2012).

In the last years, wild boar has colonized the urban and peri-urban environment in several European cities, including Barcelona, Berlin, Budapest, Genoa and Warsaw (Licoppe et al., 2013). In Barcelona, the wild boar enters the city mainly from the bordering Collserola massif, a natural area where wild boar presence was anecdotal in the 80s. Since then, and in accordance with the global trends, Collserola wild boar population has increased to reach a density over nine wild boars per 100 ha (Minuartia, 2016). This increase may have been favoured by the increased urbanisation inside the massif and therefore the increased anthropogenic food available for wild boars (domestic rubbish, vegetable material from parks and gardens, pet food and direct feeding). This might also have attracted wild boar into peri-urban and urban areas during periods of scarcity (Cahill et al., 2012).

Alternative approaches based on integrated landscape and social management are necessary to reduce wild boar populations (Cahill et al., 2003). Moreover, to specifically deal with wild boar presence in urban areas it is required to identify the main drivers, in order to establish

management measures to reduce their attractiveness. Although several studies have investigated the factors related to a species presence or distribution, only a few focus in urban wild species, and to the authors' knowledge no study has tried to identify the factors favouring wild boar presence in urban areas.

The aim of this study is identifying the factors determining the presence of wild boars inside the urban area, considering the distance to the source population, climate, food resources and urban habitat structure, to provide scientific knowledge allowing the implementation of management measures. Since wild boar adaptation to urban environments is widespread and increasing, both the analytical approach performed and the results might be useful for other urban areas where wild boar is or will shortly be conflictive.

3.1.3. Material and methods

Study area

The study area is the municipality of Barcelona (Catalonia, NE Spain) (Figure 3.1), which has a 10,100 ha area and a human population of 1,600,000 inhabitants (Institut d'Estadística de Catalunya, 2016). The urban area of Barcelona spreads between the Mediterranean seashore (SE) and the border with the Collserola massif (NW), and between the Besòs (E) and Llobregat (W) rivers. Barcelona landscape is mostly urban but green spaces represent a 35.3% (3611 ha) of the city surface, including the Collserola surface which falls within the city limits. Excluding this part of the massif, green areas represent an 18.1% (1850 ha) of Barcelona surface. Despite the existence of several large parks, the 84% of urban green spaces are less than half hectare in size and are, in general, isolated (Ajuntament de Barcelona, 2013).

Collserola massif is an 11000 ha natural area belonging to the Catalan coastal mountain range with an abundant wild boar population (DARP, 2015). It is a hilly area, altitude ranging between 60 and 512 metres above sea level. It is completely surrounded by human infrastructures and receives about two million visitors yearly. Collserola has a Mediterranean climate; mean annual temperature is 15°C (average temperature is rarely below 5°C in winter, and around 21°C in summer). The annual average rainfall is over 620 mm with two wet seasons, autumn and spring (October, 83.1 mm; May, 60.4 mm), and a dry summer period (July, 10.6 mm) (Parc de Collserola, n.d.-a). Mediterranean pine forests dominated by *Pinus halepensis* (40%) and sclerophyllous woodlands with *Quercus ilex* (15%) cover a large part of the massif, but Mediterranean and sub-Mediterranean scrubs also represent an important part of the vegetation (24%) (Pérez-Haase & Carreras, 2012).

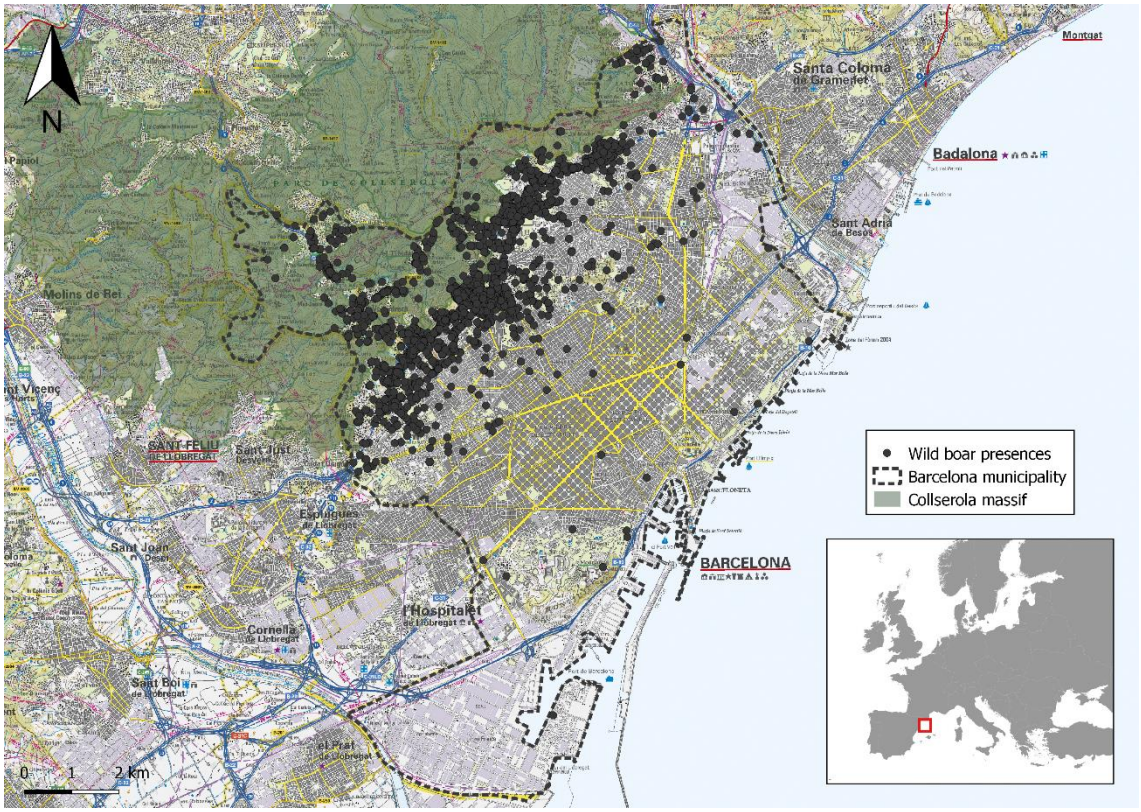

Figure 3.1. Study area. Dashed line shows the limit of the municipality of Barcelona (Catalonia, NE Spain). Shaded area corresponds to the Collserola massif. The wild boar presences are represented as dots. Map: ICGC.

Data collection

The Metropolitan Police registered the location and date of 3148 wild boar presence in the urban area of Barcelona from 2010 to 2014. A wild boar presence means that a minimum of one wild boar was present in the public street at a certain time, alive, wounded or dead (after a car accident, for instance). Most of the sightings were reported by citizens through a phone call to the local emergency number. The wild boar presences were processed to avoid pseudo-replication, retaining only one presence if two or more data occurred within 500 m from each other within a two hour frame. Therefore, only 2621 (1.4 observations/day on average) of the 3148 presences registered were finally included in the analysis. Figure 3.1 shows a map of Barcelona municipality with the refined wild boar presences.

Data creation and modelling

We created wild boar pseudoabsences as a random sample of locations to compare the presences with, following (Barbet-Massin, Jiguet, Albert, & Thuiller, 2012). Both wild boar presences and pseudoabsences were characterized with factors accounting for climate,

distance to source population, urban food resources and habitat structure. Table 3.1 shows the predictor variables constructed for the analysis. We used QGIS v2.8.1 Meng (Quantum GIS Development Team, 2015) and Fragstats v4.2.1. software (McGarigal, Cushman, & Ene, 2012).

Table 3.1. Explanatory variables initially considered in the study. Sources of the data, references which support the variable selection, mean values and standard deviation are included. 1: Variables included in the first model. 2: Variables included in the second model. * Data for cat colonies were available only from the districts bordering Collserola massif and only for 2014.

Indicator/proxy for	Explanatory variable	Source	Mean ± standard deviation	
			Presences	Pseudoabsences
Distance to source population	Distance to Collserola massif (DistC, in m)	Catalonia land cover map (CREAF and Generalitat de Catalunya 2009). Following Jaeger (2000) for landscape fragmentation.	532 (± 353)	3391 (± 1,879)
	Distance to watercourses[1,2] (DistW, in m)		602 (± 372)	3572 (± 1,861)
Urban food resources	Total surface of urban green area in a 250 m buffer (Green250, in ha)		4.7 (± 5.9)	2.9 (± 2.2)
	Total surface of urban green area in a 500 m buffer[1,2] (Green500, in ha)		17.6 (± 24)	10.6 (± 1.1)
Habitat structure	Landscape fragmentation in a 250 m buffer (Frag250, in ha)		5.9 (± 2.6)	9.9 (± 2.1)
	Landscape fragmentation in a 500 m buffer[1,2] (Frag500, in ha)		18.9 (± 6.7)	31.9 (± 9.7)
Seasonality	Date[1] (Julian day)	Unpublished data, Barcelona Metropolitan Police	183 (± 252)	228 (± 173)
	Minimum daily temperature (MinT, in ºC)	Servei Meteorològic de Catalunya 2015	14.1 (± 1.8)	11.6 (± 2.1)
	Mean daily temperature (MeanT, in ºC)		17.5 (± 0.4)	14.8 (± 2.3)
	Maximum daily temperature[1,2] (MaxT, in ºC)		22.4 (± 0.7)	19.5 (± 3.8)
	Number of days without raining over 10 mm[1,2] (Rain10)		24 (± 6.4)	25.8 (± 8.5)
Urban food resources	Proximity to cat colonies[2] (Cats, in m)*	Unpublished data, Barcelona city Council 2014	299 (± 335.2)	1159 (± 547)

Before modelling, we used R software (version 3.1.3; R Development Core Team, 2015) to perform an exploratory analysis following the protocol proposed by Zuur, Ieno, & Elphick (2010), checking for multicollinearity amongst the explanatory variables by calculating Pearson's correlation coefficients. When the variables were highly related (coefficient≥0.7; Dormann et al., 2013), only one was retained for model construction (Table 3.1).

We then built Boosted Regression Trees (BRT) using the gbm library developed by Ridgeway (2015) for R software (Elith, Leathwick, & Hastie, 2008) to identify those variables more correlated with the wild boar presence in the urban area of Barcelona. We also estimated the

relative influence of the predictor variables with the formulae developed by Friedman (2001) and implemented in the R gbm library. A first analysis included the whole urban area of Barcelona for the complete study period. However, and since 76.8% (2014 out of the 2621) of the refined wild boar presences were located in four districts bordering the Collserola massif (four districts out of ten for the whole Barcelona), a second analysis was performed to fine-tune the factors attracting wild boar in urban areas, adding to the aforementioned variables the proximity to cat colonies as an explanatory variable (Table 3.1).

In order to evaluate the resulting model, we partitioned the data into two subsets: 70% for constructing (training) and 30% for testing the model (Acevedo, Jiménez-Valverde, Lobo, & Real, 2012; Elith et al., 2008). We fitted the model with 10-fold cross-validations, testing different values for learning rate (which determines the contribution of each tree to the growing model) and tree complexity (which controls whether the interactions are fitted).

We used the area under the receiver operating characteristic (ROC) curve (AUC) to measure the discriminatory capacity of models, following (Jiménez-Valverde, 2012). To assess the model performance, we calibrated the model by testing the goodness of fit through the Hosmer-Lemeshow (HL) test (Lemeshow & Hosmer, 1982).

3.1.4. Results

The correlation coefficients amongst all the variables analysed can be found in Supporting information (Table S.3.1). The variables without collinearity retained for each one of the two models developed are shown in Table 3.1.

The learning rate (0.005) and tree complexity (10), altogether with bag fraction (0.5), determined 2100 as the number of trees necessary to achieve the lowest estimated cross-validation deviance in the BRT models (Figure 3.2a). The performance of the selected model explained 65.3% of the deviance of our data and its discrimination power as measured by the AUC was 93.1%. The H-L test did not show significant deviations between the predicted and the observed values (H-L: $\chi 2$=13.93 and p-value=0.08), so the model was well calibrated.

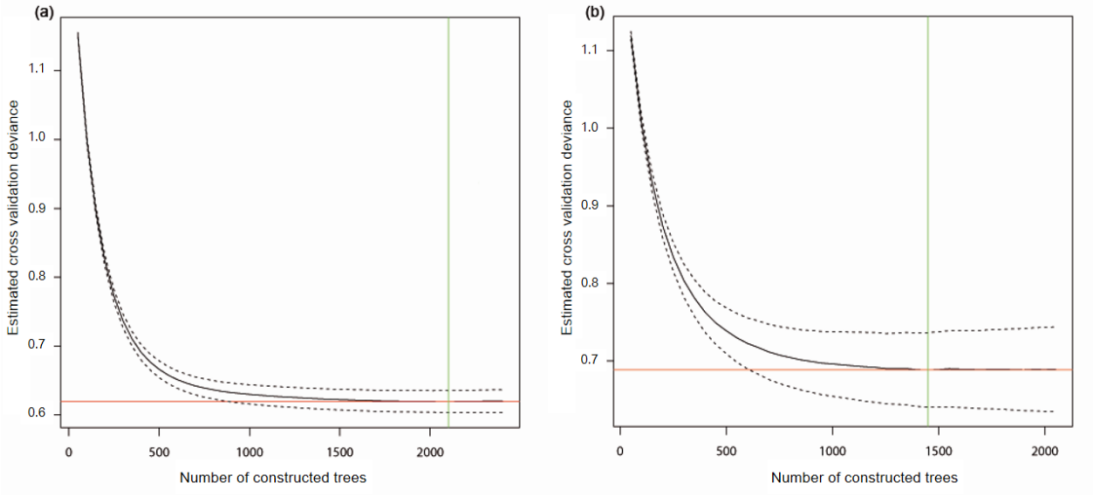

Figure 3.2. Cross-validation model-fitting for the parameters learning rate=0.005, tree complexity=10 and bag fraction=0.5. Vertical line indicates the number of trees fitted and horizontal line indicates the mean residual deviance achieved. 3.2a: 2100 trees were fitted. Mean residual deviance achieved was 0.42, which corresponds to an explained variance of the data of 65.3%. 3.2b: This model included Cats variable. 1450 trees were fitted and mean residual deviance achieved was 0.37, which corresponds to an explained variance of the data of 67.2%.

The variable with the strongest effects on predicting wild boar occurrence in the urban area of Barcelona was the distance to a watercourse (66.1% of relative importance). Landscape fragmentation and surface of urban green area (in a 500 m buffer) had a similar lower influence (10.2 and 9.2%, respectively), followed by date (6.7%), maximum daily temperature (4.8%) and accumulated days without raining over 10mm (3%) (Figure 3.4a).

When including proximity to cat colonies as a predictor variable and reducing the study area to the districts bordering Collserola massif, the model constructed 1450 trees and the explained deviance increased to 67.2% (Figure 3.2b). The AUC (discrimination power) was 91.9%. The relative importance was more distributed amongst the variables, although the distance to a watercourse continued to be the most important variable with a 35.2% of influence. Proximity to cat colonies was placed in the fifth position of importance (10.6%) and the rest of the predictors remained similar to the model without this variable (Figure 3.4b). However, this second model was not well calibrated, showing significant differences between the predicted and the observed wild boar presences (H-L: $\chi 2$=16.9 and p-value=0.03).

The partial responses of each explicative variable indicated that wild boar presences are more likely closer than 3000 m from the entry of a stream into the urban area and 1500 m from a cat colony, when included. Wild boar presences are also more common in highly fragmented urban

landscapes, with green areas around (between 8 and 25 ha in a 500 m diameter area) and from March to October (Figures 3.3 and 3.4). Maximum temperature and number of days without raining had little effect on the response in the first model, but were more important in the second one, with wild boar presence being associated to maximum temperatures between 21 and 28 ^{o}C and 10 to 40 days without raining over 10 mm (Figure 3.4). Density plots for all the predictor variables comparing presences and pseudoabsences are shown in Supporting information (Figure S.3.1).

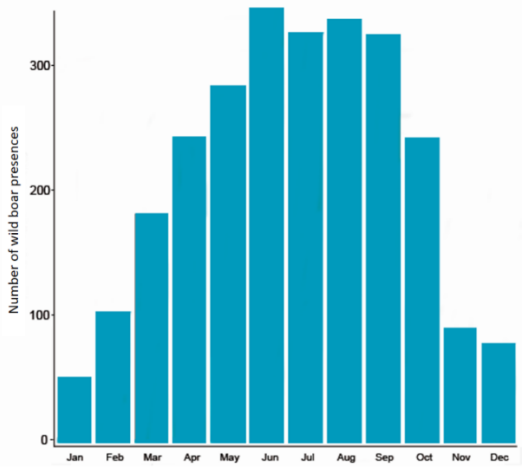

Figure 3.3. Seasonal trend of the wild boar presences in the urban area of Barcelona. It represents the monthly addition of the presences throughout the study period (2010 – 2014).

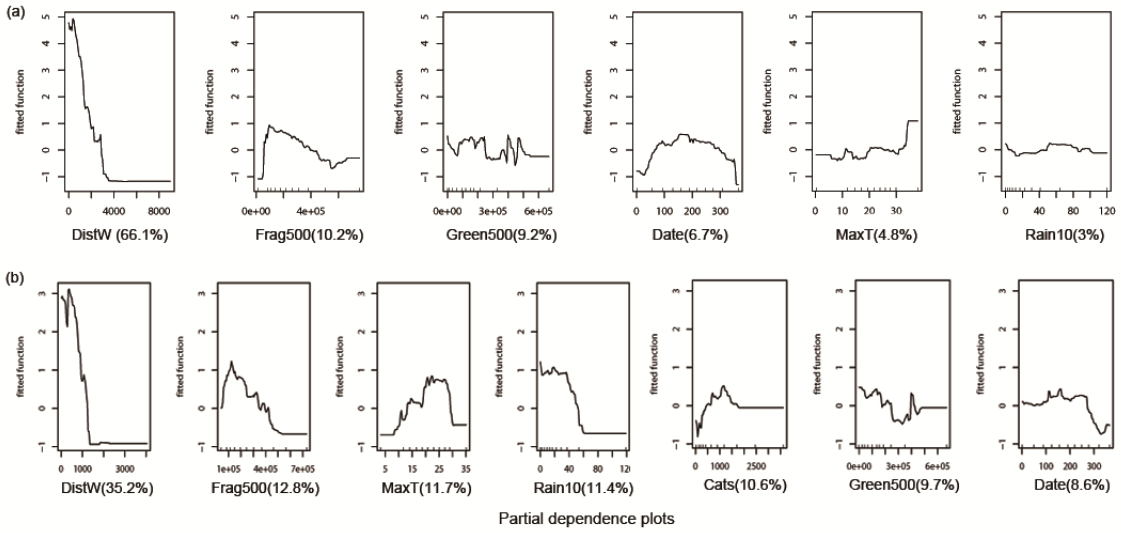

Figure 3.4. Partial dependence plots for BRT models developed using tree complexity of 10 and learning rate of 0.005, showing the dependencies of wild boar presence on each of the predictors. In brackets: summary of the relative contributions of predictor variables for the BRT models, in order of importance. 3.4a: Without including distance to cat colonies as a variable in the model, wild boar apparition is related with short distances to watercourses (DistW, < 3000 m), high landscape fragmentation (Frag500), urban green areas (Green500, mostly between 8 and 25h of surface) and from February to October (Date).

Maximum temperature (MaxT) and days without raining (Rain10) had little effect on the response. 3.4b: Including cat colonies as a variable (Cats), wild boars tend to appear near these sites (< 1500 m). The rest of predictors remained similar, except for MaxT (21 – 28 ^{o}C) and Rain10 (10 – 40 days without raining over 10 mm).

3.1.5. Discussion

This study provides evidence on the factors attracting wild boars into urban environments, therefore allowing undertaking control measures to correct and/or modify such factors. Both the methodology used and the results obtained can be useful as a reference for other cities already or near-to-be colonized by this native invader. The local rising trend in urban wild boar occurrences observed in Barcelona during the five-year study period agrees with the increasing global reports (Cahill et al., 2012; Kotulski & Koenig, 2008; Licoppe et al., 2013; Podgórski et al., 2013) to suggest that this issue will probably become still more relevant in the near future.

Spatial distribution of wild boar

Wild boars harvested in urban areas come predominantly from rural environments and not from the city (Stillfried, Fickel, et al., 2017). Accordingly, the spatial variables revealed Collserola massif as the source of wild boars entering Barcelona but, more importantly, pointed streams as corridors for this entry, even if dry in summer, most probably due to the dense vegetation cover. Humid areas with dense vegetation are important for wild boar movement (Morelle, Fattebert, Mengal, & Lejeune, 2016) and streams can serve as movement pathways or corridors (Rosenberg et al., 1997) which increase landscape connectivity (Beier & Noss, 1998) and would facilitate the entrance of wild boars into the city.

Wild boars make more displacements in urban areas due to dispersed resources (Podgórski et al., 2013). Hence, it can be easier for a wild boar to find a suitable patch (e.g. with refuge -vegetation cover-, food or water resources) in a landscape comprising different and interspersed patch types rather than a vast single patch (i.e. a built-up area with large buildings and without green areas). According to our model, the probability of wild boar occurrence is higher in the fragmented areas of Barcelona, which could be due to a higher likelihood of finding scattered resources in the urban matrix and/or to a higher change for wild boars to get disoriented. Urban green areas and cat colonies partially explained the wild boar spatial distribution within the urban area because wild boars probably use them as a source of food and water in periods of scarcity, as already suggested by (Cahill et al., 2012). Moreover, green patches can also be used as a daylight resting area, as urban adapted red foxes do in urban

environments (Adkins & Stott, 1998; Harris, 1981; Marks & Bloomfield, 2006). In comparison to Berlin, the best example of an urban wild boar city, Barcelona green spaces have less forest cover and occupy less total area (18.1% *vs*. 31.4% of city surface; Senate Department for the Environment, Transport and Climate Protection, 2017). But both cities are close to forested areas where wild boar lives, thus agreeing with the importance of the first variable selected by our models.

The attractiveness of dry cat food for urban carnivores has been previously reported (Contesse, Hegglin, Gloor, Bontadina, & Deplazes, 2004; Theimer, Clayton, Martinez, Peterson, & Bergman, 2015), but to the authors' knowledge this is the first time it is described for urban wild boars. The spatial aggregation of wild boars and cats in feeding points raises public health concerns on disease sharing and zoonosis transmission such as salmonellosis, toxoplasmosis, hepatitis E, tuberculosis and methicillin resistant *Staphylococcus aureus* (Meng et al., 2009; Mentaberre et al., 2014; Porrero et al., 2013; Tauni & Österlund, 1999).

Seasonal distribution of wild boar

In spite of the modest weight in the models of date, maximum temperature and accumulated days without raining, the seasonal pattern seems to be related with the climate. In Mediterranean climate, urban landscape primary production during summer is higher than in non-urban landscapes, due to irrigation and alteration of natural land cover by cultivation of introduced plant species (Imhoff, Tucker, Lawrence, Stutzer, & Rustin, 2000). The water availability in Mediterranean habitats is low during summer, with dry streams and hardened soils which make rooting difficult, whilst green areas are irrigated in the urban area. Furthermore, the summer food scarcity period in the natural environment agrees with the growth of family groups due to births mainly in spring and the dispersal of the yearlings born the previous year (Cahill et al., 2003; Keuling, Stier, & Roth, 2007).

Conversely, the period with less probability of wild boar records in urban areas (November to February) agrees with the higher food availability in Mediterranean habitats (Massei et al., 1996; Schley & Roper, 2003), inlcuding the acorn fall in Collserola (in October, Cahill et al., 2012). The water availability is also higher than in summer, due to autumn rains and less evaporation resulting from milder temperatures. In addition, wild boar populations have lower food needs in winter as compared to spring and summer, when piglets and juveniles are born and growing, respectively.

Management applications

The city Council of Barcelona, in collaboration with the *Servei d'Ecopatologia de Fauna Salvatge* (SEFaS) of the *Universitat Autònoma de Barcelona* (UAB), is currently undertaking the first measures aimed at decreasing the entry of wild boars in the urban area of Barcelona. Previous studies have suggested reducing rural and peri-urban wild boar population as a measure to decrease wild boar penetration in the city (González-Crespo et al., in evaluation; Stillfried et al., 2017). Therefore, an effort is currently being made in Barcelona to reduce the population of peri-urban wild boars through selective capture and euthanasia. However, reducing this population may not have a lineal effect on wild boar presence in the urban area if the city is still attractive for wild boars. Consequently, the conclusions obtained from this study are currently being also used to apply management measures in Barcelona (Table 3.2). Vegetation clearings are being carried out in a 100 m wide fringe in the limit between the Collserola massif and the urban area to create a less comfortable transition for wild boars. Also, an awareness campaign aimed at reducing anthropogenic urban food availability for wild boars is being implemented following two main axes: increasing human population knowledge about the negative consequences of wild boar presence in the city and reducing dry pet food availability by providing cat colony feeders with wild boar-proof feeding devices. In a future phase, vegetation management of selected green urban areas will be applied to try to decrease their attractiveness to wild boar. All these measures will be monitored and their effect evaluated to implement or modify them according to the results obtained in reducing wild boar presence in Barcelona.

Table 3.2. Management applications of the drivers for wild boar presence in the urban area of Barcelona identified in this study.

Factor	Variable	Measure	Feasibility	Application in Barcelona
Distance to source population	Distance to streams	Vegetation clearings in conflictive areas between Collserola massif and the city	Feasible	Currently
Habitat structure	Urban landscape fragmentation	Ecological urbanism	Difficult	No
Urban food resources	Proximity to cat colonies	Wild boar-proof feeding devices	Feasible	Currently
	Surface of green areas	Vegetation change to native species and not irrigated green areas	Feasible	Future
	Unspecific food availability (direct and indirect feeding)	Awareness campaign	Feasible	Currently

Seasonality	Environmental variables (temperature, rain)	Management of the effects of high temperatures and drought in wild boar habitat	Counter-productive	No
	Wild boar population requirements	Rural and peri-urban wild boar population management (hunting or capture)	Difficult	Currently

3.1.6. Conclusions

This study shows that wild boars appear in the urban area of Barcelona mainly through natural corridors such as streams from the bordering Collserola massif and in fragmented urban landscape. They possibly enter into the city in the search for anthropogenic food resources, including (but not only) urban green areas and dry pet food. This happens mainly during the natural food shortage period, i.e. the warmer and drier months, when food resources are more abundant in the city than in the natural Mediterranean habitat and spatial and energetic demands of the wild boar population are higher.

The conclusions obtained from this study are currently being used to address the main drivers of wild boar presence in Barcelona. The methodology used in this study can be therefore applied to identify the main factors driving wild boar or even other wildlife species in urban contexts. However, these drivers may vary according to environmental and urban circumstances for each particular case (e.g., in northern latitudes winter might be a harsher period than summer for wild boar).

3.1.7. Acknowledgements

We want to express our gratitude to the *Ajuntament de Barcelona*, *Agents Rurals* and *Guàrdia Urbana de Barcelona* for collecting and providing part of the raw data used to develop this study. We also wish to thank João Carvalho and Emmanuel Serrano for their support in statistical analyses.

This research was funded by the contracts 13/051 and 15/0174 between the Universitat Autònoma de Barcelona and the Municipality of Barcelona. Raquel Castillo-Contreras benefitted from a PhD grant (2016FI_B 00425) co-financed by Generalitat de Catalunya (Secretaria d'Universitats i Recerca, Departament d'Economia i Coneixement) and the European Social Fund. João Carvalho was supported by a PhD grant (SFRH/BD/98387/2013) co-financed by the Fundação para a Ciência e a Tecnologia (FCT), the ESF and the Ministério da Educação e Ciência (MEC). Emmanuel Serrano research activity was co-financed by the FCT through the

postdoctoral program (SFRH/BPD/96637/2013), the University of Aveiro (Department of Biology), the FCT/MEC (CESAM RU UID/AMB/50017) and the FEDER within the PT2020 Partnership Agreement.

3.1.8. Supporting information

Table S.3.1. Pearson correlation coefficients of the explicative variables: date expressed as Julian day (Date), mean, maximum and minimum daily temperature (MeanT, MaxT and MinT), days accumulated without raining over 10 mm (Rain10), surface of urban green area in 250 m and 500 m buffers (Green250 and Green500), distance to Collserola massif (DistC), distance to a watercourse or a stream (DistW) and landscape fragmentation in 250 m and 500 m buffers (Frag250 and Frag500). Coefficients of the highly related variables (≥ 0.7) appear in bold.

	Date	MeanT	MaxT	MinT	Rain10	Green 250	Green 500	DistC	DistW	Frag250	Frag500
Date		0.4	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MeanT			**1.0**	**1.0**	0.1	-0.1	-0.1	0.0	0.0	0.0	0.0
MaxT				**0.9**	0.1	-0.1	-0.1	0.0	0.0	0.0	0.0
MinT					0.1	-0.1	-0.1	0.0	0.0	0.0	0.0
Rain10						0.0	0.0	0.0	0.1	0.0	0.0
Green250							**0.8**	-0.2	-0.2	-0.1	0.0
Green500								-0.2	-0.2	-0.2	-0.1
DistC									**1.0**	0.3	0.3
DistW										0.3	0.2
Frag250											**0.8**
Frag500											

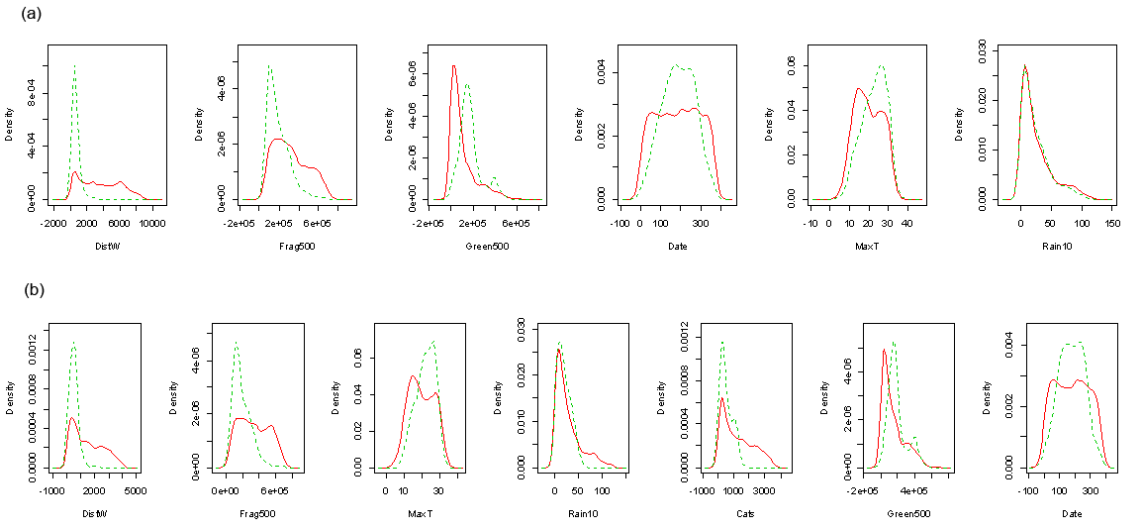

Figure S.3.1. Density plots showing the variable distribution for all predictors and wild boar presences (green dashed line) and pseudoabsences (red line). 5a: variables included in the first analysis. 5b: variables included in the second analysis. DistW: distance to a watercourse (in m), Frag500: landscape fragmentation in a 500m buffer (in ha, low values mean high fragmentation), Green500: surface of urban green area in a 500m buffer (in ha), Date: date expressed as Julian day, MaxT: maximum daily temperature (in C°), Rain10: accumulated days without raining over 10mm, Cats: distance to a cat colony (in m).

3.2. Study 2

Pigs in the city: Phenotypic responses to urban environmental change

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3.2.1. Abstract

Urbanisation is a global human-induced environmental change and one of the most important threats to biodiversity. However, some wildlife species thrive in urbanised areas even better than in natural ones. These species may adjust to the challenges posed by the urban environment either through behaviour, morphology, physiology and/or genetic changes. We aim to measure these changes in the urban wild boar (*Sus scrofa*), in the city of Barcelona (NE Spain), including body mass, growth rate, life expectancy, use of anthropogenic food, metabolic changes, female age at first reproduction and drivers of female breeding status. We compared data and samples from 445 and 183 wild boars from Barcelona (urban area) and Collserola (non-urban area), respectively, gathered from 2013 to 2019. Wild boars reached higher body mass in the urban area (mean body mass of adults 24 to 36 months old: 68.4 *vs*. 44.8 Kg), grew faster (mean growth rate: 1.8 *vs*. 1 Kg/month), but had shorter life expectancy at birth (mean age of death: 17.7 *vs*. 32.9 months). Urban wild boars used anthropogenic food sources more frequently than non-urban ones (39.2% *vs*. 4.4%), and had higher serum triglyceride (male adults) and urea (females) concentrations. First reproduction in females happened earlier in the urban area (12.5 *vs*. 16.2 months of age) and pregnancy was positively associated with body mass. Wild boars show behavioural, morphological, physiological and reproductive acclimatory responses to the urban environment. By changing their foraging behaviour to eat protein and fat-rich anthropogenic food, urban wild boars grow heavier and faster, which favours early reproduction and pregnancy probability in females. However, thriving in the urban environment might have a cost, as shown by the shorter life expectancy in the urban area.

Keywords: anthropogenic, body mass, growth, life expectancy, phenotypic plasticity, reproduction, *Sus scrofa*, weight, wild boar

3.2.2. Introduction

Urbanisation is a global, human-induced rapid environmental change (Sih, Ferrari, & Harris, 2011) and one of the most important threats to biodiversity, as it reduces species richness and increases biotic homogenisation (Chace & Walsh, 2006; Marzluff, 2001; McKinney, 2006). However, the so called synurbic species (Andrzejewski, Babińska-Werka, Gliwicz, & Goszczyński, 1978) or urban exploiters (Blair, 1996) can thrive in urban environments better than in their natural habitats and they even depend on anthropogenic resources to survive. Urban adapters, in turn, can live in urban areas but also utilise natural resources (Blair, 1996; McKinney, 2006).

On the contrary, urban avoiders cannot cope with the challenge of urbanisation and disappear from urban habitats.

Selective pressures such as predation or seasonal shortage in food and water are often less strict in urban environments (Shochat et al., 2006). However, the urban habitat entails novel challenges to wildlife such as anthropogenic disturbances of different nature (Fernández-Juricic & Tellería, 2000; Reijnen, Foppen, & Veenbaas, 1997) and increased disease transmission (Bradley & Altizer, 2007), although the effects of urban life on animals are species- and context-dependent (Birnie-Gauvin, Peiman, Gallagher, de Bruijn, & Cooke, 2016). Regarding population structure, urban environments can act as islands where a population is differentiated after an initial founder event, according to the 'urban island hypothesis' (Gloor, Bontadina, Hegglin, Deplazes, & Breitenmoser, 2001; Wandeler et al., 2003); but also become an attractive sink for rural dispersers, according to the 'population pressure hypothesis' (Gloor et al., 2001; Pulliam, 1988), if there are enough food resources and the population size is below carrying capacity due to human induced mortality (Delibes, Gaona, & Ferreras, 2001; Gundersen, Johannessen, Andreassen, & Ims, 2001).

The key to survive in human-altered habitats is adjusting to the new selection pressures (Lowry, Lill, & Wong, 2012), which can alter the behaviour, morphology, physiology and genetic structure of urban populations (McDonnell & Hahs, 2015; Shochat et al., 2006). Phenotypically plastic species achieve this by producing different phenotypes from the same genotype under different environmental conditions (DeWitt & Scheiner, 2004) and rates of phenotypic change are greater in human-related environments than in natural or non-urban anthropogenic environments (Alberti et al., 2017; Hendry, Farrugia, & Kinnison, 2008). For example, plastic species can exploit anthropogenic food sources (Lewis et al., 2015), use artificial structures as shelter (Herr, Schley, Engel, & Roper, 2010), reproduce earlier (O'Leary & Jones, 2006) or modify their communication signals (Slabbekoorn & Peet, 2003). However, the underlying mechanisms of phenotypic changes and whether they are linked to genetic adaptation, phenotypic plasticity or genetic drift are generally poorly known (Donihue & Lambert, 2015; Lowry et al., 2012). Vertebrate responses to urbanisation have been mostly studied in birds (Chace & Walsh, 2006; Marzluff, 2001), but also on small or medium sized urban mammals, such as European red foxes, European badgers *Meles meles* and raccoons *Procyon lotor* (Bateman & Fleming, 2012). However, less is known about large or medium-sized mammal changes in urbanised areas, with few studies involving the black bear *Ursus americanus* (Beckmann & Berger, 2003; Beckmann & Lackey, 2008), the white-tailed deer *Odocoileus virginianus* (Grund, McAninch, & Wiggers, 2002) and the Eurasian wild boar (Cahill et al., 2012;

Stillfried, Gras, Börner, et al., 2017). The final output –urban islands or (attractive) sinks– of urban environments will depend on the capability of the species to cope, adjust and/or adapt to specific urban challenges, both individually and at population level.

The wild boar and its domesticated forms, the feral pig and hybrids of wild boar and feral pig, are one of the most widely distributed mammals in the world (Keuling et al., 2017). Their population numbers and geographical range have been growing all over Europe for nearly five decades (Massei et al., 2015; Sáez-Royuela & Tellería, 1986), and reports on urban wild boar urbanisation are increasing accordingly (Cahill & Llimona, 2004; Cahill et al., 2012, 2003; Licoppe et al., 2013; Stillfried, Fickel, et al., 2017; Stillfried, Gras, Börner, et al., 2017). Since the wild boar fits both the description of urban adapter (Stillfried, Fickel, et al., 2017; Stillfried, Gras, Busch, et al., 2017) and phenotypically plastic (Gamelon et al., 2013; Podgórski et al., 2013), it is a suitable model to improve our knowledge about the adjustment of large mammals to urban areas (Stillfried, Gras, Börner, et al., 2017). Examples of wild boar phenotypic plasticity in urban areas are few but increasingly present in literature, mainly reporting behavioural changes (Davidson, Malkinson, & Shanas, 2018; Podgórski et al., 2013; Stillfried, Gras, Börner, et al., 2017). However, and to the best of our knowledge, there are no studies on wild boar physiological responses to urban environments such as growth and body mass gain, changes in reproduction, metabolism or life expectancy.

In our study area, in north-east Spain, wild boars have been exploring urban and peri-urban areas of Barcelona at least since 1998 (Minuartia, 2005). The city of Barcelona is next to the Collserola massif, a natural area where a growing wild boar population thrives (González-Crespo et al., 2018; Minuartia, 2017). A recent study showed that wild boars entered Barcelona mainly through streams and were attracted by green areas and cat colonies probably because of the food available there (Castillo-Contreras et al., 2018). In line with this, Cahill et al. (2012) reported that human-habituated (i.e. not afraid of people) female wild boar from peri-urban areas in Collserola were heavier than non-habituated ones and the authors attributed it to the consumption of food from anthropogenic origin.

In this study, our aim is to measure the phenotypic changes shown by an urban adapter species, the wild boar, in the city of Barcelona. The variables studied include body mass, growth rate, life expectancy, use of anthropogenic food, metabolic changes (serum concentrations of triglycerides, urea and creatinine), age at first reproduction in females and drivers of breeding status. This information will provide new insights into the ecology of urban wildlife and be useful for establishing measures to manage human-wild boar conflicts in urban areas.

3.2.3. Material and methods

Study areas

Barcelona municipality, in north-east Spain, has 1,600,000 inhabitants in 10,100 ha of surface (Idescat, 2017). It is a city predominantly urbanised, although with 1,077 ha of public urban green areas (11% of the city surface; Ajuntament de Barcelona, 2013). Barcelona is located south-east to Collserola, a massif that belongs to the Catalan coastal range. It is approximately 10,000 ha in surface (Generalitat de Catalunya, 2010), partially protected (over 8,000 ha, as Serra de Collserola Natural Park), and wild boar hunting is allowed on approximately 30% of the surface (two hunting areas: 2,309 and 767 ha, respectively; DARP, 2018) (Figure 3.5).

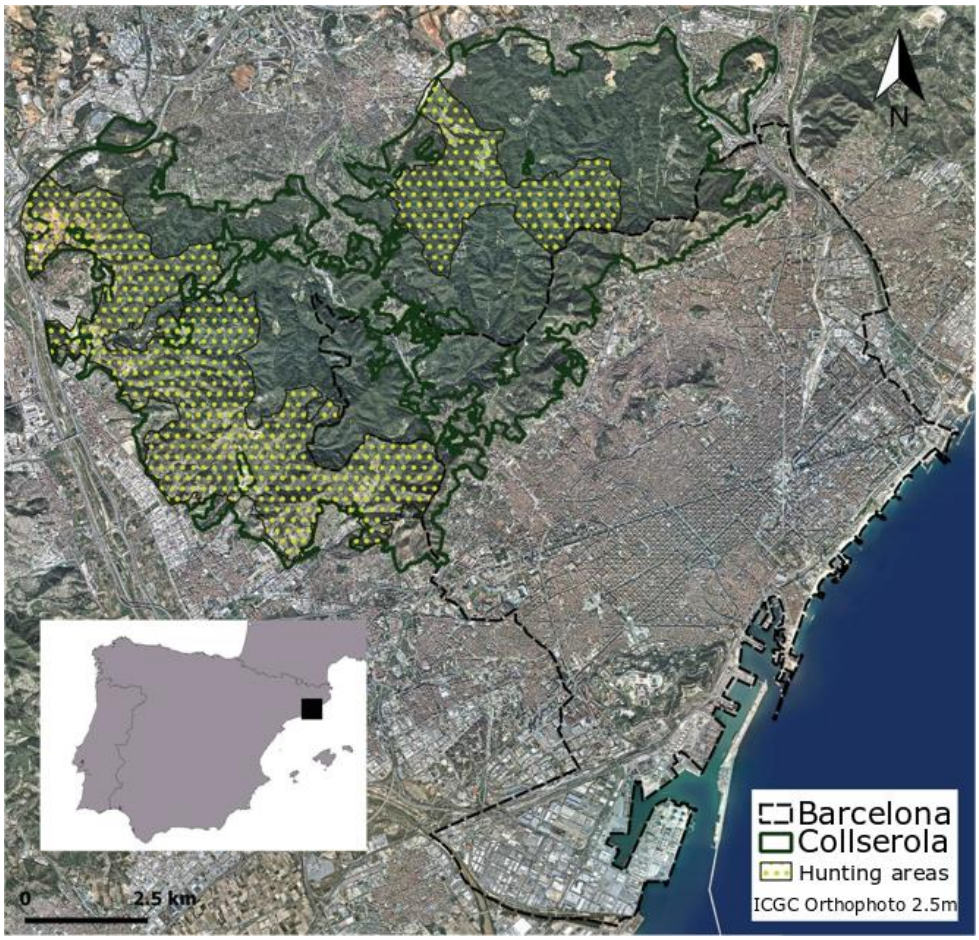

Figure 3.5. Study areas. Orthophoto: ICGC.

Data collection and sampling

Sampling and data collection took place from 2013 to 2018 in Barcelona and from 2015 to 2018 in Collserola, and we sampled three Collserola females in January and February from 2019.

We recorded location, date, sex, age, body mass, stomach contents and reproductive status (the latter only for females) and collected blood samples of 445 wild boars captured in the urban area of Barcelona and 183 wild boars harvested in the Collserola massif. For simplicity, we will call "urban" those wild boars captured within the Barcelona municipality limits, in contrast to "non-urban", which are the wild boars hunted in Collserola.

The urban wild boars were dart-anesthetized xylazine, tiletamine and zolazepam (3 mg/kg each) by a veterinarian within the framework of the contracts 13/051, 15/0174, 16/0243-00-CP/01 and 16/0243-00-PR/01 with the *Ajuntament de Barcelona*. They were captured either when causing potentially dangerous situations or in planned events in sensitive areas in order to prevent conflicts. Once captured, urban wild boars were blood sampled, euthanized (T-61®; 1.2 mL/10 kg) and transported to the necropsy room facilities in the Veterinary Faculty of the UAB to complete data and sample collection. Conversely, non-urban wild boars were shot during the regular hunting season, from October to February, in drive hunts conducted by local hunters in the Collserola hunting areas (see Figure 3.5). Once harvested, we partially sampled them in the field and transported the offal to the necropsy room facilities of the Veterinary Faculty of the UAB to finish sample processing. We kept the blood samples at 4°C in a cold box until arrival at the laboratory, and centrifuged them for serum separation at 1800 x g for 10 min. Sera were stored at −20 °C until analysis.

We estimated wild boar age through tooth eruption, replacement and wear patterns (Iff, 1978; Matschke, 1967), and classified them as piglets (0-6 months), juveniles (>6-12 months), yearlings (>12-24 months) or adults (>24 months). We classified the stomach contents as "anthropogenic" if the stomach contained food from human origin (food wrappers, food waste, pet food, exotic fruits, and other) and "natural" otherwise. As for female reproductive status, we determined if they were pregnant, lactating or neither through macroscopic examination of the reproductive tract and mammary glands, and measured the crown-rump length of foetuses, when applicable.

Statistical analyses

We performed an exploratory analysis following Zuur et al. (2010) to detect possible outliers, collinearity of explanatory variables, and to identify the type of distribution of our response variables and the relationship between response and explanatory variables.

We performed all statistical procedures using R software (version 3.5.0; R Development Core Team, 2018). In order to assess residual normality, homoscedasticity and independence, we visually explored the residuals from all the parametric models through a histogram, a normal probability plot (QQ plot) and plots of the residuals against fitted values and predictor variables. We explored residuals from non-parametric models in the same way although not looking for normality.

Effects of urbanisation on wild boar body mass and growth rate

We analysed the effect of urbanisation on wild boar body mass and growth rate in 233 wild boars, 112 from Barcelona and 121 from Collserola, between four months and three years of age, sampled from October to February. This restriction was necessary due to lack of non-urban wild boars outside this period, the lack of non-urban piglets younger than four months, and because age determination based on tooth wear may increase error margin beyond three years.

