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Factores determinantes de la transmisión y persistencia de enfermedades compartidas en ungulados silvestres: análisis de series temporales

Determinants of the transmission and persistence of shared infections in wild ungulates: analysis of broad temporal series

Tesis Doctoral

Patricia Barroso Seano

FACTORES DETERMINANTES DE LA TRANSMISIÓN Y PERSISTENCIA DE ENFERMEDADES COMPARTIDAS EN UNGULADOS SILVESTRES: ANÁLISIS DE SERIES TEMPORALES

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Determinants of the transmission and persistence of shared infections in wild ungulates: analysis of broad temporal series

Memoria presentada por

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Para optar al grado de Doctor con mención internacional

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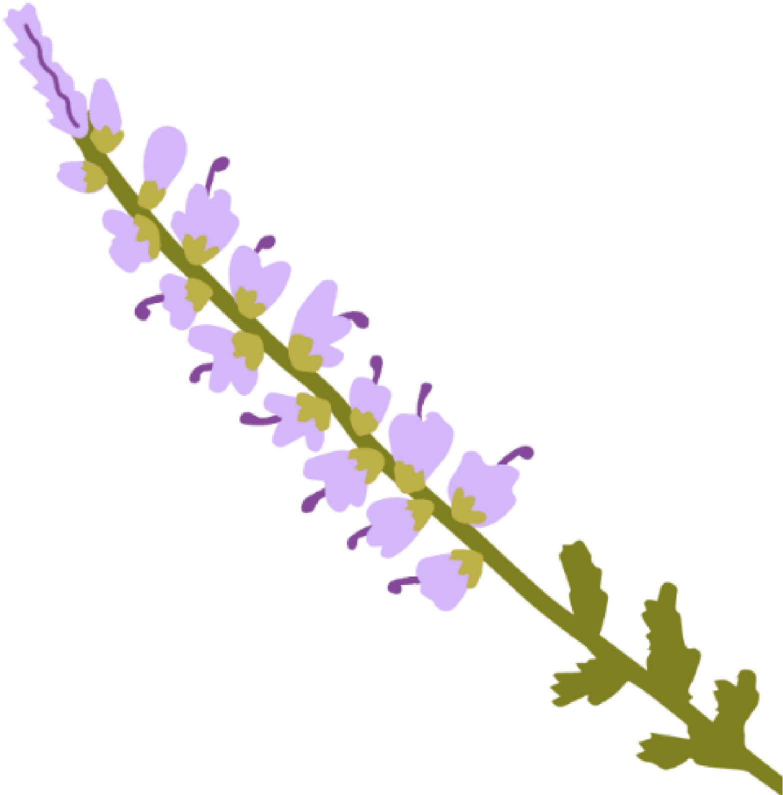


Resumen

Las infecciones compartidas en la interfaz silvestre-doméstico poseen gran relevancia para la salud pública, la economía global, la gestión de la fauna silvestre y del medio ambiente y, por último, para la conservación de la biodiversidad. Por ello, es necesario un abordaje multidisciplinar del problema mediante una perspectiva "Una sola salud". Los estudios epidemiológicos a largo plazo engloban todas las etapas que forman parte de los diferentes procesos ecológicos y epidemiológicos, lo que permite conocer cuáles son y cómo operan los principales determinantes de la dinámica temporal de las infecciones y cuáles son los principales factores implicados en la susceptibilidad y/o exposición de los hospedadores a los diferentes patógenos. Este tipo de estudios son muy escasos en el ámbito de las infecciones compartidas en fauna silvestre, debido principalmente al acceso limitado que tienen los investigadores a las poblaciones silvestres. Por tanto, generar y analizar series temporales sobre infecciones compartidas en fauna silvestre y su interfaz con el ganado es algo prioritario para poder alcanzar una mejor comprensión, control y erradicación de estas. En este contexto, mediante la presente Tesis Doctoral, se pretendió identificar los principales factores de tipo individual, poblacional, ambiental y estocástico que, operando a medio o largo plazo, intervienen en la transmisión y persistencia de las infecciones compartidas en el medio natural. Para ello, se analizó una serie temporal de trece años (2005-2018), centrándose en varios sistemas patógeno-multihospedador del Parque Nacional de Doñana. Esta aproximación a medio-largo plazo fue fundamental para identificar y comprender los patrones de transmisión y persistencia de los patógenos compartidos entre la fauna silvestre y el ganado.

Primero, se realizó una revisión bibliográfica sobre estudios epidemiológicos a largo plazo acerca de enfermedades infecciosas de la fauna silvestre. La finalidad de este capítulo fue conocer la evolución temporal de estos estudios e identificar el valor añadido que supone analizar amplias series temporales a la hora de explicar los factores determinantes y comprender sus consecuencias epidemiológicas y ecológicas. Además, se propuso una definición de "investigación a largo plazo en fauna silvestre", considerando que la escala temporal en la que intervienen los principales determinantes de la epidemiología de un patógeno es siempre dependiente del contexto (Capítulo 1).

Posteriormente, en el Capítulo 2, se determinó la dinámica temporal de la prevalencia de patógenos de diferente naturaleza (Complejo *Mycobacterium tuberculosis*, *Toxoplasma gondii*, virus de la lengua azul y virus de la hepatitis E) y se identificó el papel de los principales factores de riesgo (individuales, poblacionales, ambientales y estocásticos) en la comunidad mixta de ungulados del Parque Nacional de Doñana, hallándose valores de infección muy elevados y confirmando una exposición constante de los hospedadores a los patógenos durante el periodo de estudio.

Los resultados de esta Tesis doctoral resaltan la importancia de los estudios a largo plazo la interfaz doméstico-silvestre como herramienta fundamental para conocer la epidemiología y ecología de los patógenos, así como su respuesta ante factores de diversa naturaleza que actúan a medio y largo plazo, necesario para poder diseñar estrategias efectivas para la mitigación del riesgo.

Abstract

Shared infections at the wild-domestic interface are relevant to public health, the global economy, wildlife and environmental management, and the conservation of biodiversity. Thus, it is necessary a multidisciplinary approach to this issue from a “One Health” perspective. Long-term epidemiological studies encompass all the stages that belong to the different ecological and epidemiological processes, which allows knowing what are and how operate the main determinants of the temporal dynamics of infections, as well as what are the main factors involved in susceptibility and/or exposure to different pathogens. These studies are very scarce in the field of shared infections in wildlife, mainly due to the limited access that researchers have to wild populations. Therefore, generating and analyzing time series on shared infections in wildlife and at their interface with livestock is a priority in order to achieve a better understanding, control, and eradication of these infections. In this context, through this Thesis, we intended to identify the main individual, populational, environmental, and stochastic factors that, operating in the medium and long-term, participate in the transmission and persistence of shared infections in the natural environment. For this purpose, a thirteen-year time series (2005-2018) was analyzed, focusing on several pathogen-multi-host systems from Doñana National Park (DNP). This medium-long-term approach is essential to identify and understand the transmission and persistence patterns of pathogens shared between wildlife and livestock and, therefore, for the design and implementation of control and eradication strategies for these diseases.

Firstly, a literature review on long-term epidemiological studies on infectious diseases of wildlife was conducted. The purpose of this chapter was to understand the temporal evolution of these studies and to identify the added value that analyzing large time series provides in order to explain the determining factors and understand their epidemiological and ecological consequences. Furthermore, a definition of “long-term research in wildlife” was proposed, considering that the time scale in which the main determinants of the epidemiology of a given pathogen operates is always context-dependent (Chapter 1).

Then, in Chapter 2, the temporal dynamics of the prevalence or seroprevalence of pathogens of different nature (*Mycobacterium tuberculosis* complex, *Toxoplasma gondii*, bluetongue virus, and hepatitis E virus), and the role of the main risk factors (individual, populational, environmental, and stochastic) in the mixed community of ungulates of the

Doñana National Park, were evidenced. The elevated infection rates found confirmed a constant exposure of the hosts to the pathogens studied during the study period.

The results obtained in this thesis highlight the importance of long-term studies at the wild-domestic interface as a key tool to understand the epidemiology and ecology of pathogens, as well as their response to factors of diverse nature that operate in the medium and long-term. This knowledge is necessary to be able to design effective strategies for risk mitigation.

Introducción

1. Las infecciones compartidas en la interfaz humano-doméstico-silvestre

1.1 Las infecciones compartidas

La capacidad de los patógenos de infectar a múltiples hospedadores facilita su propagación en los ecosistemas (Power y Mitchell, 2004). Esto es un factor de riesgo esencial en la emergencia de numerosas infecciones tanto en humanos como en animales domésticos y silvestres (Cleaveland et al., 2001; Daszak et al., 2000; Taylor et al., 2001).