We applied generalized additive models (GAMs hereafter; Wood, 2011) to assess differences in the relationship between body mass (in Kg) and age (in months, as a continuous variable) in urban *vs*. non-urban wild boars. We chose this statistical approach because of a non-lineal relationship between wild boar mass and age. We also included sex as predictor. Thus, we constructed a set of GAMs with body mass as the response variable and a combination of age (smoothed), area (urban/non-urban) and sex (female/male) as predictors, with and without interactions among them. We used the gam function within the mgcv library (Wood, 2011) following Zuur (2012) instructions to construct GAMs, and selected the best model(s) by means of the Akaike's Information Criterion (AIC) (Akaike, 1973; Burnham & Anderson, 2002).

In addition to GAMs, we compared the mean body mass between areas of piglets (four to six months old), juveniles (6 to 12 months), yearlings (12 to 24 months) and adults (24 to 36 months) included in the GAM analysis by means of a t-test or a Wilcoxon rank sum test. We also calculated an overall growth rate (increase of body mass per unit of time, in this case Kg/month) per area, and a growth rate per each age class and area.

Effects of urbanisation on wild boar diet

We compared the stomach contents from 236 urban wild boars (74 from October to February, simultaneous to the hunting season, and 162 from March to September) and 180 non-urban wild boars (October through February) to look for differences in the use of anthropogenic food resources between areas.

We used a Pearson's Chi-squared test to determine whether the stomach contents (anthropogenic/non-anthropogenic) were related to the origin (urban/non-urban).

Effects of urbanisation on wild boar life expectancy

We used the wild boar age, which was the age at death, as an indicator of their life expectancy. Again, we considered the period from October to February to avoid seasonal bias, as non-urban wild boars could only be sampled during this period. We applied generalised linear models (GLMs, hereafter; McCullagh & Nelder, 1989) to explore the effects of area and sex on the wild boar age at death. We used the glm function (R stats package, version 3.7.0) for Poisson distributed errors, with "log" as the link function (Crawley, 2007b), and selected the best model(s) by means of the AIC.

Effects of urbanisation on wild boar physiology

We determined the concentration of triglycerides, urea and creatinine in sera from 100 urban wild boars using an Olympus AU400 (Olympus, Mainz, Germany) analyser. We also included sera from 54 non-urban wild boars (sampled from 2005 to 2013) from a previous study carried out in the nearby Natural Park of Sant Llorenç del Munt i Serra de l'Obac (SLMO) (Casas-Díaz et al., 2015).

We used the abovementioned GLMs (family Poisson and link function "log") to assess the concentration of triglycerides in wild boar serum, and linear models for urea and creatinine concentration. We included area (urban/non-urban), age class (adult/juvenile/piglet) and sex (female/male) as predictors in all models, as well as interactions among them. In this case, we considered as adults those wild boars above 12 months of age. To apply the aforementioned models, we needed to transform the triglycerides variable into an integer one, and to replace urea and creatinine variables by their square root for them to be normal distributed. After fitting these models, we used the package lsmeans from R to perform two-way comparisons with the Tukey adjustment method (Mangiafico, 2016).

Effects of urbanisation on female wild boar reproduction

We compared the estimated age of first conception, as indicative of first reproduction, in 16 pregnant and lactating female wild boars younger than 20 months from Barcelona (n=12) and Collserola (n=4). We used the foetuses crown-rump length to calculate their age according to Henry (1968a), or the age of piglets (in case of lactating females) to estimate how old were they at the time of conception, considering 115 days the gestational period length (Henry, 1968b). We used a Wilcoxon rank-sum test to analyse whether the age at the time of first conception depended on the area (urban/non-urban).

Furthermore, to test if urbanisation had an effect on reproduction of female wild boars in general, not only on the primiparous ones, we compared pregnant (n=28) to non-pregnant (n=191) females. We applied GLMs, using pregnant (yes/no) as binomial response and age class (juvenile/yearling/adult), body mass (Kg) and area (urban/non-urban) as predictors, as well as interactions among them. We used the abovementioned glm function from the R stats package, but for binomial distributed errors, using the link function "logit" (Crawley, 2007b). We selected the best GLM based on an AIC comparison.

3.2.4. Results

Table 3.3 contains the sample size of each analysis performed. Please see Table 3.4 for details on wild boars per area, sex and age class.

Table 3.3. Sample size of each analysis. Barcelona (urban) wild boars were sampled all year round, whereas Collserola (non-urban) wild boars were sampled from October to February (the hunting season). SLMO: Natural Park of Sant Llorenç del Munt i Serra de l'Obac.

Analysis	Sample size		Age (months)	Season	
	Urban	Non-urban		Urban	Non-urban
Body mass and growth rate	112	121	4 to 36	October to February	
Use of anthropogenic food	236 (all year round) / 74 (October to February)	180	> 4	All year round / October to February	October to February
Classification of anthropogenic food					
Triglycerides, urea and creatinine in serum	96	54	> 0.5	All year round	All year round (SLMO origin)
Life expectancy	121	185	> 4	October to February	
Female first reproduction	12	4	6-20	All year round	October to February
Drivers of reproductive status (being pregnant or not)	127	92	> 6	All year round	October to February
Total sample size	445	183	0-120	All year round	October to February

Table 3.4. Wild boars sampled per area, sex and age class. Urban wild boars from Barcelona were sampled all year round, whereas non-urban (Collserola) wild boars were sampled during the hunting season (October to February). Adults: > 24 months; yearlings: 12 to 24 months; juveniles: 6 to 12 months; piglets: 0 to 6 months.

	Urban area			Non-urban area			Total		
	Females	Males	Total	Females	Males	Total	Females	Males	Total
Adults	60	17	77	57	50	107	117	67	184
Yearlings	43	65	108	20	25	45	63	90	153
Juveniles	38	62	100	12	9	21	50	71	121
Piglets	83	77	160	7	3	10	90	80	170
Total	224	221	445	96	87	183	320	308	628

Effects of urbanisation on wild boar body mass and growth rate

The best model to explain wild boar body mass data variance (GAM 1) included age (smoothed), area and sex as predictors, with two-term interactions between the smoother of age (s(Age), hereafter) and area, and between s(Age) and sex. Two other models had an AIC value similar enough to that of the best model (Burnham & Anderson, 2002); one of them (GAM 2) did not include sex as a predictor and the other one (GAM 3) was equal to GAM 1 but included a third, non-significant interaction term between area and sex. Please see Table 3.5 for details on these models.

Table 3.5. Selected generalised additive models (GAMs) to explain wild boar body mass. Variables or interactions with significant effects ($p<0.05$) are shown in bold. The asterisk indicates an interaction between two variables. Df: degrees of freedom, AIC: Akaike's information criterion, R-sq (adj): percentage of body mass variance explained by the model adjusted by the number of predictors, s(Age): smoother of age.

Model	Selected explanatory variables and interactions	df	AIC	R-sq (adj)
GAM 1	**Area**, sex, **s(Age)*area**, **s(Age)*sex**	22.2981	1715.391	75.4%
GAM 2	**Area**, **s(Age)*area**	17.5960	1717.280	74.7%
GAM 3	**Area**, sex, area*sex, **s(Age)*area**, **s(Age)*sex**	23.2687	1717.350	75.3%

Regarding GAM 1, the main effect of area on wild boar body mass was significant, showing that non-urban wild boars had lower body mass than urban wild boars (t=-8.809 and p=4.6e-16). On the contrary, the model did not detect differences in body mass between females and males (t=35.690 and p=0.14). All the interactions between s(Age) and area and s(Age) and sex, however, resulted significant (see F and p values in Table 3.6), indicating that wild boar age had a significant effect on body mass but the relationship between age and body mass differed between urban and non-urban wild boars (see Figure 3.6) and also between females and males (see Figure 3.7). Therefore, urban wild boars reached higher body mass than non-urban wild

boars (mean body mass of adults 24 to 36 months old: 68.4 *vs*. 44.8 Kg; W=441, p<0.001) and gained body mass faster (overall growth rate: 1.8 Kg/month for urban and 1 Kg/month for non-urban wild boars), despite the body mass of piglets four to six months old was not different between areas (12.4 *vs*. 13.3 Kg; W=69, p=0.59). The difference in body mass between urban and non-urban wild boars was also observed in juveniles (24.9 *vs.* 18.13 Kg; W=748, p=0.003) and yearlings (49.75 *vs.* 37.05 Kg; t=4.48, df=65.45, p<0.001). The growth rate per age class and area was 1.9 Kg/month for urban piglets, 2.1 for urban juveniles, 2.1 for urban yearlings and 1.6 for urban adults, and 2.1, 0.8, 1.6 and 0.6 Kg/month for non-urban piglets, juveniles, yearlings and adults, respectively.

Figure 3.6. Wild boar body mass (in Kg) per area. The city (in grey) represents Barcelona and the forest (in green) represents Collserola.

Table 3.6. Approximate significance (threshold set at p<0.05) of smoothed terms of GAMs 1 to 3 in interaction (indicated by an asterisk) with area or sex. Edf: effective degrees of freedom; F: F statistic; p: p value.

Model and interactions	Edf	F	p
GAM 1			
s(Age)*Area (Urban)	0.750	10.703	0.005043
s(Age)*Area (Non-urban)	7.221	2.013	0.047043
s(Age)*Sex (Female)	1.946	4.879	0.009450
s(Age)*Sex (Male)	8.382	3.618	0.000254
GAM 2			
s(Age)*Area (Urban)	6.604	62.84	<2e-16
s(Age)*Area (Non-urban)	7.992	25.01	<2e-16
GAM 3			
s(Age)*Area (Urban)	0.750	10.648	0.005158

s(Age)*Area (Non-urban)	7.200	1.997	0.049493
s(Age)*Sex (Female)	1.939	4.771	0.010016
s(Age)*Sex (Male)	8.379	3.593	0.000276

Even though the main effect of sex in GAM 1 was non-significant, the mentioned significant interaction between s(Age) and sex showed that the body mass changed with age differently in females and males. Approximately, while males were heavier up to 16-18 months of age and reached a higher final weight from 30-32 months onwards, females showed a higher weight from 18-20 to 30 months (Figure 3.7). These shifts in the heavier gender according to age probably prevent the model from finding a significant effect of sex on body mass across all ages. GAM 1 explained the 75.4% of body mass variance.

With respect to GAM 2, the main effect of area on wild boar body mass was also significant (non-urban wild boars had lower body mass than urban ones; t=-9.267, p<2e-16), as well as the interaction between s(Age) and area (see Table 3.6), and it explained 74.7% of the variance. GAM 3 was basically the same model as GAM 1, because it maintained the significant difference between urban and non-urban wild boars (t=-6.118, p=4.55e-09) and the similarity between female and male body mass (t=1.052, p=0.294), but included a non-significant interaction term between area and sex (t=-0.059, p=0.953). This interaction indicates that body mass tended to differ between females and males between areas, as we can also see in Figure 3.7. In fact, the different growth periods for males and females tended to differ between areas, although the overall pattern was consistent in both areas. In line with GAM 1, GAM 3 also produced significant interactions between s(Age) and area, and between s(Age) and sex (Table 3.6), and explained almost the same amount of variance, 75.3% (Table 3.5). Residuals of the three GAMs met the model assumptions of normality, homoscedasticity and independence.

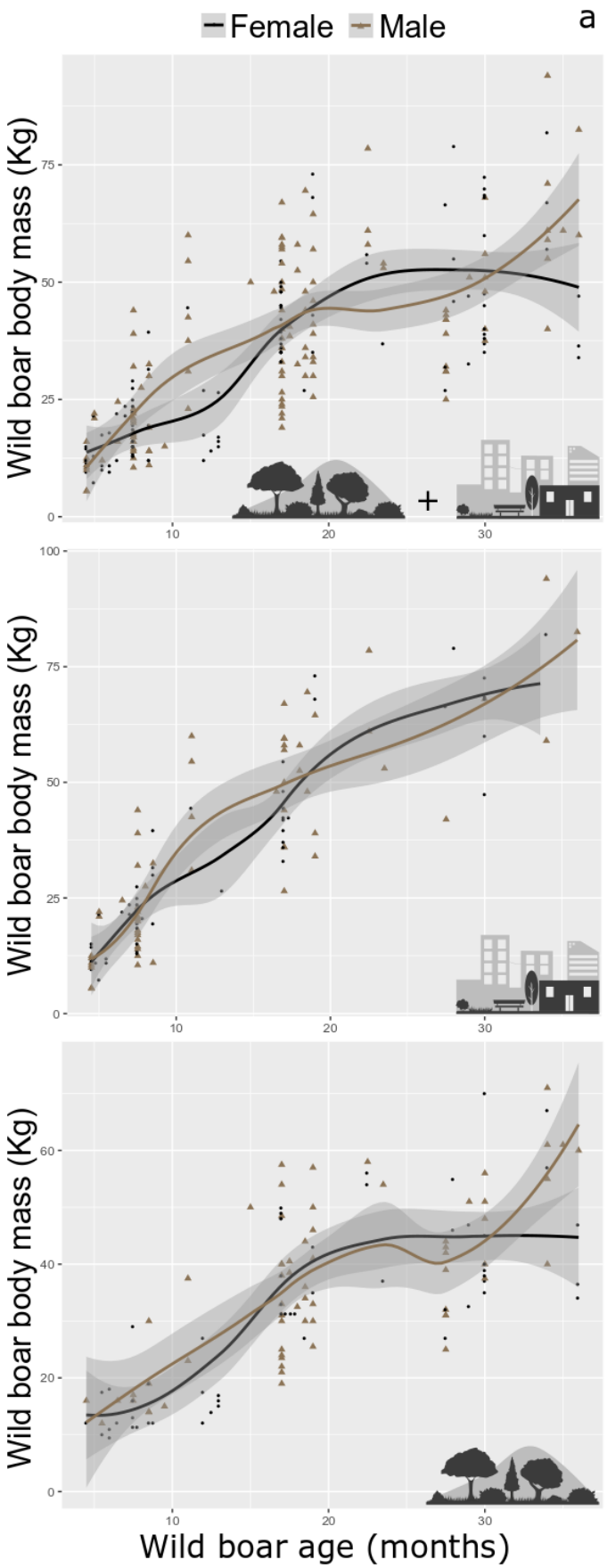

Figure 3.7. Wild boar body mass (in Kg) per sex and area. Females: black dots, males: brown triangles. a: Barcelona and Collserola wild boars altogether; b: Barcelona wild boas; c: Collserola wild boars.

Effects of urbanisation on wild boar diet

Non-urban wild boars presented anthropogenic contents in stomachs in a significantly lower proportion (4.4%, 8/180) than urban wild boars, both when considering stomachs from urban wild boars collected during the hunting season (39.2%, 29/74; X-squared 48.12, df=1, p<0.001) or collected during the whole year (48.7%, 115/236; X-squared 94.05, df=1, p<0.001). The anthropogenic contents included food or items that can be found in rubbish bins or the streets such as food waste (in almost 68% of the year-round stomachs classified as "anthropogenic"), food wrappers, plastic bags or other plastic pieces (nearly 50%) and pet food (19%), among others (Table 3.7).

Table 3.7. Classification of food and other items from anthropogenic origin found in urban (Barcelona) and non-urban (Collserola) wild boar stomachs: "food waste" included fruits, meat, cold meat, bread, rice and pasta, among others; "plastic items" included food wrappers, food labels, plastic bags and other plastic pieces; "pet food" included dry or wet food for companion animals; "other" included human hair, hair and other remains from animals found in urban areas (cat, pigeon, parrot) and non-edible items also found in urban areas.

Stomach contents	Urban area, all year round	Urban area, October to February	Non-urban area, October to February	Total
Natural	121/236 (51.3%)	45/74 (60.8%)	172/180 (95.6%)	293/416 (70.4%)
Anthropogenic	115/236 (48.7%)	29/74 (39.2%)	8/180 (4.4%)	123/416 (29.6%)
Food waste	78/115 (67.8%)	23/29 (79.3%)	4/8 (50.0%)	82/123 (66.7%)
Plastic items	57/115 (49.6%)	11/29 (37.9%)	2/8 (25.0%)	59/123 (48.0%)
Pet food	22/115 (19.1%)	3/29 (10.3%)	0/8 (0.0%)	22/123 (17.9%)
Other	19/115 (16.5%)	5/29 (17.2%)	2/8 (25.0%)	21/123 (17.1%)

Effects of urbanisation on wild boar life expectancy

The wild boar life expectancy depended on the area, as shown by the selected GLM (not shown). The area had a significant effect on the wild boar age of death, indicating that urban wild boars died younger (mean 18 and median 12 months) than non-urban wild boars (mean 33 and median 28 months; t=4.497, p<0.001; Table 3.8). There also was a marginally non-significant effect of sex, indicating that females tended to live longer than males (mean 30 *vs*. 24 months and median 23 *vs*. 18 months, respectively) for both areas altogether (t=-1.709, p=0.09). In fact, while the maximum age recorded for males was approximately four-five years in the urban area and six-seven years in the non-urban one, some females reached up to five-six years in the urban area, and nine years in the non-urban one (Table 3.8). The variance explained by this GLM was 12.3% and residuals did not show any patterns when plotted against fitted values or explanatory variables.

Table 3.8. Mean, 95% confidence interval, median and range of wild boar life expectancy (age of death, in months) per area and sex. We considered wild boars older than four months of age and sampled from October to February. Age determination is based on tooth wear and therefore approximate beyond 36 months.

	Urban		
	Females	**Males**	**Total**
Mean [95 % CI]	20.3 [15.8-24.7]	15.2 [12.6-17.9]	17.7 [15.1-20.4]
Median	12	11	11
Range	4.5-66	4.5-54	4.5-66
	Non-urban		
	Females	**Males**	**Total**
Mean [95 % CI]	35.9 [30.6-41.2]	29.4 [25.3-33.5]	32.9 [29.4-36.3]
Median	30	23	27.5
Range	4.5-108	4.5-78	4.5-108
	Total		
	Females	**Males**	**Total**
Mean [95 % CI]	30 [26.1-33.9]	23.5 [20.7-26.4]	26.9 [24.4-29.3]
Median	22.5	18	18.5
Range	4.5-108	4.5-78	4.5-108

Effects of urbanisation on wild boar physiology

GLMs or linear models and subsequent two-way comparisons showed that urban adult wild boars had significantly higher concentration of triglycerides than the non-urban ones (z=3.166, p=0.02), and that urban males had significantly higher concentration of triglycerides than the non-urban ones (z=3.550, p=0.002). Moreover, triglycerides concentration decreased with age only in non-urban male wild boars (adults *vs*. piglets: z=-2.934, p=0.039). Also, non-urban females showed higher triglyceride values than non-urban males (z=2.657, p=0.039). Regarding urea, urban females showed significantly higher values than non-urban ones (t=4.108, p=0.0004). As for creatinine, the two selected linear models agreed on that creatinine increased with age only in urban wild boars (piglets *vs*. juveniles: t=5.553, p<0.0001; juveniles *vs*. adults: t=6.433, p<0.0001), and that non-urban piglets showed higher values than urban ones (t=-7.143, p<0.0001).

Further information on the selected models can be found in Table 3.9, and please see Table 3.10 for a comprehensive list of means and 95% confidence intervals of triglycerides, urea and creatinine concentrations per wild boar age class, sex and area. Residuals from these models were normally distributed (in the case of linear models), homoscedastic and showed no patterns when plotted against fitted values and predictors.

Table 3.9. Selected generalized linear model (GLM) and linear models (LMs) explaining triglycerides, urea and creatinine concentrations in wild boar serum. Variables and interactions (indicated by an asterisk) with significant effects (p<0.05) are shown in bold. Df: degrees of freedom, AIC: Akaike's information criterion, R-sq(adj): explained variance adjusted by the number of predictors, Weight: weight of each model in the selection.

Model	Explanatory variables	df	AIC	R-sq (adj)	Weight
Triglycerides					
GLM-t	Age class + **Sex** + **Area** + **Age class*Area**	145	NA	12.48%	NA
Urea					
LM	Age class + **Sex** + **Area** + **Sex*Area**	140	126.5	12.39%	0.462
Creatinine					
LM-c 1	**Age class** + Area + **Age class*Area**	140	383.4	48.04%	0.472
LM-c 2	**Age class** + Area + Sex + **Age class*Area**	139	385.1	47.84%	0.196

Table 3.10. Mean concentration and 95% confidence interval of triglycerides, urea and creatinine in wild boar sera per age class, sex and area. Significant differences (p<0.05) obtained with two-way comparisons after the (generalized) linear models are shown in bold; asterisks and crosses indicate differences between areas, superscript letters indicate differences among age classes within each area, and superscript numbers indicate differences between sexes within each area. Piglets: 0-6 months, juveniles: 6-12 months, adults: >12 months. SLMO: Sant Llorenç del Munt i Serra de l'Obac.

	Age class	Urban (Barcelona)			Non-urban (SLMO)			Total		
		Females	Males	Total	Females	Males	Total	Females	Males	Total
Triglycerides (mmol/L) Mean [95% CI]	Adult	0.60 [0.49-0.71]	**0.50* [0.38-0.62]**	**0.54* [0.46-0.62]**	0.41 [0.11-0.71]	**0.11^{+a} [0.05-0.18]**	**0.21^{+a} [0.08-0.34]**	0.57 [0.46-0.67]	0.41 [0.30-0.52]	**0.47^{a} [0.39-0.55]**
	Juvenile	0.44 [0.28-0.61]	0.49 [0.32-0.66]	0.47 [0.35-0.59]	0.45 [0.30-0.60]	0.40 [0.24-0.56]	0.42 [0.31-0.53]	0.45 [0.33-0.56]	0.46 [0.33-0.58]	0.45 [0.37-0.53]
	Piglet	0.62 [0.43-0.81]	0.67 [0.30-1.05]	0.64 [0.46-0.82]	0.66 [0.47-0.84]	**0.54^{b} [0.41-0.67]**	**0.61^{b} [0.49-0.73]**	0.64 [0.51-0.77]	0.59 [0.43-0.75]	**0.62^{b} [0.52-0.72]**
	Total	0.55 [0.46-0.64]	**0.52* [0.42-0.61]**	0.53 [0.47-0.60]	**0.54^{1} [0.42-0.66]**	**0.36^{+2} [0.26-0.45]**	0.45 [0.37-0.53]	0.55 [0.48-0.62]	0.46 [0.39-0.53]	0.5 [0.45-0.55]
Urea (mmol/L) Mean [95% CI]	Adult	4.78 [3.98-5.59]	3.65 [3.04-4.27]	4.13 [3.62-4.65]	3.03 [2.38-3.67]	3.57 [2.78-4.36]	3.39 [2.82-3.96]	4.48 [3.75-5.21]	3.63 [3.13-4.14]	3.97 [3.55-4.40]
	Juvenile	**4.16* [3.45-4.88]**	3.59 [3.28-3.89]	3.86 [3.48-4.24]	**2.66^{+} [1.67-3.66]**	3.03 [1.88-4.18]	2.85 [2.10-3.59]	3.59 [2.95-4.23]	3.39 [2.93-3.84]	3.48 [3.1-3.87]
	Piglet	4.72 [3.64-5.80]	4.10 [3.03-5.17]	4.51 [3.72-5.31]	3.64 [3.15-4.14]	4.43 [3.20-5.66]	3.96 [3.38-4.54]	4.13 [3.54-4.72]	4.3 [3.47-5.14]	4.2 [3.72-4.67]
	Total	**4.55* [4.07-5.03]**	3.67 [3.31-4.03]	4.09 [3.78-4.40]	**3.17^{+} [2.70-3.65]**	3.63 [2.98-4.27]	3.40 [3.00-3.80]	4.05 [3.66-4.43]	3.66 [3.34-3.98]	3.85 [3.6-4.1]

Creatinine (µmol/L) Mean [95% CI]	Adult	**113.51[a] [104.15-122.86]**	**103.26[a] [97.18-109.35]**	**107.62[a] [102.19-113.06]**	112.05 [108.15-115.95]	111.53 [93.33-129.73]	111.74 [101.13-122.34]	113.3 [105.47-121.05]	104.77 [98.83-110.70]	108.34 [103.51-113.18]
	Juvenile	**84.59[b] [72.74-96.44]**	**82.26[b] [74.66-89.86]**	**83.36[b] [76.58-90.13]**	91.94 [81.04-102.83]	94.27 [86.97-101.56]	93.22 [87.04-99.39]	87.23 [78.72-95.74]	86.81 [81.02-92.61]	87.01 [82.04-91.98]
	Piglet	**56.66*[c] [50.89-62.44]**	**59.23*[c] [53.97-64.48]**	**57.52*[c] [53.35-61.68]**	**92.07[+] [85.36-98.79]**	**99.01[+] [89.87-108.15]**	**94.50[+] [88.26-100.74]**	76.68 [68.1-85.26]	82.43 [68.62-96.24]	78.65 [71.35-85.95]
	Total	91.09 [82.39-99.79]	91.30 [85.43-97.17]	91.20 [86.06-96.34]	95.10 [89.42-100.78]	99.97 [92.95-106.98]	97.43 [92.95-101.92]	92.54 [86.62-98.45]	94.11 [89.47-98.75]	93.33 [89.6-97.07]

Effects of urbanisation on female wild boar reproduction

We found 14 pregnant (14/127, 11%) and 113 non-pregnant urban females. Pregnant urban females were sampled from March to July, with peaks in March (five out of 14) and April (six out of 14). On the other hand, we found 14 pregnant (14/92, 15.2%) and 78 non-pregnant non-urban females. Non-urban pregnant females were sampled from December to February, with a peak in February (ten out of 14).

Urban females reproduced for the first time at 12.5 months of age on average, as indicated by the estimated age of conception, whereas non-urban females reproduced significantly later, at 16.2 months of age (W=6, p=0.03). Mean body mass at sampling was 60 Kg for urban and 41.8 Kg for non-urban females. Estimated ages at the time of conception suggest that the youngest urban females to become pregnant were nine months old, whereas the youngest pregnant non-urban female was 14 months old.

The two selected GLMs with pregnant as response categorical variable (Table 3.11) agreed on that being pregnant was positively influenced by body mass, although this influence was small (model estimates were close to 0). One of them (GLM-p 1, the best in terms of AIC and model weight) also detected that pregnancy was significantly more likely in the non-urban area. Moreover, the significant interaction between body mass and age class in both models indicated that the significantly higher body mass of pregnant females varied according to the age class. However, two-way comparisons following the models (either GLM-p 1 or GLM-p 2, not shown) did not show significant differences in body mass among age classes (all p values were above 0.05). Please see Table 3.12 for further information on these models.

Table 3.11. Selected generalised linear models (GLMs) with pregnant (yes/no) as the response variable. Variables and interactions (indicated by an asterisk) with significant effects (p<0.05) are shown in bold. AIC: Akaike's information criterion, Weight: weight of each model in the selection, R-sq(adj): explained variance adjusted by the number of predictors.

Model	Explanatory variables	AIC	Weight	R-sq (adj)
GLM-p 1	Age class + **Area** + **Body mass** + **Age class*Body mass**	134.8	0.507	20.46%
GLM-p 2	Age class + Area + **Body mass** + **Age class*Body mass** + Area*Body mass	136.7	0.194	20.54%

Table 3.12. Variables and interactions (indicated by an asterisk) included in the selected generalized linear models (GLMs) with pregnant (yes/no) as the response categorical variable. The reference category was "Urban" for area and "Adult" for age class. Significant effects (p<0.05) are shown in bold. sd: standard error of model estimates, z: z statistic; p: p value.

Model	Explanatory variables and interactions	Estimate	sd	z	p
GLM-p 1	**Body mass**	0.06176	0.01912	3.230	**0.00124**
	Area (Non-urban)	1.91348	0.61043	3.135	**0.00172**
	Age class (Juvenile)	-3.45002	3.66313	-0.942	0.34628
	Age class (Yearling)	-4.62374	2.71078	-1.706	0.08807
	Age class (Juvenile)*Body mass	0.12086	0.08068	1.498	0.13410
	Age class (Yearling)*Body mass	0.11546	0.04916	2.349	**0.01883**
GLM-p 2	**Body mass**	0.05289	0.02651	1.995	**0.04605**
	Area (Non-urban)	0.94207	2.09090	0.451	0.65231
	Age class (Juvenile)	-3.51929	3.79958	-0.926	0.35432
	Age class (Yearling)	-4.71981	2.74087	-1.722	0.08507
	Age class (Juvenile)*Body mass	0.11909	0.08318	1.432	0.15221
	Age class (Yearling)*Body mass	0.11646	0.04944	2.356	**0.01848**
	Area (Non-urban)*Body mass	0.01687	0.03508	0.481	0.63048

3.2.5. Discussion

In this study we describe not only a higher body mass in urban wild boars as compared to non-urban ones, as previously reported (Cahill et al., 2012), but we also demonstrate that these differences are due to differences in growth in all age classes but piglets and for both sexes. Moreover, we found that these differences in the growth pattern are accompanied by other acclimatory responses such as changes in the foraging behaviour, physiology and reproduction (McDonnell & Hahs, 2015; Ricklefs, 1990), which can help clarify the responses shown by

wildlife species in urban areas and the ecology of urban colonisation by these species as a consequence of global change urbanisation processes.

The urban wild boars were not only heavier, but also acquired body mass faster than non-urban wild boars. Although several studies have compared body mass or weight between urban and rural populations of wildlife species (Auman, Meathrel, & Richardson, 2008; Beckmann & Berger, 2003; Cypher, 2010), few describe the trend in body mass increase with age, as ours does. Post-natal growth of piglets below six months depends on the females in the family group rather than on the environment (Gaillard, Pontier, Brandt, Jullien, & Allainé, 1992; Kaminski et al., 2005), which would explain the lack of differences in body mass between urban and non-urban piglets. However, the higher body mass and growth rate in urban wild boars over six months old, and the increase with age in serum creatinine (directly related to muscular mass; Braun & Lefebvre, 2008) only in urban wild boars, suggest that urban wild boars are thriving in an environment favouring their body mass and muscular mass increase since gaining independence from their mothers (Kaminski et al., 2005). Moreover, as increases in body mass have been related to consistent increasing population trends (Ozgul et al., 2010), the higher body mass of urban wild boars may further favour the population increase and spread of this species.

The main factor favouring this higher growth, body mass body and muscular mass gain could be the higher use of anthropogenic food resources by urban wild boars, previously suggested in the study area (Cahill et al., 2012; Castillo-Contreras et al., 2018) and confirmed by our results. A similar percentage (58%) of urban wild boars in Islamabad (Pakistan) had garbage in their stomachs (Hafeez et al., 2011). Conversely, urban wild boars in Berlin selected natural over anthropogenic food resources, due to a combination of seasonality (late autumn-winter) and reduced availability of anthropogenic food achieved through supplementary feeding banning (Stillfried, Gras, Busch, et al., 2017). Being wild boar an opportunistic species (Schley & Roper, 2003), this difference in the use of anthropogenic resources could be related to differences in the relative availability of anthropogenic *vs.* "natural" resources between Berlin and Barcelona, since Berlin contains a 30.4% of public green areas and forests inside the city (Senate Department for the Environment, Transport and Climate Protection, 2017), whereas the percentage of public green areas in Barcelona is 11% (Ajuntament de Barcelona, 2013). This further emphasizes that different urban environments are part of different ecosystems and determine different uses and/or changes of wild species thriving in them.

Thus, we demonstrated that wild boars have changed their foraging behaviour in Barcelona by utilising anthropogenic food resources, which is a common behavioural response of wildlife in urban environments (Lowry et al., 2012). Moreover, the predictability in space and time of food resources in urban areas may lead to a reduction in the foraging time and improve body mass (Oro, Genovart, Tavecchia, Fowler, & Martínez-Abraín, 2013), which would also agree with our findings. Anthropogenic food and the consequent improvement in body mass could improve wild boar survival through harsh periods, i.e. hot and dry summers in Mediterranean areas (Cahill & Llimona, 2004; Massei, Genov, Staines, & Gorman, 1997), because they would be able to find food and water when these are scarce. Furthermore, a diet rich in protein and fatty acids, as the one that wild boars might be able to obtain from human organic waste (Hansen, Jansen, Spliid, Davidsson, & Christensen, 2007), could explain the higher concentration of urea and triglycerides detected in female and adult urban wild boars, respectively (Braun & Lefebvre, 2008; Bruss, 2008; Van Dam & Hunter, 2012). Plus, the significant triglyceride concentration reduction with age in the non-urban wild boars but not in the urban ones would suggest that urban wild boars maintained a fat-rich diet throughout their life.

However, studies in wildlife supplemented with food for tourism purposes have shown that long-term feeding practices can lead to reliance on supplemented food, habituation to humans, disruption of normal activities and nutritional problems (Murray, Becker, Hall, & Hernandez, 2016; Newsome & Rodger, 2008). In addition, the consumption of anthropogenic food could expose the wild boar to pollutants, poisons or toxins (Birnie-Gauvin et al., 2016; Murray et al., 2016) and increase disease contact and/or spread (Becker, Streicker, & Altizer, 2015; Bradley & Altizer, 2007; Murray et al., 2016). Habituation to humans has previously been reported in urban wild boars (Cahill et al., 2012; Stillfried, Gras, Börner, et al., 2017), and we show for the first time pathophysiological changes, such as higher urea and triglyceride concentrations, predisposing them to metabolic diseases (Braun & Lefebvre, 2008; Murray et al., 2016; Newsome & Rodger, 2008).

In addition to the differences in wild boar body mass, growth and use of anthropogenic food, we found another effect, potentially negative, of exploring the urban area, which is the shortening of urban wild boar lifespan. The life expectancy at birth of an urban wild boar was roughly half of that of a non-urban one. This could be explained by several reasons, namely 1) there is an age bias in the capture method towards young individuals (teleanaesthesia with a blowpipe in Barcelona *vs.* drive hunts in Collserola), 2) wild boar boldness (willingness to take risks), exploratory behaviour or other personality traits are more associated to young individuals, which are the ones that decide to explore the urban area, 3) the wild boar

colonisation of Barcelona is a recent phenomenon and they have not had enough time to reach the same age than in Collserola, or 4) urban wild boars die younger because mortality is higher in Barcelona than in Collserola. Since 1) teleanaesthesia using a blowpipe is an operator-activated capture system and therefore shows no bias for sex or age (Kock, Jessup, & Burroughs, 2012), 2) personality traits such as boldness and exploratory behaviour are repeatable in the wild boar (Vetter et al., 2016) and hence they do not change with age, and 3) reports of wild boar-related conflicts in Barcelona date back to 1998 (Minuartia, 2005) and probably started earlier; we would argue that the most likely explanation to the shorter life expectancy in Barcelona is that 4) wild boar mortality is higher in the urban area. This mortality is probably caused by humans, e.g., through capture and removal of problem individuals or car accidents (Ajuntament de Barcelona, 2018; Tenés, Cahill, Llimona, & Molina, 2007) and we would suggest that higher mortality or dying younger is a cost of exploring the urban area for the wild boar in our study area.

Regarding reproduction, we described for the first time that urban females started breeding earlier than non-urban ones, which would be in accordance with studies reporting that good food supply favours an early reproduction in the wild boar (Fernández-Llario & Mateos-Quesada, 1998; Massei et al., 1996; Santos et al., 2006). However, our sample size of presumably primiparous non-urban females was low, which could limit our conclusion. Nonetheless, being pregnant (when considering all females, not only the younger ones) was positively influenced by female body mass in both areas, as in previous studies (Fernández-Llario & Mateos-Quesada, 1998; Massei et al., 1996), and an early reproduction in urban females agrees with faster-increasing body mass in urban wild boars. Thus, urban females would reach the required body mass for reproduction, which is above 30 Kg in the Iberian Peninsula (Fernández-Llario & Mateos-Quesada, 1998; Fonseca et al., 2011; Rosell, Navàs, & Romero, 2012), earlier than non-urban females. The higher probability of a non-urban female from Collserola of being pregnant, however, could just reflect a bias due to the difference in sampling season between areas: all year round for urban females and October to February for non-urban females (hunting season). Since the overlap of the non-urban sampling period with the wild boar mating season in the Iberian peninsula (Fernández-Llario & Carranza, 2000; Fonseca et al., 2011) was higher than for the urban sampling period, pregnant females might be overrepresented among the non-urban Collserola wild boars.

Female wild boar reproducing earlier in their life in the urban area might collaborate to increase the number of urban wild boars, probably along with an increase in human-wild boar number and intensity of conflicts. However, survival in the urban environment has to be also accounted

for. For instance, while female black bears started reproducing earlier and had more cubs in an urban area in Nevada, mortality of females of certain age classes was higher in the urban area (Beckmann & Lackey, 2008). Provided that wild boar life expectancy was shorter in urban wild boars, we would suggest that higher mortality might counter the improved reproduction performance shown by the primiparous urban females. In fact, the probability of urban environments to act as attractive sinks or to become the place for founder populations to establish will depend on the balance between increased productivity and recruitment (for instance through higher food resources, increased fertility and increased young survival) and increased mortality (such as those due to urban-related risks and lethal management measures).

Body mass and growth rate were affected by sex together with age, while life expectancy tended to differ between males and females although without statistical significance. On one hand, and agreeing with our results, sexual morphological dimorphism in wild boar, with males showing higher body mass and/or growth rate, has been described over 18 to 30 months, depending on the study (Focardi et al., 2008; Gallo Orsi, Macchi, Perrone, & Durio, 1995; Pedone, Mattioli, & Mattioli, 1995). This could be explained by males having a longer growth period than females (Gaillard et al., 1992; Spitz, Gleize, & Duncan, 1990), or by females investing energy in both growth and reproduction instead of only growth (Gallo Orsi et al., 1995).

On the other hand, the tendency of a lower life expectancy in male wild boars could be explained by higher both non-urban and urban mortality. In Collserola, this tendency could be due to a bias towards males in hunted wild boar (Keuling et al., 2013, 2016). In Barcelona, males would suffer more from the risk of exploring urban areas, since they are more prone to quit the maternal group. This is further suggested by the life expectancy of urban males in this study (13 to 18 months), which matches the age of maximum dispersal rate in male wild boar yearlings (Truvé & Lemel, 2003).

In summary, urban wild boars had higher body mass and faster growth; changed their foraging behaviour by using fat and protein-rich anthropogenic food sources, which induced significant physiological and metabolic changes; and females reproduced earlier as compared to non-urban ones. Moreover, urban wild boars also had a shorter life expectancy, probably due to human-induced mortality (Table 3.13). We have for the first time demonstrated such simultaneous morphological, behavioural, physiological, and reproductive adaptive responses to urban environmental change in a medium-sized mammal. Although there is growing evidence of

microevolutionary changes linked to adaptive responses to urban areas (McDonnell & Hahs, 2015), whether the traits we observed in urban wild boars are due to microevolution or phenotypic plasticity is a matter that requires further study. For instance, to distinguish between plasticity and actual urban evolution, we must 1) measure phenotypic changes, 2) establish their genetic basis and fitness benefits, and 3) experimentally identify the drivers inducing these adaptations (Donihue & Lambert, 2015). We have now identified the phenotypic changes in an urban wild boar population; however, determining genetic-related fitness benefits and identifying adaption drivers would require experimental studies to be conducted.

Table 3.13. Morphological, behavioural, physiological and reproductive responses of wild boars (WBs) in the urban area with respect to the non-urban area.

Finding	Evidence	Potential consequences
Higher body mass	- Higher body mass in urban juvenile, yearling and adult WBs - Increase in serum creatinine with age in urban WBs	- Increased survival - Increased recruitment - Earlier reproduction onset - Higher productivity
Higher growth rate	- Similar urban and non-urban piglet body mass - Higher mean body mass of urban juvenile, yearling and adult WBs	- Increased survival - Increased recruitment - Earlier reproduction onset - Higher productivity
Higher use of anthropogenic food resources	- Higher proportion of stomachs with anthropogenic contents in urban WBs - Higher concentration of urea in urban female WBs - Higher concentration of triglycerides in urban male adult WBs - No reduction in triglycerides concentration with age in urban WBs	- Increased survival - Increased recruitment - Earlier reproduction onset - Higher productivity - Increased body mass and growth rate - Metabolic diseases
Shorter life expectancy	- Lower mean and median age of death in urban WBs	- Increased population turnover - Faster growth, earlier onset of reproduction and increased productivity positively selected
Earlier reproduction in females	- Lower age at first conception in urban female WBs - Higher body mass in urban female WBs (except piglets)	- Urban population increase - Selection pressures favouring WBs that utilise anthropogenic food and grow fast

Wild boars may explore urban areas for several reasons, including anthropogenic food availability (Castillo-Contreras et al., 2018). The use of predictable anthropogenic food sources and the consequent increase in body condition and growth rate may increase individual fitness and boost opportunistic species populations (Oro et al., 2013; Ozgul et al., 2010). Increased food availability could be perceived for the wild boar as an environmental proxy for good habitat quality, misleading them into an area where their life expectancy is shorter. When bad habitats are perceived as good ones (reviewed in Battin, 2018) and are selected instead of avoided, they are called attractive sinks or deceptive sources (Delibes, Gaona, et al., 2001). In

fact, sinks can occur just because the mortality is increased by animal removal by humans (Gundersen et al., 2001). Genetic studies can help to clarify whether urban populations are just rural dispersers, therefore urban areas acting as attractive sinks, or founders establishing a new population, according to the urban island hypothesis (Stillfried, Fickel, et al., 2017). This would depend on the trade-off between benefits and risks in the urban ecosystem as compared to the surrounding non-urban one.

The methodology, information gathered and results obtained in our study could be a useful model for future research on the adjustment of wildlife species to urban environments, beyond the wild boar and Barcelona context.

3.2.6. Conclusions

Wild boars from Barcelona used anthropogenic food resources more than wild boars from Collserola, showed higher body mass and grew faster. Furthermore, urban female wild boars started reproducing earlier than non-urban ones, probably as a result of achieving the required body mass earlier. However, urban wild boars had a shorter lifespan than non-urban wild boars, indicating a possible cost of exploring the urban area for the wild boar in Barcelona.

3.2.7. Acknowledgements

We want to express our gratitude to Marià Martí, Lluís Cabañeros and other of members of the Consorci del Parc Natural de la Serra de Collserola for their logistic support, as well as to local hunters from Collserola, especially Pere Martín, Joan Carles Montagut, Jesús Esquerrà and Jorge Sánchez, for allowing us access to harvested wild boar. We also wish to thank Josep Maria López, from Departament d'Agricultura, Ramaderia, Pesca i Alimentació (Generalitat de Catalunya) for serving as a liaison among public administration, management and research. Finally, we are thankful to the many SEFaS collaborators and colleagues who helped in the wild boar samplings both in the field and the necropsy room facilities, especially Johan Espunyes, Arián Ráez, Xavier Fernández, Andreu Colom, Carles Conejero and Carlos González.

This study was funded by the contracts 13/051, 15/0174, 16/0243-00-CP/01 and 16/0243-00-PR/01 between Universitat Autònoma de Barcelona and Ajuntament de Barcelona. RCC, XFA and ACC benefited from pre-doctoral grants co-financed by Agència de Gestió d'Ajuts Universitaris i de Recerca (Generalitat de Catalunya) and the European Social Fund, file numbers 2016FI_B 00425, 2017FI_B1 00040 and 2018FI_B2_00030. JE benefited from a pre-doctoral grant from the Government of Andorra, file number ATC015-AND-2015/2016, 16/17 and 17/18.

3.3. Study 3

Ticks carried by wild boar in the Metropolitan Area of Barcelona are infected with spotted fever group rickettsiae

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3.3.1. Abstract

Tick-borne pathogens (TBPs) constitute an emerging public health concern, and both the number of TBPs and the incidence of tick-borne diseases are increasing globally as a result of multidimensional global changes. Wildlife species can play a significant role in TBP epidemiology such as favouring tick abundance and acting as reservoir hosts. Eurasian wild boar populations have increased and expanded for the last decades, and since wild boar can both promote tick abundance and act as reservoir of zoonotic pathogens, we aim to assess the risk of human tick-borne diseases derived from wild boar and tick presence in a highly populated area.

Between 2014 and 2016, we collected 167 spleen samples and 2256 ticks from 261 infested wild boars in the MAB (northeast Spain). We morphologically identified four tick species, namely *Hyalomma lusitanicum* (infestation prevalence of 33.6%), *Dermacentor marginatus* (26.9%), *Rhipicephalus sanguineus* sensu lato (18.9%) and *Rhipicephalus bursa* (0.2%). Then, we pooled the ticks according to species and individual host, and screened 180 tick pools and all spleen samples by reverse line blot hybridization assay and/or real time PCR for *Ehrlichia* sp., *Anaplasma* sp., *Babesia* sp., *Theileria* sp., *Rickettsia* sp., *Borrelia burgdorferi* sensu lato and *Coxiella burnetii*. Seventy-two out of 180 tick pools were positive to *Rickettsia* spp. (minimum prevalence of 8.7%), including the species *Rickettsia massiliae*, *Rickettsia slovaca* and *Rickettsia raoultii*. We did not detect *Rickettsia* spp. in wild boar spleens, or other TBPs in ticks or wild boars.

Since the tick species found can bite humans and the spotted fever group (SFG) rickettsiae identified are emerging zoonotic pathogens, there is a risk of SFG rickettsiae transmission for MAB inhabitants. Wild boar is not a *Rickettsia* spp. reservoir in this area according to the spleen negative results. However, its abundance could be favouring tick life cycle and abundance and facilitating *Rickettsia* spp. transmission among ticks via co-feeding, which together with proximity to humans could promote the vector capacity of ticks for *Rickettsia* spp. Managers and policy makers must be aware of this risk and encourage further research and application of monitoring, prevention and management measures.

Keywords: *Dermacentor marginatus*, *Hyalomma lusitanicum*, *Rhipicephalus sanguineus*, *Sus scrofa*, tick-borne pathogen, urban

3.3.2. Introduction

Ticks are among the most important vectors of disease transmission to livestock, pets and humans (Jongejan & Uilenberg, 2004) and both the number of TBPs and the incidence of tick-borne diseases is increasing globally as a result of multidimensional global changes (Colwell, Dantas-Torres, & Otranto, 2011; Dantas-Torres et al., 2012). In fact, tick-borne diseases of humans are emerging (Doudier, Olano, Parola, & Brouqui, 2010; Mansfield et al., 2009; Parola & Raoult, 2001) and constitute a major public health concern (Estrada-Peña & Jongejan, 1999).