Las infecciones compartidas son muy complejas desde el punto de vista epidemiológico, particularmente cuando sus agentes patógenos causales afectan a una gran variedad de hospedadores, entre los que se encuentran animales domésticos, fauna silvestre, e incluso el ser humano en el caso de tratarse de infecciones zoonóticas (Gortázar et al., 2015, 2016). Este tipo de infecciones han adquirido mucha relevancia, especialmente en los últimos años, ya que implican graves consecuencias para la salud pública, la economía global, la gestión de la fauna silvestre y del medio ambiente, así como para la conservación de la biodiversidad (Cunningham et al., 2017; Daszak et al., 2000; Delibes-Mateos et al., 2014; Gortázar et al., 2006). Debido a la dificultad que supone la total erradicación de los agentes infecciosos compartidos y al conflicto que generan entre los diferentes sectores afectados, es necesario un abordaje multidisciplinar del asunto, conocido como perspectiva “Una sola salud” (del inglés “One Health”), actualmente en auge (Bonilla-Aldana et al., 2020; Davis y Sharp, 2020; Trilla, 2020). El concepto “Una sola salud” surgió en la década de los 2000 para referirse a la interconexión existente entre la sanidad animal, tanto de especies domésticas como silvestres, la del ser humano, y la vinculación de ambas con los ecosistemas en los que coexisten (Zinsstag et al., 2011). Estos tres elementos son dinámicos y se encuentran conectados formando una red compleja y estable en la que los diferentes agentes patógenos se establecen y persisten, incluso a nivel global. El enfoque “One Health”, mediante un planteamiento colaborativo global, persigue el hallazgo de métodos de evaluación del riesgo y de soluciones óptimas para preservar la salud pública frente a nuevos eventos epidemiológicos, teniendo en cuenta la interacción entre todos los elementos previamente mencionados (Dhama et al., 2013).

Por tanto, la fauna silvestre es parte del sistema y puede suponer una amenaza para la salud pública y la del ganado, mediante la transmisión de agentes patógenos compartidos

(Decker et al., 2010; Lubroth, 2013). En este sentido, numerosas especies silvestres han sido identificadas como los principales reservorios de enfermedades zoonósicas o del ganado, como la enfermedad de Aujeszky (Lari et al., 2006), la peste porcina africana (Sánchez-Vizcaíno et al., 2012), la hepatitis E (Thiry et al., 2017b), la lengua azul (Ruiz-Fons et al., 2014) o la tuberculosis bovina (Naranjo et al., 2008), entre otras. El término "reservorio" se refiere a una o más poblaciones o ambientes epidemiológicamente conectados entre sí en los que un patógeno puede ser mantenido de forma permanente, sin ninguna fuente de reinfección externa, así como ser transmitido a otra población determinada (Haydon et al., 2002). Este tipo de infecciones, en muchos casos, fueron previamente transmitidas a la fauna silvestre desde un reservorio doméstico ("spill-over"), modificándose posteriormente la direccionalidad de esta transmisión ("spill-back"), y despertando, por tanto, el interés sobre el papel que ejerce la fauna silvestre en la epidemiología de estas (Bengis et al., 2002; Gortázar et al., 2010).

En las últimas décadas, las poblaciones humanas han aumentado notablemente. Su constante demanda de proteína y otras comodidades han dado lugar a una serie de cambios de carácter antropogénico en el medio natural (Gortázar et al., 2015) que han facilitado la emergencia y/o reemergencia de numerosas infecciones compartidas como la peste porcina africana (O'Neill et al., 2020) o la fiebre hemorrágica de Crimea-Congo (Sorvillo et al., 2020). Concretamente, los cambios en los usos del suelo, en las prácticas agrícolas y ganaderas, unido a una gestión de la fauna silvestre cada vez más intensiva (vallados, alimentación suplementaria, etc.), han dado lugar a la expansión y al incesable crecimiento de las poblaciones de fauna silvestre, especialmente ungulados, en los países más desarrollados (Massei et al., 2015; Valente et al., 2020). Esta situación ha creado un ambiente favorable para la existencia de contactos cada vez más estrechos entre el ganado, la fauna silvestre y el ser humano (Bengis et al., 2004; Jones et al., 2013; Perry et al., 2013), dando lugar al concepto de "interfaz".

El término "interfaz doméstico-silvestre-humano" (Figura 1) engloba la red dinámica de conexiones existente entre la fauna silvestre, el ganado doméstico, el ser humano y el entorno que les rodea (Brahmbhatt et al., 2012; Jones et al., 2013). Esta red de conexiones está formada por los contactos o interacciones (directas o indirectas) que ocurren entre todos los elementos que constituyen la interfaz, y que no sólo facilitan la transmisión de patógenos de forma multidireccional, sino que también generan otro tipo de conflictos en numerosos sectores más allá del sanitario (Gortázar et al., 2010; Miller y Olea-Popelka, 2013). Por ejemplo, las confrontaciones surgidas por la presencia de lobo ibérico (*Canis lupus*) y oso pardo (*Ursus arctos*) en ciertos territorios de la Península Ibérica, donde convive con ganado doméstico, pueden tener graves consecuencias para la conservación de estas especies (Fernández-Gil et al., 2016). Los contactos directos ocurren cuando coinciden varias especies en un mismo territorio, produciéndose un contacto estrecho entre los individuos (Triguero-Ocaña et al., 2020). Por el contrario, los contactos indirectos se producen generalmente a través del uso compartido de algunos recursos del medio que podrían encontrarse contaminados con un determinado agente patógeno, como los puntos de agua o alimentación (Barasona et al., 2014a; Kukielka et al., 2013). Además, la presencia de vectores u hospedadores intermediarios también son considerados elementos clave para la existencia de contactos indirectos, siendo englobados como parte de la interfaz (Kock, 2005).

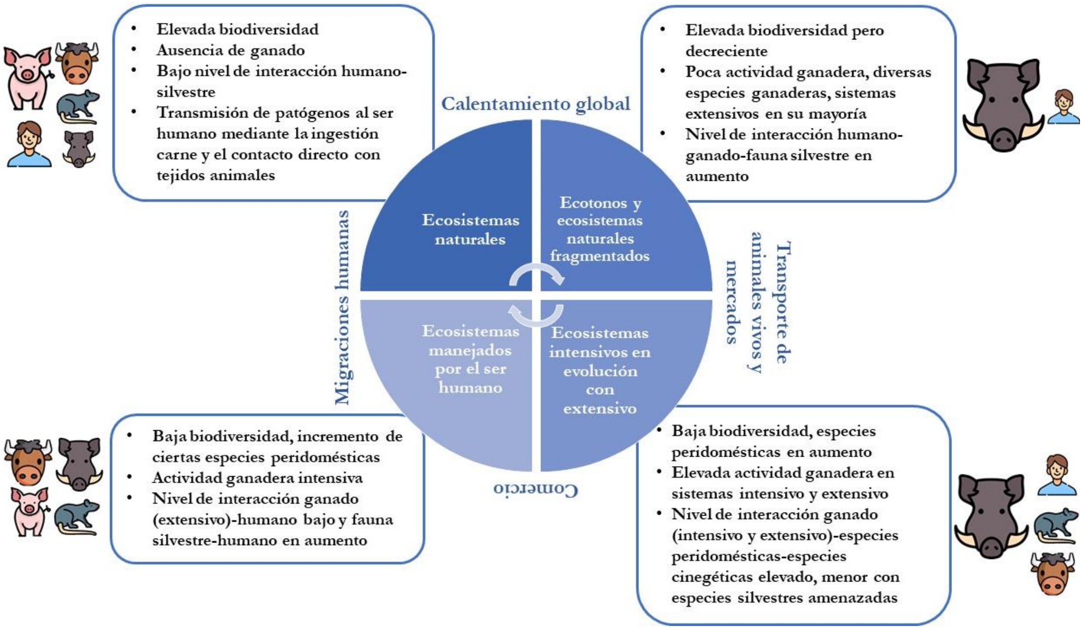


Figura 1. Características de la interfaz doméstico-silvestre-humano desde ambientes naturales hasta paisajes muy modificados por el ser humano, evaluando su nivel de biodiversidad, actividad ganadera y conectividad entre comunidades. Para cada tipo de interfaz se representa con un icono de distinto tamaño la abundancia relativa de las especies que componen la comunidad de hospedadores. Alrededor del círculo se mencionan factores determinantes asociados con el riesgo de expansión de patógenos, tanto a nivel local como global (basado en VerCauteren et al. 2021).

1.2 Tipos de hospedadores y mecanismos de transmisión de patógenos

Las interfaces conforman complejos sistemas ecológicos en los que participan varias especies que actúan como hospedadores de uno o varios patógenos (Caron et al., 2012). Por tanto, uno de los principales retos en el estudio de las infecciones compartidas en la interfaz es determinar el papel epidemiológico de cada una de las especies implicadas en su transmisión (Haydon et al., 2002). Además, la caracterización y gestión de los hospedadores, tanto conocidos como potenciales, es un elemento clave para el control efectivo de estas infecciones, ya que no todas las especies poseen la misma implicación epidemiológica.

El concepto de “reservorio” ha sido definido en el apartado anterior.