Tick ecology and therefore TBP epidemiology are driven by environmental factors including host assemblages and abundance (James et al., 2013; Randolph, 2004; Ruiz-Fons, Fernández-de-Mera, Acevedo, Gortázar, & de la Fuente, 2012). The greater the host density, the higher the probability of ticks finding a suitable host, completing their life cycle and multiplying (Estrada-Peña & de la Fuente, 2014; Randolph, 2004). Hence, wildlife species can play a significant role in TBP epidemiology, as they can act as reservoir or amplifying hosts of numerous human pathogens, can carry ticks with them elsewhere and can favour tick abundance (Dantas-Torres et al., 2012; James et al., 2013; Ruiz-Fons et al., 2012). Moreover, with the increasing number of human-wildlife interactions in urban or highly populated areas, we face new scenarios where zoonotic pathogens, TBPs among them, can be transmitted and even favoured by urban-adapted hosts (Bradley & Altizer, 2007; Mackenstedt, Jenkins, & Romig, 2015).

We can assess the risk of transmission of TBPs to people through the study of ticks carried by sympatric species, and the Eurasian wild boar may be a good sentinel. Wild boar is commonly infested by hard ticks (Ortuño et al., 2007; Ruiz-Fons et al., 2006), its populations have increased across Europe since 1965 (Massei et al., 2015; Sáez-Royuela & Tellería, 1986) and it is increasingly close to humans as it is using urbanised and/or highly populated areas (Licoppe et al., 2013). These conditions occur in the MAB, in northeast Spain, where wild boars have grown in numbers for the last 20 years (Minuartia, 2017) and they are often seen in urban areas including the city of Barcelona (Cahill, Llimona, Cabañeros, & Calomardo, 2009; Castillo-Contreras et al., 2018). Most of these areas are within or around the Collserola massif, a natural space with high densities of wild boar (González-Crespo et al., 2018; Minuartia, 2017) and intensively used by MAB inhabitants for leisure activities (Parc de Collserola, n.d.-c).

Previous studies report different tick species on wild boars in Spain (Ortuño et al., 2007; Ruiz-Fons et al., 2006), the most common being *Hyalomma marginatum marginatum, Rhipicephalus bursa* and *Dermacentor marginatus* (Ruiz-Fons et al., 2006). However, *Dermacentor reticulatus*,

Rhipicephalus turanicus and *Ixodes ricinus* can also parasitize them in north Spain (Astobiza et al., 2011; García-Pérez et al., 2016; Ortuño et al., 2007; Ruiz-Fons et al., 2006).

Regarding the pathogens that can be transmitted by these ticks, some of them have been previously detected in ticks collected from wild boar, for instance *Ehrlichia* sp., *Anaplasma* sp., *Rickettsia* sp. and *Babesia* sp., along with *Borrelia burgdorferi* sensu lato (de la Fuente et al., 2004; Estrada-Peña, Osácar, Pichon, & Gray, 2005; Iori et al., 2010; Ortuño et al., 2006; Toledo et al., 2009). Most of these and other TBPs such as *Theileria* sp. and *Coxiella burnetii* have been also identified in wild boar tissues or sera in Czech Republic, Italy, Spain and Portugal (Astobiza et al., 2011; Faria et al., 2015; Petrovec et al., 2003; Selmi, Martello, Bertolotti, Bisanzio, & Tomassone, 2009; Tampieri et al., 2008; Zanet et al., 2014). However, to the best of our knowledge, studies on most of these pathogens have yielded negative results in tissues from wild boars from Spain (de La Fuente et al., 2005; García-Pérez et al., 2016; Gimenez, Casado, Criado-Fornelio, Álvarez de Miguel, & Dominguez-Peñafiel, 2009; Lledó et al., 2014).

At this point, an increased risk of TBP infection for MAB inhabitants could be suspected as a consequence of direct and indirect effects of wild boar expansion and proximity to humans. Our aim is to make a first assessment of this risk through two specific objectives: 1) identifying the tick species parasitizing wild boars and 2) describing the TBPs infecting ticks and their wild boar hosts inhabiting the MAB.

3.3.3. Material and methods

Study area

The study area comprises several locations within the MAB, in Catalonia, northeast Spain (Figure 3.8). The MAB comprises 36 municipalities, has 3,239,337 inhabitants and occupies 63,600 ha; this means that 42.8% of the whole Catalan population is concentrated in around 2% of the territory, resulting in an average density of 5,093 inhabitants per 100 ha (AMB, 2012). We performed this study in three different areas within the MAB: the Collserola massif, the city of Barcelona and the campus of Bellaterra from UAB, in the municipality of Cerdanyola del Vallès (Figure 3.8).

Collserola is a massif that belongs to the Catalan coastal range, roughly 10,000 ha in size, located in the centre of the MAB and with its highest point at 510 m above sea level (Generalitat de Catalunya, 2010). It includes two areas where wild boar hunting is allowed since 1995 (DARP, 2018) and recent estimations indicate that the wild boar population in Collserola

increased by almost ten times (from 165 to 1500 individuals) in the core area of the massif (8,000 ha) from 2000 to 2015 (González-Crespo et al., 2018). Collserola landscape is composed by a mixture of forests, scrublands, meadows, croplands and also human infrastructures such as roads, recreational spaces and residential areas. The vegetation is typically Mediterranean, with abundant and diverse scrub species (Generalitat de Catalunya, 2010). Collserola is also used by MAB inhabitants for leisure activities such as running, biking, hiking, walking or going on a picnic and it receives approximately three million visitors every year (Parc de Collserola, n.d.-c).

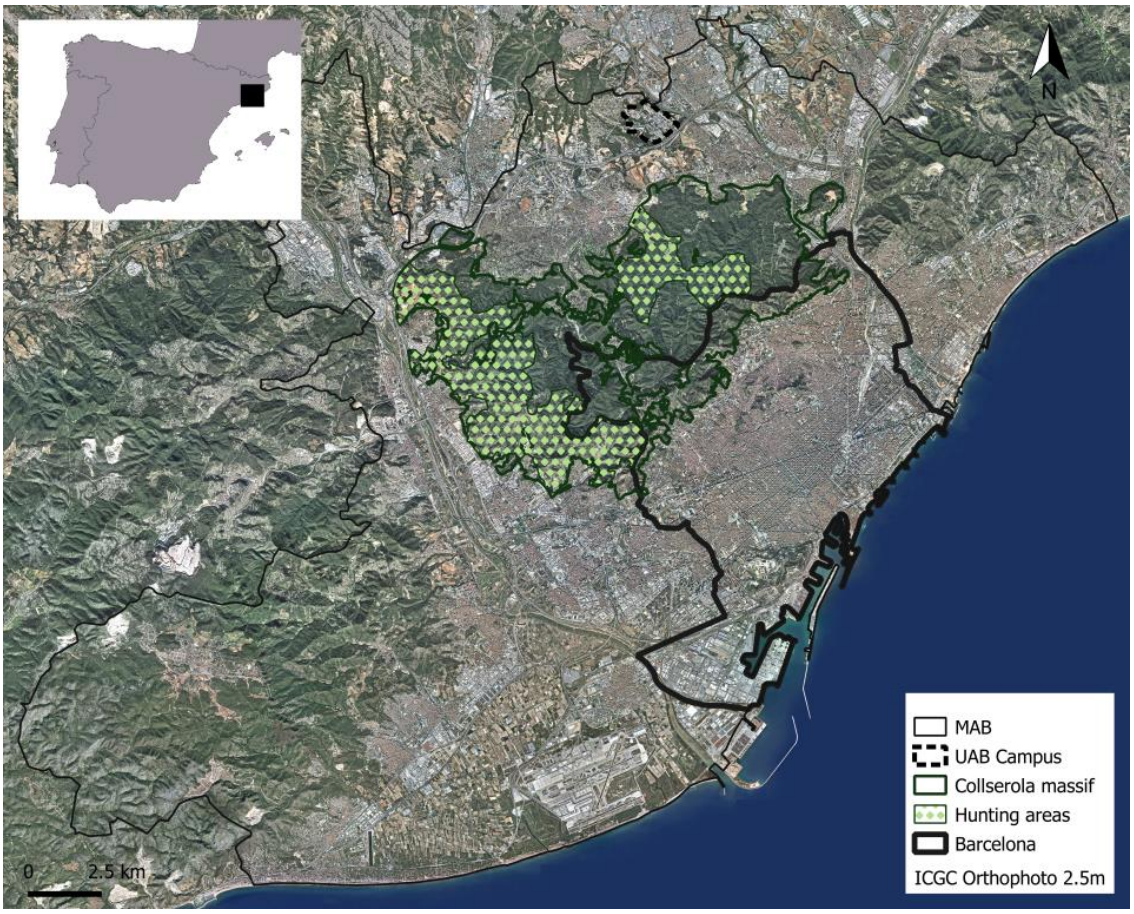

Figure 3.8. Study areas: Collserola (solid green line), Barcelona (heavy black line) and UAB (dashed black line), in the Metropolitan Area of Barcelona (MAB, thin black line). Top left: location of MAB (black square) in the Iberian peninsula. Orthophoto: ICGC.

The city of Barcelona is located southeast to Collserola, with a population of 1,600,000 inhabitants in 10,100 ha of surface (Idescat, 2017). In contrast to Collserola, Barcelona is mostly urbanised, although it comprises 1,077 ha of public green areas (Ajuntament de Barcelona, 2013). In addition, the northern districts of Barcelona are adjacent to Collserola and frequently visited by wild boars, which in the whole city cause 700 incidents (phone calls to local emergency number) a year on average (Ajuntament de Barcelona, 2018).

The UAB campus is located north to Collserola, roughly 260 ha in size, and regularly used by more than 45,000 people, including the student body and staff (UAB, 2018). It is surrounded by urbanised, forestry and agricultural areas, and forestry and agricultural patches cover approximately 60% of its surface (UAB, 2019a). Moreover, there are several gardens, some of them with ornamental plants and irrigation (UAB, 2019b). The wild boar is present in the UAB campus and between 15 and 30 individuals are removed every year as part of the wild boar management plan aimed at preventing damages in gardens, crops and other negative interactions with people (vehicle collisions, attacks) (Lavín et al., 2017).

The climate in the MAB is Mediterranean, with mild winters, hot dry summers and two rainy seasons, spring and autumn. Mean annual temperatures range from 6 to 11°C in winter and from 20 to 23°C in summer, and mean annual precipitation is 600-650 mm (Servei Meteorològic de Catalunya, 2016a, 2016b).

Sampling

Between 2014 and 2016, we examined 438 wild boars, either hunted or captured and euthanized, from Collserola (n=122, mainly from October to February), Barcelona (n=230, all year round), UAB campus (n=80, mainly from May to September) and other locations within the MAB (n= 12, from May to December).

Wild boars were removed for either population or conflict management purposes, not for research, and according to national laws. Hunted wild boars were shot by authorized local hunters, and euthanized wild boars were previously anesthetized with a blowpipe by a veterinarian within the framework of the contracts 13/051, 15/0174, 16/0243-00-CP/01 and 16/0243-00-PR/01 with the *Ajuntament de Barcelona* (Barcelona City Council) and the authorisation of *Generalitat de Catalunya* (Government of Catalunya), reference numbers AC/059, AC/190 and AC/215.

We performed a post-mortem external and internal examination of wild boar carcasses. We manually removed all ticks feeding on each wild boar and stored them in sterile 5 ml transport tubes, one per wild boar host. We also collected spleen samples and stored them in 5 ml tubes at -20ºC until further processing. To study wild boar parasitization, we recorded wild boar age class, sampling area, month and year of collection. We determined wild boar age using dentition patterns and wear (Iff, 1978; Matschke, 1967) and assigned the corresponding age class: piglet (up to 6 months), juvenile (6 to 12 months), yearling (12 to 24 months) and adult (over 2 years). We assigned a season according to the month of collection: winter (January,

February, March), spring (April, May, June), summer (July, August, September) and autumn (October, November, December).

Tick identification and pooling

We identified tick specimens with binocular lens according to morphological identification keys (Estrada-Peña, 2000; Estrada-Peña, Bouattour, Camicas, & Walker, 2004; Manilla, 1998). We did not differentiate between two *Rhipicephalus* species included within the *sanguineus* complex (*Rhipicephalus sanguineus* sensu stricto and *Rhipicephalus turanicus*) due to their morphological similarities and because of the impracticality of using the molecular techniques required for accurate classification (Zahler, Filippova, Morel, Gothe, & Rinder, 1997). We also determined tick life stage (adult, nymph or larva) and sex (female or male). We made pools (n=575) by wild boar host, tick species, life stage and sex, and stored them into sterile 1.5 ml microcentrifuge tubes at -20°C until further processing.

Tick-borne pathogen analyses

For TBP analyses, we selected 180 out of 575 tick pools, containing 827 ticks in total, in order to obtain representation of the four tick species found, the different areas, seasons and wild boar age classes. We also included 167 spleen samples belonging to the wild boar hosts from which the selected tick pools were collected. The selected tick pools comprised a variable number of adult ticks (one to six) of the same species, with no sex discrimination. In the case of wild boars co-infested with more than one tick species, we selected only one tick species per host. And in the case of wild boars with both male and female ticks from the same species, we mixed both sexes into the same pool.

We processed tick pools individually and washed each pool three times with sterile water and once with 70% ethanol. We air dried the tick specimens and collected them in sterile tubes. We then physically disrupted the ticks using sterilized scissors and conical tissue grinders in 200 ml of sterile phosphate buffered saline (PBS). We also mechanically disrupted and homogenized 10 mg of each of the 167 wild boar spleen samples in 200 ml of PBS.

Next, we used the QIAamp cador Pathogen Mini Kit (Qiagen, Hilden, Germany) to extract DNA from ticks and spleen samples in a single step (Ammazzalorso, Zolnik, Daniels, & Kolokotronis, 2015; Halos et al., 2004). We followed the manufacturer instructions and stored the resulting DNA extracts at -20°C until further analysis.

We screened the extracted 180 tick pools and 167 wild boar spleen samples by Real Time PCR (RT-PCR) for *Rickettsia* spp., *C. burnetii* and *B. burgdorferi* (s.l.), and by Reverse line blot hybridization assay (RLB) for *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. If the same pathogen was targeted by two different assays (RT-PCR and RLB), we considered as positive those samples yielding a positive results in both. Information on amplification mixtures and conditions can be found in Supporting Information S.3.3 to S.3.6, while target regions, expected length of the PCR products and oligonucleotide sequences of primers and probes are detailed in Table 3.14.

Table 3.14. Tick-borne pathogens (TBPs) targeted in this study. RT-PCR: Real Time PCR, RLB: Reverse line blot hybridization assay, PCR: conventional PCR, bp: base pairs, F: forward primer, R: reverse primer. Probes were labelled at the 5' and 3' ends with 6-carboxy-fluorescein (6-FAM) and 6-carboxyl-tetramethyl-rhodamine (TAMRA), respectively.

TBP (type of assay)	Target gene	Length (bp)	Oligonucleotide sequence of primers and probes (5'-3')	Reference
Rickettsia (RT-PCR)	gtlA	165	RKND03F: GTGAATGAAAGATTACACTATTTAT RKND03R: GTATCTTAGCAATCATTCTAATAGC RKND03: 6FAM-CTATTATGCTTGCGGCTGTCGGTTC-TAMRA	Rolain et al. (2009)
C. burnetii (RT-PCR)	IS1111	200	IS1111F: GCGTCATAATGCGCCAACATA IS1111R: CGCAGCCCACCTTAAGACTG IS1111: 6FAM-TGCTCAGTATGTATCCACCG-TAMRA	Brouqui, Rolain, Foucault, & Raoult (2005)
C. burnetii (RT-PCR)	IS30a	120	Cbis30aF: AATGTCTGCGGGAAATAGGC Cbis30aR: GAGGCCTTTTACCGGAATTC IS30a: 6FAM-TCGAGATCATAGCGTCATT-TAMRA	Brouqui et al. (2005)
B. burgdorferi (RT-PCR)	23S rRNA	75	Bb23Sf: CGAGTCTTAAAAGGGCGATTTAGT Bb23Sr: GCTTCAGCCTGGCCATAAATAG Bb23Sp: 6FAM-AGATGTGGTAGACCCGAAGCCGAGTG-TAMRA	Courtney, Kostelnik, Zeidner, & Massung (2004)
Rickettsia (RLB)	16S rDNA	350-400	Rick-F1: GAACGCTATCGGTATGCTTAACACA Rick-R2: Biotin-CATCACTCACTCGGTATTGCTGGA	Lorusso et al. (2016)
Ehrlichia/ Anaplasma (RLB)	16S rDNA	460-520	16S8FE: GGAATTCAGAGTTGGATC(A/C)TGG(C/T)TCAG BGA1B-new: Biotin-CGGGATCCCGAGTTTGCCGGGACTT(C/T)TTCT	Lorusso et al. (2016)
Theileria/ Babesia (RLB)	18S rDNA	460-540	RLB-F2: GACACAGGGAGGTAGTGACAAG RLB-R2: Biotin-CTAAGAATTTCACCTCTGACAGT	Lorusso et al. (2016)
Rickettsia (PCR)	gltA	850	CS409d: CCTATGGCTATTATGCTTGC Rp1258n: ATTGCAAAAAGTACAGTGAACA	Roux, Rydkina, Eremeeva, & Raoult (1997); Tijsse-Klasen et al. (2011)

For DNA amplification of *Rickettsia* sp., *C. burnetii* and *B. burgdorferi* (s.l.) by RT-PCR, we used a DNA Engine Opticon 2 Continuous Fluorescence Detector CFD-3220 (MJ Research, Canada). For DNA amplification of the rest of pathogens, plus *Rickettsia* sp., by RLB, we used a Prime Elite Thermal Cycler (Techne, UK). A detailed RLB protocol for membrane preparation, hybridization and detection can be found in O'Sullivan, Zhou, Sintchenko, Kong, & Gilbert (2011), and further details on the specific membrane used and the oligonucleotide probes included are available in Lorusso et al. (2016).

For *Rickettsia* sp. sequencing, we used the protocol described in Tijsse-Klasen et al. (2011) to amplify a 850 bp fragment of the *glt*A gene, which encodes for a citrate synthase protein. Oligonucleotide sequences of primers can be found in Table 3.14 and amplification mixture and conditions, in S5. Sanger sequencing was performed at the Servei de Genòmica i Bioinformàtica (Bellaterra, Spain), using an ABI 3130XL sequencer (Applied Biosystems, California). We aligned sequenced data in MEGA (version X; Kumar, Stecher, Li, Knyaz, & Tamura, 2018) and identified the species by comparison with the nucleotide collection (GenBank, EMBL, DDBJ, PDB and RefSeq sequences) through NCBI BLAST (http://www.ncbi.nlm.nih.gov/blast). We accepted a result when both the BLAST query cover and identity were equal to or above 99%.

Statistical analyses

We used the R software (version 3.5.0; R Development Core Team, 2018), to perform all statistical analyses. For 95% confidence intervals, we used the binconf function from the Hmisc package (Harrel Jr, 2018).

We looked for patterns in the spatio-temporal distribution of the different tick species identified, as well as for wild boar age-related patterns, in the infested wild boar from Collserola (n=82), Barcelona (n=139) and UAB (n=38), through GLMs (McCullagh & Nelder, 1989). We applied GLMs using the glm function within the stats package (R Core Team, 2019), with binomial family and logit link function. The response variable was the presence/absence of each tick species on a specific wild boar, and the predictors were area (Collserola, Barcelona or UAB), sampling year (2014 to 2016), season (winter, spring, summer or autumn) and wild boar age class (piglet, juvenile, yearling or adult). We did not include in this analysis ticks from seven wild boars sampled within the MAB but in areas other than the three previous ones due to the low available sample size.

Regarding TBPs, we applied another GLM to explore the *Rickettsia* sp. positivity in tick pools; the response variable was the positive or negative result obtained from each tick pool from the laboratory tests, and the predictors were tick species, area, sampling year, season and wild boar age class. Moreover, to test whether there was a relationship between the tick species and the *Rickettsia* species identified, we applied a Fisher's exact test for count data with the function fisher.test.

For model selection, we used the function dredge from the package MuMIn (Bartoń, 2018) to choose the best GLM(s) according to their AIC value (Burnham & Anderson, 2002). We also used

the rsq function from the package with the same name to estimate the percentage of data variation explained by the independent variables (adjusted R-squared) of our models.

3.3.4. Results

Ticks

We collected 2256 ticks feeding on 261 out of 438 wild boars examined (59.6%). We identified four different tick species, namely *Hyalomma lusitanicum* (1156/2256, 51.2%), *Rhipicephalus sanguineus* sensu lato (557/2256, 24.7%), *D. marginatus* (542/2256, 24%) and *R. bursa* (1/2256, 0.04%). See Table 3.15 for details on the life stage and sex of these ticks.

Table 3.15. Ticks collected from wild boar per tick species, life stage and sex, and relative frequency of each.

Tick species	Nymphs (%)	Adults (%)	Adult females (%)	Adult males (%)
H. lusitanicum	94 (93.1)	1062 (49.3)	265 (32.4)	797 (59.6)
R. sanguineus (s.l.)	7 (6.9)	550 (25.5)	305 (37.3)	245 (18.3)
D. marginatus	0 (0)	542 (25.2)	248 (30.3)	294 (22)
R. bursa	0 (0)	1 (0.05)	0 (0.0)	1 (0.07)
Sum	101	2155	818	1337

At host level, the average number of ticks per wild boar was 8.6 and the median was five, ranging from one to 70 ticks per wild boar. The species parasitizing most wild boars was *H. lusitanicum* (infestation prevalence of 33.6%, 95% CI: 29.3-38.1%), followed by *D. marginatus* (26.9%, 95% CI: 23-31.3%) and *R. sanguineus* (s.l.) (18.9%, 95% CI: 16.4-23.9%), while *R. bursa* was present on one wild boar (0.2%, 95% CI: 0.01-1.3%). Most wild boars were infested by one tick species, but there were also two and three tick-species infestations (see Table 3.16 for further details).

Table 3.16. Wild boars parasitized by each tick or combination of tick species with respect to the total amount of infested wild boars (n=261).

Tick species	Wild boars infested (%)
H. lusitanicum	147 (56.3)
D. marginatus	118 (45.2)
R. sanguineus (s.l.)	87 (33.3)
R. bursa	1 (0.4)
One tick species infestation	173 (66.3)
Only *H. lusitanicum*	67 (25.7)
Only *D. marginatus*	68 (26.1)
Only *R. sanguineus* (s.l.)	38 (14.6)
Only *R. bursa*	0 (0)
Two tick species infestation	84 (32.2)
H. lusitanicum + *D. marginatus*	38 (14.6)

H. lusitanicum + *R. sanguineus* (s.l.)	37 (14.2)
D. marginatus + *R. sanguineus* (s.l.)	8 (3.1)
H. lusitanicum + *R. bursa*	1 (0.4)
Three tick species infestation	4 (1.5)
H. lusitanicum + *D. marginatus* + *R. sanguineus* (s.l.)	4 (1.5)
Total wild boars infested	261 (100)

With regard to sampling areas, *H. lusitanicum* parasitized over half of the infested wild boar from Barcelona and over 30% of those from Collserola. One of the two selected GLMs confirmed that its presence was significantly higher in wild boars from Barcelona than in wild boars from UAB (GLM-h1: Z=-5.24, p<0.001) and both GLMs agreed on that *H. lusitanicum* was more frequently collected from Collserola than from UAB wild boars (GLM-h1: Z=3.72, p=0.0002; GLM-h2: Z=3.48, p=0.0005). *Dermacentor marginatus* selected GLM, in turn, revealed that this tick was more frequently found on wild boars from Collserola and UAB than from Barcelona (Collserola *vs*. Barcelona: Z=2.58, p=0.01; UAB *vs*. Barcelona; Z=3.37, p=0.0008). As for *R. sanguineus* (s.l.), over 60% of the infested wild boars from UAB carried this tick, but the corresponding GLMs could not find significant differences among areas.

Regarding seasonality, *H. lusitanicum* parasitized over 50% of the infested wild boars from April to October, and the two selected GLMs confirmed a seasonal pattern, with a maximum in summer and a minimum in winter (summer *vs*. autumn, GLM-h1: Z=4.52, p<0.001, and GLM-h2: Z=3.74, p=0.0002; winter *vs*. autumn, GLM-h1: Z=-3.07, p=0.002, and GLM-h2: Z=-3.88, p=0.0001; spring *vs*. autumn, GLM-h1: Z=3.24, p=0.001). *D. marginatus* was mainly found from October to February, when almost all wild boars carried this tick, and the corresponding GLM proved that it was significantly more frequent on infested wild boars during autumn, followed by winter, than during spring and summer (spring *vs*. autumn: Z=-4.83, p<0.001; summer *vs*. autumn: Z=-5.23, p<0.001; winter *vs*. autumn: Z=-2.23, p=0.03). On the other hand, *R. sanguineus* (s.l.) ticks were collected primarily from February to July, and most wild boars were infested with this tick from March to June, but the corresponding GLMs could not prove a season-related pattern. The single *R. bursa* was collected in September.

Furthermore, and according to one of the selected *H. lusitanicum* GLMs, its presence significantly increased from piglet to adult wild boars (GLM-h1: Z=-2.76, p=0.006), and also from 2014 to 2016 (GLM-h1: Z=3.17, p=0.002). On the contrary, *R. sanguineus* (s.l.) presence seemed to decrease with wild boar age, as indicated by one of the three selected *R. sanguineus* (s.l.) GLMs (juveniles *vs*. adults, GLM-r3: Z=2.49, p=0.013). Piglets and yearlings showed the same pattern although without statistical significance.

The explained variance was 41.3% and 44.5% for the two *H. lusitanicum* selected models, 54.4% for the *D. marginatus* selected model and 74.1%, 75.3% and 62.4% for the three *R. sanguineus* (s.l.) selected models.

Tick-borne pathogens

We found 72 out of the 180 tick pools (40%) to be positive for *Rickettsia* spp., which yields an overall minimum prevalence of 8.7% (95% CI: 7-10.8). The minimum prevalence per tick species was 14.7% for *R. sanguineus*, 12.2% (95% CI: 9.1-16.1) for *D. marginatus* and 0.7% (95% CI: 0.2-2.5) for *H. lusitanicum* (see Table 3.17 for further information). Since we selected one tick pool per wild boar host, the number of wild boars with positive tick pools was 72 (72/180, 40%, 95% CI: 33.1-47.3).

Table 3.17. *Rickettsia*-positive tick pools, minimum prevalence and *Rickettsia* species identified per tick species.

Tick species	Positive tick pools (%)	Minimum positive ticks[a] (minimum prevalence, 95% CI)	*Rickettsia* species identified
D. marginatus	40/74 (54.1)	40/329 (12.2; 9.1-16.1)	*R. slovaca* (24), *R. raoultii* (9), *Rickettsia* sp. (7)
R. sanguineus (s.l.)	30/43 (69.8)	30/204 (14.7; 10.5-20.2)	*R. massiliae* (28), *Rickettsia* sp. (2)
H. lusitanicum	2/62 (3.2)	2/293 (0.7; 0.2-2.5)	*R. slovaca* (1), *Rickettsia* sp. (1)
R. bursa	0/1 (0)	-	-
Total	72/180 (40)	72/827 (8.7; 6.97-10.82)	*R. massiliae* (28), *R. slovaca* (25), *R. raoultii* (9), *Rickettsia* sp. (10)

[a] Minimum number of positive ticks within the total amount of ticks included in the pools.

Being positive to *Rickettsia* spp. strongly depended on the tick species. More precisely, the selected GLM revealed that *Rickettsia* spp. was positively associated to *R. sanguineus* (s.l.) (Z=2.82, p=0.005) and negatively associated to *H. lusitanicum* (Z=-3.96, p<0.001), when compared to *D. marginatus* tick pools. Positivity to *Rickettsia* spp. also depended on the sampling area; there were less positive tick pools in UAB than in Barcelona (Z=-2.53, p=0.01) and Collserola (Z=-2.795, p=0.007), although Collserola tick pools were no different from Barcelona ones (Z=0.954, p=0.3). However, as tick species were not equally distributed per area, we must point out that most positive tick pools from UAB and Barcelona wild boars belonged to *R. sanguineus* (s.l.) species, while almost all positives from Collserola wild boars came from *D. marginatus* pools (see Figure 3.9). Sampling year, season and wild boar age class were not included in the selected GLM and the explained variance was 32.95%.

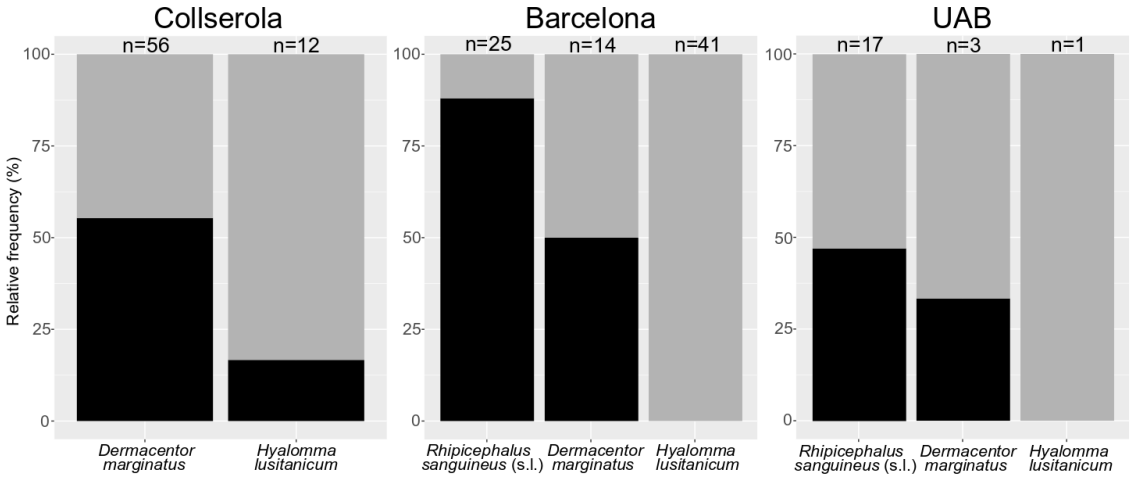

Figure 3.9. *Rickettsia* spp. positive (black) and negative (grey) tick pools per tick species and sampling area. Eleven tick pools are not included in the figure because they were obtained in areas other than Collserola, Barcelona or UAB. UAB: Universitat Autònoma de Barcelona.

Among the *Rickettsia*-positive pools, 62 of them could be sequenced. The Sanger sequencing revealed three different *Rickettsia* species, namely *Rickettsia massiliae* (28/62, 45.2%), *Rickettsia slovaca* (25/62, 40.3%) and *Rickettsia raoultii* (9/62, 14.5%). Ten other positive pools could not be identified at the species level (Table 3.17). Tick species significantly affected the *Rickettsia* species identified in them (Fisher's test, $p<2.2e^{-16}$). *Rickettsia massiliae* was only detected in *R. sanguineus* (s.l.) tick pools, while *R. slovaca* and *R. raoultii* were both detected in *D. marginatus* pools. *R. slovaca* was also the species identified in the one *H. lusitanicum* pool that could be sequenced (Table 3.17).

We did not find *Rickettsia* spp. DNA in wild boar spleens. As for the other TBPs included in our study, we did not detect *C. burnetii*, *B. burgdorferi* (s.l.), *Ehrlichia* sp., *Anaplasma* sp., *Babesia* sp. or *Theileria* sp. either in the 180 tick pools or the 167 wild boar spleen samples analysed.

3.3.5. Discussion

Ticks

The prevalence of tick infestation on wild boars in this study, close to 60%, was within the range of prevalence previously found on Spanish wild boars, which varies from 9 to 70% depending on the region (Ortuño et al., 2007; Ruiz-Fons et al., 2006). The four tick species identified are commonly found in areas with Mediterranean climate and there are several domestic animals among their hosts (Estrada-Peña et al., 2004). All four species have been collected from wild boars in Spain (de la Fuente et al., 2004; Márquez, 2009; Ruiz-Fons et al., 2006) but, to our

knowledge, only *D. marginatus* had been previously reported on wild boars from our region, in northeast Spain (Ortuño et al., 2007, 2006). The anecdotal observation of one *R. bursa*, which is common in livestock from Mediterranean areas (Estrada-Peña et al., 2004), could be related to the marginal presence of free-ranging domestic ruminants in our study area (Parc de Collserola, n.d.-b).

We did not find *I. ricinus*, which is commonly reported to parasitize wild boars in our country (García-Pérez et al., 2016; Márquez, 2009; Ruiz-Fons et al., 2006) and central Portugal (Pereira et al., 2018). We did not find *D. reticulatus* either, which has been found on wild boars from north and northwest Spain (Astobiza et al., 2011; Ruiz-Fons et al., 2006), or *H. marginatum*, already described on wild boars from central Spain (Ruiz-Fons et al., 2006) and central Portugal (Pereira et al., 2016).

Regarding the spatial distribution of the tick species collected, *H. lusitanicum* significantly differed among areas, being wild boars from UAB less frequently parasitized than wild boars from Barcelona and Collserola. On one hand, the difference detected between Barcelona and UAB comes primarily from the comparison during spring-summer, which was the sampling period shared by these areas. And this difference could be related to food resource aggregation in urbanised areas, as Wright & Gompper (2005) showed through an experiment with urban raccoons. In that study, they proved that clumped food resources made the raccoons aggregate, so their contact rates were higher and, as a result, the infection prevalence with endoparasites increased. It would be possible that wild boars from Barcelona aggregate in specific locations where food is available (garbage bins, green areas or pet food dispensers; Castillo-Contreras et al., 2018; Licoppe et al., 2013), therefore increasing direct contact and tick transmission. Since *H. lusitanicum* can be found in large numbers in rock crevices and rabbit burrows (Estrada-Peña et al., 2004), the lesser availability of resting areas in the more urbanised Barcelona could be also favouring wild boar aggregation. Finally, other factors that have not been evaluated in this study, such as differences in wild boar abundance or in the composition of the community of hosts (Estrada-Peña & de la Fuente, 2014), could also be limiting *H. lusitanicum* presence in UAB wild boars. On the other hand, the difference in *H. lusitanicum* infestation between Collserola and UAB wild boars arises from its comparison during autumn-winter, provided that this is the sampling period shared by the two areas. However, as the sample size of UAB wild boars during autumn-winter was very low (n=5), we would attribute the difference found by the model to a bias in the sampling strategy.

Regarding seasonality, the intra-annual variation of *H. lusitanicum* infestation is probably due to its questing behaviour, as adults reach a peak in their questing activity in May-July and again in October-November (Estrada-Peña et al., 2004; Requena-García, Cabrero-Sañudo, Olmeda-García, González, & Valcárcel, 2017; Valcárcel, González, Pérez-Sánchez, Tercero-Jaime, & Olmeda, 2015). The preferred host size of this tick, e.g. large and medium-sized domestic and wild ungulates (Apanaskevich, Santos-Silva, & Horak, 2008), is possibly the reason why we found more wild boars parasitized by this tick as they grew older. Last, there was also an increase in the number of wild boars parasitized by *H. lusitanicum* from 2014 to 2016. A recent study in central Spain has revealed that *H. lusitanicum* questing activity is positively related to temperature but negatively related to humidity (Requena-García et al., 2017). Mean annual temperature in Catalonia was higher in 2016 than in 2014, and annual precipitation was lower in 2016 than in 2014 (Servei Meteorològic de Catalunya, 2015, 2017). Therefore, 2016 might have been better, in terms of temperature and humidity, than 2014 for *H. lusitanicum* activity.

As for *D. marginatus*, this tick usually prefers areas with dense bushes and tree cover (Estrada-Peña et al., 2004) and this could explain why the wild boars from Collserola and UAB were more parasitized than the Barcelona ones. Moreover, *D. marginatus* seasonal pattern agrees with the period of activity of this tick, as adults are active at the end of autumn and throughout winter (Estrada-Peña et al., 2004; Rubel et al., 2016).

Regarding *R. sanguineus* (s.l.), its presence was related to wild boar age class, and while *H. lusitanicum* preferred older wild boars, *R. sanguineus* (s.l.) apparently selected younger ones. This ticks might avoid adult wild boars because of some physical feature such as skin or hair that is not suitable for the proper attachment to the host or because it is easier for the wild boars to remove the attached ticks by grooming. In this regard, *Rhipicephalus* ticks have a hypostome shorter than usual so they attach more superficially than other ticks (Dantas-Torres et al., 2012), and Welch, Samuel, & Wilke (1991) suggested that differences in hair coat among ungulates could affect their ability to remove ticks by grooming.

Concerning the almost absence of *R. sanguineus* (s.l.) ticks in Collserola wild boars, although the selected GLMs did not show a significant difference with respect to area, we would argue that the hunting season is probably the cause, as spring-summer was the period when we collected most ticks of this species in the other areas but hunting in Collserola is limited to autumn and winter.

Tick-borne pathogens

The minimum prevalence of *Rickettsia* spp. that we obtained for *R. sanguineus* (s.l.), nearly 15%, falls within the range previously described, despite it is difficult to draw any conclusions from our result because the *sanguineus* group includes more than one species with different vector competence (Zahler et al., 1997). In fact, *Rickettsia* spp. prevalence in *R. sanguineus* (s.l.) varies depending on the region and/or the host. For instance, 13% of *R. sanguineus* (s.l.) collected from domestic and wild animals in Portugal harboured rickettsial DNA (Pereira et al., 2018), 25% of *R. sanguineus* (s.s.), also from domestic and wild animals, were *Rickettsia*-positive in central Spain (Toledo et al., 2009), whereas less than 2% of *R. turanicus* ticks from goats were positive in Sardinia (Chisu et al., 2014). Conversely, several studies on *D. marginatus* ticks from wild boars have reported a *Rickettsia* spp. infection prevalence higher than the 12% reported here: nearly 34% in south Italy (Selmi et al., 2009), over 50% in south Spain (Márquez, 2009) or 65% in northeast Spain – our region – (Ortuño et al., 2007). However, we must take into consideration that our estimations are minimum prevalences, assuming that each of the *Rickettsia*-positive tick pools just contained one positive tick. Hence, the actual prevalence values in our study are probably higher. As for *H. lusitanicum*, none of the 94 specimens collected in a study with domestic and wild animals in central Spain carried rickettsial DNA (Toledo et al., 2009), and just one out of 50 was positive in another study in Portugal (Pereira et al., 2018), which is in accordance with our low minimum prevalence (less than 1%) and might indicate that this tick is a less competent vector of *Rickettsia* spp., as already suggested by (Toledo et al., 2009).

Regarding the spatial distribution of *Rickettsia*-positive ticks, the proportion of positive tick pools was lower in ticks collected from wild boars from UAB than from the other areas. The fact that *Rickettsia* positivity depended on the tick species and that almost all positive tick pools from Collserola wild boars were *D. marginatus* prevent us from comparing Collserola with the other two areas. Hence, the difference between Barcelona and UAB, where most positive pools belonged to *R. sanguineus* (s.l.) ticks, could be explained by the dilution effect (Ostfeld & Keesing, 2000). These authors observed that an increase in the diversity of host species caused a decrease in *B. burgdorferi* prevalence in ticks because these fed on inefficient disease reservoirs rather than focusing on white-footed mice (*Peromyscus leucopus*), the most competent reservoir hosts in North America. Since UAB contains patches of forest and scrubland and is also surrounded by this type of vegetation, there might be a greater variety of *R. sanguineus* (s.l.) potential hosts in UAB than in Barcelona, allowing the ticks to feed on different hosts (ones with better competence as reservoirs than others) and thus decreasing the

number of *Rickettsia*-positive ticks. In the case that some or all ticks that we classified as *R. sanguineus* (s.l.) were *R. sanguineus* (s.s.), the higher abundance of positive tick pools in Barcelona could be associated to a higher abundance of dogs, its main host, in Barcelona. More contact between wild boars and dogs could lead to an increased transmission of ticks and, therefore, of the pathogens they carry.

With regard to the *Rickettsia* species identified, previous studies support a strong relationship between them and the hosting tick species. *R. massiliae* has been detected in *R. sanguineus* (s.l.) ticks collected from wild boar (Chisu et al., 2014; Leulmi et al., 2016), whereas *R. slovaca* and *R. raoultii* have both been identified in *D. marginatus* ticks, also from wild boar (Márquez, 2009; Selmi et al., 2009). On the contrary, and to the best of our knowledge, this is the first time that *R. slovaca* is reported in *H. lusitanicum* ticks, either collected from wild boar, other hosts or the environment. Nonetheless, the detection of DNA of a certain pathogen in ticks might demonstrate that they have been exposed but not their role in pathogen transmission, so studies to confirm *H. lusitanicum* competence as a vector of *R. slovaca* should be performed.

In contrast to our negative results, *Ehrlichia*, *Anaplasma*, *Babesia* and *B. burgdorferi* (s.l.) species have been previously detected in ticks collected from wild boar, either in Spain (de la Fuente et al., 2004; Estrada-Peña et al., 2005) or in other countries such as Czech Republic, Italy or Germany (Honig et al., 2017; Iori et al., 2010; Silaghi, Pfister, & Overzier, 2014). Conversely, our negative results for *Theileria* sp. and *C. burnetii* agree with previous findings in wild boar ticks (Astobiza et al., 2011; Iori et al., 2010; Leulmi et al., 2016).

Regarding TBPs in wild boars, to the best of our knowledge, there are no reports of *Anaplasma*, *Rickettsia*, *Theileria* or *Babesia* sp. in wild boar tissues in Spain, which agrees with our results. Conversely, *C. burnetii*, the agent of Q fever, has been found in wild boar tissues in northern Spain and the Canary Islands (Astobiza et al., 2011; Jado et al., 2012), which differs from our results. Some of these pathogens have also been detected in wild boar tissues in other countries. For instance, *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis and tick-borne fever of ruminants, has been reported in wild boars from Czech Republic, Romania, Germany and Slovakia (Kazimírová et al., 2018; Kiss, Cadar, Krupaci, Bordeanu, & Spînu, 2014; Petrovec et al., 2003; Silaghi et al., 2014), and different species of *Rickettsia*, *Theileria* and *Babesia*, capable of causing disease in humans or domestic animals, have been detected in wild boars from Italy or Algeria (Selmi et al., 2009; Tampieri et al., 2008; Zanet et al., 2014; Zeroual, Leulmi, Bitam, & Benakhla, 2018). Lastly, *B. burgdorferi* (s.l.), responsible for Lyme disease in humans, and *Ehrlichia* sp., some of which cause disease in

humans and domestic animals, have not been reported yet in wild boar tissues (Kazimírová et al., 2018; Pereira et al., 2016; Silaghi et al., 2014), in accordance with our results.

The negative results obtained from wild boar tissues prevent us from concluding a reservoir role of this species for *Rickettsia* spp. in our study area. These results contrast with previous findings of specific spotted fever group (SFG) *Rickettsia* antibodies in wild boars from central and northeastern Spain (Fernández de Mera et al., 2013; Ortuño et al., 2007), proving exposure and suggesting that wild boars can serve as hosts of *Rickettsia* spp. Altogether this might indicate both susceptibility of wild boar to *Rickettsia* infection and its ability to control the infection through undetermined immune responses. This ability has been previously suggested for *A. phagocytophilum* through an experimental infection in wild boars (de la Fuente & Gortázar, 2012).

Therefore, since *Rickettsia* spp. can be transmitted trans-stadially (from one life stage to the next) and transovarially (from females to their eggs), ticks in our study could have acquired *Rickettsia* spp. while feeding on a previous infected host, most probably during immature stages (Azad & Beard, 1998). Similarly, the study of Millán et al. (2016) showed that *Rhipicephalus* ticks collected from carnivores were infected with *Rickettsia* spp. but their carnivore hosts were not, suggesting that the infection occurred when feeding on small mammals as immature ticks. Also, the positive ticks in our study could have been infected via co-feeding, as this way of transmission has already been proven for some *Rickettsia* species (Moraes-Filho, Costa, Gerardi, Soares, & Labruna, 2018; Zemtsova, Killmaster, Mumcuoglu, & Levin, 2010).

Despite the wild boar does not seem to be a *Rickettsia* spp. reservoir in our study area, both the wild boar abundance and expansion into highly populated areas could be acting as promoter factors of the vector capacity of ticks for *Rickettsia* spp. It has already been suggested that the vector capacity of ticks – the real ability to transmit a pathogen under natural conditions – is determined, either upwards or downwards, by factors other than mere vector competence (Duron, Sidi-Boumedine, Rousset, Moutailler, & Jourdain, 2015; Varela-Castro et al., 2018). On one hand, the increasing trend of wild boar populations during the last years (Massei et al., 2015) is probably facilitating the life cycle of ticks and, therefore, their abundance (Estrada-Peña & de la Fuente, 2014). Moreover, wild boars could be favouring the *Rickettsia* spp. transmission among ticks via co-feeding, even if wild boars are not (Moraes-Filho et al., 2018; Zemtsova et al., 2010). On the other hand, human-wildlife coexistence is generating new paradigms of interactions (Soulsbury & White, 2015) due to increased wildlife populations, bidirectional fearless behaviours (Martínez-Abraín et al., 2019) and different perceptions of people towards

wildlife (Conejero et al., 2019). This may acquire bigger dimensions in a scenario where wild species and humans live in sympatry, human population at risk is considerable, and hence there is an increased health risk for people (Arce et al., 2013), such as in the MAB.

The three *Rickettsia* species identified – *R. massiliae*, *R. slovaca* and *R. raoultii* – belong to the SFG, which represents a public health concern as these pathogens are emerging and cause rickettsioses in humans (Brouqui, Parola, Fournier, & Raoult, 2007; Oteo & Portillo, 2012). Both R. *slovaca* and *R. raoultii*, for instance, cause tick-borne lymphadenopathy (TIBOLA), also known as *Dermacentor*-borne necrosis erythema and lymphadenopathy (DEBONEL; Parola et al., 2009; Raoult, Berbis, Roux, Xu, & Maurin, 1997), the most prevalent tick-borne rickettsiosis in Europe after Mediterranean spotted fever (Oteo & Portillo, 2012). *R. massiliae* infection, although less common, has also been described as a cause of disease in humans since its first description (Vitale, Mansueto, Rolain, & Raoult, 2006); see Eldin et al. (2018) for a comprehensive list of cases.

In north Spain, most ticks collected from people attending a health care centre were identified as *D. marginatus*. Some of these specimens were found infected with *Rickettsia* spp., exposing the risk for transmission to people in that area (Merino et al., 2005). Another study carried out in our region, Catalonia, revealed the presence of antibodies against *R. slovaca* in 5.5% of patients attending a hospital for reasons other than infectious diseases, also suggesting an exposure of people to this pathogen (Antón et al., 2008). Since the three *Rickettsia* species identified in our study are zoonotic and the ticks found on wild boars can bite humans (mainly *D. marginatus*; Estrada-Peña & Jongejan, 1999), there is a risk of *Rickettsia* spp. transmission for people living in the MAB. In fact, between 2012 and 2017, and despite we cannot discard imported cases, 99 people attended a health care centre in the MAB and were diagnosed with some kind of rickettsiosis, and 13 of them required hospital care (AQuAS, 2018a, 2018b).

Many MAB inhabitants may be at risk when practising their daily or leisure activities either in Barcelona, Collserola or UAB, and information to visitors should be provided through informative or warning panels and information campaigns. Nevertheless, the risk may spread further, since hosts can disperse infected ticks (Palomar et al., 2012). Wild boars can travel distances of several kilometres daily (Podgórski et al., 2013) and, as we already mentioned, some of them are colonising urban and peri-urban areas such as the city of Barcelona (Cahill et al., 2012; Castillo-Contreras et al., 2018; Licoppe et al., 2013). In the particular case of Barcelona, wild boar presence occurs within and around the city such as in urban parks, private and public gardens (Castillo-Contreras et al., 2018), so ticks and TBPs may reach places where

the risk is supposed to be low or non-existent and hence more difficult to predict. Managers and policy makers must be aware of this risk in order to encourage the design and application of monitoring, prevention and management measures.

To better characterise tick ecology, TBPs epidemiology and improve risk prevention, further studies should be directed at collection and identification of questing ticks from vegetation, on one hand, to assess the relationship between wild boar abundance and tick abundance, and on the other hand, to screen them for TBPs, especially *Rickettsia* spp. This would allow us to identify other tick species present in our study area and, since we would presumably collect nymphs and larvae, TBP results would also help us elucidate in which life stage do *D. marginatus* and *R. sanguineus* (s.l.) acquire *Rickettsia* spp.

3.3.6. Conclusions

The MAB is home to three million people that live in sympatry with wild boars, some of which carry ticks infected with zoonotic *Rickettsia* species. In this study we described the presence of four tick species, three of which had not been previously collected from wild boars in our region. Moreover, we identified three emerging zoonotic pathogens belonging to the SFG, namely *R. massiliae*, *R. slovaca* and *R. raoultii*, in ticks parasitizing wild boars. However, we did not detect these pathogens in wild boar tissues, and thus we cannot conclude a reservoir role of wild boar for *Rickettsia* spp. In spite of this, the increasing trend of wild boar populations could be promoting tick abundance in natural areas, as well as the wild boar presence in urbanised areas could be favouring the dispersion of ticks into these areas. Moreover, wild boars could be facilitating the *Rickettsia* spp. transmission among ticks through co-feeding. Therefore, a tangible risk of human exposure to *Rickettsia* spp. can be expected, even in urban locations where both the presence of ticks and the infection risk is supposedly low or non-existent, and hence more difficult to predict.

3.3.7. Acknowledgements

We want to express our gratitude to Marià Martí, Lluís Cabañeros and other of members of the Consorci del Parc Natural de la Serra de Collserola, as well as to Josep Maria López Martín, from Departament d'Agricultura, Ramaderia, Pesca i Alimentació (Generalitat de Catalunya), for their logistic support. Also, we wish to thank local hunters from Collserola and all friends and colleagues from SEFaS that collaborated in the sample collection, processing and/or TBP analyses, especially Luis Magen and Oscar Cabezón. Our special thanks to thank Richard Birtles,

Jessica Hall and Isabel G. Fernández de Mera for their support in laboratory analyses, and to all people involved in the wild boar monitoring and management on UAB campus, especially Anna Florensa.

This study was funded by the contracts 13/051, 15/0174, 16/0243-00-CP/01 and 16/0243-00-PR/01 between Universitat Autònoma de Barcelona and Ajuntament de Barcelona. RCC, XFA and ACC benefited from pre-doctoral grants co-financed by Agència de Gestió d'Ajuts Universitaris i de Recerca (Generalitat de Catalunya) and the European Social Fund, file numbers 2016FI_B 00425, 2017FI_B1 00040 and 2018FI_B2_00030.

3.3.8. Supporting information

S.3.3. Molecular detection of *Rickettsia* spp. DNA was performed using a total PCR volume of 20 µL, which comprised 5 µL of extracted DNA and 15µL of PCR mixture. PCR mixture and conditions were designed following manufacturer instructions and included 10 µL of MyTaq™ Mix (Bioline), 0.5 µl (20 pmol/µl) of forward primer RKND03F, 0.5 µl (20 pmol/µl) of reverse primer RKND03R, 2 µl (2 pmol/µl) of FAM and TAMRA-labelled probe RKND03R (Taqman®), and 2 µL of distilled water. Amplification conditions started with a first step at 95°C for 3 minutes, followed by 40 cycles of denaturation at 92°C for 1 second and annealing and extension at 60°C for 35 seconds, and one last cycle at 42°C for 30 seconds. Samples with cycle threshold (C_t) values lower than 35 were considered positive. Distilled water was used as negative control and a laboratory-cultured *Rickettsia conorii* strain was the positive control.

S.3.4. Molecular detection of *Coxiella burnetii* was performed following a protocol described in Brouqui et al. (2005), which consisted on the amplification of two different target regions through two different PCRs. For the first one, a total volume of 20 µL including 5 µL of extracted DNA and 15µL of PCR mix was used. The PCR mix included: 10 µl of MyTaq™ Mix (Bioline), 0.5µl (10 pmol/µL) of forward primer IS1111F, 0.5µl (10 pmol/µL) of reverse primer IS1111R, 2 µl (2 µmol/µL) of FAM and TAMRA-labelled probe IS1111 and 2 µL of distilled water. The same mix was used for the second PCR, but the primers and probe were replaced by Cbis30aF, Cbis30aR and IS30a, respectively. Amplification conditions included a first step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C for 1 second, and annealing and extension at 60°C for 60 seconds. Samples with C_t values lower than 35 for both genes were considered positive. Distilled water was used as negative control and a known *C. burnetii* strain served as positive control.

S.3.5. Molecular detection of *B. burgdorferi* (s.l.) was performed following a modified protocol from Courtney et al. (2004). The total PCR volume was 20 µL and comprised 2 µL of extracted DNA and 18 µL of PCR mix. The mix included 10 µL of MyTaq™ Mix (Bioline), 1 µl (10 pmol/µl) of forward primer Bb23Sf, 1 µl (10 pmol/µl) of reverse primer Bb23Sr, 1 µl (10 pmol/µl) of FAM and TAMRA-labelled probe Bb23Sp-FAM and 5 µL of distilled water. Amplification conditions included a first step at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 60 seconds. Samples with C_t values lower than 35 were considered positive. Distilled water was used as negative control and a known *B. burgdorferi* strain served as positive control.

S.3.6. Molecular amplification of *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. DNA through RLB consisted on three different amplifications, one for *Rickettsia* spp., one for *Ehrlichia*/*Anaplasma* spp. and one for *Babesia*/*Theileria* spp. The total volume of all three PCRs was 25 µL and comprised 2.5 µL of extracted DNA and 22.5 µL of PCR mix. The mix included 10 µL of MyTaq™ Red Mix (Bioline), 1 µl (20 pmol/µl) of forward primer, 1 µl (20 pmol/µl) of reverse primer and 10.5 µL of distilled water. There were three different sets of primers; one pair for *Rickettsia* sp., one pair for *Ehrlichia*/*Anaplasma* sp. and another pair for *Theileria*/*Babesia* sp. PCR amplification conditions included a first denaturation step at 98°C for 30 seconds, followed by 10 cycles of: denaturation at 98°C for 5 seconds, annealing at 67°C for 5 seconds (with a 1°C decrease per cycle) and extension at 72°C for 7 seconds, followed by 40 cycles of: denaturation at 98°C for 5 seconds, annealing at 57°C for 5 seconds and extension at 72°C for 7 seconds, with a final extension step at 72°C for 60 seconds. Distilled water was used as negative control and a laboratory-cultured *Rickettsia conorii* strain, a known *Ehrlichia ruminantium* and a known *Babesia bigemina* served as positive controls for *Rickettsia* sp., *Ehrlichia/Anaplasma* sp. and *Theileria/Babesia* sp. assays, respectively.

S.3.7. Molecular amplification of an 850 bp fragment from the *glt*A gene from *Rickettsia* sp. was performed following a protocol modified from Tijsse-Klasen et al. (2011). A total volume of 20 µL including 2 µL of extracted DNA and 18µL of PCR mix was used. The PCR mix was designed according to manufacturer instructions and included: 10 µl MyTaq™ Red Mix (Bioline), 1µl (10 pmol/µL) of forward primer CS409d, 1µl (10 pmol/µL) of reverse primer Rp1258n and 6 µL of distilled water. PCR conditions consisted on a first step at 94°C for 15 minutes, followed by 40 cycles of: 94°C for 30 seconds, 54°C for 30 seconds and 72°C for 55 seconds, and a final elongation step at 72°C for 7 minutes. Distilled water was used as negative control and a laboratory-cultured *Rickettsia conorii* strain served as positive control.

3.4. Study 4

Zoonotic *Campylobacter* spp. and *Salmonella* sp. carried by wild boars in a metropolitan area: occurrence, antimicrobial susceptibility and public health relevance

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3.4.1. Abstract

Campylobacteriosis is the most commonly reported zoonosis in Europe, followed by salmonellosis, and both are among the four most reported causes of foodborne illness worldwide. Although wildlife is not the main source of human infection, both *Campylobacter* and *Salmonella* can be transmitted from wildlife to humans. Wild boars can harbour a wide variety of zoonotic pathogens shared with livestock, companion animals and humans, including these two zoonotic bacteria. In the MAB (northeast Spain), wild boars enter the city of Barcelona from the bordering Serra de Collserola Natural Park, where the wild boar population has increased in recent years. In order to assess the potential public health risk of this wild boar population, from June 2015 to February 2016 we collected stool samples from 130 wild boars from three distinct areas in the MAB, to determine the *Campylobacter* spp. and *Salmonella* sp. occurrence and the antimicrobial susceptibility of the isolates. Also, we investigated the genetic diversity and virulence potential of *Campylobacter* isolates. Most wild boars carried *Campylobacter* spp. (60.8%, 95% CI: 52.2-68.7); among these, 46.2% carried *C. lanienae* (95% CI: 37.8-54.7) and 16.2% carried *C. coli* (95% CI: 10.8-23.4). Although *Salmonella enterica* subsp. *enterica* was only detected in four wild boars (3%, 95% CI: 1.2-7.6), notably one of them carried the multidrug resistant (ASSuT) monophasic *S.* Typhimurium clone associated with human infections and pig meat in several EU countries, including Spain. We observed a high genetic diversity of *Campylobacter* species using pulse-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), and identified new sequence types. *Campylobacter lanienae* were not typeable with *fla*A-RFLP, and the restriction enzyme SmaI was more suitable than KpnI for typing both *Campylobacter* species by PFGE. Thirty per cent of *C. coli* and 12.5% of *C. lanienae* isolates showed a high virulence potential. None of the *Campylobacter* isolates tested were susceptible to all the antimicrobials and 58.8% of *C. coli* isolates were multiresistant. Wild boars could be a reservoir of *Campylobacter* spp. or simply carry and spread these foodborne zoonotic bacteria in urban and peri-urban areas in the MAB, posing a threat to people since the wild boar population, their presence in urban and peri-urban areas and interaction with humans are increasing.

Keywords: *Campylobacter coli*, *Campylobacter lanienae*, MLST, PFGE, *Sus scrofa*, urban

3.4.2. Introduction

Zoonoses such as campylobacteriosis and salmonellosis are an important cause of human morbidity worldwide and an obstacle to socio-economic development (WHO, 2015).

Campylobacteriosis has been the most commonly reported zoonosis in Europe since 2005, representing almost 70% of all reported human cases in 2017, followed by salmonellosis (EFSA and ECDC, 2018), and they are the second and fourth most reported causes of foodborne illness worldwide, respectively (WHO, 2015).

The vast majority of human cases of campylobacteriosis are caused by *Campylobacter jejuni* (84.4%), followed by *Campylobacter coli* (9.2%) (EFSA and ECDC, 2018). Handling, preparation and consumption of broiler meat account for 20-30% of human cases, whereas 50-80% might have their origin in chicken not only in the food chain but also through the environment or by direct contact (EFSA, 2010). Other sources include raw milk and water (Silva et al., 2011), and wildlife has been associated to the transmission of *Campylobacter* spp. into the food chain (Greig et al., 2015). Some species of *Campylobacter* have been associated to disease or lesions in food-producing and companion animals, but no clinical disease has been described in free-ranging wild animals (Schweitzer et al., 2011; Speck, 2012).

Typing of bacterial isolates is useful for tracing the source and routes of infection, which is key to design effective control measures and prevent further infections (Dingle et al., 2002). Furthermore, determining the presence of genes encoding for putative virulence factors may help understanding their potential to cause disease (Bang et al., 2003; Datta, Niwa, & Itoh, 2003). Complications associated to campylobacteriosis only occur in 1% of cases, including the Guillain-Barré syndrome, reactive arthritis, or irritable bowel syndrome (Chlebicz & Śliżewska, 2018).

The genus *Salmonella* contains two species, *S. bongori* and *S. enterica*, the latter comprises six subspecies, which are further subtyped into serovars according to flagellar antigens (Grimont & Weill, 2007; Tindall, Grimont, Garrity, & Euzéby, 2005). The main sources of human infection are food (poultry meat, eggs, milk) and vegetables or water contaminated with manure (EFSA and ECDC, 2017; WHO, 2018). *Salmonella* can also be found in the environment (water, soil, foods, etc.) (Winfield & Groisman, 2003), and wildlife species can be the source of human infection via different routes (Greig et al., 2015; Hilbert, Smulders, Chopra-Dewasthaly, & Paulsen, 2012). A few wild bird and mammal species are susceptible to *Salmonella* infection, whereas many others simply carry the organism in their intestine without showing clinical signs, as with *Campylobacter* spp. (Gaffuri & Holmes, 2012).

Food can also be a source of antimicrobial resistant bacteria and resistance genes for humans, and the presence of such resistance genes in pathogenic bacteria such as *Campylobacter* spp. and *Salmonella* spp. pose a risk of infection following ingestion or handling of contaminated

products (Newell et al., 2010). Moreover, human activities strongly affect the carriage of antimicrobial resistant bacteria by wildlife through their impact on natural habitats, and omnivorous species such as the wild boar are among the ones at a higher risk of being carriers and potential spreaders these bacteria (Vittecoq et al., 2016).

Wild boars may harbour a wide variety of zoonotic pathogens shared with livestock, companion animals and humans, including *Campylobacter* and *Salmonella* spp. (Meng et al., 2009; Ruiz-Fons, 2017). Wild boar populations have been increasing and expanding across Europe for the last decades (Massei et al., 2015), which increases the disease transmission potential due to increased contacts (Ruiz-Fons, 2017).

Moreover, wild boars inhabit a wide range of habitats (Abaigar et al., 1994; Meriggi & Sacchi, 2001; Virgós, 2002), including urban and peri-urban areas (Licoppe et al., 2013), where they cause conflicts with humans such as traffic accidents, damage to green spaces and even attacks on people and pets, in addition to the risk they pose of disease transmission (Fernández-Aguilar et al., 2018; Soulsbury & White, 2015; Wang et al., 2019). In these areas, people are at risk because wild boars may defecate, feed and drink water from public spaces. Furthermore, hunters are at risk of direct transmission when handling wild boar carcasses (Brown, Bowen, & Bosco-Lauth, 2018).

In the MAB (northeast Spain), wild boars enter the city of Barcelona from the bordering Serra de Collserola Natural Park (Castillo-Contreras et al., 2018), where the wild boar population has increased in recent years (González-Crespo et al., 2018). Similarly, in recent years wild boars have been entering and causing damages in the Bellaterra campus of Universitat Autònoma de Barcelona (UAB) (Lavín et al., 2017), located north of Collserola and Barcelona.

Given the presence of wild boar in urban and peri-urban areas in the MAB and their potential role as reservoir hosts or carriers of numerous zoonotic pathogens, our aim is to assess the occurrence of zoonotic *Campylobacter* and *Salmonella* spp. in wild boar faeces from the MAB, as well as the antimicrobial susceptibility of the isolates, and to characterise *Campylobacter* isolates in terms of genetic diversity and virulence potential.

3.4.3. Material and methods

Sampling

From June 2015 to February 2016, we collected stool samples from 130 wild boars from three distinct areas in the MAB (northeast Spain); a natural area (Collserola massif, n=73) and two

urbanised ones (Barcelona municipality, n=32, and the Bellaterra Campus of the Universitat Autònoma de Barcelona-UAB, n=25) (Figure 3.10).

We obtained sterile swabs from the wild boar rectum, and then placed the swabs in Amies transport medium containing charcoal (Deltalab, Barcelona Spain). We kept the swabs refrigerated until arrival and processing at the laboratory, which occurred within the next 24h.

Wild boars were harvested by local hunters in Collserola, or anaesthetised with a blowpipe by a veterinarian in Barcelona (tele-anaesthesia, contracts 15/0174, 16/0243 and 16/0243-00-PR/01 with *Ajuntament de Barcelona*-Barcelona city council) and UAB (using cage traps prior to anaesthesia, authorization AC/190 from *Generalitat de Catalunya*-Government of Catalonia). In Barcelona and UAB, we used an anaesthetic combination of xylazine, tiletamine and zolazepam (3 mg/kg each). Wild boars were captured or hunted for the regular population management purposes, not for research, and according to national and local legislation.

Study area

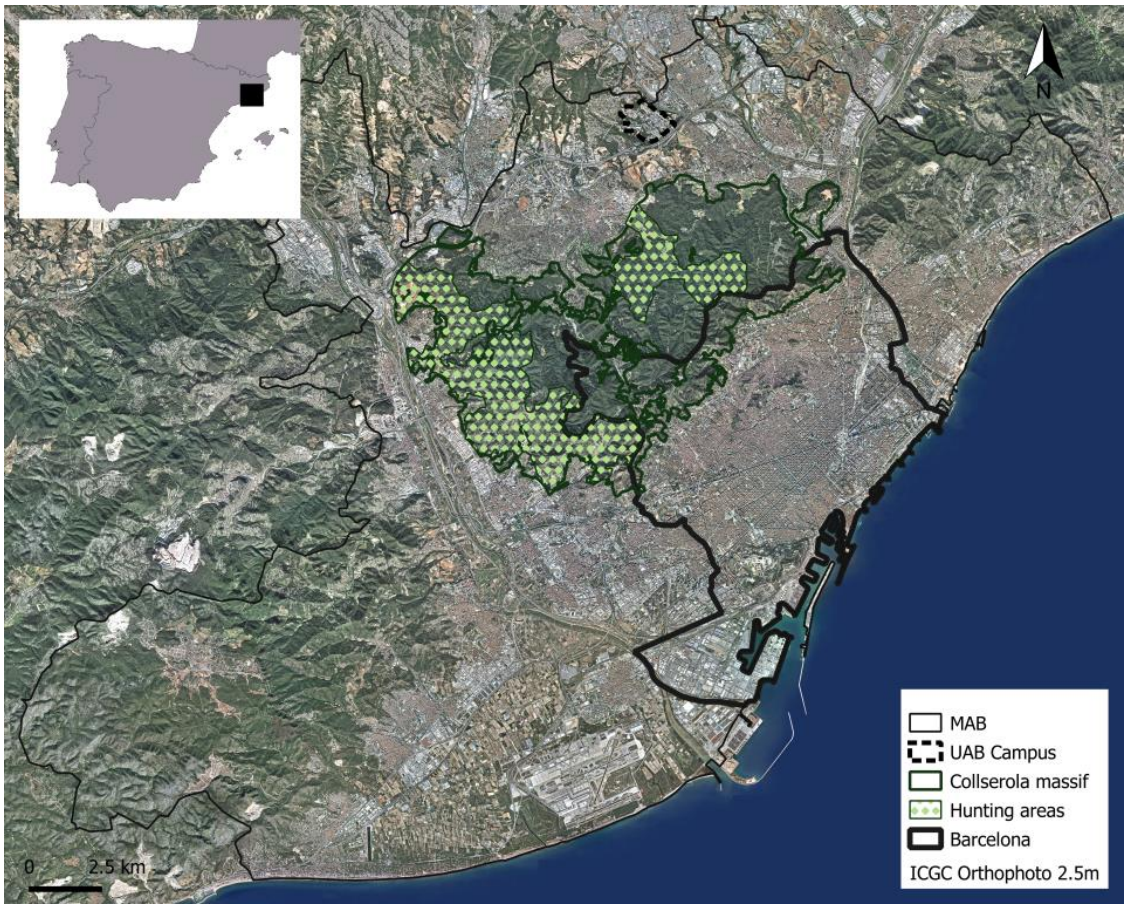


Figure 3.10. Study area: Collserola (solid green line), Barcelona (heavy black line) and UAB (dashed black line), in the Metropolitan Area of Barcelona (MAB, thin black line). Top left: location of MAB (black square) in the Iberian Peninsula. Orthophoto: ICGC.

Collserola massif, Barcelona municipality and UAB (Figure 3.10) campus belong to the MAB (Catalonia, northeastern Spain), which is home to over 3,200,000 people (Institut d'Estadística de Catalunya, 2015). Collserola massif is roughly 10,000 ha in surface, mostly covered by scrubland, forest and meadows, but it also contains recreational spaces, roads, built-up areas and roads (Generalitat de Catalunya, 2010). Moreover, it receives 3 million visitors each year (Parc de Collserola, n.d.-c). On the contrary, Barcelona is a 10,100 ha city inhabited by 1,600,000 people (Institut d'Estadística de Catalunya, 2019), and UAB, in turn, is a 260 ha university campus regularly used by 45,000 people (UAB, 2018).

Campylobacter and *Salmonella* isolation and identification

We performed *Campylobacter* sp. isolation and identification as described by Urdaneta, Dolz, & Cerdà-Cuéllar (2015). We preserved *Campylobacter* sp. isolates in brain heart infusion broth with 20% glycerol at -75ºC until further analysis. For species identification, we used a multiplex PCR targeting lipid A gene *lpx*A (Klena et al., 2004), with forward primers lpxA-*C. coli* 5'-AGACAAATAAGAGAGAATCAG-3' and lpxA-*C. jejuni* 5'-ACAACTTGGTGACGATGTTGTA-3', and a reverse primer lpxA-RKK2m 5'-CAATCATGDGCDATATGASAATAHGCCAT-3' for both *C. coli* and *C. jejuni*. For *C. lanienae*, we used another PCR using the primers CLAN76F (5'-GTAAGAGCTTGCTCTTATGAG-3') and CLAN1021R (5'-TCTTATCTCTAAGAGGTTCTTA-3'), as described by Logan, Burnens, Linton, Lawson, & Stanley (2000).

We performed *Salmonella* sp. isolation and identification following Antilles, Sanglas, & Cerdà-Cuéllar (2015). We preserved the *Salmonella* sp. isolates in brain heart infusion broth with 20% glycerol at -75ºC until further analysis. *Salmonella* sp. isolates were serotyped at the Laboratori Agroalimentari (Cabrils, Spain) from the Catalan Government (Departament d'Agricultura, Ramaderia, Pesca i Alimentació), according to White-Kauffmann-Le Minor scheme (Grimont & Weill, 2007).

Molecular typing of the isolates

In order to assess the genotypic diversity of *Campylobacter* strains, we first used two different typing methods, namely restriction fragment length polymorphism of the *fla*A gene (*fla*A-RFLP) for *C. coli*, and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) for *Campylobacter lanienae*. We considered as the same strain those isolates from the same wild boar host showing identical band patterns (either from *fla*A-RFLP or ERIC-PCR), and selected one of each (83 *C. lanienae* and 70 *C. coli* isolates) for further typing by pulse-field gel

electrophoresis (PFGE). Based on PFGE results, we selected 29 isolates (8 *C. lanienae* and 21 *C. coli*) for further analysis by multilocus sequence typing (MLST).

ERIC-PCR

We performed ERIC-PCR as described by Antilles et al. (2015), using primers ERIC-1R 5'-ATGTAAGCTCCTGGGGATTCAC-3' and ERIC-2 5'-AAGTAAGTGACTGGGGTGAGCG-3' (Versalovic, Koeuth, & Lupski, 1991). We resolved the PCR products by electrophoresis in 2% agarose gel in 1x tris-acetate-electrophoresis (TAE) buffer at 60V for 3h.

*Fla*A-RFLP

We performed *fla*A-RFLP according to the CAMPYNET protocol (Harrington, Moran, Ridley, Newell, & Madden, 2003). For amplification of the *fla*A gene we used the forward A1 (5'-GGATTTCGTATTAACACAAATGGTGC-3') and reverse A2 (5'-CTGTAGTAATCTTAAAACATTTTG-3') primers (Nachamkin, Bohachick, & Patton, 1993). The amplified PCR product (1.7 kb) was digested with the restriction enzyme *Dde*I (*Hyp*F3I, FastDigest®, Thermo Fisher Scientific, Waltham, MA, USA), and separated by electrophoresis in 2.5% agarose gel in 1x TAE buffer at 90V for 3h.

PFGE

We followed the standard operating protocol from PulseNet (www.pulsenetinternational.org/protocols/pfge/) for PFGE typing of the selected *Campylobacter* isolates (83 *C. lanienae* and 70 *C. coli*). We digested the genomic DNA with SmaI and KpnI restriction enzymes (Roche Applied Science, Indianapolis, IN, USA). Electrophoresis was performed in a CHEF-DR III System (Bio-Rad, Hercules, CA, USA).

We analysed PFGE band patterns with Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA), as previously described (Moré et al., 2017). We obtained a dendrogram per each *Campylobacter* species and restriction enzyme used, and another dendrogram combining SmaI and KpnI enzymes. Similarity matrices were calculated using the Dice coefficient with tolerance and optimization values of 1.0%. Dendrograms were constructed using an unweighted-pair group method with arithmetic mean (UPGMA). We considered PFGE band patterns with a similarity ≥ 90% to be the same pulsotype and named them according to the restriction enzyme used.

MLST

Based on PFGE results, we selected 29 isolates (8 *C. lanienae* and 21 *C. coli*) for MLST typing, which was performed according to Miller et al. (2012) for *C. lanienae*. For *C. coli* isolates, we followed the procedure and primers reported by Miller et al. (2005) and when no amplicon was obtained, we used those primers reported by Korczak, Zurfluh, Emler, Kuhn-Oertli, & Kuhnert (2009). Primer sets are shown in Tables 3.18 and 3.19. Sanger sequencing of the PCR purified products was undertaken by Geneservice Source BioScience (Nottingham, United Kingdom).

Table 3.18. *C. coli* primer sets used for MLST.

Locus	Primer	Forward 5'-3'	Primer	Reverse 3'-5'	Reference
aspA	aspAF1	GAGAGAAAAGCWGAAGAATTTAAAGAT	aspAR1	TTTTTTCATTWGCRSTAATACCATC	Miller et al. (2005)
	aspA_Cjc-L	CAACTKCAAGATGCWGTACC	aspA_Cjc-R	ATCWGCTAAAGTATRCATTGC	Korczak et al. (2009)
atpA	atpAF	GWCAAGGDGTTATYTGTATWTATGTTGC	atpAR	TTTAADAVYTCAACCATTCTTTGTCC	Miller et al. (2005)
	atpA_Cjc-L	CAAAAGCAAAGYACAGTGGC	atpA_Cjc-R	CTACTTGCCTCATCYAAATCAC	Korczak et al. (2009)
glnA	glnAF	TGATAGGMACTTGGCAYCATATYAC	glnAR	ARRCTCATATGMACATGCATACCA	Miller et al. (2005)
	glnA_Cjc-L	ACWGATATGATAGGAACTTGGC	glnA_Cjc-R	GYTTTGGCATAAAAGTKGCAG	Korczak et al. (2009)
gltA	gltAF	GARTGGCTTGCKGAAAAYAARCTTT	gltAR	TATAAACCCTATGYCCAAAGCCCAT	Miller et al. (2005)
	gltA_Cjc-L	TATCCTATAGARTGGCTTGC	gltA_Cjc-R	AAGCGCWCCAATACCTGCTG	Korczak et al. (2009)
glyA	glyAF	ATTCAGGTTCTCAAGCTAATCAAGG	glyAR	GCTAAATCYGCATCTTTKCCRCTAAA	Miller et al. (2005)
	glyA_Cjc-L	AGGTTCTCAAGCTAATCAAGG	glyA_Cjc-R	CATCTTTTCCRCTAAAYTCACG	Korczak et al. (2009)
pgm	pgmF1	CATTGCGTGTDGTTTTAGATGTVGC	pgmR1	AATTTTCHGTBCCAGAATAGCGAAA	Miller et al. (2005)
	glmM_Cjc-L	GCTTATAAGGTAGCWCCKACTG	glmM_Cjc-R	AATTTTCHGTTCCAGAATAGCG	Korczak et al. (2009)
tkt	tktF1	GCAAAYTCAGGMCAYCCAGGTGC	tktR1	TTTTAATHAVHTCTTCRCCCAAAGGT	Miller et al. (2005)
	tkt_Cjc-L	AAAYCCMACTTGGCTAAACCG	tkt_Cjc-R	TGACTKCCTTCAAGCTCTCC	Korczak et al. (2009)

Table 3.19. *Campylobacter lanienae* primer sets used for MLST (Miller et al., 2012).

Locus	Primer	Forward 5'-3'	Primer	Reverse 3'-5'
aspA	LANaspF	TTTAGCCACAGCTATGGAGTATCTCAA	LANaspR	ATATGGGTTRAAWGCTGTAACRATACC
	*HFLaspXF	AAYATGAAYGCAAACGAAGTTATAGC	LANaspR	ATATGGGTTRAAWGCTGTAACRATACC
atpA	LANatpF	AACCAAAAAGGTCAAGATGTTATATG	LANatpR	ATTTTCTACTGGAAGTGGGCTATAAGG
glnA	LANglnF	TGGCAYCAYGTATCWTATAATATAAAAGC	LANglnR	ATGGACRTGCATACCRCTWCCATTATC
	*HFLglnXF	TTTYGAATWTTGTRAWGAAAATGAAGT	*HFLglnXF	AGAGTAWGTWAGAATGCTTGGKGCTTC
gltA	LANgltF	ATGCATAGMGGMTATGATATAGCGTGG	LANgltR	CATCAACTCTATCTGGAGTWCCKATCA
glyA	LANglyF	TGCWAATGTTCAGCCAAATAGCG	LANglyR	CAAGAGCGATATCRGCRTCTTTACC
pgm	LANpgmF	GCTTACYTTAAAAGGCCTRMGAGTTGT	LANpgmR	AAGAAGCAGYCTAATCAAATTYTCTGT
tkt	LANtktF	CATCTAAAKCAYAATCCMAAAAATCC	LANtktR	ATCTCWKCGCCAAGMGGAGC

*Alternative MLST primer.

We used the Fingerprinting II v3.0 software to edit and analyse the Sanger sequencing results, assigned alleles and sequence types (STs) based on the MLST scheme provided on the

Campylobacter PubMLST database (http://pubmlst.org/campylobacter), and submitted novel alleles and STs to this database. Moreover, to assess the phylogenetic relationship among *C. lanienae* isolates we constructed a maximum likelihood tree based on concatenated MLST loci, using the MEGA X software (Kumar et al., 2018).

Virulence-associated genes

In total, we tested 8 *C. lanienae* and 20 *C. coli* isolates by PCR for the presence of 14 genes encoding putative virulence factors. These included genes related to motility (*fla*A and *fla*B), adhesion and colonization (*cad*F, *dna*J, *rac*R, *pld*A, *vir*B11), invasion (*ceu*E, *cia*B), cytotoxin production (*cdt*A, *cdt*B, *cdt*C and *wla*N) and the type 6 secretion system (T6SS) (Bolton, 2015; Dasti, Tareen, Lugert, Zautner, & Groß, 2010). Primer sets and corresponding annealing temperatures are indicated in Table 3.20.

Table 3.20. Primers of virulence factors used and annealing temperatures.

Locus	Primer	Sequence 5'- 3'	Primer	Sequence 5'- 3'	Tª	Reference
flaA	flaA664	AATAAAAATGCTGATAAAACAGGTG	flaA1494	TACCGAACCAATGTCTGCTCTGATT	55	Datta et al. (2003)
flaB	flaB-F	AAGGATTTAAAATGGGTTTTAGAATAAACACC	flaB-R	GCTCATCCATAGCTTTATCTGC	55	Goon et al. (2003)
cadF	cadF-F2B	TTGAAGGTAATTTAGATATG	cadF-R1B	CTAATACCTAAAGTTGAAAC	55	Datta et al. (2003)
ceuE (Cc)	COL1	ATGAAAAAATATTTAGTTTTTGCA	COL2	GATCTTTTTGTTTTGTGCTGC	55	Bang et al. (2003)
racR	racR-25	GATGATCCTGACTTTG	racR-593	TCTCCTATTTTTACCC	40	Datta et al. (2003)
dnaJ	dnaJ-299	AAGGCTTTGGCTCATC	dnaJ-1003	CTTTTTGTTCATCGTT	40	Datta et al. (2003)
virB11	virB-232	TCTTGTGAGTTGCCTTACCCCTTTT	virB-701	CCTGCGTGTCCTGTGTTATTTACCC	45	Datta et al. (2003)
ciaB	ciaB-403	TTTTTATCAGTCCTTA	ciaB-1373	TTTCGGTATCATTAGC	45	Datta et al. (2003)
pldA	pldA-84	AAGCTTATGCGTTTTT	pld-981	TATAAGGCTTTCTCCA	45	Datta et al. (2003)
cdtA	DS-18	CCTTGTGATGCAAGCAATC	DS-15	ACACTCCATTTGCTTTCTG	55	Hickey et al. (2000)
cdtB	cdtB-113	CAGAAAGCAAATGGAGTGTT	cdtB-713	AGCTAAAAGCGGTGGAGTAT	55	Datta et al. (2003)
cdtC	cdtC-192	CGATGAGTTAAAACAAAAAGATA	cdtC-351	TTGGCATTATAGAAAATACAGTT	55	Datta et al. (2003)
wlaN	wlaN-DL 39	TTAAGAGCAAGATATGAAGGTG	wlaN-DL 41	CCATTTGAATTGATATTTTTG	53	Linton et al. (2000)
hcp	hcp-F	CAAGCGGTGCATCTACTGAA	hcp-R	TAAGCTTTGCCCTCTCTCCA	56	Corcionivoschi et al. (2015)
	*gltA-F	GCCCAAAGCCCATCATGCACA	*gltA-R	GCGCTTTGGGGTCATGCACA	56	Corcionivoschi et al. (2015)

* Internal positive control of the PCR. ª Annealing tempertaure (ºC).

Antimicrobial susceptibility testing

We tested one *Campylobacter* isolate per pulsotype and area (n=7 *C. lanienae* and n=17 *C. coli* isolates) and all *Salmonella* isolates (n=4) for antimicrobial susceptibility according to the

National Veterinary Institute (Uppsala, Sweden) minimum inhibitory concentration (MIC) system. We followed the VetMIC Camp EU protocol (https://www.sva.se/globalassets/redesign2011/pdf/analyser_produkter/vetmic/anvandarinstruktioner/vetmic-camp-eu.pdf) for *Campylobacter* isolates and the VetMIC GN-mo protocol (https://www.sva.se/globalassets/redesign2011/pdf/analyser_produkter/vetmic/vetmic_gn.pdf) for *Salmonella* isolates. After incubation, we checked each well for growth (as a pellet in the bottom of the well and/or turbidity) and established the MIC at the lowest concentration inhibiting visible growth. Then, we designated each isolate as wild type (susceptible) or non-wild type (non-susceptible) depending on the break-points determined by EUCAST for each antimicrobial and bacterial species (https://mic.eucast.org/Eucast2/SearchController/search.jsp?action=init), which correspond to cut-off values appropriate to detect biological resistance, not therapeutic efficacy. When such break-points were not available, we used those from *Escherichia coli* for *Salmonella* sp., and those from *C. coli* for *C. lanienae* isolates.

For *Campylobacter* isolates, antimicrobials included erythromycin (1-128 µg/ml), ciprofloxacin (0.12-16 µg/ml), nalidixic acid (1-64 µg/ml), tetracycline (0.5-64 µg/ml), streptomycin (0.25-32 µg/ml) and gentamicin (0.12-16 µg/ml). For *Salmonella* isolates, the antimicrobials tested were ampicillin (1-128 µg/ml), cefotaxime (0.016-2 µg/ml), ceftazidime (0.25-16 µg/ml), ciprofloxacin (0.008-1 µg/ml), nalidixic acid (1-128 µg/ml), gentamicin (0.12-16 µg/ml), streptomycin (2-256 µg/ml), kanamycin (8-16 µg/ml), tetracycline (1-128 µg/ml), florfenicol (4-32 µg/ml), chloramphenicol (2-64 µg/ml), colistin (0.5-4 µg/ml), sulfamethoxazole (8-1024 µg/ml) and trimethoprim (0.12-16 µg/ml).

Statistical analyses

We used the R software, version 3.5.0 (R Development Core Team, 2018). For 95 % confidence interval (CI) calculation, we used the binconf function from the Hmisc package (Harrel Jr, 2018). To compare the number of *Campylobacter*-carrying wild boars (any species) among areas, we applied a chi-squared test. We also used a 3-sample test for equality of proportions (Crawley, 2007a) to compare the prevalence of *C. lanienae*, *C. coli* and mixed infections, including post-hoc pairwise comparisons with the holm adjustment method. We used the same methodology to address differences in the prevalence of each *Campylobacter* species among areas, and we considered a result as statistically significant at p values below 0.05.

3.4.4. Results

Campylobacter and *Salmonella* prevalence

We obtained *Campylobacter* sp. isolates from 79 out of the 130 wild boars (60.8%, see Table 3.21). *Campylobacter lanienae* was more frequently isolated than *C. coli* (X-squared=74.4, df=2, p=7.2e-7), mixed infections involving both *C. lanienae* and *C. coli* were the least frequent (*C. lanienae vs*. mixed infection: p=7.2e-15; *C. coli vs*. mixed infection: p=0.0008), and *C. jejuni* was not detected (Table 3.21).

There were not significant differences among the three study areas, either in *Campylobacter* spp. (X-squared=3.5, df=2, p=0.2) or *C. coli* prevalence (X-squared=5.3, df=2, p=0.07). The prevalence of *C. lanienae*, on the contrary, differed among areas (X-squared=6.4, df=2, p=0.04), although post-hoc pairwise comparisons only showed a tendency in *C. lanienae* prevalence towards being more frequent in wild boars from Collserola than from Barcelona (p=0.06) (Table 3.21).

Table 3.21. *Campylobacter* spp. isolates from wild boars; relative frequency, prevalence and 95% confidence interval per species and area.

	Positive wild boar/Analysed wild boar (prevalence, %) [95% confidence interval]			
	Barcelona	Collserola	UAB	Total
C. lanienae	9/32 (28.1) [15.6- 45.4	40/73 (54.8) [43.4 65.7]	11/25 (44) [26.7-62.9]	60/130 (46.2) [37.8- 54.7]
C. coli	8/32 (25) [13.3- 42.1]	7/73 (9.6) [4.7- 18.5]	6/25 (24) [11.5- 43.4]	21/130 (16.2) [10.8- 23.4]
C. jejuni	0/32 (0) [0-10.7]	0/73 (0) [0-5]	0/25 (0) [0-13.3]	0/130 (0) [0-2.9]
Mixed infection	2/32 (6.3) [1.7- 20.1]	1/73 (1.4) [0.07- 7.4]	1/25 (4) [0.2- 19.5]	4/130 (3.1) [1.2- 7.6]
Campylobacter sp.	0/32 (0) [0-10.7]	1/73 (1.4) [0.07-7.4]	1/25 (4) [0.2- 19.5]	2/130 (1.5) [0.4 5.4]
Total	15/32 (46.9) [30.9- 63.6]	47/73 (64.4) [52.9- 74.4]	17/25 (68) [48.4-82.8]	79/130 (60.8) [52.2- 68.7]

We isolated *Salmonella enterica* subsp. *enterica* from four out of the 130 wild boars (3.1%, 95% CI: 1.2-7.6%), three from Collserola and one from Barcelona, belonging to three different serovars, namely *S.* Typhimurium monophasic (in Collserola), *S.* Bardo (in Collserola), and *S.* Enteritidis (in Barcelona); the last *Salmonella* isolate was not typeable.

Genetic diversity

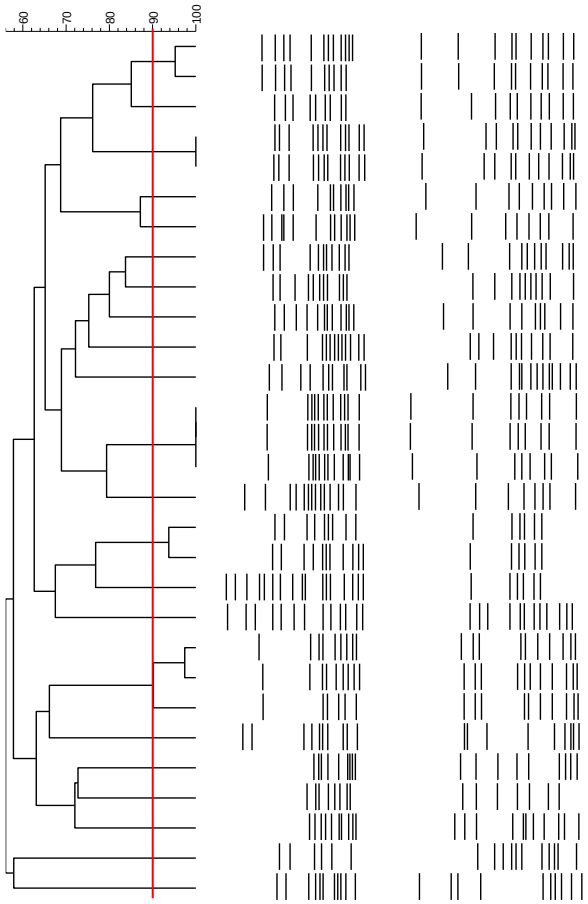
ERIC-PCR and *fla*A-RFLP

We initially attempted the typing of 187 *C. lanienae* isolates (obtained from 60 wild boars) by *fla*A-RFLP, but most isolates were not typeable. Typing by ERIC-PCR revealed 83 different profiles. Typing of 70 *C. coli* isolates (from 21 wild boars) by *fla*A-RFLP revealed 31 different profiles, with eight isolates not typeable with this method. One isolate per profile was selected to further type them by PFGE.

PFGE

Among the 83 *C. lanienae* profiles, we obtained 23 different PFGE pulsotypes with the restriction enzyme SmaI and nine with the restriction enzyme KpnI. Among the 31 *C. coli* isolates, we obtained 15 different pulsotypes with SmaI and 14 with KpnI. With both restriction enzymes combined, PFGE typing revealed 22 different pulsotypes, six for *C. lanienae* and 16 for *C. coli*. However, as happened with *fla*A-RFLP, not all isolates were typeable by PFGE; we were able to obtain band patterns from 42.2% (35/83) of the *C. lanienae* isolates and all (31/31) the *C. coli* isolates with SmaI, whereas these numbers were reduced to 20.5% (17/83) and 74.2% (23/31), respectively, when using KpnI.

The combined dendrogram of SmaI and KpnI PFGE profiles grouped all the *Campylobacter* isolates into four clusters with a similarity of approximately 62% (Figure 3.11). Three of these clusters comprised only *C. coli* isolates, whilst the fourth one only contained *C. lanienae* isolates. Two other isolates, one from each *Campylobacter* species, were grouped separately with a 58% similarity. Most wild boars carried a single *Campylobacter* pulsotype, except one of them which carried two different *C. lanienae* pulsotypes (SK17 and SK19), and three other which carried two different *C. coli* pulsotypes each (SK8 and SK14, SK9 and SK11, and SK11 and SK12). Despite this high genotypic diversity, we found two pulsotypes in wild boars from different sampling areas: pulsotype SK13 both in Collserola and UAB, and SK16 in Collserola and Barcelona (Figure 3.11). Other pulsotypes recovered from several individuals were all from the same sampling area (SK1, SK3, SK11).

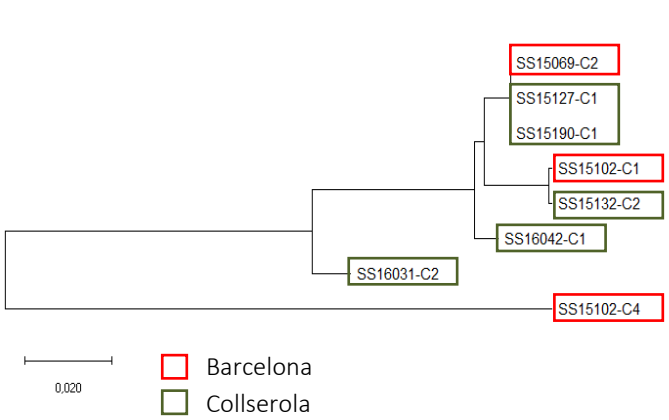


Isolate	Sp	Year	Area	Puls	ST	CC
SS16036-C1	*C.c*	2016	B	SK1	2814	828
SS16009-C3	*C.c*	2016	B	SK1	**9237**	-
SS16030-C1	*C.c*	2016	C	SK2	854	828
SS15115-C1	*C.c*	2015	B	SK3	854	828
SS15117-C1	*C.c*	2015	B	SK3	828	828
SS15188-C1	*C.c*	2015	C	SK4	**9235**	-
SS15100-C1	*C.c*	2015	B	SK5	**9235**	-
SS15091-C1	*C.c*	2015	UAB	SK6	854	828
SS15077-C2	*C.c*	2015	UAB	SK6	2097	828
SS15068-C1	*C.c*	2015	UAB	SK7	854	828
SS16005-C2	*C.c*	2016	UAB	SK8	854	828
SS15118-C1	*C.c*	2015	C	SK9	3020	828
SS15083-C1	*C.c*	2015	UAB	SK10	-	-
SS16005-C3	*C.c*	2016	UAB	SK11	**9238**	-
SS15080-C3	*C.c*	2015	UAB	SK11	**9236**	-
SS15080-C2	*C.c*	2015	UAB	SK11	-	-
SS15078-C1	*C.c*	2015	UAB	SK12	827	828
SS15185-C1	*C.c*	2015	C	SK13	827	828
SS15068-C4	*C.c*	2015	B	SK14	827	828
SS15070-C3	*C.c*	2015	UAB	SK15	1058	828
SS15190-C1	*C.l*	2015	C	SK16	-	-
SS15069-C2	*C.l*	2015	B	SK16	-	-
SS15127-C1	*C.l*	2015	C	SK16	-	-
SS15102-C4	*C.l*	2015	B	SK17	-	-
SS15132-C2	*C.l*	2015	C	SK18	-	-
SS15102-C1	*C.l*	2015	B	SK19	-	-
SS16042-C1	*C.l*	2016	C	SK20	-	-
SS16037-C1	*C.c*	2016	C	SK21	5006	828
SS16031-C2	*C.l*	2016	C	SK22	-	-

Figure 3.11. Combined dendrogram of SmaI and KpnI PFGE profiles of the *C. lanienae* and *C. coli* isolates, MLST sequence type (ST) and MLST clonal complex (CC) of *C. coli* isolates. Sp: species, Puls: PFGE pulsotype. Novel STs are shown in bold. B: Barcelona, C: Collserola, U: UAB.

MLST

A total of 29 isolates (*C. coli* n=21, *C. lanienae* n=8) from the 22 different pulsotypes obtained with the combined PFGE dendrogram (Figure 3.11) were selected for the MLST analysis. For *C. lanienae*, all but four alleles (*unc*A 7, *unc*A 12, *gln*A 12, *gly*A 21) from two different genes were novel, and we used a maximum likelihood tree to assess the phylogenetic relationship among the *C. lanienae* isolates (Figure 3.12). Three isolates from two different areas showed 100% similarity (SS15069-C2 from Barcelona, SS15127-C1 and SS15190-C1 from Collserola) and belonged to the same pulsotype SK16, while two other isolates (SS15102-C1 and SS15132-C2) belonging to two different pulsotypes (SK19 and SK18) were closely related and differed only in six nucleotides. Isolate SS15102-C4, from a Barcelona wild boar, was the most divergent.



Isolate	Year	Location	Pulsotype
SS15069-C2	2015	Barcelona	SK16
SS15127-C1	2015	Collserola	SK16
SS15190-C1	2015	Collserola	SK16
SS15102-C1	2015	Barcelona	SK19
SS15132-C2	2015	Collserola	SK18
SS16042-C1	2016	Collserola	SK20
SS16031-C2	2016	Collserola	SK22
SS16031-C2	2016	Barcelona	SK17

Figure 3.12. Maximum likelihood tree based on concatenated MLST loci of *C. lanienae* isolates. Branch lengths are based on the number of substitutions per site.

We obtained the complete MLST profile and therefore the corresponding ST (Figure 3.11 and Table 3.22) for 19 out of the 21 *C. coli* isolates. Overall, we found 12 different STs, four of which (ST 9235, ST 9236, ST 9237 and ST 9238) were new. These novel STs consisted on new allele combinations (ST 9236, ST 9237 and ST 9238) or new allele sequences (ST 9235, new allele *unc*A 588). All the *C. coli* isolates with existing ST and the novel ST 9236 and ST 9238 belonged to the same clonal complex (CC 828). The most frequent ST among *C. coli* isolates was ST 854, which we found in five individuals (isolates SS15068-C1, SS15091-C1, SS15115-C1, SS16005-C2 and SS16030-C1) from all three studied areas. The second most common ST was ST 827 (isolates SS15185-C1, SS15078-C1 and SS15068-C4), also found in all the areas. We also identified one of the novel STs (ST 9235) in two isolates, from Barcelona (SS15100-C1) and from Collserola (SS15188-C1). The nine remaining STs (828, 1058, 2097, 2814, 3020, 5006, 9236, 9237 and 9238) belonged to single isolates and were distributed heterogeneously among the three studied areas.

Table 3.22. MLST allelic profiles and sequence types (ST) of *C. coli* isolates. Novel STs are shown in bold. Isolates are grouped by studied areas. CC: MLST clonal complex.

Isolate	Year	Area	aspA	glnA	gltA	glyA	pgm	tkt	uncA	ST[a]	CC
SS15100-C1	2015	Barcelona	33	39	44	82	118	35	588	**9235**	-
SS15115-C1	2015	Barcelona	33	38	30	82	104	43	17	854	828
SS15117-C1	2015	Barcelona	33	39	30	82	104	43	17	828	828
SS16009-C3	2016	Barcelona	32	38	30	82	104	44	36	**9237**	-
SS16036-C1	2016	Barcelona	32	38	30	82	104	43	36	2814	828
SS15068-C4	2015	Barcelona	33	39	30	82	104	56	17	827	828
SS15118-C1	2015	Collserola	33	179	30	79	113	43	17	3020	828
SS15185-C1	2015	Collserola	33	39	30	82	104	56	17	827	828
SS15188-C1	2015	Collserola	33	39	44	82	118	35	588	**9235**	-

SS16030-C1	2016	Collserola	33	38	30	82	104	43	17	854	828
SS16037-C1	2016	Collserola	33	66	30	192	189	43	17	5006	828
SS15068-C1	2015	UAB	33	38	30	82	104	43	17	854	828
SS15070-C3	2015	UAB	33	39	30	82	104	35	17	1058	828
SS15077-C2	2015	UAB	33	38	30	238	104	43	36	2097	828
SS15078-C1	2015	UAB	33	39	30	82	104	56	17	827	828
SS15080-C3	2015	UAB	33	4	30	115	104	85	17	**9236**	828
SS15080-C2	2015	UAB	33	38	30	115	104	85	-	nd	-
SS15083-C1	2015	UAB	-	-	-	-	-	-	-	nd	-
SS15091-C1	2015	UAB	33	38	30	82	104	43	17	854	828
SS16005-C2	2016	UAB	33	38	30	82	104	43	17	854	828
SS16005-C3	2016	UAB	33	39	30	82	118	44	17	**9238**	828

[a] nd: no sequence obtained and thus no allele number could be assigned.

We obtained a higher number of pulsotypes (16) than STs (12) for *C. coli* isolates (Figure 3.11 and Table 3.22). We found different pulsotypes grouped as the same ST (ST 827 in SK13 and SK14; ST 854 in SK2, SK3, SK6, SK8 and SK9; ST 9235 in SK4 and SK5) and vice versa, isolates with the same pulsotype but different ST (SK3 in ST 854 and ST 828; SK1 in ST 2814 and ST 9237; SK11 in ST 9236 and 9238).

When compared to the *C. jejuni* and *C. coli* isolates available in the PubMLST, most of the STs from *C. coli* found in the present study had been previously isolated from a wide range of domestic animals such as sheep, cattle and pigs, but most frequently from chickens (Table 3.23). Besides the novel STs, all STs obtained in our study have also been isolated from human stools. In addition, ST 827, 828 and 854 have been found in environmental waters. The main source of ST 827 is human stools, which represent a 46.6% of all isolates within this ST in the PubMLST database. In the case of ST 828, the predominant source is chicken (64%), while ST 854 and ST 1058 come primarily from pigs (39.2% and 68.9%, respectively).

Table 3.23. Sequence type (ST) of C. coli isolates obtained from wild boars in the present study compared to the wider population of STs in the PubMLST database. The most frequent source of each ST is shown in bold.

ST	PubMLST isolates	Chicken	Chicken meat	Sheep	Farm environment	Cattle	Pig	Environmental waters	Human stool	Other sources
827	1562	209 (14.7%)	72 (5.8%)	138 (9.7%)	155 (10.9%)	82 (5.8%)	5 (0.4%)	12 (0.8%)	**663 (46.6%)**	226 (14.5%)
828	500	**298 (64%)**	32 (6.9%)	1 (0.21%)	-	4 (0.9%)	27 (5.8%)	6 (1.29%)	70 (15.02%)	62 (12.4%)
854	433	150 (36.5%)	20 (4.87%)	3 (0.7%)	-	8 (2%)	**161 (39.2%)**	7 (1.7%)	36 (8.8%)	48 (11.1%)
1058	45	2 (4.4%)	5 (11.1%)	-	-	-	**31 (68.9%)**	-	3 (6.7%)	4 (8.9%)
2097	8	**3 (37.5%)**	-	-	-	-	-	-	**3 (37.5%)**	2 (25%)
2814	4	-	-	-	-	-	1 (25%)	-	3 (75%)	-

3020	8	4 (50%)	-	-	-	-	-	-	3 (37.5%)	-
5006	1	-	-	-	-	-	-	-	**1 (100%)**	-
9235	1	-	-	-	-	-	-	-	-	1 (100%)
9236	1	-	-	-	-	-	-	-	-	1 (100%)
9237	1	-	-	-	-	-	-	-	-	1 (100%)
9238	1	-	-	-	-	-	-	-	-	1 (100%)

Virulence-associated genes

One *C. coli* isolate from Collserola (SS16030-C1, ST 854) and another one from UAB (SS15070-C3, ST 1058) showed the highest number of virulence determinants (12). Six other isolates, five *C. coli* from the three studied areas and one *C. lanienae* from Barcelona, carried ten or eleven virulence determinants (Table 3.24).

Table 3.24. Presence of virulence-associated genes depicted as blue squares.

Isolate	Species[a]	ST	Virulence genes														Area[b]
			Motility		Adhesion and colonization					T6SS	Invasion		Toxin production				
			flaA	*flaB*	*cadF*	*racR*	*dnaJ*	*pldA*	*virB11*	*hcp*	*ceuE*	*ciaB*	*cdtA*	*cdtB*	*cdtC*	*wlaN*	
SS15068-C1	*C.c*	854	■	■	■				■	■	■		■	■	■		B
SS15068-C4	*C.c*	827	■	■	■	■	■	■		■	■		■	■	■		B
SS15100-C1	*C.c*	9235	■	■	■				■	■	■			■			B
SS15115-C1	*C.c*	854	■	■	■				■	■	■			■			B
SS15117-C1	*C.c*	828	■	■	■		■		■	■	■		■	■	■		B
SS16009-C3	*C.c*	9237	■	■	■		■			■	■		■	■	■		B
SS16036-C1	*C.c*	2814	■	■	■					■	■		■	■			B
SS15118-C1	*C.c*	3020	■	■	■					■	■		■	■	■		C
SS15185-C1	*C.c*	827	■	■						■	■			■			C
SS15188-C1	*C.c*	9235	■	■	■				■	■	■	■		■			C
SS16030-C1	*C.c*	854	■	■	■	■		■	■	■	■	■	■	■	■		C
SS16037-C1	*C.c*	5006	■	■	■	■	■	■		■	■		■	■	■		C
SS15070-C3	*C.c*	1058	■	■	■	■	■	■	■	■	■		■	■	■		U
SS15077-C2	*C.c*	2097	■	■	■		■		■	■	■		■	■	■		U
SS15078-C1	*C.c*	827	■	■	■		■		■	■	■		■	■	■		U
SS15080-C2	*C.c*	-		■	■			■	■	■	■	■	■	■			U
SS15080-C3	*C.c*	9236	■	■	■					■	■						U
SS15091-C1	*C.c*	854	■	■	■					■	■	■	■	■	■		U
SS16005-C2	*C.c*	854	■	■	■				■	■	■			■			U
SS16005-C3	*C.c*	9238	■		■				■	■	■	■	■	■	■		U
SS15069-C2	*C.l*	-		■	■	■	■	■		■	■		■	■	■		B
SS15102-C1	*C.l*	-		■				■		■	■						B
SS15102-C4	*C.l*	-		■					■	■	■						B
SS15127-C1	*C.l*	-		■				■		■	■						C
SS15132-C2	*C.l*	-		■			■	■		■							C
SS15190-C1	*C.l*	-		■	■	■	■			■	■		■	■	■		C
SS16031-C2	*C.l*	-		■					■	■	■						C
SS16042-C1	*C.l*	-		■						■	■			■			C

[a] C.c*: C. coli,* C.l*: C. lanienae*, [b] B: Barcelona, C: Collserola, U: UAB.

We detected the motility gene *fla*B in all the *C. lanienae* isolates analysed, and both *fla*A and *fla*B in all but two *C. coli* isolates. Regarding genes involved in invasion, we found *ceu*E in all the *C. lanienae* and *C. coli* isolates. On the contrary, we detected *cia*B in only five out of the 20 *C. coli* isolates analysed, two from Collserola and three from UAB, and in none of the *C. lanienae* isolates.

With regard to adherence and colonization genes, we found the gene *cad*F in two out of the eight *C. lanienae* isolates and in all but one *C. coli* ones, and *vir*B11 (located in the virulence related plasmid pVir) in two out of the eight *C. lanienae* and over half of the *C. coli* isolates analysed, mainly from Barcelona and UAB. Conversely, the presence of *rac*R (response regulation protein), *dna*J (heat shock protein) and *pld*A (phospholipase A) was low in both species. We also detected the *hcp* gene, encoding for a host surface adhesion protein (component of T6SS) associated with virulence, in all the *C. lanienae* and *C. coli* isolates analysed.

Finally, only two *C. lanienae* isolates were positive to *cdt*A, *cdt*B and *cdt*C genes, coding for the synthesis and deliver of the cytolethal distending toxin (CDT). Almost all the *C. coli* isolates, on the contrary, were positive for all or some of these CDT genes; twelve out of 20 carried all three genes, and only one isolate was negative for the three of them. The only virulence-associated gene that was not detected in any of *C. lanienae* and *C. coli* isolates was *wla*N, which is associated to Guillain-Barré syndrome.

Antimicrobial susceptibility

All the *C. lanienae* and *C. coli* isolates were resistant to at least one antimicrobial agent tested (Table 3.25). In the case of *C. lanienae*, most isolates (5/7) were resistant to nalidixic acid only, and another one (1/7) was resistant to nalidixic acid and to streptomycin. Regarding the *C. coli* isolates, most of them (14/17) showed resistance to quinolones (nalidixic acid and ciprofloxacin) and tetracycline (13/17), and 10/17 were multidrug resistant (MDR, resistant to 3 or more classes of antimicrobials). MDR isolates were recovered from wild boars from the three studied areas (four isolates in UAB, four in Barcelona and two in Collserola) (Table 3.25). We could not determine the antimicrobial susceptibility of one *C. lanienae* and two *C. coli* isolates, since they poorly grew in the culture medium.

With regard to *Salmonella*, two out of the four isolates (*S.* Enteritidis and *S.* Bardo) from Barcelona and Collserola were pansusceptible, whilst a third isolate from Collserola (SS15133-S1, *S*. Typhimurium monophasic) was resistant to four agents (ampicillin, streptomycin,

tetracycline and sulphametoxazole, ASSuT). The antimicrobial susceptibility of the fourth *Salmonella* isolate could not be determined.

Table 3.25. Antimicrobial susceptibility of *Campylobacter* isolates.

Isolate	Species	Pulsotype	ST	Antimicrobial agents[a]						Area
				Em	Nal	Ci	Tc	S	Gm	
SS15068-C1	*C. coli*	SK8	854							Barcelona
SS15068-C4	*C. coli*	SK14	827							Barcelona
SS15100-C1	*C. coli*	SK5	9235							Barcelona
SS15115-C1	*C. coli*	SK3	854							Barcelona
SS16036-C1	*C. coli*	SK1	2814							Barcelona
SS15118-C1	*C. coli*	SK10	3020							Collserola
SS15185-C1	*C. coli*	SK13	827							Collserola
SS15188-C1	*C. coli*	SK4	9235							Collserola
SS16030-C1	*C. coli*	SK2	854	nd						Collserola
SS16037-C1	*C. coli*	SK21	5006							Collserola
SS15070-C3	*C. coli*	SK15	1058							UAB
SS15077-C2	*C. coli*	SK7	2097							UAB
SS15078-C1	*C. coli*	SK13	827							UAB
SS15080-C2	*C. coli*	SK12	-							UAB
SS15083-C1	*C. coli*	SK11	-							UAB
SS15091-C1	*C. coli*	SK6	854							UAB
SS16005-C2	*C. coli*	SK9	854	nd						UAB
SS15069-C2	*C. lanienae*	SK16	-	nd						Barcelona
SS15102-C1	*C. lanienae*	SK19	-							Barcelona
SS15102-C4	*C. lanienae*	SK17	-							Barcelona
SS15132-C2	*C. lanienae*	SK18	-							Collserola
SS15190-C1	*C. lanienae*	SK16	-							Collserola
SS16031-C2	*C. lanienae*	SK22	-							Collserola
SS16042-C1	*C. lanienae*	SK20	-							Collserola

[a] Em: erythromycin, Nal: nalidixic acid, Ci: ciprofloxacin, Tc: tetracycline, S: streptomycin, Gm: gentamicin. nd: could not be determined.

3.4.5. Discussion

Campylobacter and *Salmonella* prevalence

In the present study, we report the carriage by wild boars of the two most relevant zoonotic bacteria in Europe (EFSA and ECDC, 2018). Moreover, the overall *Campylobacter* prevalence (over 60%) is among the highest reported in wild boars. Previous studies in wild boars describe a prevalence ranging from zero to 66% depending on the sample analysed and the country of origin (Díaz-Sánchez et al., 2013; Kanai et al., 1997; Stella, Tirloni, Castelli, Colombo, & Bernardi, 2018). Furthermore, several studies report the occurrence of *C. coli* and *C. jejuni* in wild boars or feral pigs (Cummings et al., 2018; Díaz-Sánchez et al., 2013; Wahlström et al., 2003), but uncommon species such as *C. lanienae* are less frequent in literature, possibly because it is a difficult species to culture (Inglis & Kalischuk, 2003) and *Campylobacter* isolates are not always identified at the species level.

In the present study, *C. lanienae* was more frequently isolated from wild boar than *C. coli*, which could be explained because *C. lanienae* is predominantly found in wild boars and domestic pigs, although it has also been found in other domestic animals (Guévremont, Normand, Lamoureux, & Côté, 2008; Navarro-González et al., 2014; Oporto & Hurtado, 2011; Schweitzer et al., 2011). *C. lanienae* prevalence found in this study (46%) is, to our knowledge, the highest reported to date in wild boars, which usually ranges between 10 and 27% (Carbonero et al., 2014; Navarro-González et al., 2014; Sasaki et al., 2013). In addition to the high prevalence, this species has already been linked to human diarrhoeal disease (Lévesque, Lemay, Bekal, Frost, & Michaud, 2016), after its discovery in humans working at an abattoir (Logan et al., 2000).

The *C. coli* prevalence found in our study (16%) is also above the ones previously reported in wild boars or feral pigs (Atanassova, Apelt, Reich, & Klein, 2008; Carbonero et al., 2014; Cummings et al., 2018; Navarro-González et al., 2014), including previous reports from the MAB (Navarro-González et al., 2013). Furthermore, the finding of *C. coli* in wild boars from such a highly populated area -the MAB- is especially relevant to public health concern, since *C. coli* is far more relevant in veterinary and human medicine than *C. lanienae* (Horrocks, Anderson, Nisbet, & Ricke, 2009) and is the principal cause causative agent of campylobacteriosis in humans after *C. jejuni* (EFSA and ECDC, 2018).

Regarding the spatial distribution of *Campylobacter*-carrying wild boars, we could not detect significant differences among areas. This could be either because such differences do not exist, or because the spatiotemporal distribution of samples did not allow us to detect any patterns. In this regard, although *C. lanienae* was more frequent than *C. coli*, we must consider that it is not completely accurate to compare the *Campylobacter* species without taking area and season into account, since *Campylobacter* prevalence varies with both (Carbonero et al., 2014; Gürtler, Alter, Kasimir, & Fehlhaber, 2005).

The low *Salmonella* prevalence found in the wild boars from this study (3%) is in accordance with previous studies in wild boar (Navarro-González et al., 2013; Sasaki et al., 2013), but in contrast to others reporting prevalence over 20% (in Portugal) and up to 36% (in Spain) (Navarro-González et al., 2012; Vieira-Pinto et al., 2011). *S.* Enteritidis is one the most frequently isolated serovars from game animals in Europe including the wild boar (Paulsen, Smulders, & Hilbert, 2012), *S.* Typhimurium monophasic has also been reported in wild boars in Italy and Sweden (Sannö, Jacobson, Sterner, Thisted-Lambertz, & Aspán, 2018; Zottola et al., 2013), and *S.* Bardo in wild boars from Spain and Poland (Díaz-Sánchez et al., 2013; Wisniewski, 2001). Despite the latter serovar is rare, it can cause disease to humans (Schmid & Baumgartner,

2013), in addition to *S*. Enteritidis and *S*. Typhimurium monophasic, which are the first and third most commonly reported serovars in human cases acquired in the EU during 2017, respectively (EFSA and ECDC, 2018).

Campylobacter coli, C. jejuni and different serovars of *S. enterica* have been reported in wildlife from urban and peri-urban areas, for instance in kelp gulls (*Larus dominicanus*) from Cape Town (Moré et al., 2017), different wild bird species from the vicinity of Vienna and Brno (Troxler et al., 2017), racoons from Central Park in New York (Rainwater et al., 2017), feral pigeons (*Columba livia* var. *domestica*) from Barcelona (Casanovas, de Simón, Ferrer, Arqués, & Monzón, 1995) and wild boars also from Barcelona (Navarro-González et al., 2013). However, to the best of our knowledge, this is the first report of *C. lanienae* in wild boars or other wildlife species from (peri-)urban areas. According to our results, previous report of a 20% prevalence of *Campylobacter* sp. in urban wild boars from Barcelona and neighbouring areas (Navarro-González et al., 2013) probably detected *C. lanienae* but did not perform identification at the species level.

Genetic diversity

*Fla*A-RFLP is usually used for typing *C. coli* and *C. jejuni* isolates, but no amplification occurred in the case of *C. lanienae* isolates. This can be explained by the absence of the flagellar gene *fla*A in these *C. lanienae* isolates, or by the lack of recognition of the target region by the primers used. As for PFGE, the restriction enzyme SmaI was more suitable than KpnI for typing both *C. coli* and *C. lanienae*. Nevertheless, it worked with less than half (42.2%) of the *C. lanienae* isolates. This is opposite to previous reports of similar success with both restriction enzymes or KpnI being more useful than SmaI when typing by PFGE *C. lanienae* isolates obtained from domestic pigs (Kérouanton, Chidaine, Rose, Samson, & Denis, 2015; Schweitzer et al., 2011). In our case, the worse performance of restriction enzymes with *C. lanienae* isolates might be related to the lack of restriction sites, as seen in *C. jejuni* isolates (Oyarzabal et al., 2008).

Based on the PFGE and MLST results, we observed a high genetic diversity among *Campylobacter* isolates from both species. This might be attributable to the frequent genetic rearrangements that occur in *Campylobacter* genome (de Boer et al., 2002; Wassenaar, Geilhausen, & Newell, 1998), which seem to improve phenotypic fitness to survive and colonize different hosts (Ridley, Toszeghy, Cawthraw, Wassenaar, & Newell, 2008). Despite the high genetic diversity, most wild boars carried a single pulsotype each, either from *C. lanienae* or *C. coli*. This agrees with previous reports of some *C. jejuni* PFGE types showing dominance and persistence over others, even in experimental infection with a mixture of two or three types

(Ridley et al., 2008). This was explained by some strains having a greater capacity to overcome the environmental pressures and colonise the gut of the host, which might also be true for *C. lanienae* and *C. coli* strains in the present study.

We found no coincidences between the *C. lanienae* alleles from wild boars and those published in the pubMLST database, probably due to the few *C. lanienae* STs (n=171) in the MLST database, compared with that of *C. coli/jejuni* which currently contains 9,986 STs. The small number of previously described *C. lanienae* isolates in the pubMLST database can be explained by the difficulties to isolate this species, as in most cases conventional culturing methods are not successful (Inglis & Kalischuk, 2003; Schweitzer et al., 2011), by the relatively recent discovery of this species (Logan et al., 2000), and by the recent association to foodborne human disease (Lévesque et al., 2016), driving to a lower research interest.

In the case of *C. coli*, we described four new STs and a new allele. The diversity across MLST loci is important for the potential identification of alleles or STs that may correlate with animal host or geographical location (Dingle et al., 2002; Miller et al., 2006), so we submitted the novel MLST data to the corresponding PubMLST, contributing to the understanding of *C. coli* epidemiology. MLST analysis showed a predominant ST(ST 854) which is overrepresented in pigs but it has also been isolated from chicken, livestock and the environment (Sheppard et al., 2009). All existing STs described in this study have been associated with human disease, and some of them (ST 827, ST 828, ST 854 and ST 1058) have a worldwide distribution, thus highlighting the role that wild boars can potentially play in human infection. Despite the high diversity of STs described among the *C. coli* isolates, all belonged to the same clonal complex (CC 828), indicating a close relationship among them. The high prevalence of this clonal complex was consistent with its prevalence in other studies (Cantero, 2017; Sheppard et al., 2009). Contrary to the more genetically diverse *C. jejuni*, few *C. coli* CCs have been described to date and most existing isolates belong to CC 828.

The differences observed between PFGE and MLST results may be explained by the different discriminatory power of these techniques and, as abovementioned, by the high genetic instability of *Campylobacter* genome (Wassenaar et al., 1998). In some cases, same pulsotypes belonged to different STs, probably as a result of single nucleotide changes that do not alter the restriction sites and therefore are not reflected in band profiles. Conversely, some STs belonged to different pulsotypes, possibly indicating that nucleotide changes may have changed the restriction sites.

We detected certain *C. coli* and *C. lanienae* genotypes during the two sampled years in the different studied areas, suggesting a possible circulation of strains among areas. This might be a consequence of wild boar movement (Jerina, Pokorny, & Stergar, 2014; Podgórski et al., 2014), as well as the existence of a common source of infection including other wildlife species (Jones, 2001).

Virulence potential

The present study also provides information on the virulence potential of *C. lanienae* and *C. coli* isolates. For colonization to take place in the intestine, the bacteria need motility, adhesion to intestinal mucosa, invasion and production of toxins (Ketley, 1997; Wassenaar, 1997). On average, virulence genes were less prevalent in the *C. lanienae* isolates than in the *C. coli* isolates. The primers used in both species for PCR amplification of virulence factors were designed for *C. jejuni* and *C. coli*. Therefore, the specificity of primers along with possible significant differences between the *C. lanienae* and the *C. coli* alleles could explain the lack of amplification of some virulence genes in *C. lanienae* isolates, rather than their absence. Among the flagellar genes, *fla*B was present in both *Campylobacter* species, whilst *fla*A was only present in *C. coli* isolates. Flagellar genes are important for *Campylobacter* motility, which is essential for intestine colonization, as the flagellar filament helps the bacteria to resist gut peristalsis and survive in the hostile environment of the stomach (Nachamkin, Yang, & Stern, 1993). Genes involved in adhesion (*cad*F, *rac*R, *dna*J; Brás, Chatterjee, Wren, Newell, & Ketley, 1999; Ziprin et al., 2001) and invasion (*ceu*E, *vir*B11, *cia*B and *pld*A; Bacon et al., 2000; Bang et al., 2003; Konkel, Kim, Rivera-Amill, & Garvis, 1999; Ziprin et al., 2001) were less conserved than other genes and presented a heterogeneous distribution within C. *lanienae* and *C. coli* isolates as previously reported in *C. jejuni* (Datta et al., 2003).

In contrast to other studies, the prevalence of *vir*B11 in *C. coli* isolates was high (70%) (Koolman, Whyte, Burgess, & Bolton, 2015). This gene encodes a putative type IV secretion system, a mutation of which can reduce the adherence of the bacteria in the intestinal tract, resulting in a significant reduced virulence (Bacon et al., 2000). The absence or low prevalence of the *wla*N gene, involved in the Guillain-Barré syndrome, agrees with previous studies (Datta et al., 2003; Koolman et al., 2015). The gene *hcp* is a component of the T6SS and it is associated with more severe forms of campylobacteriosis as it can confer cytotoxicity towards red blood cells (Bleumink-Pluym, van Alphen, Bouwman, Wösten, & van Putten, 2013). Moreover, *hcp*+ strains have increased abilities to adhere and invade the host intestine, conferring more virulence to strains (Corcionivoschi et al., 2015), and this gene was found in all the *C. coli* and *C. lanienae*

isolates analysed in our study. Whilst this is the first report of T6SS in *C. lanienae*, high prevalence among *C. coli* isolates has previously been reported (Corcionivoschi et al., 2015).

Cytolethal distending toxin genes (*cdt*ABC) had a high prevalence among *C. coli* isolates, as previously reported (Koolman et al., 2015). While *cdt*B was detected in all the *C. coli* isolates analysed, *cdt*A and *cdt*C were detected at a lower frequency, which also agrees with previous studies (Bang et al., 2003; Koolman et al., 2015). *Cdt*A and *cdt*C are necessary for binding to the host cell and *cdt*B is the active subunit that enters to the cell and causes cell death (Konkel et al., 1999), but the three of them seem necessary because isolates without either *cdt*A or *cdt*C genes produce little or no CDT (Bang et al., 2003).

Antimicrobial susceptibility

Notably, none of the *Campylobacter* isolates were pansusceptible. However, all but one *C. lanienae* were only resistant to nalidixic acid, similarly to previous studies in *C. lanienae* isolated from domestic pigs (Schweitzer et al., 2011). The remaining *C. lanienae* isolate was resistant to nalidixic acid and streptomycin, a phenotype which has been previously reported in isolates from wild artiodactyls and domestic pigs (Carbonero et al., 2014; Schweitzer et al., 2011).

Conversely, more diverse antimicrobial resistance profiles were found in *C. coli* isolates including MDR of ten isolates. These differences between *C. coli* and *C. lanienae* susceptibility to antimicrobials was also observed in wild artiodactyls (Carbonero et al., 2014), which could be related to a lower exposure to antimicrobials of *C. lanienae* isolates, and hence selection pressures have not favoured the survival of resistant strains (Holmes et al., 2016). Similarly to our findings, a high frequency of *C. coli* isolates resistant to ciprofloxacin, streptomycin and tetracycline, and a low frequency of erythromycin-resistant isolates have been reported (Carbonero et al., 2014). The resistance to ciprofloxacin is especially relevant in *C. coli* since it is one of the two antimicrobials regarded as critically important for treatment of human campylobacteriosis (EFSA and ECDC, 2019). Moreover, a high proportion of *Campylobacter* isolates (particularly *C. coli*) from humans are resistant to ciprofloxacin and tetracycline in Europe in 2017 (EFSA and ECDC, 2019), which we also frequently found in our *C. coli* isolates. The antimicrobial resistance profiles found in this study, particularly in *C. coli*, suggests an anthropogenic origin of certain strains, as suggested in previous studies for resistant *E. coli* (Dolejska et al., 2016; Mukerji et al., 2019).

Regarding the *Salmonella* isolates, one of them (*S.* Typhimurium monophasic) was MDR, with the typical ASSuT resistance profile of the clone that has emerged in several European countries

(Dionisi et al., 2009; Lucarelli et al., 2010; Mossong et al., 2007). Pigs are the likely reservoir of infection of this ASSuT resistant serovar for humans (Hopkins et al., 2010), and although *Salmonella* prevalence in our study was low, the multiresistant isolate was found in a hunted wild boar, which poses a risk for hunters because of handling and consumption.

We suggest that wild boars might have become infected with *Campylobacter* and *Salmonella* strains of anthropogenic origin (human waste or animal products), that is, there has been an event of reverse zoonosis, given that wild boars exploit anthropogenic food resources -including rubbish- in urban and peri-urban areas (Dolejska et al., 2016; García-Sánchez, Melero, Diez, Jaime, & Rovira, 2018; Hafeez et al., 2011). Moreover, all the *C. coli* STs identified from wild boars in the present study have been previously isolated from human stools; two of the *Salmonella* serovars found are among the most reported in human cases; and antimicrobial resistance in *C. coli* and monophasic *S*. Typhimurium agreed with those most frequently found in the respective species or serovar from humans in Spain or Europe. Since there is a strong link between the impact of human activities on natural habitats and the carriage of antimicrobial resistant bacteria by wildlife (Vittecoq et al., 2016), it is likely that humans have influenced the carriage of *Campylobacter* and *Salmonella* isolates by wild boars in our study, as well as their virulence potential and antimicrobial resistance. Moreover, wild boars can now act as carriers and spread these bacteria, which have great impact on human health (EFSA and ECDC, 2018, 2019).

Wild boars infected with *Campylobacter* and *Salmonella* species are a potential public health threat since wild boar populations are expanding and increasing their presence in urban areas (Licoppe et al., 2013; Massei et al., 2015). Although the overall influence of wild boars on human campylobacteriosis and salmonellosis may be small, hunters may be at high risk of exposure when handling wild boar carcasses and through the consumption of contaminated meat (Ruiz-Fons, 2017), and wild boars may contaminate the urban environment when feeding or defecating in public spaces, since they can bring several zoonotic pathogens into cities (Fernández-Aguilar et al., 2018; Jansen et al., 2007; Wang et al., 2019). Besides humans, wild boars could also transmit *Campylobacter* and *Salmonella* species to companion animals or to free-ranging domestic animals present in the MAB (Navarro-González et al., 2014), and contaminate fruits and vegetables in agricultural fields (Jay-Russell, Bates, Harden, Miller, & Mandrell, 2012).

To control campylobacteriosis and salmonellosis, which are the most commonly reported zoonoses in Europe and within the top four foodborne diseases worldwide (EFSA and ECDC,

2017; WHO, 2015), it is necessary to better understand the global epidemiology of the pathogens, its reservoirs and the pathogenicity of the different strains. Furthermore, special emphasis should be made on wildlife species, since they constitute a reservoir mainly for *Campylobacter* spp.

3.4.6. Conclusions

Our results provide further evidence on the role of wild boars as reservoirs of zoonotic thermophilic *Campylobacter* species. We observed a high genetic diversity among both *C. lanienae* and *C. coli*, and the same clones are circulating in wild boars from urban, peri-urban and natural areas. In addition, all the *C. coli* STs had previously been reported in humans, with some isolates showing a high virulence potential, and a number of *Campylobacter* isolates being resistant or MDR. We also report the presence of three zoonotic *Salmonella* serovars, two of them commonly isolated from humans. Notably, one of them was the recently emerged and widely spread mutiresistant monophasic *S.* Thyphimurium. Wild boars infected with *Campylobacter* spp. and *Salmonella* sp. are a potential public health threat since wild boar populations are expanding worldwide and increasing their presence in urbanised areas.

3.4.7. Acknowledgements

We want to express our gratitude to all people from SEFaS and CReSA involved in the study design, sample collection, processing and/or analyses, especially Marta Cerdà, Xavier Fernández, Andreu Colom, Teresa Ayats and Marta Marín. Also, we would like to thank the Consorci del Parc Natural de la Serra de Collserola, local hunters and Josep Maria López Martín, from Departament d'Agricultura, Ramaderia, Pesca i Alimentació (Generalitat de Catalunya), for their support in sampling wild boars from Collserola. Finally, we wish to thank Anna Florensa and the rest of people involved in the wild boar monitoring and management on UAB campus.

This study was funded by the contracts 13/051, 15/0174, 16/0243-00-CP/01 and 16/0243-00-PR/01 between Universitat Autònoma de Barcelona and Ajuntament de Barcelona. RCC, XFA and ACC benefited from pre-doctoral grants co-financed by Agència de Gestió d'Ajuts Universitaris i de Recerca (Generalitat de Catalunya) and the European Social Fund, file numbers 2016FI_B 00425, 2017FI_B1 00040 and 2018FI_B2_00030.

4. DISCUSSION

4.1. Wild boars in the city: drivers and responses

This thesis reports the existence of drivers of the wild boar presence in the city of Barcelona (Study 1), as well as phenotypic changes in individuals exploring this area (Study 2). Streams determined the urban location of wild boars probably due to their role as wildlife corridors (Rosenberg et al., 1997), which increase landscape connectivity and might allow the wild boars to approach the city under a dense vegetation cover (Beier & Noss, 1998; Morelle et al., 2016). Landscape fragmentation was another important driver, in line with the findings in urban wild boars in the city of Haifa (Toger et al., 2018). This may be explained because the patches resulting from a fragmented landscape might be heterogeneous enough for the wild boars to find food, water and shelter, although they spend little time foraging in each patch (Podgórski et al., 2013).

Food resources have been previously suggested as attracting factors for the wild boar in urban areas both in our study site and in other cities (Cahill et al., 2012; Toger et al., 2018). Moreover, the finding of pet food in wild boar stomachs (Study 2) supports the selection of cat colonies as drivers (Study 1), and the finding of food waste and plastic items in urban wild boar stomachs (Study 2) suggests that rubbish bins and containers should also be considered factors favouring the wild boar presence in Barcelona. Regarding green spaces, plant material can be used by urban wild boars, and it is sometimes preferred over anthropogenic food as seen in Berlin (Stillfried, Gras, Busch, et al., 2017).

Through exploring the urban area, wild boar behaviour, morphology and reproduction have changed (Study 2; Cahill et al., 2012; Podgórski et al., 2013; Stillfried, Gras, Börner, et al., 2017). The availability of human-derived food seems to contribute to faster growth and higher body mass in urban wild boars, as occurs in rural populations with higher production of natural food resources (Massei et al., 1996). Higher food supply also favours an early reproduction in wild boar females (Fernández-Llario & Mateos-Quesada, 1998; Massei et al., 1996; Santos et al., 2006), as detected in wild boar females in the urban area of Barcelona (Study 2). Nevertheless, visiting the urban area might also have a great cost involving a shorter lifespan, which may be directly caused by humans through capture and removal of problem individuals (a measure currently applied in Barcelona; Ajuntament de Barcelona, 2018) as well as by increased risks related to the urban environment (Tenés et al., 2007; Zuberogoitia et al., 2014).

Benefits of exploring the urban environment are apparently clear, but the shorter lifespan, together with other potential costs such as disruption of normal activities and nutritional

problems seen in wildlife fed by tourists (Newsome & Rodger, 2008) and exposure to pollutants, poisons or toxins (Birnie-Gauvin et al., 2016; Murray et al., 2016), could eventually be detrimental for urban wild boars. Furthermore, the output of such complex situation might depend on the specific area and wild boar population, since urban wild boars do not show the same responses in all urban areas. For example, Barcelona wild boars use anthropogenic food resources (Studies 1 and 2), whereas Berlin wild boars prefer natural food resources (Stillfried, Gras, Busch, et al., 2017). Whether this process of adjustment to the urban area stays as a series of phenotypic changes with or without genetic basis, or continues under the effects of urban selection pressures to become urban evolution, remains to be studied in the future.

Moreover, Studies 1 and 2 provide further evidence on the great plasticity of wild boars, as previously suggested in both rural and urban areas (Gamelon et al., 2013, 2017; Podgórski et al., 2013; Schley & Roper, 2003; Stillfried, Gras, Börner, et al., 2017). Research on urban wildlife ecology is not recent (Adams, 2005; DeStefano & DeGraaf, 2003), but the wild boar is a newcomer to the urban environment, with most cases arising from 1992 to 1997 (Licoppe et al., 2013). Using wild boar as a model of urban adapter can allow scientists to advance in the knowledge of the process of adjustment (or maybe evolutionary adaptation, if proven in the future) of a wild species to the urban environment.

4.2. Zoonotic diseases of wild boar in urban and non-urban areas

The wild boar is a reservoir host of several zoonotic pathogens, frequently without showing clinical signs of disease (Meng et al., 2009). This thesis (Study 3) provides further evidence that wild boars are commonly parasitized by ticks, which can be infected with emerging zoonotic *Rickettsia* species (Brouqui et al., 2007; Oteo & Portillo, 2012). The Study 4 shows that wild boars can serve as reservoirs of zoonotic *Campylobacter* spp., as previously suggested (Carbonero et al., 2014; Stella et al., 2018), including strains with high virulence potential and multiresistance.

Considering that direct and indirect contacts between wild boar and humans probably occur in the MAB (Rosell & Llimona, 2012), the zoonotic pathogens and parasites found in this thesis pose a risk for MAB inhabitants. On one hand (Study 3), urban and peri-urban wild boars from Barcelona carry ticks that commonly bite humans, such as *D. marginatus* (Estrada-Peña & Jongejan, 1999), infected with two *Rickettsia* species that cause TIBOLA/DEBONEL in humans (Parola et al., 2009; Raoult et al., 1997). This is the second most prevalent tick-borne rickettsiosis in Europe (Oteo & Portillo, 2012). Moreover, increasing ungulate population

densities, such as the Barcelona and Collserola wild boar population, is a concern for TBP transmission among wild ungulates, domestic animals and humans (Ruiz-Fons et al., 2006). On the other hand (Study 4), wild boars can spread zoonotic *Campylobacter* spp. and *Salmonella* sp. in urban and non-urban areas, which is concerning since these two pathogens are transmitted through contaminated food or water and cause the majority of human cases of zoonoses in Europe (EFSA and ECDC, 2018). Both *Campylobacter* spp. and *Salmonella* sp. have moderate-to-high transmissibility potential to humans (Ruiz-Fons, 2017), but according to the prevalences found in wild boars from the studied areas, wild boars pose a higher risk of transmission of *Campylobacter* sp. than *Salmonella* sp.

Hunters are traditionally considered as the group at higher risk of exposition to tick-borne and foodborne pathogens, since they handle wild boar carcasses and share the meat (Franco-Paredes et al., 2017; Ruiz-Fons, 2017). Tick and/or disease transmission, however, could also happen when people practise outdoor activities such as running or biking in the MAB, especially in the Serra de Collserola Natural Park (Bradley & Altizer, 2007; Parc de Collserola, n.d.-c; Ruiz-Fons, 2017), or indirectly from wild boars to humans through companion animals (Grech-Angelini et al., 2019, 2016), free-ranging livestock (Iori et al., 2010; Jori et al., 2016) and contaminated food (Jay et al., 2007); the contribution of the two latter is probably minor since livestock presence in Collserola is restricted to only a few sheep herds and there are few agricultural fields (Parc de Collserola, n.d.-b). However, colonisation of the urban environment by wild boars and the increase in both direct and indirect interaction between humans and wild boars (Studies 1 and 2; Cahill et al., 2012), coupled with the pathogen carrier status of wild boar (Studies 3 and 4; Fernández-Aguilar et al., 2018; Navarro-González et al., 2013; Wang et al., 2019), create new epidemiological scenarios with new risks for zoonosis transmission.

The risk of transmission of zoonotic pathogens is not limited to those reported in this thesis or detected in wild boars from the same study area (*S. suis*, *Campylobacter* and *Salmonella* spp., HEV; Fernández-Aguilar et al., 2018; Navarro-González et al., 2013; Wang et al., 2019) and from other urbanised areas (*C. burnetii*, *Leptospira* spp.; Henning et al., 2015; Jansen et al., 2007), but also to a series of pathogens not investigated up to date. In rural areas, there have been a number of human cases of disease transmission associated to wild boar (Franco-Paredes et al., 2017; Halaby et al., 2000; Matsuda et al., 2003), as well as local disease outbreaks (Fichi et al., 2015). Since a host species with an already established pathogen is a potential source of an emerging pathogen (Woolhouse, Haydon, & Antia, 2005), wild boar is a potential source for emerging human diseases and it is likely that human cases of disease linked to wild boar will

occur also in urban areas such as Barcelona in the near future. It is also possible that these cases already exist but the wild boar origin has not been clarified due to the difficulty of identifying indirect routes of transmission (Jansen et al., 2006). Global urbanisation and wild boar populations are expected to continue growing (Massei et al., 2015; United Nations, 2018), therefore, the space where wildlife and people interact (either in or outside cities) will enlarge and there will be increasing interactions between wild boars and humans. All these factors raise the need for assessing and monitoring the health status of urban and peri-urban wild boar populations, including the one in Barcelona and Collserola.

Notifiable diseases relevant for livestock, such as ASF, have not been assessed in this thesis, and domestic pig farms are not present in the study area. However, since the most significant geographic advances in the spread of ASF are presumed to be human-driven (Beltran-Alcrudo, Falco, Raizman, & Dietze, 2019; Chenais et al., 2019), the new human-wild boar interface described in the Barcelona area (Studies 1 and 2) may pose a potential hazard for the introduction of ASF and other human-driven diseases in the wild boar population, becoming a hotspot for disease transmission. The consequences of the introduction of ASF in the wild boar populations in Spain would have severe detrimental consequences not only for the wild boar population (in light of the high mortality observed in experimental infections; Barasona et al., 2019; Blome, Gabriel, Dietze, Breithaupt, & Beer, 2012; Gabriel et al., 2011), but also for domestic pig farming and trade (Halasa et al., 2016). Therefore, no matter how little the hazard is, active surveillance for ASF is recommended also in the Barcelona wild boar population.

4.3. Management recommendations for urban wild boars

Wild boar presence in urban areas is a current cause of concern and topic of interest, as evidenced by the recent and almost simultaneous publication of two studies in 2018 (Study 1; Toger et al., 2018) addressing the drivers of wild boar presence in a city. Therefore, research on this topic is useful and timely, since urban and peri-urban areas are suffering from wild boar colonisation when there is not a consensus on the best management strategy yet (Licoppe et al., 2013). Managers should seek assistance from researchers to ensure objectivity in designing, implementing and evaluating wild boar management strategies in urbanised areas.

Despite this thesis does not focus on assessing wild boar management measures in urban areas, all four studies yielded results useful to provide recommendations for management purposes, especially the identification of drivers of wild boar presence in urban areas and the wild boar responses to urbanisation. In fact, some of the measures derived from Study 1 are already being

applied in Barcelona, but their evaluation is still ongoing. The management recommendations are designed along four main lines: reducing food availability, controlling conflictive individuals, hinder the access of wild boar to the urban area, and monitoring the health status of the wild boar population.

Based on the knowledge resulting from this thesis (Studies 1 and 2), together with other studies (González-Crespo et al., 2018; Lewis et al., 2015; Minuartia, 2005), reducing the food availability for wild boars in urban and peri-urban areas could be one of the most important measures to undertake. Wild boar are opportunistic, taking advantage of supplemental feeding (Study 2; Ballari & Barrios-García, 2014; Fournier-Chambrillon, Maillard, & Fournier, 1995), and high availability of food enhances reproductive success and reduces juvenile mortality (Geisser & Reyer, 2005). Therefore, appropriate food-related measures in high-risk areas would involve 1) educating people to prevent them from feeding (intentionally or not) the wild boars (Figure 4.1), followed by applying proactive enforcement (e.g. warnings, fines) (Baruch-Mordo, Breck, Wilson, & Broderick, 2011; Farrar, 2007); 2) securing rubbish bins and containers to the ground to prevent access by wild boars (Figure 4.2), similarly to what is done to address the same problem with urban bears (Lewis et al., 2015); 3) installing wild boar-proof feeding devices in feral cat colonies also to prevent access (Figure 4.3); and 4) considering non-irrigated vegetation when creating or restoring green spaces to reduce the food and water available for wild boar, since irrigated urban green areas are used by them (Study 1; Lavín et al., 2017; Licoppe et al., 2013). Food-related measures 1 through 3 are currently being applied in Barcelona.

Figure 4.1. Information booklet used in Barcelona to educate people on how to prevent wild boars from entering the urban area.

Figure 4.2. Rubbish containers secured to the ground.

Figure 4.3. Wild boar-proof feeding device in a cat colony.

Capture and euthanasia of the individuals causing a potentially dangerous situation are needed to resolve these kind of situations and prevent risks for people, such as injuries from attacks and car accidents (Carrillo, Schmidt, Bergman, & Paz, 2007; Jones & Thomas, 1999; Rowden, Steinhardt, & Sheehan, 2008; Zuberogoitia et al., 2014) (Figure 4.4). Specifically targeting

problem individuals increases the efficacy of management measures to reduce human-wildlife conflicts (Carrillo et al., 2007; López et al., 2010; Swan, Redpath, Bearhop, & McDonald, 2017). Furthermore, the removal of bold individuals (those daring most to explore the urban area), mainly in the edge area between the natural and urban habitat, could also be useful to reduce the number of wild boar-human conflicts by maintaining shyer individuals in the population (Honda, Iijima, Tsuboi, & Uchida, 2018). Lethal control of problem animals in urban areas is mostly disapproved by the general public (Kotulski & Koenig, 2008; Vaske & Needham, 2007). However, translocations are not advised since translocated animals can resume their problem behaviour in the new location, especially if they were causing problems in or near residential areas (see review by Massei, Quy, Gurney, & Cowan, 2010). The removal of individuals, either causing potentially dangerous situations or in planned events in sensitive areas to prevent further conflicts, is also currently being applied in Barcelona.

Figure 4.4. A potentially dangerous situation caused by a wild boar in Barcelona that was resolved by capturing and euthanasing this individual.

Furthermore, since streams were identified as corridors used by wild boars to approach Barcelona, clearings could reduce the vegetation cover that provides food and shelter (Dexter, 1998) and allow wild boars to get closer to the urban area without being exposed, as has been suggested for ungulates regarding traffic collisions (Seiler et al., 2016). While vegetation clearings have been proven effective in reducing the number of traffic and train accidents involving moose (Andreassen, Gundersen, & Storaas, 2005; Jaren, Andersen, Ulleberg, Pedersen, & Wiseth, 1991; Lavsund & Sandegren, 1991), other studies have not found any effect (Eriksson, 2014; Seiler et al., 2016). This measure has not been previously tested to drive wildlife away from urban areas and it is also currently being applied in Barcelona.

Managers should also take public health into consideration, since both urban and non-urban wild boars and ticks can carry zoonotic pathogens in all the study areas included in this thesis, as well as in other urban areas (Studies 3 and 4; Fernández-Aguilar et al., 2018; Jansen et al., 2006; Navarro-González et al., 2013; Wang et al., 2019). Addressing this issue should include health surveillance and monitoring of urban wild boar populations, and public health information should be provided in the abovementioned education campaigns in urban areas, and at the information centre in the Serra de Collserola Natural Park, as well as through informative or warning panels. Emphasis should be put into not bringing food from foreign countries, especially those currently affected by ASF (Gavier-Widén et al., 2015).

4.4. Ongoing and future research

The present thesis provides novel results and raises questions for further research that will be part of ongoing theses. Concerning the wild boar responses to urbanisation, genetic studies (e.g. microsatellite typing; Jarne & Lagoda, 1996) are useful to address the wild boar population structure and to therefore determine whether there is continuous immigration from Collserola to Barcelona (Barcelona could be acting as an attractive sink; Delibes, Ferreras, & Gaona, 2001), or there is already an established urban population in Barcelona ("urban islands" theory; Gloor et al., 2001; Wandeler et al., 2003), or both, as occurs in Berlin wild boars (Stillfried, Fickel, et al., 2017). This is an ongoing study, in collaboration with the Department of Ecological Dynamics from the Leibniz Institute for Zoo and Wildlife Research (Berlin).

Moreover, the study of the spatiotemporal behaviour of urban wild boars by using GPS tracking would complement the previous genetic studies and would help gathering information required to design management strategies, such as their daily activity pattern, their resting and feeding preferences according to the season and daytime, the relationship between the use of natural, peri-urban and urban environments, whether there is individual variability and whether their spatial ecology is in accordance with population structure results. This is another ongoing study in collaboration with the Leibniz Institute for Zoo and Wildlife Research.

In addition, it would be interesting to continue the monitoring of female reproduction both in Barcelona and Collserola to complement the results from Study 2. For instance, studies on wild boar reproduction usually involve the number of foetuses or the litter size as a proxy of reproductive success (Fonseca et al., 2011), which we could not address due to lack or incomplete data.

Lastly, regarding health implications of wild boar presence in urban and peri-urban areas, it would be useful to collect questing ticks in order to better characterise tick ecology and TBP(s) epidemiology. Moreover, the molecular characterization of the rickettsiae found in Study 3 would be needed to search for epidemiological connections to human cases. To give continuity to Study 4 and obtain a more comprehensive picture on the circulation of foodborne pathogens in Barcelona, other sympatric wildlife species could be studied, given that *Campylobacter* and *Salmonella* have been previously reported in urban species such as pigeons and raccoons (Casanovas et al., 1995; Rainwater et al., 2017). Also, it would be advisable to monitor other pathogens that could eventually jump from wild boars to humans (Fernández-Aguilar et al., 2018; Jansen et al., 2007) to further assess the risk that wild species pose to humans in urban areas and help preventing their emergence.

4.5. Closing remarks

The Eurasian wild boar (and derived forms) show a wide distribution (Oliver & Leus, 2008), high habitat and diet adaptability (Santos et al., 2004; Schley & Roper, 2003), high fecundity (Focardi et al., 2008; Fonseca et al., 2011), resistance to high hunting pressure (Toïgo, Servanty, Gaillard, Brandt, & Baubet, 2008), and low natural mortality (Keuling et al., 2013). It is no wonder why, according to hunting bags, wild boar populations have been increasing all over Europe for the last 50 years and will probably continue to do so in the near future (Massei et al., 2015; Sáez-Royuela & Tellería, 1986).

The wild boar is currently regarded as a species that can exploit the resources offered by urbanised areas, as red foxes, racoons or bears have been doing for a longer time in other locations (Beckmann & Berger, 2003; Doncaster, Dickman, & Macdonald, 1990; Prange, Gehrt, & Wiggers, 2003). The wild boar adjustment to urban environments is still an ongoing process, with limited (although increasing) scientific evidence and a lot of questions yet to be answered. The present thesis contributes to improve the incipient knowledge of urban wild boars with an ecological, epidemiological and applied management approach. It can also serve as starting point for future research, as wild boars will probably continue exploiting resources from urban areas and the number of wild boar-human conflicts will continue escalating, due to the expansion and increasing numbers of the former and the ubiquitous nature of the latter.

In an urbanising world (United Nations, 2018), more research into urban wildlife and the ecology of wildlife diseases is needed, since urbanisation is responsible for global environmental change at different scales (Grimm et al., 2015) and ecological and epidemiological science

cannot be directly extrapolated from non-urban areas (Mackenstedt et al., 2015). Overall, this thesis provides relevant insights into this developing and necessary field of knowledge.

5. CONCLUSIONS

1. Wild boars use streams as natural corridors to enter Barcelona from the natural bordering area, in the search for food resources available in a fragmented landscape with green areas and cat colonies.

2. Wild boars appear in Barcelona mainly from March to October, probably due to a combination of low availability of natural food resources, high availability of human-derived food resources, and spatial and energetic demands of the wild boar population.

3. Wild boars respond to the urban environment in Barcelona with higher use of anthropogenic food, higher body mass, higher growth rate and early female reproduction, but have shorter lifespan.

4. To mitigate wild boar-human conflicts in Barcelona, the potentially most efficient management measures include the reduction of food available for wild boars and the removal of problem individuals.

5. The wild boars in the Metropolitan Area of Barcelona (MAB) are infested with *Hyalomma lusitanicum*, *Dermacentor marginatus* and *Rhipicephalus sanguineus* sensu lato ticks, which can be infected with emerging zoonotic *Rickettsia* species (*R. massiliae, R. slovaca* and *R. raoultii*).

6. The wild boars from the MAB could promote *Rickettsia* spp. circulation among ticks, by supporting abundant tick populations and facilitating the transmission via co-feeding, although a reservoir role could not be confirmed.

7. Zoonotic *Campylobacter lanienae*, *C. coli* and *Salmonella* sp. are carried by wild boars in the MAB.

8. The *C. coli* genotypes isolated from wild boars in the MAB show high virulence potential and multiresistant profiles.

9. The wild boars carrying *Campylobacter* spp., *Salmonella* sp. and *Rickettsia*-infected ticks are a potential public health threat, given the increasing trend in wild boar population, urbanisation and human-wild boar interactions.

6. REFERENCES

Abaigar, T., Del Barrio, G., & Vericad, J. R. (1994). Habitat preference of wild boar (*Sus scrofa* L., 1758) in a mediterranean environment. Indirect evaluation by signs. *Mammalia*, *58*(2), 201–210. doi: 10.1515/mamm.1994.58.2.201

Acevedo, P., Jiménez-Valverde, A., Lobo, J. M., & Real, R. (2012). Delimiting the geographical background in species distribution modelling. *Journal of Biogeography*, *39*, 1383–1390. doi: 10.1111/j.1365-2699.2012.02713.x/full

Adams, L. W. (2005). Urban wildlife ecology and conservation: A brief history of the discipline. *Urban Ecosystems*, *8*, 139–156.

Adkins, C., & Stott, P. (1998). Home ranges, movements and habitat associations of red foxes *Vulpes vulpes* in suburban Totonto, Ontario. *Journal of Zoology*, *244*, 335–346. doi: 10.1111/ j.1469-7998.1998.tb00038.x

Ajuntament de Barcelona. (2013). *Diagnosis del verde (2009-2019). En: Plan del verde y de la biodiversidad de Barcelona 2020*. Barcelona.

Ajuntament de Barcelona. (2018). *Informe pla d'acció senglars 2017.* Unpublished report. Barcelona.

Akaike, H. (1973). Information theory and the maximum likelihood principle. In B. N. Petrov & F. Csàki (Eds.), *2nd International Symposium on Information Theory*. Budapest: Akademiai Kiàdo.

Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., … Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences*, *114*(34), 8951–8956. doi: 10.1073/pnas.1606034114

Amendolia, S., Lombardini, M., Pierucci, P., & Meriggi, A. (2019). Seasonal spatial ecology of the wild boar in a peri-urban area. *Mammal Research*, *64*(3), 387–396.

Ammazzalorso, A. D., Zolnik, C. P., Daniels, T. J., & Kolokotronis, S.-O. (2015). To beat or not to beat a tick: Comparison of DNA extraction methods for ticks (*Ixodes scapularis*). *PeerJ*, *3*, e1147. doi: 10.7717/peerj.1147

Andraud, M., Halasa, T., Boklund, A., & Rose, N. (2019). Threat to the French swine industry of African swine fever: Surveillance, spread, and control perspectives. *Frontiers in Veterinary Science*, *6*, 248. doi: 10.3389/fvets.2019.00248

Andreassen, H. P., Gundersen, H., & Storaas, T. (2005). The effect of scent-marking, forest clearing, and supplemental feeding on moose–train collisions. *Journal of Wildlife Management*, *69*(3), 1125–1132. doi: 10.2193/0022-541x(2005)069[1125:teosfc]2.0.co;2

Andrzejewski, R., Babińska-Werka, J., Gliwicz, J., & Goszczyński, J. (1978). Synurbization processes in population of *Apodemus agrarius* I. Characteristics of populations in an urbanization gradient. *Acta Theriologica*, *23*, 341–358. doi: 10.4098/AT.arch.78-24

Angelici, F. (2016). Problematic wildlife at the beginning of the twenty-first century: Introduction. In F. Angelici (Ed.), *Problematic wildlife: A cross-disciplinary approach* (pp. 3–20). doi: 10.1007/978-3-319-22246-2

Antilles, N., Sanglas, A., & Cerdà-Cuéllar, M. (2015). Free-living waterfowl as a source of zoonotic bacteria in a dense wild bird population area in Northeastern Spain. *Transboundary and Emerging Diseases*,

62(5), 516–521. doi: 10.1111/tbed.12169

Antón, E., Nogueras, M. M., Pons, I., Font, B., Muñoz, T., Sanfeliu, I., & Segura, F. (2008). *Rickettsia slovaca* infection in humans in the Northeast of Spain: Seroprevalence study. *Vector-Borne and Zoonotic Diseases*, *8*(5), 689–694. doi: 10.1089/vbz.2007.0246

Apanaskevich, D. A., Santos-Silva, M. M., & Horak, I. G. (2008). The genus Hyalomma Koch, 1844. IV. Redescription of all parasitic stages of H. (Euhyalomma) lusitanicum Koch, 1844 and the adults of H. (E.) franchinii Tonelli Rondelli, 1932 (Acari: Ixodidae) with a first description of its immature stages. *Folia Parasitologica*, *55*, 61–74. doi: 10.14411/fp.2008.009

Apollonio, M., Andersen, R., & Putman, R. (2010). Present status and future challenges for European ungulate management. In M. Apollonio, R. Andersen, & R. Putman (Eds.), *European ungulates and their management in the 21st century* (pp. 578–604). Cambridge: Cambridge University Press.

Apollonio, M., Belkin, V. V., Borkowski, J., Borodin, O. I., Borowik, T., Cagnacci, F., … Yanuta, G. (2017). Challenges and science-based implications for modern management and conservation of European ungulate populations. *Mammal Research*, *62*(3), 209–217. doi: 10.1007/s13364-017-0321-5

Agència de qualitat i avaluació sanitàries de Catalunya (AQuAS). (2018a). *Malalties transmeses per paparres a l'Àrea Metropolitana de Barcelona, atenció hospitalària. Servei de petició de dades*. Website: http://aquas.gencat.cat/ca/ambits/analitica-dades/padris/

Agència de qualitat i avaluació sanitàries de Catalunya (AQuAS). (2018b). *Malalties transmeses per paparres a l'Àrea Metropolitana de Barcelona, atenció primària. Servei de petició de dades*. Website: http://aquas.gencat.cat/ca/ambits/analitica-dades/padris/

Arce, A., Estirado, A., Ordobas, M., Sevilla, S., García, N., Moratilla, L., … Vilas, F. (2013). Re-emergence of Leishmaniasis in Spain: Community outbreak in Madrid, Spain, 2009 to 2012. *Euro Surveillance*, *18*(30), 20546.

Àrea Metropolitana de Barcelona (AMB). (2012). The metropolitan area. Retrieved February 16, 2019, from Getting to know the metropolitan area : Population, location and land uses website : http://www.amb.cat/en/web/area-metropolitana/coneixer-l-area-metropolitana

Artois, M., Depner, K. R., Guberti, V., Hars, J., Rossi, S., & Rutili, D. (2002). Classical swine fever (hog cholera) in wild boar in Europe. *Revue Scientifique et Technique (International Office of Epizootics)*, *21*(2), 287–303. doi: 10.1053/tvjl.2001.0601

Astobiza, I., Barral, M., Ruiz-Fons, F., Barandika, J. F., Gerrikagoitia, X., Hurtado, A., & García-Pérez, A. L. (2011). Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. *Veterinary Microbiology*, *147*, 190–194. doi: 10.1016/j.vetmic.2010.05.046

Atanassova, V., Apelt, J., Reich, F., & Klein, G. (2008). Microbiological quality of freshly shot game in Germany. *Meat Science*, *78*(4), 414–419. doi: 10.1016/j.meatsci.2007.07.004

Auman, H. J., Meathrel, C. E., & Richardson, A. (2008). Supersize me: Does anthropogenic food change the body condition of silver gulls? A comparison between urbanized and remote, non-urbanized areas. *Waterbirds*, *31*(1), 122–126. doi: 10.1675/1524-4695(2008)31[122:SMDAFC]2.0.CO;2

Azad, A. F., & Beard, C. B. (1998). Rickettsial pathogens and their arthropod vectors. *Emerging Infectious*

Diseases, *4*(2), 179–186. doi: 10.3201/eid0402.980205

Bacon, D. J., Alm, R., Burr, D. H., Hu, L., Kopecko, D. J., Ewing, C. P., … Guerry, P. (2000). Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. *Infection and Immunity*, *68*(8), 4384–4390. doi: 10.1128/IAI.68.8.4384-4390.2000

Ballari, S. A., & Barrios-García, M. N. (2014). A review of wild boar *Sus scrofa* diet and factors affecting food selection in native and introduced ranges. *Mammal Review*, *44(2)*, 124-134. doi: 10.1111/mam.12015

Bang, D. D., Nielsen, E. M., Scheutz, F., Pedersen, K., Handberg, K., & Madsen, M. (2003). PCR detection of seven virulence and toxin genes of *Campylobacter jejuni* and *Campylobacter coli* isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. *Journal of Applied Microbiology*, *94*(6), 1003–1014. doi: 10.1046/j.1365-2672.2003.01926.x

Barasona, J. A., Gallardo, C., Cadenas-Fernández, E., Jurado, C., Rivera, B., Rodríguez-Bertos, A., … Sanchez-Vizcaino, J. M. (2019). First oral vaccination of Eurasian wild boar against African swine fever virus genotype II. *Frontiers in Veterinary Science*, *6*, 137. doi: 10.3389/fvets.2019.00137

Barbet-Massin, M., Jiguet, F., Albert, C. H., & Thuiller, W. (2012). Modelling species distributions to map the road towards carnivore conservation in the tropics. *Methods in Ecology and Evolution*, *3*(2), 327–338. doi: 10.1111/j.2041-210X.2011.00172.x

Barrios-Garcia, M. N., & Ballari, S. A. (2012). Impact of wild boar (Sus scrofa) in its introduced and native range: A review. *Biological Invasions*, *14*, 2283–2300. doi: 10.1007/s10530-012-0229-6

Bartoń, K. (2018). *Package "MuMIn": Multi-Model Inference*. Retrieved from MuMIn_1.42.1.tar.gz

Baruch-Mordo, S., Breck, S. W., Wilson, K. R., & Broderick, J. (2011). The carrot or the stick? Evaluation of education and enforcement as management tools for human-wildlife conflicts. *PLoS ONE*, *6*(1), e15681. doi: 10.1371/journal.pone.0015681

Bateman, P. W., & Fleming, P. A. (2012). Big city life: Carnivores in urban environments. *Journal of Zoology*, *287*(1), 1–23. doi: 10.1111/j.1469-7998.2011.00887.x

Battin, J. (2018). When good animals love bad habitats: Ecological traps and the conservation of animal populations. *Conservation Biology*, *18*(6), 1482–1491. doi: 10.1111/j.1523-1739.2004.00417.x

Becker, D. J., Streicker, D. G., & Altizer, S. (2015). Linking anthropogenic resources to wildlife-pathogen dynamics: A review and meta-analysis. *Ecology Letters*, *18*(5), 483–495. doi: 10.1111/ele.12428

Beckmann, J. P., & Berger, J. (2003). Using black bears to test ideal-free distribution models experimentally. *Journal of Mammalogy*, *84*(2), 594–606. doi: 10.1644/1545-1542(2003)084<0594:UBBTTI>2.0.CO;2

Beckmann, J. P., & Lackey, C. W. (2008). Carnivores, urban landscapes, and longitudinal studies: A case history of black bears. *Human-Wildlife Conflicts*, *2*(2), 168–174. Retrieved from https://www.jstor.org/stable/24875662

Beier, P., & Noss, R. F. (1998). Do habitat corridors provide connectivity? *Conservation Biology*, *12*(6), 1241–1252. doi: 10.1111/j.1523-1739.1998.98036.x

Beltran-Alcrudo, D., Falco, J. R., Raizman, E., & Dietze, K. (2019). Transboundary spread of pig diseases:

The role of international trade and travel. *BMC Veterinary Research*, *15*(1), 64. doi: 10.1186/s12917-019-1800-5

Bieber, C., & Ruf, T. (2005). Population dynamics in wild boar *Sus scrofa*: Ecology, elasticity of growth rate and implications for the management of pulsed resource consumers. *Journal of Applied Ecology*, *42*(6), 1203–1213. doi: 10.1111/j.1365-2664.2005.01094.x

Birnie-Gauvin, K., Peiman, K. S., Gallagher, A. J., de Bruijn, R., & Cooke, S. J. (2016). Sublethal consequences of urban life for wild vertebrates. *Environmental Reviews*, *24*(4), 416–425. doi: 10.1139/er-2016-0029

Birtles, R. (2012). Rickettsiales infections. In D. Gavier-Widén, J. Duff, & A. Meredith (Eds.), *Infectious diseases of wild mammals and birds in Europe* (pp. 363–371). 1 doi: 0.1002/9781118342442.ch12

Blair, R. (1996). Land use and avian species diversity along an urban gradient. *Ecological Adaptations*, *6*(2), 506–519. doi: 10.2307/2269387

Bleumink-Pluym, N. M. C., van Alphen, L. B., Bouwman, L. I., Wösten, M. M. S. M., & van Putten, J. P. M. (2013). Identification of a functional type VI secretion system in *Campylobacter jejuni* conferring capsule polysaccharide sensitive cytotoxicity. *PLoS Pathogens*, *9*(5), e1003393. doi: 10.1371/journal.ppat.1003393

Blome, S., Gabriel, C., Dietze, K., Breithaupt, A., & Beer, M. (2012). High virulence of African swine fever virus Caucasus isolate in European wild boars of all ages. *Emerging Infectious Diseases*, *18*(4), 708. doi: 10.3201/eid1804.111813

Boitani, L., Mattei, L., Nonis, D., & Corsi, F. (1994). Spatial and activity patterns of wild boars in Tuscany , Italy. *Journal of Mammalogy*, *75*(3), 600–612. doi: 10.2307/1382507

Bolton, D. J. (2015). *Campylobacter* virulence and survival factors. *Food Microbiology*, *48*, 99–108. doi: 10.1016/j.fm.2014.11.017

Bradley, C. A., & Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends in Ecology and Evolution*, *22*(2), 95–102. doi: 10.1016/j.tree.2006.11.001

Brás, A. M., Chatterjee, S., Wren, B. W., Newell, D. G., & Ketley, J. M. (1999). A novel *Campylobacter jejuni* two-component regulatory system important for temperature-dependent growth and colonization. *Journal of Bacteriology*, *181*(10), 3298–3302.

Braun, J.-P., & Lefebvre, H. P. (2008). Kidney function and damage. In M. L. Kaneko, Jerry J.; Harvey, John W.; Bruss (Ed.), *Clinical biochemistry of domestic animals* (6th ed, pp. 485–528). doi: 10.1016/B978-0-12-370491-7.00016-7

Brivio, F., Grignolio, S., Brogi, R., Benazzi, M., Bertolucci, C., & Apollonio, M. (2017). An analysis of intrinsic and extrinsic factors affecting the activity of a nocturnal species: The wild boar. *Mammalian Biology*, *84*, 73–81. doi: 10.1016/j.mambio.2017.01.007

Brouqui, P, Rolain, J. M., Foucault, C., & Raoult, D. (2005). Q fever and Plasmodium falciparum malaria co-infection in a patient returning from the Comoros archipelago. *American Journal of Tropical Medicine and Hygiene*, *73*(6), 1028–1030.

Brouqui, Philippe, Parola, P., Fournier, P. E., & Raoult, D. (2007). Spotted fever rickettsioses in southern

and eastern Europe. *FEMS Immunology and Medical Microbiology*, *49*(1), 2–12. doi: 10.1111/j.1574-695X.2006.00138.x

Brown, V. R., Bowen, R. A., & Bosco-Lauth, A. M. (2018). Zoonotic pathogens from feral swine that pose a significant threat to public health. *Transboundary and Emerging Diseases*, *65*(3), 649–659. doi: 10.1111/tbed.12820

Bruinderink, G., & Hazebroek, E. (1996). Ungulate traffic collisions in Europe. *Conservation Biology*, *10*(4), 1059–1067. doi: 10.1046/j.1523-1739.1996.10041059.x

Bruss, M. L. (2008). Lipids and ketones. In M. L. Kaneko, Jerry J.; Harvey, John W.; Bruss (Ed.), *Clinical biochemistry of domestic animals* (6th ed., pp. 81–115). doi: 10.3233/JAD-2012-111385

Burnham, K. P., & Anderson, D. R. (2002). Information and likelihood theory: A basis for model selection and inference. In *Model selection and multimodel inference. A practical information-theoretic approach* (2nd ed, pp. 49–97). doi: 10.1007/b97636

Cahill, S., & Llimona, F. (2004). Demofraphics of a wild boar *Sus scrofa* Linnaeus, 1758 population in a metropolitan park in Barcelona. *Galemys*, *16*, 37–52.

Cahill, S., Llimona, F., Cabañeros, L., & Calomardo, F. (2009). Habituation of wild boar (Sus scrofa) in a metropolitan area. Characterisation, conflicts and solutions in Collserola Park, Barcelona. In N. A & T. T (Eds.), *7th International symposium on wild boar (Sus scrofa) and on suborder Suiformes* (pp. 25–27).

Cahill, S., Llimona, F., Cabañeros, L., & Calomardo, F. (2012). Characteristics of wild boar (*Sus scrofa*) habituation to urban areas in the Collserola Natural Park (Barcelona) and comparison with other locations. *Animal Biodiversity and Conservation*, *35*(2), 221–233. Retrieved from http://abc.museucienciesjournals.cat/files/ABC_35-2_pp_221-233.pdf

Cahill, S., Llimona, F., & Gràcia, J. (2003). Spacing and nocturnal activity of wild boar *Sus scrofa* in a Mediterranean metropolitan park. *Wildlife Biology*, *9*(Suppl.1), 3–13. doi: 10.2981/wlb.2003.058

Calero-Bernal, R., Pérez-Martín, J. E., Reina, D., Serrano, F. J., Frontera, E., Fuentes, I., & Dubey, J. P. (2015). Detection of zoonotic protozoa *Toxoplasma gondii* and *Sarcocystis suihominis* in wild boars from Spain. *Zoonoses and Public Health*, *63*(5), 346–350. doi: 10.1111/zph.12243

Caminiti, A., Pelone, F., La Torre, G., De Giusti, M., Saulle, R., Mannocci, A., … Scaramozzino, P. (2016). Control and eradication of tuberculosis in cattle: A systematic review of economic evidence. *Veterinary Record*, *179*, 70–75. doi: 10.1136/vr.103616

Cantero, J. G. (2017). *Campylobacter spp. en granjas de pollos de engorde: diversidad genética, resistencia antimicrobiana y factores de virulencia* (doctoral Thesis). Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.

Carbonero, A., Paniagua, J., Torralbo, A., Arenas-Montes, A., Borge, C., & García-Bocanegra, I. (2014). *Campylobacter* infection in wild artiodactyl species from southern Spain: Occurrence, risk factors and antimicrobial susceptibility. *Comparative Immunology, Microbiology and Infectious Diseases*, *37*(2), 115–121. doi: 10.1016/j.cimid.2014.01.001

Carey, M. P., Sanderson, B. L., Barnas, K. A., & Olden, J. D. (2012). Native invaders - Challenges for science,

management, policy, and society. *Frontiers in Ecology and the Environment*, *10*(7), 373–381. doi: 10.1890/110060

Carrillo, C. D., Schmidt, J., Bergman, D., & Paz, G. (2007). Management of urban coyotes and attacks in Green Valley, Pima County, Arizona. In D. Nolte, W. Arjo, & D. Stalman (Eds.), *12th Wildlife Damage Management Conferences* (p. 60). Retrieved from http://digitalcommohttps//digitalcommons.unl.edu/icwdm_wdmconfproc/60/

Casanovas, L., de Simón, M., Ferrer, M. D., Arqués, J., & Monzón, G. (1995). Intestinal carriage of campylobacters, salmonellas, yersinias and listerias in pigeons in the city of Barcelona. *Journal of Applied Bacteriology*, *78*(1), 11–13. doi: 10.1111/j.1365-2672.1995.tb01666.x

Casas-Díaz, E., Closa-Sebastià, F., Marco, I., Lavín, S., Bach-Raich, E., & Cuenca, R. (2015). Hematologic and biochemical reference intervals for wild boar (*Sus scrofa*) captured by cage trap. *Veterinary Clinical Pathology*, *44*(2), 215–222. doi: 10.1111/vcp.12250

Castillo-Contreras, R., Carvalho, J., Serrano, E., Mentaberre, G., Fernández-Aguilar, X., Colom, A., … López-Olvera, J. R. (2018). Urban wild boars prefer fragmented areas with food resources near natural corridors. *Science of the Total Environment*, *615*, 282–288. doi: 10.1016/j.scitotenv.2017.09.277

Cay, A., & Letellier, C. (2009). Isolation of Aujeszky's disease virus from two hunting dogs in Belgium after hunting wild boars. *Vlaams Diergeneeskundig Tijdschrift*, *78*(3), 194–195.

Chace, J. F., & Walsh, J. J. (2006). Urban effects on native avifauna: a review. *Landscape and Urban Planning*, *74*, 46–69. doi: 10.1016/j.landurbplan.2004.08.007

Charrier, F., Rossi, S., Jori, F., Maestrini, O., Richomme, C., Casabianca, F., … Le Potier, M.-F. (2018). Aujeszky's disease and Hepatitis E viruses transmission between domestic pigs and wild boars in Corsica: Evaluating the importance of wild/domestic interactions and the efficacy of management measures. *Frontiers in Veterinary Science*, *5*, 1. doi: 10.3389/fvets.2018.00001

Chenais, E., Depner, K., Guberti, V., Dietze, K., Viltrop, A., & Ståhl, K. (2019). Epidemiological considerations on African swine fever in Europe 2014-2018. *Porcine Health Management*, *5*, 6. doi: 10.1186/s40813-018-0109-2

Chisu, V., Masala, G., Foxi, C., Socolovschi, C., Raoult, D., & Parola, P. (2014). *Rickettsia conorii israelensis* in *Rhipicephalus sanguineus* ticks, Sardinia, Italy. *Ticks and Tick-Borne Diseases*, *5*(4), 446–448. doi: 10.1016/j.ttbdis.2014.02.003

Chlebicz, A., & Śliżewska, K. (2018). Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: A review. *International Journal of Environmental Research and Public Health*, *15*(5), 863. doi: 10.3390/ijerph15050863

Colwell, D. D., Dantas-Torres, F., & Otranto, D. (2011). Vector-borne parasitic zoonoses: Emerging scenarios and new perspectives. *Veterinary Parasitology*, *182*(1), 14–21. doi: 10.1016/j.vetpar.2011.07.012

Conejero, C., Castillo-Contreras, R., González-Crespo, C., Serrano, E., Mentaberre, G., Lavín, S., & López-Olvera, J. R. (2019). Past experiences drive citizen perception of wild boar in urban areas. *Mammalian Biology*, *96*, 68–72. doi: 10.1016/j.mambio.2019.04.002

Contesse, P., Hegglin, D., Gloor, S., Bontadina, F., & Deplazes, P. (2004). The diet of urban foxes (*Vulpes vulpes*) and the availability of anthropogenic food in the city of Zurich, Switzerland. *Mammalian Biology*, *69*(2), 81–95. doi: 10.1078/1616-5047-00123

Corcionivoschi, N., Gundogdu, O., Moran, L., Kelly, C., Scates, P., Stef, L., … Madden, R. H. (2015). Virulence characteristics of hcp+ *Campylobacter jejuni* and *Campylobacter coli* isolates from retail chicken. *Gut Pathogens*, *7*, 20. doi: 10.1186/s13099-015-0067-z

Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J. M., & Pfeiffer, D. U. (2013). Epidemiology of African swine fever virus. *Virus Research*, *173*, 191–197. doi: 10.1016/j.virusres.2012.10.030

Côté, S. D., Rooney, T. P., Tremblay, J. P., Dussault, C., & Waller, D. M. (2004). Ecological impacts of deer overabundance. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 113–147. doi: 10.1146/annurev.ecolsys.35.021103.105725

Courtney, J. W., Kostelnik, L. M., Zeidner, N. S., & Massung, R. F. (2004). Multiplex Real-Time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. *Journal of Clinical Microbiology*, *42*(7), 3164–3168. doi: 10.1128/JCM.42.7.3164-3168.2004

Crawley, M. J. (2007a). Classical tests. In *The R book* (pp. 279–322). Chippenham: John Wiley & Sons.

Crawley, M. J. (2007b). Generalized linear models. In *The R book* (pp. 511–526). Chippenham: John Wiley & Sons.

Cságola, A., Kecskeméti, S., Kardos, G., Kiss, I., & Tuboly, T. (2006). Genetic characterization of type 2 porcine circoviruses detected in Hungarian wild boars. *Archives of Virology*, *151*, 495–507. doi: 10.1007/s00705-005-0639-1

Cummings, K. J., Rodriguez-Rivera, L. D., McNeely, I., Suchodolski, J. S., Mesenbrink, B. T., Leland, B. R., & Bodenchuk, M. J. (2018). Fecal shedding of *Campylobacter jejuni* and *Campylobacter coli* among feral pigs in Texas. *Zoonoses and Public Health*, *65*(1), 215–217. doi: 10.1111/zph.12390

Cypher, B. L. (2010). Kit foxes (*Vulpes macrotis*). In S. D. Gehrt, S. P. D. Riley, & B. L. Cypher (Eds.), *Urban Carnivores: Ecology, conflict and conservation* (pp. 49–60). Baltimore: The John Hopkins University Press.

Dantas-Torres, F., Chomel, B. B., & Otranto, D. (2012). Ticks and tick-borne diseases: a One Health perspective. *Trends in Parasitology*, *28*(10), 437–446. doi: 10.1016/j.pt.2012.07.003

Dardaillon, M. (1988). Wild boar social groupings and their seasonal changes in the Camargue, southern France. *Zeitschrift Für Säugetierkunde*, *53*(1), 22–30.

Dasti, J. I., Tareen, A. M., Lugert, R., Zautner, A. E., & Groß, U. (2010). *Campylobacter jejuni*: A brief overview on pathogenicity-associated factors and disease-mediating mechanisms. *International Journal of Medical Microbiology*, *300*(4), 205–211. doi: 10.1016/j.ijmm.2009.07.002

Datta, S., Niwa, H., & Itoh, K. (2003). Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. *Journal of Medical Microbiology*, *52*(4), 345–348. doi: 10.1099/jmm.0.05056-0

Davidson, A., Malkinson, D., & Shanas, U. (2018). Urban boars show lower perceived risk of humans compare to boars from agricultural areas and nature reserves. In J. Drimaj & J. Kamler (Eds.), *12th*

International Symposium on wild boar and other suids (p. 25). Lázně Bělohrad, Czech Republic.

de Boer, P., Wagenaar, J. A., Achterberg, R. P., Van Putten, J. P. M., Schouls, L. M., & Duim, B. (2002). Generation of *Campylobacter jejuni* genetic diversity in vivo. *Molecular Microbiology*, *44*(2), 351–359. doi: 10.1046/j.1365-2958.2002.02930.x

de la Fuente, J., & Gortázar, C. (2012). Wild boars as hosts of human-pathogenic *Anaplasma phagocytophilum* variants. *Emerging Infectious Diseases*, *18*(12), 2094–2095. doi: 10.3201/eid1806.110997

de La Fuente, J., Naranjo, V., Ruiz-Fons, F., Höfle, U., Fernández De Mera, I. G., Villanúa, D., … Gortázar, C. (2005). Potential vertebrate reservoir hosts and invertebrate vectors of *Anaplasma marginale* and *A. phagocytophilum* in Central Spain. *Vector-Borne and Zoonotic Diseases*, *5*(4), 390–401. doi: 10.1089/vbz.2005.5.390

de la Fuente, J., Naranjo, V., Ruiz-Fons, F., Vicente, J., Estrada-Peña, A., Almazán, C., … Gortázar, C. (2004). Prevalence of tick-borne pathogens in ixodid ticks (Acari: Ixodidae) collected from European wild boar (*Sus scrofa*) and Iberian red deer (*Cervus elaphus hispanicus*) in central Spain. *European Journal of Wildlife Research*, *50*, 187–196. doi: 10.1007/s10344-004-0060-1

Deinet, S., Ieronymidou, C., McRae, L., Burfield, I. J., Foppen, R. P., Collen, B., & Böhm, M. (2013). *Wildlife comeback in Europe: The recovery of selected mammal and bird species.* Final report to Rewilding Europe by ZSL, BirdLife International and the European Bird Census Council. London, UK: ZSL.

Delibes, M., Ferreras, P., & Gaona, P. (2001). Attractive sinks, or how individual behavioural decisions determine source-sink dynamics. *Ecology Letters*, *4*(5), 401–403. doi: 10.1046/j.1461-0248.2001.00254.x

Delibes, M., Gaona, P., & Ferreras, P. (2001). Effects of an attractive sink leading into maladaptive habitat selection. *The American Naturalist*, *158*(3), 277–285. doi: 10.1086/321319

Departament d'Agricultura, Ramaderia, Pesca i Alimentació (DARP), Generalitat de Catalunya. (2015). *Pla tècnic de Gestió Cinegètica. Zona de Caça Controlada de Collserola. Temporada 2015/2016*. Barcelona.

Departament d'Agricultura, Ramaderia, Pesca i Alimentació (DARP), Generalitat de Catalunya. (2018). *Pla tècnic de gestió cinegètica. Zona de caça controlada de Collserola. Temporada 2018/2019.* Barcelona.

DeStefano, S., & DeGraaf, R. M. (2003). Exploring the ecology of suburban wildlife. *Frontiers in Ecology and the Environment*, *1*(2), 95–101. doi: 10.1890/1540-9295(2003)001[0095:ETEOSW]2.0.CO;2

DeWitt, T. J., & Scheiner, S. M. (2004). *Phenotypic plasticity: Functional and conceptual approaches* (1st edition). Oxford: Oxford University Press.

Dexter, N. (1998). The influence of pasture distribution and temperature on habitat selection by feral pigs in a semi-arid environment. *Wildlife Research*, *25*, 547–559. doi: 10.1071/WR97119

Díaz-Sánchez, S., Sánchez, S., Herrera-León, S., Porrero, C., Blanco, J., Dahbi, G., … Vidal, D. (2013). Prevalence of Shiga toxin-producing *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp. in large game animals intended for consumption: Relationship with management practices and

livestock influence. *Veterinary Microbiology*, *163*(3–4), 274–281. doi: 10.1016/j.vetmic.2012.12.026

Dingle, K. E., Colles, F. M., Ure, R., Wagenaar, J. A., Duim, B., Bolton, F. J., … Maiden, M. C. J. (2002). Molecular characterization of *Campylobacter jejuni* clones: A basis for epidemiologic investigation. *Emerging Infectious Diseases*, *8*(9), 949–955. doi: 10.3201/eid0809.020122

Dionisi, A. M., Graziani, C., Lucarelli, C., Filetici, E., Villa, L., Owczarek, S., … Luzzi, I. (2009). Molecular characterization of multidrug-resistant strains of *Salmonella enterica* serotype Typhimurium and monophasic variant (*S*. 4,[5],12:i:-) isolated from human infections in Italy. *Foodborne Pathogens and Disease*, *6*(6), 711–717. doi: 10.1089/fpd.2008.0240

Dolejska, M., Masarikova, M., Dobiasova, H., Jamborova, I., Karpiskova, R., Havlicek, M., … Literak, I. (2016). High prevalence of *Salmonella* and IMP-4-producing Enterobacteriaceae in the silver gull on Five Islands, Australia. *Journal of Antimicrobial Chemotherapy*, *71*(1), 63–70. doi: 10.1093/jac/dkv306

Doncaster, C. P., Dickman, C. R., & Macdonald, D. W. (1990). Feeding ecology of red foxes (*Vulpes vulpes*) in the city of Oxford, England. *Journal of Mammalogy*, *71*(2), 188–194. doi: 10.2307/1382166

Donihue, C. M., & Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio*, *44*, 194–203. doi: 10.1007/s13280-014-0547-2

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., … Lautenbach, S. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, *36*(1), 027–046. doi: 10.1111/j.1600-0587.2012.07348.x

Doudier, B., Olano, J., Parola, P., & Brouqui, P. (2010). Factors contributing to emergence of *Ehrlichia* and *Anaplasma* spp. as human pathogens. *Veterinary Parasitology*, *167*, 149–154. doi: 10.1016/j.vetpar.2009.09.016

Dubey, J. (2001). Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. *Journal of Parasitology*, *87*(1), 215–219. doi: 10.2307/3285204

Dubey, J., Kotula, A., Sharar, A., Andrews, C., & Lindsay, D. (1990). Effect of high temperature on infectivity of *Toxoplasma gondii* tissue cysts in pork. *The Journl of Parasitology*, *76*(2), 201–204. doi: 10.2307/3283016

Dupouy-Camet, J. (2000). Trichinellosis: A worldwide zoonosis. *Veterinary Parasitology*, *93*, 191–200. doi: 10.1016/s0304-4017(00)00341-1

Duron, O., Sidi-Boumedine, K., Rousset, E., Moutailler, S., & Jourdain, E. (2015). The importance of ticks in Q Fever transmission: What has (and has not) been demonstrated? *Trends in Parasitology*, *31*(11), 536–552. doi: 10.1016/j.pt.2015.06.014

Eales, K. M., Norton, R. E., & Ketheesan, N. (2010). Short report: Brucellosis in Northern Australia. *American Journal of Tropical Medicine and Hygiene*, *83*(4), 876–878. doi: 10.4269/ajtmh.2010.10-0237

ECDC (European Centre for Disease Prevention and Control). (2013). *Epidemiological situation of rickettsioses in EU/EFTA countries* (H. Zeller & W. Van Bortel, Eds.). doi: 10.2900/92039

EFSA. (2010). Scientific opinion on quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. *EFSA Journal*, *8*(1), 1437. doi: 10.2903/j.efsa.2010.1437

EFSA and ECDC. (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. In *EFSA Journal* 15(12). doi: 10.2903/j.efsa.2017.5077

EFSA and ECDC. (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal*, *16*(12), 5500. doi: 10.2903/j.efsa.2018.5500

EFSA and ECDC. (2019). The European union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. In *EFSA Journal* 17(2). doi: 10.2903/j.efsa.2019.5598

Eldin, C., Virgili, G., Attard, L., Edouard, S., Viale, P., Raoult, D., & Parola, P. (2018). *Rickettsia massiliae* infection after a tick bite on the eyelid. *Travel Medicine and Infectious Disease*, *26*, 66–68. doi: 10.1016/j.tmaid.2018.08.002

Elith, J., Leathwick, J. R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology*, *77*, 802–813. doi: 10.1111/j.1365-2656.2008.01390.x

Emerson, S. U., & Purcell, R. H. (2003). Hepatitis E virus. *Reviews in Medical Virology*, *13*, 145–154. doi: doi.org/10.1002/rmv.384

Eriksson, C. (2014). *Does tree removal along railways in Sweden influence the risk of train accidents with moose and roe deer?* (Master thesis). Swedish University of Agricultural Sciences, Uppsala, Sweden.

Estrada-Peña, A. (2000). *Ixodoidea (Acarina) en la peninsula ibérica*. Barcelona: Edigraf.

Estrada-Peña, A., Bouattour, A., Camicas, J. ., & Walker, A. . (2004). *Ticks of domestic animals in the Mediterranean region. A guide to identification of species*. doi: 10.1016/0044-8486(94)00383-Y

Estrada-Peña, A., & de la Fuente, J. (2014). The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral Research*, *108*, 104–128. doi: 10.1016/j.antiviral.2014.05.016

Estrada-Peña, A., & Jongejan, F. (1999). Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission. *Experimental and Applied Acarology*, *23*, 685–715. doi: 10.1023/A:1006241108739

Estrada-Peña, A., Osácar, J. J., Pichon, B., & Gray, J. S. (2005). Hosts and pathogen detection for immature stages of *Ixodes ricinus* (Acari: Ixodidae) in North-Central Spain. *Experimental and Applied Acarology*, *37*, 257–268. doi: 10.1007/s10493-005-3271-6

Faria, A. S., Paiva-Cardoso, M. das N., Nunes, M., Carreira, T., Vale-Gonçalves, H. M., Veloso, O., … Vieira, M. L. (2015). First detection of *Borrelia burgdorferi* sensu lato DNA in serum of the wild boar (*Sus scrofa*) in Northern Portugal by Nested-PCR. *EcoHealth*, *12*(1), 183–187. doi: 10.1007/s10393-014-0973-4

Farrar, R. O. (2007). Assessing the impact of urban coyote on people and pets in Austin, Travis County, Texas. In D. Nolte, W. Arjo, & D. Satlman (Eds.), *12th Wildlife Damage Management Conferences* (p. 62). Retrieved from https://digitalcommons.unl.edu/icwdm_wdmconfproc/62/

Fernández-Aguilar, X., Aragon, V., Galofré-Milà, N., Cabezón, O., Velarde, R., Castillo-Contreras, R., … Ardanuy, C. (2018). Urban wild boars and risk for zoonotic *Streptococcus suis*, Spain. *Emerging Infectious Diseases*, *24*(6), 1083–1086. doi: 10.3201/eid2406.171271

Fernández-Juricic, E., & Tellería, J. L. (2000). Effects of human disturbance on spatial and temporal feeding patterns of Blackbird *Turdus merula* in urban parks in madrid, Spain. *Bird Study*, *47*, 13–21. doi: 10.1080/00063650009461156

Fernández-Llario, P., & Carranza, J. (2000). Reproductive performance of the wild boar in a mediterranean ecosystem under drought conditions. *Ethology Ecology and Evolution*, *12*, 335–343. doi: 10.1080/08927014.2000.9522791

Fernández-Llario, P., & Mateos-Quesada, P. (1998). Body size and reproductive parameters in the wild boar *Sus scrofa*. *Acta Theriologica*, *43*(4), 439–444. doi: 10.4098/AT.arch.98-54

Fernández de Mera, I. G., Ruiz-Fons, F., de la Fuente, G., Mangold, A. J., Gortázar, C., & de la Fuente, J. (2013). Spotted fever group rickettsiae in questing ticks, central Spain. *Emerging Infectious Diseases*, *19*(7), 1163–1165. doi: 10.3201/eid1907.130005

Fichi, G., Stefanelli, S., Pagani, A., Luchi, S., De Gennaro, M., Gómez-Morales, M. A., … Pozio, E. (2015). Trichinellosis outbreak caused by meat from a wild boar hunted in an Italian region considered to be at negligible risk for *Trichinella*. *Zoonoses and Public Health*, *62*(4), 285–291. doi: 10.1111/zph.12148

Focardi, S., Gaillard, J.-M., Ronchi, F., & Rossi, S. (2008). Survival of wild boars in a variable environment: Unexpected life-history variation in an unusual ungulate. *Journal of Mammalogy*, *89*(5), 1113–1123. doi: 10.1644/07-MAMM-A-164.1

Fonseca, C., da Silva, A. A., Alves, J., Vingada, J., & Soares, A. M. V. M. (2011). Reproductive performance of wild boar females in Portugal. *European Journal of Wildlife Research*, *57*, 363–371. doi: 10.1007/s10344-010-0441-6

Fournier-Chambrillon, C., Maillard, D., & Fournier, P. (1995). Diet of the wild boar (*Sus scrofa* L.) inhabiting the Montpellier garrigue. *Journal of Mountain Ecology*, *3*, 174–179.

Franco-Paredes, C., Chastain, D., Taylor, P., Stocking, S., & Sellers, B. (2017). Boar hunting and brucellosis caused by *Brucella suis*. *Travel Medicine and Infectious Disease*, *16*, 18–22. doi: 10.1016/j.tmaid.2017.03.006

Frauendorf, M., Gethöffer, F., Siebert, U., & Keuling, O. (2016). The influence of environmental and physiological factors on the litter size of wild boar (*Sus scrofa*) in an agriculture dominated area in Germany. *Science of the Total Environment*, *541*, 877–882. doi: 10.1016/j.scitotenv.2015.09.128

Fredriksson-Ahomaa, M. (2019). Wild boar: A reservoir of foodborne zoonoses. *Foodborne Pathogens and Disease*, *16*(3), 153–165. doi: 10.1089/fpd.2018.2512

Friedman, J. H. (2001). Greedy function approximation: a gradient boosting machine. *Annals of Statistics*, *29*, 1189–1232.

Fulgione, D., Rippa, D., Buglione, M., Trapanese, M., Petrelli, S., & Maselli, V. (2016). Unexpected but welcome. Artificially selected traits may increase fitness in wild boar. *Evolutionary Applications*,

9(6), 769–776. doi: 10.1111/eva.12383

Gabriel, C., Blome, S., Malogolovkin, A., Parilov, S., Kolbasov, D., Teifke, J. P., & Beer, M. (2011). Characterization of African swine fever virus Caucasus isolate in European wild boars. *Emerging Infectious Diseases*, *17*(12), 2342–2345. doi: 10.3201/eid1712.110430

Gaffuri, A., & Holmes, J. P. (2012). *Salmonella* infections. In D. Gavier-Widén, J. Duff, & A. Meredith (Eds.), *Infectious diseases of wild mammals and birds in Europe* (pp. 386–397). Blackwell Publishing Ltd.

Gaillard, J. M., Pontier, D., Brandt, S., Jullien, J. M., & Allainé, D. (1992). Sex differentiation in postnatal growth rate: A test in a wild boar population. *Oecologia*, *90*(2), 167–171. doi: 10.1007/BF00317173

Gallo Orsi, U., Macchi, E., Perrone, A., & Durio, P. (1995). Biometric data and growth rates of a wild boar population living in the Italian Alps. *Journal of Mountain Ecology*, *3*, 60–63.

Gamelon, M., Douhard, M., Baubet, E., Gimenez, O., Brandt, S., & Gaillard, J. (2013). Fluctuating food resources influence developmental plasticity in wild boar. *Biology Letters*, *9*, 20130419. doi: 10.5061/dryad.8hf1c.2.

Gamelon, M., Focardi, S., Baubet, E., Brandt, S., Franzetti, B., Ronchi, F., … Gaillard, J.-M. (2017). Reproductive allocation in pulsed-resource environments: A comparative study in two populations of wild boar. *Oecologia*, *183*(4), 1065–1076. doi: 10.1007/s00442-017-3821-8

García-Pérez, A. L., Oporto, B., Espí, A., del Cerro, A., Barral, M., Povedano, I., … Hurtado, A. (2016). Anaplasmataceae in wild ungulates and carnivores in northern Spain. *Ticks and Tick-Borne Diseases*, *7*, 264–269. doi: 10.1016/j.ttbdis.2015.10.019

García-Sánchez, L., Melero, B., Diez, A. M., Jaime, I., & Rovira, J. (2018). Characterization of *Campylobacter* species in Spanish retail from different fresh chicken products and their antimicrobial resistance. *Food Microbiology*, *76*, 457–465. doi: 10.1016/j.fm.2018.07.004

Gavier-Widén, D., Ståhl, K., Neimanis, A., Hård Av Segerstad, C., Gortázar, C., Rossi, S., & Kuiken, T. (2015). African swine fever in wild boar in Europe: A notable challenge. *Veterinary Record*, *176*, 199–200. doi: 10.1136/vr.h699

Geisser, H., & Reyer, H. U. (2005). The influence of food and temperature on population density of wild boar *Sus scrofa* in the Thurgau (Switzerland). *Journal of Zoology*, *267*, 89–96.

Generalitat de Catalunya. (2010). Decret 146/2010, de 19 d'octubre, de declaració del Parc Natural de la Serra de Collserola i de les reserves naturals parcials de la Font Groga i de la Rierada-Can Balasc. In *Diari Oicial de La Generalitat de Catalunya*. Retrieved from http://cido.diba.cat/legislacio/1380945/decret-1462010-de-19-doctubre-de-declaracio-del-parc-natural-de-la-serra-de-collserola-i-de-les-reserves-naturals-parcials-de-la-font-groga-i-de-la-rierada-can-balasc

Generalitat de Catalunya. (2016). *Programa de vigilància sanitària de la fauna salvatge a Catalunya*. Retrieved from http://agricultura.gencat.cat/web/.content/07-ramaderia/sanitat-animal/vigilancia-sanitaria-fauna-salvatge/enllacos-documents/fitxers-binaris/pla-vigilancia-sanitaria-fauna-salvatge-catalunya.pdf

Gethöffer, F., Sodeikat, G., & Pohlmeyer, K. (2007). Reproductive parameters of wild boar (*Sus scrofa*) in

three different parts of Germany. *European Journal of Wildlife Research*, *53*(4), 287–297. doi: 10.1007/s10344-007-0097-z

Giménez-Anaya, A., Herrero, J., Rosell, C., Couto, S., & García-Serrano, A. (2008). Food habits of wild boars (*Sus Scrofa*) in a Mediterranean coastal wetland. *Wetlands*, *28*(1), 197–203. doi: 10.1672/07-18.1

Gimenez, C., Casado, N., Criado-Fornelio, Á., Álvarez de Miguel, F., & Dominguez-Peñafiel, G. (2009). A molecular survey of Piroplasmida and *Hepatozoon* isolated from domestic and wild animals in Burgos (northern Spain). *Veterinary Parasitology*, *162*, 147–150. doi: 10.1016/j.vetpar.2009.02.021

Gloor, S., Bontadina, F., Hegglin, D., Deplazes, P., & Breitenmoser, U. (2001). The rise of urban fox populations in Switzerland. *Mammal Biology*, *66*, 155–164. doi: 10.5167/uzh-141504

González-Crespo, C., Serrano, E., Cahill, S., Castillo-Contreras, R., Cabañeros, L., López-Martín, J. M., … López-Olvera, J. R. (2018). Stochastic assessment of management strategies for a Mediterranean peri-urban wild boar population. *PLoS ONE*, *13*(8), e0202289. doi: 10.1371/journal.pone.0202289

Goon, S., Kelly, J., Logan, S., Ewing, C., & Guerry, P. (2003). Pseudaminic acid, the major modification on *Campylobacter* flagellin, is synthesized via the Cj1293 gene. *Molecular Microbiology*, *50*(2), 659–671.

Gortázar, C., Delahay, R., Mcdonald, R., Boadella, M., Wilson, G., Gavier-Widén, D., & Acevedo, P. (2012). The status of tuberculosis in European wild mammals. *Mammal Review*, Vol. 42, pp. 193–206. doi: 10.1111/j.1365-2907.2011.00191.x

Gortázar, C., Vicente, J., Fierro, Y., León, L., Cubero, M., & González, M. (2002). Natural Aujeszky's disease in a Spanish wild boar population. *Annals of the New York Academy of Sciences*, *969*, 210–212.

Gottstein, B., Pozio, E., & Nöckler, K. (2009). Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Reviews*, *22*(1), 127–145. doi: 10.1128/CMR.00026-08

Goyette-Desjardins, G., Auger, J. P., Xu, J., Segura, M., & Gottschalk, M. (2014). *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent-an update on the worldwide distribution based on serotyping and sequence typing. *Emerging Microbes and Infections*, *3*, e45. doi: 10.1038/emi.2014.45

Grech-Angelini, S., Richomme, C., Peytavin de Garam, C., Boucher, J. M., Maestrini, O., Grenouillet, F., … Umhang, G. (2019). Identification and molecular characterization of *Echinococcus canadensis* G6/7 in dogs from Corsica, France. *Parasitology Research*, *118*(4), 1313–1319. doi: 10.1007/s00436-019-06261-6

Grech-Angelini, S., Stachurski, F., Lancelot, R., Boissier, J., Allienne, J.-F., Marco, S., … Uilenberg, G. (2016). Ticks (Acari: Ixodidae) infesting cattle and some other domestic and wild hosts on the French Mediterranean island of Corsica. *Parasites and Vectors*, *9*, 582. doi: 10.1186/s13071-016-1876-8

Greig, J., Rajić, A., Young, I., Mascarenhas, M., Waddell, L., & Lejeune, J. (2015). A scoping review of the role of wildlife in the transmission of bacterial pathogens and antimicrobial resistance to the food chain. *Zoonoses and Public Health*, *62*(4), 269–284. doi: 10.1111/zph.12147

Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., … Briggs, J. M. (2015). Global change and the ecology of cities. *Science*, *319*(2008), 756–760. doi: 10.1126/science.1150195

Grimont, P. A., & Weill, F.-X. (2007). *Antigenic formulae of the Salmonella serovars. 9th edition*. Paris.

Groves, C. (2007). Current view on the taxonomy and zoogeography of the genus *Sus*. In U. Albarella, K. Dobney, A. Ervynck, & P. Rowley-Conwy (Eds.), *Pigs and humans: 10,000 years of interaction*. Oxford: Oxford University Press.

Groves, C., & Grubb, P. (1993). The Eurasian suids (*Sus* and *Babyrousa*). In W. L. R. Oliver (Ed.), *Pigs, Peccaries, and Hippos. Status survey and conservation action plan*. Gland, Switzerland: IUCN.

Groves, C., & Grubb, P. (2011). *Ungulate taxonomy*. Baltimore: Johns Hopkins University Press.

Grund, M. D., McAninch, J. B., & Wiggers, E. P. (2002). Seasonal movements and habitat use of female white-tailed deer associated with an urban park. *Journal of Wildlife Management*, *66*(1), 123–130. doi: 10.2307/3802878

Guévremont, E., Normand, V., Lamoureux, L., & Côté, C. (2008). Genetic detection of *Campylobacter lanienae* in fecal matter and stored manure from swine and dairy cattle. *Foodborne Pathogens and Disease*, *5*(3), 361–364. doi: 10.1089/fpd.2007.0054

Gundersen, G., Johannessen, E., Andreassen, H. P., & Ims, R. A. (2001). Source-sink dynamics: How sink affect demography of sources. *Ecology Letters*, *4*, 14–21. doi: 10.1046/j.1461-0248.2001.00182.x

Gürtler, M., Alter, T., Kasimir, S., & Fehlhaber, K. (2005). The importance of *Campylobacter coli* in human campylobacteriosis: Prevalence and genetic characterization. *Epidemiology and Infection*, *133*(6), 1081–1087. doi: 10.1017/S0950268805004164

Hafeez, S., Abbas, M., Khan, Z. H., & Rehman, E. (2011). Preliminary analysis of the diet of wild boar (*Sus scrofa* L., 1758) in Islamabad, Pakistan. *Turkish Journal of Zoology*, *35*(1), 115–118. doi: 10.3906/zoo-0806-24

Halaby, T., Hoitsma, E., Hupperts, R., Spanjaard, L., Luirink, M., & Jacobs, J. (2000). *Streptococcus suis* meningitis, a poacher's risk. *European Journal of Clinical Microbiology and Infectious Diseases*, *19*, 943–945. doi: 10.1007/PL00011230

Halasa, T., Bøtner, A., Mortensen, S., Christensen, H., Toft, N., & Boklund, A. (2016). Control of African swine fever epidemics in industrialized swine populations. *Veterinary Microbiology*, *197*, 142–150. doi: 10.1016/j.vetmic.2016.11.023

Halos, L., Jamal, T., Vial, L., Maillard, R., Suau, A., Le Menach, A., … Vayssier-Taussat, M. (2004). Determination of an efficient and reliable method for DNA extraction from ticks. *Veterinary Research*, *35*, 709–713. doi: 10.1051/vetres:2004038

Hansen, T. L., Jansen, J. la C., Spliid, H., Davidsson, A. sa, & Christensen, T. H. (2007). Composition of source-sorted municipal organic waste collected in Danish cities. *Waste Management*, *27*, 510–518. doi: 10.1016/j.wasman.2006.03.008

Harrel Jr, F. E. (2018). *Package "Hmisc."* Retrieved from http://biostat.mc.vanderbilt.edu/Hmisc

Harrington, C. S., Moran, L., Ridley, A. M., Newell, D. G., & Madden, R. H. (2003). Inter-laboratory evaluation of three flagellin PCR/RFLP methods for typing *Campylobacter jejuni* and *C. coli*: The CAMPYNET experience. *Journal of Applied Microbiology*, *95*(6), 1321–1333. doi: 10.1046/j.1365-2672.2003.02101.x

Harris, S. (1981). An estimation of the number of foxes (*Vulpes vulpes*) in the city of Bristol, and some possible factors affecting their distribution. *Journal of Applied Ecology*, *18*(2), 455–465. doi: 10.2307/2402406

Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. (2008). Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology*, *17*(1), 20–29. doi: 10.1111/j.1365-294X.2007.03428.x

Henning, V., Angela, H., & Wittstatt, U. (2015). Seroepidemiological study on Q fever in wild boars in Berlin. *Tierarztliche Umschau*, *70*(1–2), 41–42.

Henry, V. G. (1968a). Fetal development in European wild hogs. *The Journal of Wildlife Management*, *32*(4), 966–970. doi: 10.2307/3799577

Henry, V. G. (1968b). Length of estrous cycle and gestation in European wild hogs. *The Journal of Wildlife Management*, *32*(2), 406–408. doi: 10.2307/3798986

Herr, J., Schley, L., Engel, E., & Roper, T. J. (2010). Den preferences and denning behaviour in urban stone martens (*Martes foina*). *Mammalian Biology*, *75*, 138–145. doi: 10.1016/j.mambio.2008.12.002

Herrero, J., García-Serrano, A., Couto, S., Ortuño, V. M., & García-González, R. (2006). Diet of wild boar *Sus scrofa* L. and crop damage in an intensive agroecosystem. *European Journal of Wildlife Research*, *52*, 245–250. doi: 10.1007/s10344-006-0045-3

Herrero, J., García-serrano, A., & García-gonzález, R. (2008). Reproductive and demographic parameters in two Iberian wild boar *Sus scrofa* populations. *Acta Theriologica*, *53*(4), 355–364.

Hickey, T. E., Mcveigh, A. L., Scott, D. A., Michielutti, R. E., Bixby, A., Carroll, S. A., … Bourgeois, A. L. (2000). *Campylobacter jejuni* cytolethal distending toxin mediates release of Interleukin-8 from intestinal epithelial cells. *68*(12), 6535–6541. doi: 10.1128/IAI.68.12.6535-6541.2000.Updated

Hilbert, F., Smulders, F. J. M., Chopra-Dewasthaly, R., & Paulsen, P. (2012). *Salmonella* in the wildlife-human interface. *Food Research International*, *45*(2), 603–608. doi: 10.1016/j.foodres.2011.08.015

Hill, D., & Dubey, J. P. (2002). *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clinical Microbiology and Infection*, *8*, 634–640. doi: 10.1046/j.1469-0691.2002.00485.x

Hobbs, N. T. (1996). Modification of ecosystems by ungulates. *The Journal of Wildlife Managment*, *60*(4), 695–713. doi: 196.21.233.72

Holmes, A. H., Moore, L. S. P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., … Piddock, L. J. V. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, *387*(10014), 176–187. doi: 10.1016/S0140-6736(15)00473-0

Honda, T., Iijima, H., Tsuboi, J., & Uchida, K. (2018). A review of urban wildlife management from the animal personality perspective: The case of urban deer. *Science of the Total Environment*, *644*, 576–582. doi: 10.1016/j.scitotenv.2018.06.335

Honig, V., Carolan, H. E., Vavruskova, Z., Massire, C., Mosel, M. R., Crowder, C. D., … Eshoo, M. W. (2017). Broad-range survey of vector-borne pathogens and tick host identification of *Ixodes ricinus* from Southern Czech Republic. *FEMS Microbiology Ecology*, *93*(11), 1–13. doi: 10.1093/femsec/fix129

Hopkins, K. L., Kirchner, M., Guerra, B., Granier, S. A., Lucarelli, C., Porrero, M. C., … Mevius, D. J. (2010). Multiresistant *Salmonella enterica* serovar 4,[5],12:i:- in Europe: A new pandemic strain?

Eurosurveillance, *15*(22), 19580. Retrieved from http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19580

Horrocks, S. M., Anderson, R. C., Nisbet, D. J., & Ricke, S. C. (2009). Incidence and ecology of *Campylobacter jejuni* and coli in animals. *Anaerobe*, *15*(1–2), 18–25. doi: 10.1016/j.anaerobe.2008.09.001

Idescat (Institut Català d'Estadística). (2017). Barcelona. El municipio en cifras. Retrieved from https://www.idescat.cat/emex/?id=080193&lang=es

Iff, U. (1978). Détermination de l'âge chez le Sanglier. *Diana*, *95*(10), 377–381.

Imhoff, M. L., Tucker, C. J., Lawrence, W. T., Stutzer, D. C., & Rustin, R. J. (2000). The use of multi-source satellite and geospatial data to study the effect of urbanization on primary productivity in the United States. *IEEE Trans. Geosci. Remote Sens.*, *38*, 2549–2556.

Inglis, G. D., & Kalischuk, L. D. (2003). Use of PCR for direct detection of *Campylobacter* species in bovine feces. *Applied and Environmental Microbiology*, *69*(6), 3435–3447. doi: 10.1128/AEM.69.6.3435-3447.2003

Institut d'Estadística de Catalunya (Idescat). (2015). *Evolució de la població de l'Àrea Metropolitana de Barcelona segons sexe, 1991-2014*. Retrieved from http://www.amb.cat/s/es/web/area-metropolitana/dades-estadistiques/demografia/serie-historica.html

Institut d'Estadística de Catalunya (Idescat). (2016). El municipi en xifres: Barcelona.

Institut d'Estadística de Catalunya. (2019). *The municipality in figures. Barcelona*. Retrieved from https://www.idescat.cat/emex/?id=080193&lang=en

Iori, A., Gabrielli, S., Calderini, P., Moretti, A., Pietrobelli, M., Tampieri, M. P., … Cancrini, G. (2010). Tick reservoirs for piroplasms in central and northern Italy. *Veterinary Parasitology*, *170*, 291–296. doi: 10.1016/j.vetpar.2010.02.027

Jado, I., Carranza-Rodríguez, C., Barandika, J. F., Toledo, Á., García-Amil, C., Serrano, B., … Anda, P. (2012). Molecular method for the characterization of *Coxiella burnetii* from clinical and environmental samples: variability of genotypes in Spain. *BMC Microbiology*, *12*(91). doi: 10.1186/1471-2180-12-91

James, M. C., Bowman, A. S., Forbes, K. J., Lewis, F., McLeod, J. E., & Gilbert, L. (2013). Environmental determinants of *Ixodes ricinus* ticks and the incidence of *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in Scotland. *Parasitology*, *140*, 237–246. doi: 10.1017/S003118201200145X

Jansen, A., Luge, E., Guerra, B., Wittschen, P., Gruber, A. D., Loddenkemper, C., … Nöckler, K. (2007). Leptospirosis in urban wild boars, Berlin, Germany. *Emerging Infectious Diseases*, *13*(5), 739–742.

Jansen, A., Nöckler, K., Schönberg, A., Luge, E., Ehlert, D., & Schneider, T. (2006). Wild boars as possible source of hemorrhagic leptospirosis in Berlin, Germany. *European Journal of Clinical Microbiology and Infectious Diseases*, *25*(8), 544–546. doi: 10.1007/s10096-006-0174-3

Jaren, V., Andersen, R., Ulleberg, M., Pedersen, P. H., & Wiseth, B. (1991). Moose-train collisions: the effects of vegetation removal with a cost-benefit analysis. *Alces*, *27*, 93–99.

Jarne, P., & Lagoda, P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends in*

Ecology and Evolution, *11*(10), 424–429. doi: 10.1016/0169-5347(96)10049-5

Jay-Russell, M. T., Bates, A., Harden, L., Miller, W. G., & Mandrell, R. E. (2012). Isolation of *Campylobacter* from feral swine (*Sus scrofa*) on the ranch associated with the 2006 *Escherichia coli* O157:H7 spinach outbreak investigation in California. *Zoonoses and Public Health*, *59*(5), 314–319. doi: 10.1111/j.1863-2378.2012.01465.x

Jay, M. T., Cooley, M., Carychao, D., Wiscomb, G. W., Sweitzer, R. A., Crawford-Miksza, L., … Mandrell, R. E. (2007). *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, central California oast. *Emerging Infectious Diseases*, *13*(12), 1908–1911. doi: 10.3201/eid1312.070763

Jerina, K., Pokorny, B., & Stergar, M. (2014). First evidence of long-distance dispersal of adult female wild boar (*Sus scrofa*) with piglets. *European Journal of Wildlife Research*, *60*(2), 367–370. doi: 10.1007/s10344-014-0796-1

Jiménez-Valverde, A. (2012). Insights into the area under the receiver operating characteristic curve (AUC) as a discrimination measure in species distribution modelling. *Global Ecology and Biogeography*, *21*, 498–507.

Jones, D. N., & Thomas, L. K. (1999). Attacks on humans by Australian magpies: Management of an extreme suburban human-wildlife conflict. *Wildlife Society Bulletin*, *27*(2), 473–478.

Jones, K. (2001). Campylobacters in water, sewage and the environment. *Journal of Applied Microbiology*, *90*(S), 68S-79S. doi: 10.1046/j.1365-2672.2001.01355.x

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, *451*(7181), 990–993. doi: 10.1038/nature06536

Jongejan, F., & Uilenberg, G. (2004). The global importance of ticks. *Parasitology*, *129*, S3–S14. doi: 10.1093/ps/86.6.1057

Jori, F., Laval, M., Maestrini, O., Casabianca, F., Charrier, F., & Pavio, N. (2016). Assessment of domestic pigs, wild boars and feral hybrid pigs as reservoirs of hepatitis E virus in Corsica, France. *Viruses*, *8*, 236. doi: 10.3390/v8080236

Kaminski, G., Brandt, S., Baubet, E., & Baudoin, C. (2005). Life-history patterns in female wild boars (*Sus scrofa*): Mother–daughter postweaning associations. *Canadian Journal of Zoology*, *83*(3), 474–480. doi: 10.1139/z05-019

Kanai, Y., Hayashidani, H., Kaneko, K. I., Ogawa, M., Takahashi, T., & Nakamura, M. (1997). Occurrence of zoonotic bacteria in retail game meat in Japan with special reference to *Erysipelothrix*. *Journal of Food Protection*, *60*(3), 328–331. doi: 10.4315/0362-028X-60.3.328

Kansky, R., & Knight, A. T. (2014). Key factors driving attitudes towards large mammals in conflict with humans. *Biological Conservation*, *179*, 93–105. doi: 10.1016/j.biocon.2014.09.008

Kanzaki, N., Perzanowski, K., & Nowosad, M. (1998). Factors affecting wild boar (*Sus scrofa*) population dynamics in Bieszczady, Poland. *Gibier Fane Sauvage - Game and Wildlife*, *15*(2–3), 1171–1178.

Kazimírová, M., Hamšíková, Z., Špitalská, E., Minichová, L., Mahríková, L., Caban, R., … Kocianová, E. (2018). Diverse tick-borne microorganisms identified in free-living ungulates in Slovakia. *Parasites and Vectors*, *11*(495), 1–18. doi: 10.1186/s13071-018-3068-1

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., … Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, *468*, 647–652. doi: 10.1038/nature09575

Kenward, R., & Putman, R. (2011). Ungulate management in Europe: Towards a sustainable future. In R. Putman, M. Apollonio, & R. Andersen (Eds.), *Ungulate management in Europe: Problems and practices* (pp. 376–395). Cambridge University Press.

Kérouanton, A., Chidaine, B., Rose, V., Samson, V., & Denis, M. (2015). Direct detection of *Campylobacter* from feces of organic and conventional pigs highlighted the presence of *Campylobacter lanienae*. *Epidemiology and Control of Hazards in Pork Production Chain – SAFEPORK*, 53–57. doi: 10.31274/safepork-180809-281

Ketley, J. M. (1997). Pathogeneis of enteric infection by *Campylobacter*. *Microbiology*, *143*(1), 5–21. doi: 10.1099/00221287-143-1-5

Keuling, O., Baubet, E., Duscher, A., Ebert, C., Fischer, C., Monaco, A., … Thurfjell, H. (2013). Mortality rates of wild boar *Sus scrofa* L. in central Europe. *European Journal of Wildlife Research*, *59*(6), 805–814. doi: 10.1007/s10344-013-0733-8

Keuling, O., Lauterbach, K., Stier, N., & Roth, M. (2010). Hunter feedback of individually marked wild boar *Sus scrofa* L.: Dispersal and efficiency of hunting in northeastern Germany. *European Journal of Wildlife Research*, *56*, 159–167. doi: 10.1007/s10344-009-0296-x

Keuling, O., Podgórski, T., Monaco, A., Melletti, M., Merta, D., Albrycht, M., … Gongora, J. (2017). Eurasian wild boar *Sus scrofa* (Linnaeus, 1758). In M. Melletti & E. Meijaard (Eds.), *Ecology, conservation and management of wild pigs and peccaries* (pp. 202–233). doi: 10.1017/9781316941232.023

Keuling, O., Stier, N., & Roth, M. (2007). Annual and seasonal space use of different age classes of female wild boar *Sus scrofa* L. *European Journal of Wildlife Research*, *54*(3), 403–412. doi: 10.1007/s10344-007-0157-4

Keuling, O., Stier, N., & Roth, M. (2008). How does hunting influence activity and spatial usage in wild boar *Sus scrofa* L.? *European Journal of Wildlife Research*, *54*(4), 729–737. doi: 10.1007/s10344-008-0204-9

Keuling, O., Strauß, E., & Siebert, U. (2016). Regulating wild boar populations is "somebody else's problem"! - Human dimension in wild boar management. *Science of the Total Environment*, *554–555*(April), 311–319. doi: 10.1016/j.scitotenv.2016.02.159

Kim, Y. M., Jeong, S. H., Kim, J. Y., Song, J. C., Lee, J. H., Kim, J. W., … Kim, J. S. (2011). The first case of genotype 4 hepatitis E related to wild boar in South Korea. *Journal of Clinical Virology*, *50*(3), 253–256. doi: 10.1016/j.jcv.2010.11.005

Kiss, T., Cadar, D., Krupaci, F. A., Bordeanu, A. D., & Spînu, M. (2014). Prevalence of *Anaplasma phagocytophilum* infection in European wild boar (*Sus scrofa*) populations from Transylvania, Romania. *Epidemiology and Infection*, *142*(2), 246–250. doi: 10.1017/S0950268813000812

Klena, J. D., Parker, C. T., Knibb, K., Claire Ibbitt, J., Devane, P. M. L., Horn, S. T., … Konkel, M. E. (2004). Differentiation of *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter*

upsaliensis by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpx*A. *Journal of Clinical Microbiology*, *42*(12), 5549–5557. doi: 10.1128/JCM.42.12.5549-5557.2004

Kock, M. D., Jessup, D., & Burroughs, R. (2012). Ballistics and projectile darting systems. In M. D. Kock & R. Burroughs (Eds.), *Chemical and physical restraint of wild animals, a training and field manual for African species* (2nd ed., pp. 271–285). Retrieved from http://wildlifecaptureafrica.com/wp-content/uploads/2013/10/chap_11.pdf

Konkel, M. E., Kim, B. J., Rivera-Amill, V., & Garvis, S. G. (1999). Identification of proteins required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. *Advances in Experimental Medicine and Biology*, *32*(4), 691–701.

Koolman, L., Whyte, P., Burgess, C., & Bolton, D. (2015). Distribution of virulence-associated genes in a selection of *Campylobacter* isolates. *Foodborne Pathogens and Disease*, *12*(5), 424–432. doi: 10.1089/fpd.2014.1883

Korczak, B. M., Zurfluh, M., Emler, S., Kuhn-Oertli, J., & Kuhnert, P. (2009). Multiplex strategy for multilocus sequence typing, *fla* typing, and genetic determination of antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates collected in Switzerland. *Journal of Clinical Microbiology*, *47*(7), 1996–2007. doi: 10.1128/JCM.00237-09

Kotula, A., Murrell, K., Acosta-Stein, L., Lamb, L., & Douglass, L. (1983). *Trichinella spiralis*: effect of high temperature on infectivity in pork. *Experimental Parasitology*, *56*(1), 15–19.

Kotulski, Y., & Koenig, A. (2008). Conflicts, crises and challenges: wild boar in the Berlin city - A social empirical and statistical survey. *Natura Croatica*, *17*(4), 233–246. doi.org/york.kotulski@gmx.de

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549.

Lagos, L., Picos, J., & Valero, E. (2012). Temporal pattern of wild ungulate-related traffic accidents in northwest Spain. *European Journal of Wildlife Research*, *58*(4), 661–668. doi: 10.1007/s10344-012-0614-6

Lavín, S., López-Olvera, J. R., Castillo-Contreras, R., Mentaberre, G., Florensa, A., & Reina, J. (2017). *Informe sobre la presència de senglar (*Sus scrofa*) al campus de la UAB, any 2016.* Unpublished report. Bellaterra, Barcelona, Spain.

Lavsund, S., & Sandegren, F. (1991). Moose-vehicle relations in Sweden. *Alces, 27*, 118–126.

Lemeshow, S., & Hosmer, D. J. (1982). A review of goodness of fit statistics for use in the development of logistic regression models. *American Journal of Epidemiology*, *115*(1), 92–106.

Leschnik, M., Gruber, A., Kobber-Heiss, A., Bago, Z., Revilla-Fernandez, S., Wodak, E., … Deutz, A. (2012). Epidemiological aspects of Aujeszky's disease in Austria by the means of six cases in dogs. *Wiener Tierarztliche Monatsschrift*, *99*(3–4), 82–90.

Leulmi, H., Aouadi, A., Bitam, I., Bessas, A., Benakhla, A., Raoult, D., & Parola, P. (2016). Detection of *Bartonella tamiae*, *Coxiella burnetii* and *rickettsiae* in arthropods and tissues from wild and domestic animals in northeastern Algeria. *Parasites and Vectors*, *9*, 27. doi: 10.1186/s13071-016-1316-9

Lévesque, S., Lemay, F., Bekal, S., Frost, E. H., & Michaud, S. (2016). First reported case of *Campylobacter lanienae* enteritis in a human. *JMM Case Reports*, *3*(3), e005045. doi: 10.1099/jmmcr.0.005045

Levett, P. N. (2001). Leptospirosis. *Clinical Microbiology Reviews*, *14*(2), 296–326. doi: 10.1128/CMR.14.2.296

Lewis, D. L., Baruch-Mordo, S., Wilson, K. R., Breck, S. W., Mao, J. S., & Broderick, J. (2015). Foraging ecology of black bears in urban environments: Guidance for human-bear conflict mitigation. *Ecosphere*, *6*(8), 1–18. doi: 10.1890/ES15-00137.1

Licoppe, A., Prévot, C., Heymans, M., Bovy, C., Casaer, J., & Cahill, S. (2013). Wild boar/feral pig in (peri-) urban areas. Managing wild boar in human-dominated landscapes. In *International Union of Game Biologists - Congress IUGB 2013*. Brussels, Belgium.

Linden, A., Licoppe, A., Volpe, R., Paternostre, J., Lesenfants, C., Cassart, D., … Cay, A. B. (2019). Summer 2018: African swine fever virus hits north-western Europe. *Transboundary and Emerging Diseases*, *66*(1), 54–55. doi: 10.1111/tbed.13047

Linnell, J. D. C., & Zachos, F. E. (2011). Status and distribution patterns of European ungulates: genetics, population history and conservation. In R. Putman, M. Apollonio, & R. Andersen (Eds.), *Ungulate management in Europe: Problems and practices* (pp. 12–53). Cambridge University Press.

Linton, D., Gilbert, M., Hitchen, P. G., Dell, A., Morris, H. R., Warren, W., … Wren, B. W. (2000). Phase variation of a b-1,3 galactosyltransferase involved in generation of the ganglioside GM1-like lipo-oligosaccharide of *Campylobacter jejuni*. *Molecular Microbiology*, *37*(3), 501–514.

Lledó, L., Domínguez-Peñafiel, G., Giménez-Pardo, C., Gegúndez, I., González, R., & Saz, J. V. (2014). Molecular and serological study of rickettsial infection in humans, and in wild and farm animals, in the province of Burgos, Spain. *Vector-Borne and Zoonotic Diseases*, *14*(6), 383–388. doi: 10.1089/vbz.2013.1513

Llimona, F., Cahill, S., Tenés, A., Camps, D., Bonet-Arbolí, V., & Cabañeros, L. (2007). El estudio de los mamíferos en relación a la gestión de áreas periurbanas. El caso de la región metropolitana de Barcelona. *Galemys*, *19*, 215–234.

Logan, J. M. J., Burnens, A., Linton, D., Lawson, A. J., & Stanley, J. (2000). *Campylobacter lanienae* sp. nov., a new species isolated from workers in an abattoir. *International Journal of Systematic and Evolutionary Microbiology*, *50*(2), 865–872. doi: 10.1099/00207713-50-2-865

Long, J. (2003). *Introduced mammals of the world*. Melbourne: CABI/CSIRO Publishing.

López, R., López, J., Gavela, J., Bosch, J., & Ballesteros, C. (2010). Wild boar capture methodology (*Sus scrofa*, Linnaeus 1758) in a suburban area: The case of Las Rozas de Madrid (central Spain). *Abstracts of the 8th International Symposium on Wild Boar and Other Suids.*, 62–63. York.

Lorusso, V., Wijnveld, M., Majekodunmi, A. O., Dongkum, C., Fajinmi, A., Dogo, A. G., … Picozzi, K. (2016). Tick-borne pathogens of zoonotic and veterinary importance in Nigerian cattle. *Parasites and Vectors*, *9*, 217. doi: 10.1186/s13071-016-1504-7

Lowry, H., Lill, A., & Wong, B. B. M. (2012). Behavioural responses of wildlife to urban environments. *Biological Reviews*, *88*(3), 537–549. doi: 10.1111/brv.12012

Lucarelli, C., Dionisi, A. M., Torpdahl, M., Villa, L., Graziani, C., Hopkins, K., … Luzzi, I. (2010). Evidence for a second genomic island conferring multidrug resistance in a clonal group of strains of *Salmonella enterica* serovar Typhimurium and its monophasic variant circulating in Italy, Denmark, and the United Kingdom. *Journal of Clinical Microbiology*, *48*(6), 2103–2109. doi: 10.1128/JCM.01371-09

Mackenstedt, U., Jenkins, D., & Romig, T. (2015). The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. *International Journal for Parasitology: Parasites and Wildlife*, *4*, 71–79. doi: 10.1016/j.ijppaw.2015.01.006

Madden, F. (2004). Creating coexistence between humans and wildlife: Global perspectives on local efforts to address human–wildlife conflict. *Human Dimensions of Wildlife*, *9*(4), 247–257. doi: 10.1080/10871200490505675

Maillard, D., & Fournier, P. (1995). Effects of shooting with hounds on size of resting range of wild boar (*Sus scrofa* L.) groups in Mediterranean habitat. *Proceedings of 2nd International Symposium on Wild Boar (Sus Scrofa) and on Order Suiformes*, *3*(January), 102–107.

Mangiafico, S. S. (2016). *Summary and analysis of extension program evaluation in R, version 1.17.11*. Retrieved from rcompanion.org/documents/RHandbookProgramEvaluation.pdf

Manilla, G. (1998). Acari, Ixodida. In *Fauna d'Italia Vol. 36* (p. 280). Bologna: Edizioni Calderoni.

Mansfield, K. L., Johnson, N., Phipps, L., Stephenson, J. R., Fooks, A. R., & Solomon, T. (2009). Tick-borne encephalitis virus - a review of an emerging zoonosis. *Journal of General Virology*, *90*(8), 1781–1794. doi: 10.1099/vir.0.011437-0

Marks, C. a., & Bloomfield, T. E. (2006). Home-range size and selection of natal den and diurnal shelter sites by urban red foxes (*Vulpes vulpes*) in Melbourne. *Wildlife Research*, *33*(4), 339–347. doi: 10.1071/WR04058

Márquez, F. J. (2009). Rickettsiae in ticks from wild ungulates of Sierra Nevada and Doñana national parks (Spain). *Clinical Microbioly and Infection*, *15*(2), 227–229. doi: 10.1111/j.1469-0691.2008.02148.x Rickettsiae

Martínez-Abraín, A., Jiménez, J., & Oro, D. (2019). Pax Romana: 'refuge abandonment' and spread of fearless behavior in a reconciling world. *Animal Conservation*, *22*(1), 3–13. doi: 10.1111/acv.12429

Marzluff, J. M. (2001). Worldwide urbanization and its effects on birds. In J. M. Marzluff, R. Bowman, & R. Donnelly (Eds.), *Avian ecology and conservation in an urbanizing world* (pp. 19–47). doi: 10.1007/978-1-4615-1531-9

Massei, G., Genov, P., Staines, B., & Gorman, M. (1997). Mortality of wild boar, *Sus scrofa*, in a Mediterranean area in relation to sex and age. *Journal of Zoology*, *242*, 394–400. doi: 10.1111/j.1469-7998.1997.tb05813.x

Massei, G., & Genov, P. V. (2004). The environmental impact of wild boar. *Galemys*, *16*, 135–145.

Massei, G., Genov, P. V, & Staines, B. W. (1996). Diet, food availability and reproduction of wild boar in a Mediterranean coastal area. *Acta Theriologica*, *41*(3), 307–320. doi: 10.4098/AT.arch.96-29

Massei, G., Kindberg, J., Licoppe, A., Gačić, D., Šprem, N., Kamler, J., … Náhlik, A. (2015). Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. *Pest*

Management Science, *71*(4), 492–500. doi: 10.1002/ps.3965

Massei, G., Quy, R. J., Gurney, J., & Cowan, D. P. (2010). Can translocations be used to mitigate humanwildlife conflicts? *Wildlife Research*, *37*(5), 428–439. doi: 10.1071/WR08179

Massei, G., Roy, S., & Bunting, R. (2011). Too many hogs? A review of methods to mitigate impact by wild boar and feral hogs. *Human-Wildlife Interactions*, *5*(1), 79–99.

Matschke, G. H. (1967). Aging European wild hogs by dentition. *The Journal of Wildlife Management*, *31*(1), 109–113.

Matsuda, H., Okada, K., Takahashi, K., & Mishiro, S. (2003). Severe hepatitis E virus infection after ingestin of uncooked liver from a wild boar. *The Journal of Infectious Diseases*, *188*(6), 944. doi: 10.1086/378074

Maurin, M., & Raoult, D. (1999). *Q Fever*. *12*(4), 518–553.

McCullagh, P., & Nelder, J. A. (1989). *Generalized linear models* (2nd edition). doi: 10.1007/978-1-4899-3242-6

McDermott, J. J., Grace, D., & Zinsstag, J. (2013). Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique de l'OIE*, *32*(1), 249–261. doi: 10.20506/rst.32.1.2197

McDonnell, M. J., & Hahs, A. K. (2015). Adaptation and adaptedness of organisms to urban environments. *The Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 261–280. doi: 10.1146/annurev-ecolsys-112414-054258

McGarigal, K., Cushman, S., & Ene, E. (2012). *FRAGSTATS v4: Spatial pattern analysis program for categorical and continuous maps*. Retrieved from http://www.umass.edu/landeco/research/fragstats/fragstats.html

McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological Conservation*, *127*(3), 247–260. doi: 10.1016/j.biocon.2005.09.005

Melis, C., Szafrańska, P. A., Jędrzejewska, B., & Bartoń, K. (2006). Biogeographical variation in the population density of wild boar (*Sus scrofa*) in western Eurasia. *Journal of Biogeography*, *33*(5), 803–811. doi: 10.1111/j.1365-2699.2006.01434.x

Meng, X. J., Lindsay, D. S., & Sriranganathan, N. (2009). Wild boars as sources for infectious diseases in livestock and humans. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *364*, 2697–2707. doi: 10.1098/rstb.2009.0086

Mentaberre, G., Romero, B., De Juan, L., Navarro-González, N., Velarde, R., Mateos, A., … Serrano, E. (2014). Long-term assessment of wild boar harvesting and cattle removal for bovine tuberculosis control in free ranging populations. *PLoS ONE*, *9*(2), e88824. doi: 10.1371/journal.pone.0088824

Meriggi, A., & Sacchi, O. (2001). Habitat requirements of wild boars in the northern apennines (N Italy): A multi-level approach. *Italian Journal of Zoology*, *68*(1), 47–55. doi: 10.1080/11250000109356382

Merino, F. J., Nebreda, T., Serrano, J. L., Fernández-Soto, P., Encinas, A., & Pérez-Sánchez, R. (2005). Tick species and tick-borne infections identified in population from a rural area of Spain. *Epidemiology and Infection*, *133*, 943–949. doi: 10.1017/S0950268805004061

Millán, J., Proboste, T., Fernández de Mera, I. G., Chirife, A. D., de la Fuente, J., & Altet, L. (2016). Molecular detection of vector-borne pathogens in wild and domestic carnivores and their ticks at the human-wildlife interface. *Ticks and Tick-Borne Diseases*, *7*, 284–290. doi: 10.1016/j.ttbdis.2015.11.003

Miller, W. G., Chapman, M. H., Yee, E., On, S. L. W., McNulty, D. K., Lastovica, A. J., … Mandrell, R. E. (2012). Multilocus sequence typing methods for the emerging *Campylobacter* species *C. hyointestinalis*, *C. lanienae*, *C. sputorum*, *C. concisus*, and *C. curvus*. *Frontiers in Cellular and Infection Microbiology*, *2*, 45. doi: 10.3389/fcimb.2012.00045

Miller, W. G., Englen, M. D., Kathariou, S., Wesley, I. V., Wang, G., Pittenger-Alley, L., … Mandrell, R. A. (2006). Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. *Microbiology*, *152*(1), 245–255. doi: 10.1099/mic.0.28348-0

Miller, W. G., On, S. L. W., Wang, G., Fontanoz, S., Lastovica, A. J., & Mandrell, R. E. (2005). Extended multilocus sequence typing system for *Campylobacter coli*, *C. lari*, *C. upsaliensis*, and *C. helveticus*,. *Journal of Clinical Microbiologyinical Microbiology*, *43*(5), 2315–2329. doi: 10.1128/JCM.43.5.2315

Minuartia. (2005). *Pla de control del senglar (*Sus scrofa*) a Collserola. Situació actual i avaluació d'alternatives d'actuació*.

Minuartia. (2016). *Programa de seguiment de les poblacions de senglar a Catalunya. Temporada 2015-2016*. Barcelona.

Minuartia. (2017). *Programa de seguiment de les poblacions de senglar a Catalunya. Temporada 2016-2017*. Retrieved from http://agricultura.gencat.cat/web/.content/06-medi-natural/caca/enllacos-documents/informes-tecnics/programa-seguiment-poblacions-senglar-sus-scrofa/fitxers-binaris/seguiment_senglar_cat_2017.pdf

Minuartia. (2018). *Programa de seguiment de les poblacions de senglar a Catalunya. Temporada 2017-2018*. Retrieved from http://agricultura.gencat.cat/web/.content/06-medi-natural/caca/enllacos-documents/informes-tecnics/programa-seguiment-poblacions-senglar-sus-scrofa/fitxers-binaris/programa-seguiment-poblacions-senglar-sus-scrofa-temporada-2015-2016.pdf

Moraes-Filho, J., Costa, F. B., Gerardi, M., Soares, H. S., & Labruna, M. B. (2018). *Rickettsia rickettsii* co-feeding transmission among *Amblyomma aureolatum* ticks. *Emerging Infectious Diseases*, *24*(11), 2041–2048. doi: 10.3201/eid2411.180451

Moré, E., Ayats, T., Ryan, P. G., Naicker, P. R., Keddy, K. H., Gaglio, D., … Cerdà-Cuéllar, M. (2017). Seabirds (Laridae) as a source of *Campylobacter* spp., *Salmonella* spp. and antimicrobial resistance in South Africa. *Environmental Microbiology*, *19*(10), 4164–4176. doi: 10.1111/1462-2920.13874

Morelle, K., Fattebert, J., Mengal, C., & Lejeune, P. (2016). Invading or recolonizing? Patterns and drivers of wild boar population expansion into Belgian agroecosystems. *Agriculture, Ecosystems and Environment*, *222*, 267–275. doi: 10.1016/j.agee.2016.02.016

Morelle, K., Podgórski, T., Prévot, C., Keuling, O., Lehaire, F., & Lejeune, P. (2015). Towards understanding wild boar *Sus scrofa* movement: A synthetic movement ecology approach. *Mammal Review*, *45*(1), 15–29. doi: 10.1111/mam.12028

Mossong, J., Marques, P., Ragimbeau, C., Huberty-Krau, P., Losch, S., Meyer, G., … Schneider, F. (2007). Outbreaks of monophasic *Salmonella enterica* serovar 4,[5],12:i:- in Luxembourg, 2006. *Eurosurveillance: European Communicable Disease Bulletin*, *12*(3–6), E11-2.

Mukerji, S., Stegger, M., Truswell, A. V., Laird, T., Jordan, D., Abraham, R. J., … Abraham, S. (2019). Resistance to critically important antimicrobials in Australian silver gulls (*Chroicocephalus novaehollandiae*) and evidence of anthropogenic origins. *Journal of Antimicrobial Chemotherapy*, *74*, 2566–2574. doi: 10.1093/jac/dkz242

Müller, T., Hahn, E. C., Tottewitz, F., Kramer, M., Klupp, B. G., Mettenleiter, T. C., & Freuling, C. (2011). Pseudorabies virus in wild swine: A global perspective. *Archives of Virology*, *156*, 1691–1705. doi: 10.1007/s00705-011-1080-2

Mur, L., Boadella, M., Martínez-López, B., Gallardo, C., Gortazar, C., & Sánchez-Vizcaíno, J. (2012). Monitoring of African swine fever in the wild boar population of the most recent endemic area of Spain. *Transboundary and Emerging Diseases*, *59*, 526–531. doi: 10.1111/j.1865-1682.2012.01308.x

Murray, M. H., Becker, D. J., Hall, R. J., & Hernandez, S. M. (2016). Wildlife health and supplemental feeding: A review and management recommendations. *Biological Conservation*, *204*, 163–174. doi: 10.1016/j.biocon.2016.10.034

Nachamkin, I., Bohachick, K., & Patton, C. M. (1993). Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology*, *31*(6), 1531–1536.

Nachamkin, I., Yang, X. H., & Stern, N. J. (1993). Role of *Campylobacter jejuni* flagella as colonization factors for three- day-old chicks: Analysis with flagellar mutants. *Applied and Environmental Microbiology*, *59*(5), 1269–1273.

Náhlik, A., Cahill, S., Cellina, S., Gál, J., Jánoska, F., Rosell, C., … Massei, G. (2017). Wild boar management in Europe: Knowledge and practice. In M. Melletti & E. Meijaard (Eds.), *Ecology, Conservation and Management of Wild Pigs and Peccaries* (pp. 339–353). doi: 10.1017/9781316941232.033

Navarro-González, N., Casas-Díaz, E., Porrero, C. M., Mateos, A., Domínguez, L., Lavín, S., & Serrano, E. (2013). Food-borne zoonotic pathogens and antimicrobial resistance of indicator bacteria in urban wild boars in Barcelona, Spain. *Veterinary Microbiology*, *167*(3–4), 686–689. doi: 10.1016/j.vetmic.2013.07.037

Navarro-González, N., Mentaberre, G., Porrero, C. M., Serrano, E., Mateos, A., López-Martín, J. M., … Domínguez, L. (2012). Effect of cattle on *Salmonella* carriage, diversity and antimicrobial resistance in free-ranging wild boar (*Sus scrofa*) in Northeastern Spain. *PLoS ONE*, *7*(12), e51614. doi: 10.1371/journal.pone.0051614

Navarro-González, N., Ugarte-Ruiz, M., Porrero, M. C., Zamora, L., Mentaberre, G., Serrano, E., … Domínguez, L. (2014). *Campylobacter* shared between free-ranging cattle and sympatric wild ungulates in a natural environment (NE Spain). *EcoHealth*, *11*(3), 333–342. doi: 10.1007/s10393-014-0921-3

Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., … Kruse, H. (2010). Food-

borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, *139*(SUPPL. 1), S3–S15. doi: 10.1016/j.ijfoodmicro.2010.01.021

Newsome, D., & Rodger, K. (2008). To feed or not to feed: A contentious issue in wildlife tourism. In D. Lunney, A. Munn, & W. Meikle (Eds.), *Too close for comfort: Contentious issues in human-wildlife encounters* (pp. 255–270). Mosman: Royal Zoological Society of New South Wales.

O'Leary, R., & Jones, D. N. (2006). The use of supplementary foods by Australian magpies *Gymnorhina tibicen*: Implications for wildlife feeding in suburban environments. *Austral Ecology*, *31*, 208–216. doi: 10.1111/j.1442-9993.2006.01583.x

O'Reilly, L., & Daborn, C. (1995). The epidemiology of *Mycobacterium bovis* infections in animals and man: A review. *Tubercle and Lung Disease*, *76*(1), 1–46. doi: 10.1016/0962-8479(95)90591-X

O'Sullivan, M. V. N., Zhou, F., Sintchenko, V., Kong, F., & Gilbert, G. L. (2011). Multiplex PCR and Reverse Line Blot Hybridization Assay (mPCR/RLB). *Journal of Visualized Experiments*, *54*(e2781), 1–5. doi: 10.3791/2781

Oli, M. K. (2004). The fast-slow continuum and mammalian life-history patterns: An empirical evaluation. *Basic and Applied Ecology*, *5*(5), 449–463. doi: 10.1016/j.baae.2004.06.002

Oliver, W., & Leus, K. (2008). *Sus scrofa. The IUCN Red list of threatened species 2008*. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T41775A10559847.en

Oporto, B., & Hurtado, A. (2011). Emerging thermotolerant *Campylobacter* species in healthy ruminants and swine. *Foodborne Pathogens and Disease*, *8*(7), 807–813. doi: 10.1089/fpd.2010.0803

Oro, D., Genovart, M., Tavecchia, G., Fowler, M. S., & Martínez-Abraín, A. (2013). Ecological and evolutionary implications of food subsidies from humans. *Ecology Letters*, *16*(12), 1501–1514. doi: 10.1111/ele.12187

Ortuño, A., Quesada, M., López-Claessens, S., Castellà, J., Sanfeliu, I., Antón, E., & Segura-Porta, F. (2007). The role of wild boar (*Sus scrofa*) in the eco-epidemiology of *R. slovaca* in Northeastern Spain. *Vector-Borne and Zoonotic Diseases*, *7*(1), 59–64. doi: 10.1089/vbz.2006.0576

Ortuño, A., Quesada, M., López, S., Miret, J., Cardeñosa, N., Castellà, J., … Segura, F. (2006). Prevalence of *Rickettsia slovaca* in *Dermacentor marginatus* ticks removed from wild boar (*Sus scrofa*) in Northeastern Spain. *Annals of the New York Academy of Sciences*, *1078*, 324–327. doi: 10.1196/annals.1374.061

Ostfeld, R. S., & Keesing, F. (2000). Biodiversity and disease risk: the case of Lyme disease. *Conservation Biology*, *14*(3), 722–728. doi: 10.1046/j.1523-1739.2000.99014.x

Oteo, J. A., & Portillo, A. (2012). Tick-borne rickettsioses in Europe. *Ticks and Tick-Borne Diseases*, *3*, 271–278. doi: 10.1016/j.ttbdis.2012.10.035

Oyarzabal, O. A., Backert, S., Williams, L. L., Lastovica, A. J., Miller, R. S., Pierce, S. J., … Rebollo-Carrato, F. (2008). Molecular typing, serotyping and cytotoxicity testing of *Campylobacter jejuni* strains isolated from commercial broilers in Puerto Rico. *Journal of Applied Microbiology*, *105*(3), 800–812. doi: 10.1111/j.1365-2672.2008.03809.x

Ozgul, A., Childs, D. Z., Oli, M. K., Armitage, K. B., Blumstein, D. T., Olson, L. E., … Coulson, T. (2010). Coupled dynamics of body mass and population growth in response to environmental change. *Nature*, *466*(7305), 482–485. doi: 10.1038/nature09210

Palomar, A. M., Santibáñez, P., Mazuelas, D., Roncero, L., Santibáñez, S., Portillo, A., & Oteo, J. A. (2012). Role of birds in dispersal of etiologic agents of tick-borne zoonoses, Spain, 2009. *Emerging Infectious Diseases*, *18*(7), 1188–1191. doi: 10.3201/eid1807.111777

Panda, S., Thakral, D., & Rehman, S. (2007). Hepatitis E virus. *Reviews in Medical Virology*, *17*, 151–180. doi: 10.1002/rmv.522

Parc de Collserola. (n.d.-a). Clima y meteorología. Retrieved from https://www.parcnaturalcollserola.cat/es/clima-y-meteorologia/

Parc de Collserola. (n.d.-b). Catàleg de la pagesia de Collserola. Retrieved May 24, 2019, from https://www.parcnaturalcollserola.cat/pla-agropecuari/cataleg-de-productors/

Parc de Collserola. (n.d.-c). Uso público, divulgación y educación ambiental. Retrieved February 16, 2019, from Estrategia de uso público 2016-2020 website: https://www.parcnaturalcollserola.cat/es/uso-publico-divulgacion-y-educacion-ambiental/

Parola, P., & Raoult, D. (2001). Ticks and tickborne bacterial diseases in humans: An emerging infectious threat. *Clinical Infectious Diseases*, *32*, 897–928. doi: 10.1086/319347

Parola, P., Rovery, C., Rolain, J. M., Brouqui, P., Davoust, B., & Raoult, D. (2009). *Rickettsia slovaca* and *R. raoultii* in Tick-borne Rickettsioses. *Emerging Infectious Diseases*, *15*(7), 1105–1108. doi: 10.3201/eid1507.081449

Paulsen, P., Smulders, F. J. M., & Hilbert, F. (2012). *Salmonella* in meat from hunted game: A Central European perspective. *Food Research International*, *45*(2), 609–616. doi: 10.1016/j.foodres.2011.06.055

Pedone, P., Mattioli, S., & Mattioli, L. (1995). Body size and growth patterns in wild boars of Tuscany, Central Italy. *IBEX Journal of Mountain Ecology*, *3*, 66–68. Retrieved from http://www.mountainecology.org/index.php/me/article/view/64/53

Pereira, A., Parreira, R., Cotão, A. J., Nunes, M., Vieira, M. L., Azevedo, F., … Maia, C. (2018). Tick-borne bacteria and protozoa detected in ticks collected from domestic animals and wildlife in central and southern Portugal. *Ticks and Tick-Borne Diseases*, *9*(2), 225–234. doi: 10.1016/j.ttbdis.2017.09.008

Pereira, A., Parreira, R., Nunes, M., Casadinho, A., Vieira, M. L., Campino, L., & Maia, C. (2016). Molecular detection of tick-borne bacteria and protozoa in cervids and wild boars from Portugal. *Parasites and Vectors*, *9*, 251. doi: 10.1186/s13071-016-1535-0

Pérez-Haase, A., & Carreras, J. (2012). *Creació d'una Geobase de dades dels hàbitats CORINE del Parc de Collserola a escala 1:10.000*. Retrieved from https://www.parcnaturalcollserola.cat/pdfs/HabitatsCORINE_PNCollserola_2012_Memoria.pdf

Petrovec, M., Sixl, W., Schweiger, R., Mikulašek, S., Elke, L., Wüst, G., … Avšič-Županc, T. (2003). Infections of wild animals with *Anaplasma phagocytophila* in Austria and the Czech Republic. *Annals of the New York Academy of Sciences*, *990*, 103–106. doi: 10.1111/j.1749-6632.2003.tb07345.x

Podgórski, T., Baś, G., Jędrzejewska, B., Sönnichsen, L., Śnieżko, S., Jędrzejewski, W., & Okarma, H. (2013). Spatiotemporal behavioral plasticity of wild boar (*Sus scrofa*) under contrasting conditions of human pressure: Primeval forest and metropolitan area. *Journal of Mammalogy*, *94*(1), 109–119. doi: 10.1644/12-MAMM-A-038.1

Podgórski, T., Scandura, M., & Jedrzejewska, B. (2014). Next of kin next door - philopatry and socio-genetic population structure in wild boar. *Journal of Zoology*, *294*(3), 190–197. doi: 10.1111/jzo.12167

Porrero, M. C., Mentaberre, G., Sánchez, S., Fernández-Llario, P., Gómez-Barrero, S., Navarro-González, N., … Domínguez, L. (2013). Methicillin resistant *Staphylococcus aureus* (MRSA) carriage in different free-living wild animal species in Spain. *The Veterinary Journal*, *198*(1), 127–130. doi: 10.1016/j.tvjl.2013.06.004

Prange, S., Gehrt, S. D., & Wiggers, E. P. (2003). Demographic factors contributing to high racoon densities in urban landscapes. *Journal of Wildlife Management*, *67*(2), 324–333. doi: 10.2307/3802774

Pulliam, R. H. (1988). Sources, sinks, and population regulation. *The American Naturalist*, *132*(5), 652–661. doi: 10.1086/284880

Putman, R. J. (1996). Ungulates in temperate forest ecosystems: Perspectives and recommendations for future research. *Forest Ecology and Management*, *88*(1–2), 205–214. doi: 10.1016/S0378-1127(96)03878-9

Quantum GIS Development Team. (2015). *Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project.*

R Core Team. (2019). *The R stats package*. Retrieved from https://stat.ethz.ch/R-manual/R-devel/library/stats/html/00Index.html

R Development Core Team. (2015). *R software, version 3.1.3*.

R Development Core Team. (2018). *R software version 3.5.0*. Retrieved from www.r-project.com

Rainwater, K. L., Marchese, K., Slavinski, S., Humberg, L. A., Dubovi, E. J., Jarvis, J. A., … Calle, P. P. (2017). Health survey of free-ranging raccoons (*Procyon lotor*) in Central Park, New York, New York, USA: Implications for human and domestic animal health. *Journal of Wildlife Diseases*, *53*(2), 272–284. doi: 10.7589/2016-05-096

Randolph, S. E. (2004). Tick ecology: Processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology*, *129*, S37–S65. doi: 10.1017/S0031182004004925

Raoult, D., Berbis, P., Roux, V., Xu, W., & Maurin, M. (1997). A new tick-transmitted disease due to *Rickettsia slovaca*. *Lancet*, *350*, 1996–1997. doi: 10.1016/S0140-6736(05)61814-4

Reijnen, R., Foppen, R., & Veenbaas, G. (1997). Disturbance by traffic of breeding birds: Evaluation of the effect and considerations in planning and managing road corridors. *Biodiversity and Conservation*, *6*, 567–581. doi: 10.1023/A:1018385312751

Requena-García, F., Cabrero-Sañudo, F., Olmeda-García, S., González, J., & Valcárcel, F. (2017). Influence of environmental temperature and humidity on questing ticks in central Spain. *Experimental and*

Applied Acarology, *71*, 277–290. doi: 10.1007/s10493-017-0117-y

Ricklefs, R. (1990). *Ecology* (3rd ed.). New York: Freeman.

Ridgeway, G. (2015). *Package "gbm". Generalized Boosted Regression Models*.

Ridley, A. M., Toszeghy, M. J., Cawthraw, S. A., Wassenaar, T. M., & Newell, D. G. (2008). Genetic instability is associated with changes in the colonization potential of *Campylobacter jejuni* in the avian intestine. *Journal of Applied Microbiology*, *105*(1), 95–104. doi: 10.1111/j.1365-2672.2008.03759.x

Risco, D., García, A., Serrano, E., Fernandez-Llario, P., Benítez, J. M., Martínez, R., … Hermoso de Mendoza, J. (2014). High-density dependence but low impact on selected reproduction parameters of *Brucella suis* biovar 2 in wild boar hunting estates from South-Western Spain. *Transboundary and Emerging Diseases*, *61*(6), 555–562. doi: 10.1111/tbed.12060

Rolain, J. M., Bitam, I., Buffet, S., Marié, J. L., Bourry, O., Portelli-Clerc, C., … Raoult, D. (2009). Presence or absence of plasmid in *Rickettsia felis* depending on the source of fleas. *Clinical Microbiology and Infection*, *15*(Suppl. 2), 296–297. doi: 10.1111/j.1469-0691.2008.02245.x

Rooney, T. P., & Waller, D. M. (2003). Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest Ecology and Management*, *181*(1–2), 165–176. doi: 10.1016/S0378-1127(03)00130-0

Rosell, C., & Llimona, F. (2012). Human-wildlife interactions. *Animal Biodiversity and Conservation*, *35*(2), 219–220.

Rosell, C., Navàs, F., & Romero, S. (2012). Reproduction of wild boar in a cropland and coastal wetland area: Implications for management. *Animal Biodiversity and Conservation*, *35*(2), 209–217.

Rosenberg, D. K., Noon, B. R., Meslow, E. C., Rosenberg, D. K., Noon, B. R., & Meslow, E. C. (1997). Biological corridors: form, function, and efficacy. *BioScience*, *47*(10), 677–687.

Rosenkranz, M., Elsner, H. A., Stürenburg, H. J., Weiller, C., Röther, J., & Sobottka, I. (2003). *Streptococcus suis* meningitis and septicemia contracted from a wild boar in Germany. *Journal of Neurology*, *250*, 869–870. doi: 10.1007/s00415-003-1103-3

Roux, V., Rydkina, E., Eremeeva, M., & Raoult, D. (1997). Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. *International Journal of Systematic Bacteriology*, *47*(2), 252–261. doi: 10.1099/00207713-47-2-252

Rowden, P., Steinhardt, D., & Sheehan, M. (2008). Road crashes involving animals in Australia. *Accident Analysis and Prevention*, *40*(6), 1865–1871. doi: 10.1016/j.aap.2008.08.002

Rubel, F., Brugger, K., Pfeffer, M., Chitimia-Dobler, L., Didyk, Y. M., Leverenz, S., … Kahl, O. (2016). Geographical distribution of *Dermacentor marginatus* and *Dermacentor reticulatus* in Europe. *Ticks and Tick-Borne Diseases*, *7*(1), 224–233. doi: 10.1016/j.ttbdis.2015.10.015

Ruiz-Fons, F. (2017). A review of the current status of relevant zoonotic pathogens in wild swine (*Sus scrofa*) populations: Changes modulating the risk of transmission to humans. *Transboundary and Emerging Diseases*, *64*(1), 68–88. doi: 10.1111/tbed.12369

Ruiz-Fons, F., Fernández-de-Mera, I. G., Acevedo, P., Gortázar, C., & de la Fuente, J. (2012). Factors driving the abundance of *Ixodes ricinus* ticks and the prevalence of zoonotic *I. ricinus*-borne

pathogens in natural foci. *Applied and Environmental Microbiology*, *78*(8), 2669–2676. doi: 10.1128/aem.06564-11

Ruiz-Fons, F., Fernández-de-Mera, I. G., Acevedo, P., Höfle, U., Vicente, J., de la Fuente, J., & Gortazár, C. (2006). Ixodid ticks parasitizing Iberian red deer (*Cervus elaphus hispanicus*) and European wild boar (*Sus scrofa*) from Spain: Geographical and temporal distribution. *Veterinary Parasitology*, *140*, 133–142. doi: 10.1016/j.vetpar.2006.03.033

Ruiz-Fons, F., Segalés, J., & Gortázar, C. (2008). A review of viral diseases of the European wild boar: Effects of population dynamics and reservoir rôle. *The Veterinary Journal*, *176*, 158–169. doi: 10.1016/j.tvjl.2007.02.017

Sabrina, S., Jean-Michel, G., Carole, T., Serge, B., & Eric, B. (2009). Pulsed resources and climate-induced variation in the reproductive traits of wild boar under high hunting pressure. *Journal of Animal Ecology*, *78*(6), 1278–1290. doi: 10.1111/j.1365-2656.2009.01579.x

Sáez-Royuela, C., & Tellería, J. L. (1986). The increased population of the wild boar (*Sus scrofa* L.) in Europe. *Mammal Review*, *16*(2), 97–101.

Sannö, A., Jacobson, M., Sterner, S., Thisted-Lambertz, S., & Aspán, A. (2018). The development of a screening protocol for *Salmonella* spp. and enteropathogenic *Yersinia* spp. in samples from wild boar (*Sus scrofa*) also generating MLVA–data for *Y. enterocolitica* and *Y. pseudotuberculosis*. *Journal of Microbiological Methods*, *150*, 32–38. doi: 10.1016/j.mimet.2018.05.014

Santos, P., Fernández-Llario, P., Fonseca, C., Monzón, A., Bento, P., Soares, A., … Petrucci-Fonseca, F. (2006). Habitat and reproductive phenology of wild boar (*Sus scrofa*) in the western Iberian Peninsula. *European Journal of Wildlife Research*, *52*(3), 207–212. doi: 10.1007/s10344-005-0025-z

Santos, P., Mexia-de-Almeida, L., & Petrucci-Fonseca, F. (2004). Habitat selection by wild boar *Sus scrofa* L. in Alentejo, Portugal. *Galemys*, *16*(1), 167–184.

Sanz, B. (2000). Mamíferos urbanos en Zaragoza. *Galemys*, *12*(1), 55–57.

Sasaki, Y., Goshima, T., Mori, T., Murakami, M., Haruna, M., Ito, K., & Yamada, Y. (2013). Prevalence and antimicrobial susceptibility of foodborne bacteria in wild boars (*Sus scrofa*) and wild deer (*Cervus nippon*) in Japan. *Foodborne Pathogens and Disease*, *10*(11), 985–991. doi: 10.1089/fpd.2013.1548

Savard, J. L., Clergeau, P., & Mennechez, G. (2000). Biodiversity concepts and urban ecosystems. *Landscape and Urban Planning*, *48*, 131–142.

Scandura, M., Iacolina, L., & Apollonio, M. (2011). Genetic diversity in the European wild boar *Sus scrofa*: Phylogeography, population structure and wild x domestic hybridization. *Mammal Review*, *41*(2), 125–137. doi: 10.1111/j.1365-2907.2010.00182.x

Schley, L., Dufrêne, M., Krier, A., & Frantz, A. C. (2008). Patterns of crop damage by wild boar (*Sus scrofa*) in Luxembourg over a 10-year period.pdf. *European Journal of Wildlife Research*, *54*, 589–599. doi: 10.1007/s10344-008-0183-x

Schley, L., & Roper, T. J. (2003). Diet of wild boar *Sus scrofa* in western Europe, with particular reference to consumption of agricultural crops. *Mammal Review*, *33*(1), 43–56. doi: 10.1046/j.1365-2907.2003.00010.x

Schmid, H., & Baumgartner, A. (2013). Epidemiology of infections with enteric salmonellae in Switzerland with particular consideration of travelling activities. *Swiss Medical Weekly*, *143*, w13842. doi: 10.4414/smw.2013.13842

Schweitzer, N., Damjanova, I., Kaszanyitzky, É., Ursu, K., Samu, P., Tóth, Á. G., … Dán, Á. (2011). Molecular characterization of *Campylobacter lanienae* strains isolated from food-producing animals. *Foodborne Pathogens and Disease*, *8*(5), 615–621. doi: 10.1089/fpd.2010.0754

Segalés, J., Allan, G., & Domingo, M. (2005). Porcine circovirus diseases. *Animal Health Research Reviews*, *6*(2), 119–142. doi: 10.1079/ahr2005106

Seiler, A., Sjölund, M., Rosell, C., Torrellas, M., Rolandsen, M., Solberg, E. J., … Ringsby, T.-H. (2016). *SAFEROAD. Safe roads for wildlife and people. Case studies on the effect of local road and verge features on ungulate-vehicle collisions*. Retrieved from https://www.saferoad-cedr.org/upload_mm/9/5/7/3d796e12-d228-4c32-9f51-fda91958c248_CEDR Call 2013_SAFEROAD_Technical Report 7.pdf

Selmi, M., Martello, E., Bertolotti, L., Bisanzio, D., & Tomassone, L. (2009). *Rickettsia slovaca* and *Rickettsia raoultii* in *Dermacentor marginatus* ticks collected on wild boars in Tuscany, Italy. *Journal of Medical Entomology*, *46*(6), 1490–1493. doi: 10.1603/033.046.0636

Senate Department for the Environment, Transport and Climate Protection. (2017). *Public parks (green and recreation spaces). Data and facts*. Retrieved from https://www.berlin.de/senuvk/umwelt/stadtgruen/gruenanlagen/en/daten_fakten/index.shtml

Servei Meteorològic de Catalunya. (2015). *Butlletí climàtic de l'any 2014*. Retrieved from http://static-m.meteo.cat/wordpressweb/wp-content/uploads/2015/06/08120043/Butlleti-anual-2014.pdf

Servei Meteorològic de Catalunya. (2016a). *Climatologia. El Barcelonès. 1971-200*. Retrieved from http://static-m.meteo.cat/wordpressweb/wp-content/uploads/2014/11/13083422/Barcelones.pdf

Servei Meteorològic de Catalunya. (2016b). *Climatologia. El Vallès Occidental*. Retrieved from http://static-m.meteo.cat/wordpressweb/wp-content/uploads/2014/11/13083422/VallesOccidental.pdf

Servei Meteorològic de Catalunya. (2017). *Butlletí climàtic anual del 2016*. Retrieved from http://static-m.meteo.cat/wordpressweb/wp-content/uploads/2015/06/13120413/Butlletí-climàtic-2016_v5.pdf

Sheppard, S. K., Dallas, J. F., MacRae, M., McCarthy, N. D., Sproston, E. L., Gormley, F. J., … Forbes, K. J. (2009). *Campylobacter* genotypes from food animals, environmental sources and clinical disease in Scotland 2005/6. *International Journal of Food Microbiology*, *134*(1–2), 96–103. doi: 10.1016/j.ijfoodmicro.2009.02.010

Shochat, E., Warren, P. S., Faeth, S. H., McIntyre, N. E., & Hope, D. (2006). From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology & Evolution*, *21*(4), 186–191. doi: 10.1016/j.tree.2005.11.019

Sih, A., Ferrari, M. C. O., & Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications*, *4*, 367–387. doi: 10.1111/j.1752-

4571.2010.00166.x

Silaghi, C., Pfister, K., & Overzier, E. (2014). Molecular investigation for bacterial and protozoan tick-borne pathogens in wild boars (*Sus scrofa*) from southern Germany. *Vector-Borne and Zoonotic Diseases*, *14*(5), 371–373. doi: 10.1089/vbz.2013.1495

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). *Campylobacter* spp. as a foodborne pathogen: A review. *Frontiers in Microbiology*, *2*, 200. doi: 10.3389/fmicb.2011.00200

Silwal, T., Kolejka, J., & Sharma, R. (2016). Severities of wildlife attacks on humans in the vicinity of Chitwan National Park, Nepal. In L. Lnenicka (Ed.), *23rd Central European Conference on Central Europe Area in View of Current Geography. October 2015* (pp. 82–98). Brno, Czech Republic: Central Europe Area in view of current geography.

Simberloff, D. (2011). Native Invaders. In D. Simberloff & M. Rejmánek (Eds.), *Encyclopedia of biological invasions*. Berkeley and Los Angeles, CA: University of California Press.

Slabbekoorn, H., & Peet, M. (2003). Birds sing at a higher pitch in urban noise. *Nature*, *424*, 267. doi: doi: 10.1038/424267a

Snow, N. P., Jarzyna, M. A., & VerCauteren, K. C. (2017). Interpreting and predicting the spread of invasive wild pigs. *Journal of Applied Ecology*, *54*(6), 2022–2032. doi: 10.1111/1365-2664.12866

Soulsbury, C. D., & White, P. C. L. (2015). Human – wildlife interactions in urban areas: A review of conflicts, benefits and opportunities. *Wildlife Research*, *42*, 541–553. doi: 10.1071/WR14229

Speck, S. (2012). *Campylobacter* infections. In D. Gavier-Widén, J. Duff, & A. Meredith (Eds.), *Infectious diseases of wild mammals and birds in Europe* (1st edition, pp. 398–401). Blackwell Publishing Ltd.

Spitz, F., Gleize, J.-C., & Duncan, P. (1990). Particularités de la croissance pondérale du sanglier: cas des populations de Camargue (sud de la France). *Mammalia*, *54*(3), 405–414.

Spitz, F., & Janeau, G. (1995). Daily selection of habitat in wild boar (*Sus scrofa*). *Journal of Zoology*, *237*(3), 423–434. doi: 10.1111/j.1469-7998.1995.tb02772.x

Šprem, N., Piria, M., Prđun, S., Novosel, H., & Treer, T. (2016). Variation of wild boar reproductive performance in different habitat types: Implications for management. *Russian Journal of Ecology*, *47*(1), 96–103. doi: 10.1134/s106741361506017x

Stella, S., Tirloni, E., Castelli, E., Colombo, F., & Bernardi, C. (2018). Microbiological evaluation of carcasses of wild boar hunted in a hill area of northern Italy. *Journal of Food Protection*, *81*(9), 1519–1525. doi: 10.4315/0362-028X.JFP-18-077

Stillfried, M., Fickel, J., Börner, K., Wittstatt, U., Heddergott, M., Ortmann, S., … Frantz, A. C. (2017). Do cities represent sources, sinks or isolated islands for urban wild boar population structure? *Journal of Applied Ecology*, *54*, 272–281. doi: 10.1111/1365-2664.12756

Stillfried, M., Gras, P., Börner, K., Göritz, F., Painer, J., Röllig, K., … Kramer-Schadt, S. (2017). Secrets of success in a landscape of fear: urban wild boar adjust risk perception and tolerate disturbance. *Frontiers in Ecology and Evolution*, *5*(157), 1–12. doi: 10.3389/fevo.2017.00157

Stillfried, M., Gras, P., Busch, M., Börner, K., Kramer-Schadt, S., & Ortmann, S. (2017). Wild inside: Urban wild boar select natural, not anthropogenic food resources. *PLoS ONE*, *12*(4), 1–21. doi:

10.1371/journal.pone.0175127

Swan, G. J. F., Redpath, S. M., Bearhop, S., & McDonald, R. A. (2017). Ecology of problem individuals and the efficacy of selective wildlife management. *Trends in Ecology and Evolution*, *32*(7), 518–530. doi: 10.1016/j.tree.2017.03.011

Szulowski, K., Iwaniak, W., Zlotnicka, Da, M., Weiner, M., Lipowski, A., & Jablonski, A. (2015). Survey of the anti-*Brucella* antibody status determined by ELISA testing in wild boars in Poland. *Medycyna Weterynaryjna*, *71*(4), 215–218.

Tampieri, M. P., Galuppi, R., Bonoli, C., Cancrini, G., Moretti, A., & Pietrobelli, M. (2008). Wild ungulates as *Babesia* hosts in Northern and Central Italy. *Vector-Borne and Zoonotic Diseases*, *8*(5), 667–674. doi: 10.1089/vbz.2008.0001

Tauni, M. A., & Österlund, A. (1999). Outbreak of *Salmonella typhimurium* in cats and humans associated with infection in wild birds. *Journal of Small Animal Practice*, *41*, 339–341. doi: 10.1111/j.1748-5827.2000.tb03214.x

Tenés, A., Cahill, S., Llimona, F., & Molina, G. (2007). Atropellos de mamíferos y tráfico en la red viaria de un espacio natural en el Área Metropolitana de Barcelona: quince años de seguimiento en el parque de Collserola. *Galemys*, *19*, 169–188.

Terpstra, C., & de Smit, A. (2000). The 1997/1998 epizootic of swine fever in the Netherlands: Control strategies under a non-vaccination regimen. *Veterinary Microbiology*, *77*, 3–15. doi: 10.1016/S0378-1135(00)00252-2

Theimer, T. C., Clayton, A. C., Martinez, A., Peterson, D. L., & Bergman, D. L. (2015). Visitation rate and behavior of urban mesocarnivores differs in the presence of two common anthropogenic food sources. *Urban Ecosystems*, *18*, 895–906. doi: 10.1007/s11252-015-0436-x

Tijsse-Klasen, E., Jameson, L. J., Fonville, M., Leach, S., Sprong, H., & Medlock, J. M. (2011). First detection of spotted fever group rickettsiae in *Ixodes ricinus* and *Dermacentor reticulatus* ticks in the UK. *Epidemiology and Infection*, *139*, 524–529. doi: 10.1017/S0950268810002608

Tindall, B. J., Grimont, P. A. D., Garrity, G. M., & Euzéby, J. P. (2005). Nomenclature and taxonomy of the genus *Salmonella*. *International Journal of Systematic and Evolutionary Microbiology*, *55*(1), 521–524. doi: 10.1099/ijs.0.63580-0

Toger, M., Benenson, I., Wang, Y., Czamanski, D., & Malkinson, D. (2018). Pigs in space: An agent-based model of wild boar (*Sus scrofa*) movement into cities. *Landscape and Urban Planning*, *173*, 70–80. doi: 10.1016/j.landurbplan.2018.01.006

Toïgo, C., Servanty, S., Gaillard, J. M., Brandt, S., & Baubet, E. (2008). Disentangling natural from hunting mortality in an intensively hunted wild boar population. *Journal of Wildlife Management*, *72*(7), 1532–1539. doi: 10.2193/2007-378

Toledo, Á., Olmeda, A. S., Escudero, R., Jado, I., Valcárcel, F., Casado-Nistal, M. A., … Anda, P. (2009). Tick-borne zoonotic bacteria in ticks collected from central Spain. *American Journal of Tropical Medicine and Hygiene*, *81*(1), 67–74. doi: 10.4269/ajtmh.2009.81.67

Troxler, S., Hess, C., Konicek, C., Knotek, Z., Barták, P., & Hess, M. (2017). Microdilution testing reveals

considerable and diverse antimicrobial resistance of *Escherichia coli*, thermophilic *Campylobacter* spp. and *Salmonella* spp. isolated from wild birds present in urban areas. *European Journal of Wildlife Research*, *63*, 68. doi: 10.1007/s10344-017-1125-2

Truvé, J., & Lemel, J. (2003). Timing and distance of natal dispersal for wild boar *Sus scrofa* in Sweden. *Wildlife Biology*, *9*(4), 51–57. doi: 10.2981/wlb.2003.056

UAB. (2018). The UAB in figures. Retrieved February 16, 2019, from https://www.uab.cat/web/about-the-uab/the-uab/the-uab-in-figures-1345668682835.html

UAB. (2019a). UAB Natural Heritage. Retrieved February 16, 2019, from Wooded areas website: https://www.uab.cat/web/natural-heritage/wooded-areas-1345676863802.html

UAB. (2019b). UAB Natural Heritage. Retrieved February 16, 2019, from Landscape gardens website: https://www.uab.cat/web/natural-heritage/description-1345676863978.html

United Nations. (2018). *World Urbanization Prospects 2018*. Retrieved from https://www.un.org/development/desa/publications/2018-revision-of-world-urbanization-prospects.html

Urdaneta, S., Dolz, R., & Cerdà-Cuéllar, M. (2015). Assessment of two different types of sample for the early detection and isolation of thermophilic *Campylobacter* in broiler farms. *Avian Pathology*, *44*(2), 103–105. doi: 10.1080/03079457.2015.1006576

Valcárcel, F., González, J., Pérez-Sánchez, J. L., Tercero-Jaime, J. M., & Olmeda, A. S. (2015). Long-term ecological study of host-seeking adults of *Hyalomma lusitanicum* (Acari: Ixodidae) in a Meso-Mediterranean climate. *Journal of Medical Entomology*, *53*(1), 221–224. doi: doi: 10.1093/jme/tjv152

Van Dam, R. M., & Hunter, D. (2012). Biochemical indicators of dietary intake. In W. Willett (Ed.), *Nutritional Epidemiology* (3rd ed., pp. 150–212). doi: 10.1093/acprof:oso/9780199754038.003.0008

Varela-Castro, L., Zuddas, C., Ortega, N., Serrano, E., Salinas, J., Castellà, J., … Mentaberre, G. (2018). On the possible role of ticks in the eco-epidemiology of *Coxiella burnetii* in a Mediterranean ecosystem. *Ticks and Tick-Borne Diseases*, *9*, 687–694. doi: 10.1016/j.ttbdis.2018.02.014

Vaske, J., & Needham, M. D. M. (2007). Segmenting public beliefs about conflict with coyotes in an urban recreation setting. *Journal of Park and Recreation Administration*, *25*(4), 79–98. Retrieved from http://nature.forestry.oregonstate.edu/sites/default/files/2007-1 JPRA - Vaske Needham (2007).pdf

Versalovic, J., Koeuth, T., & Lupski, R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to finerpriting of bacterial enomes. *Nucleic Acids Research*, *19*(24), 6823–6831. doi: 10.1093/nar/19.24.6823

Vetter, S. G., Brandstätter, C., Macheiner, M., Suchentrunk, F., Gerritsmann, H., & Bieber, C. (2016). Shy is sometimes better: Personality and juvenile body mass affect adult reproductive success in wild boars, *Sus scrofa*. *Animal Behaviour*, *115*, 193–205. doi: 10.1016/j.anbehav.2016.03.026

Vetter, S. G., Ruf, T., Bieber, C., & Arnold, W. (2015). What is a mild winter? Regional differences in within-species responses to climate change. *PLoS ONE*, *10*(7), 1–17. doi:

10.1371/journal.pone.0132178

Vicente, J., Segalés, J., Höfle, U., Balasch, M., Plana-Durán, J., Domingo, M., & Gortázar, C. (2004). Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar (*Sus scrofa*). *Veterinary Research*, *35*(2), 243–253. doi: 10.1051/ve- tres:2004008

Vieira-Pinto, M., Morais, L., Caleja, C., Themudo, P., Torres, C., Igrejas, G., … Martins, C. (2011). *Salmonella* sp . in game (*Sus scrofa* and *Oryctolagus cuniculus*). *Foodborne Pathogens and Disease*, *8*(6), 739–741. doi: 10.1089/fpd.2010.0742

Virgós, E. (2002). Factors affecting wild boar (*Sus scrofa*) occurrence in highly fragmented Mediterranean landscapes. *Canadian Journal of Zoology*, *80*(3), 430–435. doi: 10.1139/z02-028

Vitale, G., Mansueto, S., Rolain, J.-M., & Raoult, D. (2006). *Rickettsia massiliae* human isolation. *Emerging Infectious Diseases*, *12*(1), 174–175. doi: 10.3201/eid1201.050850

Vittecoq, M., Godreuil, S., Prugnolle, F., Durand, P., Brazier, L., Renaud, N., … Renaud, F. (2016). Antimicrobial resistance in wildlife. *Journal of Applied Ecology*, *53*(2), 519–529. doi: 10.1111/1365-2664.12596

Wahlström, H., Tysén, E., Olsson Engvall, E., Brändström, B., Eriksson, E., Mörner, T., & Vagsholm, I. (2003). Survey of *Campylobacter* species, VTEC 01 57 and *Salmonella* species in Swedish wildlife. *Veterinary Record*, *153*(3), 74–80. doi: 10.1136/vr.153.3.74

Wandeler, P., Funk, S. M., Largiadèr, C. R., Gloor, S., & Breitenmoser, U. (2003). The city-fox phenomenon: Genetic consequences of a recent colonization of urban habitat. *Molecular Ecology*, *12*(3), 647–656. doi: 10.1046/j.1365-294X.2003.01768.x

Wang, H., Castillo-Contreras, R., Saguti, F., López-Olvera, J. R., Karlsson, M., Mentaberre, G., … Norder, H. (2019). Genetically similar hepatitis E virus strains infect both humans and wild boars in the Barcelona area, Spain, and Sweden. *Transboundary and Emerging Diseases*, *66*(2), 978–985. doi: 10.1111/tbed.13115

Wassenaar, T. M. (1997). Toxin production by *Campylobacter* spp. *Clinical Microbiology Reviews*, *10*(3), 466–476.

Wassenaar, T. M., Geilhausen, B., & Newell, D. G. (1998). Evidence of genomic instability in *Campylobacter jejuni* isolated from poultry. *Applied and Environmental Microbiology*, *64*(5), 1816–1821.

Wedlock, D. N., Skinner, M. A., De Lisle, G. W., & Buddle, B. M. (2002). Control of *Mycobacterium bovis* infections and the risk to human populations. *Microbes and Infection*, *4*, 471–480. doi: 10.1016/S1286-4579(02)01562-9

Welch, D. A., Samuel, W. M., & Wilke, C. J. (1991). Suitability of moose, elk, mule deer, and white-tailed deer as hosts for winter ticks (*Dermacentor albipictus*). *Canadian Journal of Zoology*, *69*(9), 2300–2305. doi: 10.1139/z91-323

WHO. (2015). *WHO estimates of the global burden of foodborne diseases*. Retrieved from https://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/

WHO. (2018). *Fact sheet:* Salmonella *(non-typhoidal)*. Retrieved from https://www.who.int/en/news-

room/fact-sheets/detail/salmonella-(non-typhoidal)

Wichmann, O., Schimanski, S., Koch, J., Kohler, M., Rothe, C., Plentz, A., … Stark, K. (2008). Phylogenetic and case-control study on Hepatitis E virus infection in Germany. *The Journal of Infectious Diseases*, *198*(12), 1732–1741. doi: 10.1086/593211

Winfield, M. D., & Groisman, E. A. (2003). Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology*, *69*(7), 3687–3694. doi: 10.1128/AEM.69.7.3687

Wisniewski, J. (2001). The incidence of *Salmonella* spp. in wild boars in Poland. *Medycyna Weterynaryjna*, *57*(6), 399–401.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society. Series B (Statistical Methodology)*, *73*(1), 3–36. doi: 10.2307/41057423

Woolhouse, M. E. J., Haydon, D. T., & Antia, R. (2005). Emerging pathogens: The epidemiology and evolution of species jumps. *Trends in Ecology and Evolution*, *20*(5), 238–244. doi: 10.1016/j.tree.2005.02.009

Wright, A. N., & Gompper, M. E. (2005). Altered parasite assemblages in raccoons in response to manipulated resource availability. *Oecologia*, *144*(1), 148–156. doi: 10.1007/s00442-005-0018-3

Wu, N., Abril, C., Hinic, V., Brodard, I., Thür, B., Fattebert, J., … Ryser-Degiorgis, M. P. (2011). Free-ranging wild boar: A disease threat to domestic pigs in Switzerland? *Journal of Wildlife Diseases*, *47*(4), 868–879. doi: 10.7589/0090-3558-47.4.868

Wyckoff, A. C., Henke, S. E., Campbell, T. A., Hewitt, D. G., & VerCauteren, K. C. (2009). Feral swine contact with domestic swine: A serologic survey and assessment of potential for disease transmission. *Journal of Wildlife Diseases*, *45*(2), 422–429.

Zahler, M., Filippova, N. A., Morel, P. C., Gothe, R., & Rinder, H. (1997). Relationships between species of the *Rhipicephalus sanguineus* group: a molecular approach. *The Journal of Parasitology*, *83*(2), 302–306. doi: 10.2307/3284460

Zanet, S., Trisciuoglio, A., Bottero, E., Garcia Fernández de Mera, I., Gortazar, C., Carpignano, M. G., & Ferroglio, E. (2014). Piroplasmosis in wildlife: *Babesia* and *Theileria* affecting free-ranging ungulates and carnivores in the Italian Alps. *Parasites and Vectors*, *7*(1), 1–7. doi: 10.1186/1756-3305-7-70

Zemtsova, G., Killmaster, L. F., Mumcuoglu, K. Y., & Levin, M. L. (2010). Co-feeding as a route for transmission of *Rickettsia conorii israelensis* between *Rhipicephalus sanguineus* ticks. *Experimental and Applied Acarology*, *52*, 383–392. doi: 10.1007/s10493-010-9375-7

Zeroual, F., Leulmi, H., Bitam, I., & Benakhla, A. (2018). Molecular evidence of *Rickettsia slovaca* in spleen of wild boars in northeastern Algeria. *New Microbes and New Infections*, *24*, 17–20. doi: 10.1016/j.nmni.2018.03.008

Ziprin, R. L., Young, C. R., Byrd, J. A., Stanker, L. H., Hume, M. E., Gray, S. A., … Konkel, M. E. (2001). Role of *Campylobacter jejuni* potential virulence genes in cecal colonization. *Avian Diseases*, *45*(3), 549. doi: 10.2307/1592894

Zottola, T., Montagnaro, S., Magnapera, C., Sasso, S., De Martino, L., Bragagnolo, A., … Pagnini, U. (2013). Prevalence and antimicrobial susceptibility of *Salmonella* in European wild boar (*Sus scrofa*); Latium Region - Italy. *Comparative Immunology, Microbiology and Infectious Diseases*, *36*(2), 161–168. doi: 10.1016/j.cimid.2012.11.004

Zuberogoitia, I., Del Real, J., Torres, J. J., Rodríguez, L., Alonso, M., & Zabala, J. (2014). Ungulate vehicle collisions in a peri-urban environment: Consequences of transportation infrastructures planned assuming the absence of ungulates. *PLoS ONE*, *9*(9), e107713. doi: 10.1371/journal.pone.0107713

Zuur, A. F. (2012). Introduction to additive models using deep-sea fisheries data. In *A beginner's guide to generalized additive models with R* (pp. 33–68). Newburgh: Highland statistics.

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3–14. doi: 10.1111/j.2041-210X.2009.00001.x