En cambio, un “hospedador accidental” es aquel capaz de mantener la infección únicamente en presencia de una fuente de reinfección externa, que suele ser otro hospedador o el medio ambiente (Martin et al., 2011). Estos hospedadores pueden llegar a ser esenciales para la epidemiología del patógeno. También, pueden convertirse en verdaderos reservorios si el patógeno es capaz de adaptarse y circular activamente en la población (Rodriguez-Prieto, 2014).

El “hospedador fondo de saco epidemiológico” es aquel capaz de infectarse, pero incapaz de transmitir la infección, finalizando por tanto el ciclo epidemiológico en ellos (Konjević et al., 2017; Vicente-Santos et al., 2017).

Por último, un hospedador “portador asintomático” es aquel que se infecta y excreta el patógeno, pero no desarrolla sintomatología clínica (Hernandez et al., 2016).

En ocasiones, resulta muy complicado clasificar a las especies implicadas en la epidemiología de un determinado patógeno en las categorías de hospedadores existentes. De hecho, Martin et al. (2011), tras una revisión bibliográfica sobre infecciones de la fauna silvestre, hallaron que únicamente el 34,2% de los artículos revisados determinaron el papel epidemiológico ejercido por las especies estudiadas para el ciclo del patógeno. Además, el tipo de hospedador y, por tanto, el rol epidemiológico de las especies no es algo estático. Son muchos los factores que condicionan que una misma especie actúe como reservorio u hospedador accidental, o que pueda cambiar su estatus de hospedador, tales como el hábitat, los mecanismos de transmisión de patógenos y las interacciones de dicha especie con los distintos elementos del ecosistema (MAPA, 2017). La determinacion del tipo de hospedador también depende del sistema epidemiológico en el que se engloba, incluso para la misma especie y patógeno, como por ejemplo, el caso del jabalí (*Sus scrofa*) y el tejón (*Meles meles*) para el Complejo *Mycobacterium tuberculosis* (CMT; Gortázar et al., 2012, 2016).

Respecto a los mecanismos de transmisión de patógenos, conocer su direccionalidad, es decir, “quién infecta a quién”, es un elemento clave para la predicción y el control de la emergencia de determinados patógenos en las diferentes comunidades de hospedadores (Cabaret et al., 2012). Esta transmisión puede ser intra o interespecífica, lo que condicionará el rol de hospedador que ejercerá una especie en la circulación del patógeno (Caley y Hone, 2004). El proceso por el que un patógeno es transmitido de forma interespecífica de un reservorio a un hospedador accidental se denomina salto de especie o “spill-over”, y suele ocurrir cuando éste alcanza tasas elevadas de prevalencia en el primero, como pudo ocurrir con la tuberculosis bovina (Palmer et al., 2012) o la sarna sarcóptica (Smith et al., 2009). Dentro de los mecanismos de transmisión de patógenos, otro punto clave para alcanzar una mayor comprensión sobre la epidemiología de los patógenos en sistemas multihospedador son las vías de transmisión (Jones et al 2011). Éstas determinan la circulación de patógenos y su capacidad de persistencia en los ecosistemas, y a su vez dependen de numerosos factores intrínsecos y extrínsecos que condicionan la exposición y susceptibilidad de los hospedadores a los patógenos (Corner, 2006; Nugent, 2011). Los factores intrínsecos son aquellos que determinan la capacidad del patógeno de persistir en las especies hospedadoras. Entre ellos destacan la virulencia, especificidad, capacidad de replicación, grado de supervivencia ambiental del patógeno, así como los factores genéticos y estado inmunitario del hospedador. Los factores extrínsecos, por su parte, son aquellos que determinan la frecuencia, intensidad y dirección de las interacciones espaciotemporales entre patógenos y hospedadores (Cabaret et al., 2012), haciendo que el contacto entre dos especies sea o no efectivo para la transmisión del patógeno. Dentro de este grupo se encuentran los factores poblacionales, principalmente densidades de hospedadores y vectores, factores climáticos, ecológicos, de manejo y etológicos.

1.3 Monitorización y gestión integral en la interfaz doméstico-silvestre

Para llevar a cabo un control adecuado de las infecciones compartidas debe considerarse la interfaz humano-doméstico-silvestre en su conjunto. Esta aproximación es la mejor forma de optimizar los recursos disponibles y alcanzar un buen estatus zoosanitario (Gortázar et al., 2015). De forma ideal, los programas de lucha contra las infecciones compartidas tendrían que estar compuestos por una parte enfocada a la monitorización integrada (MI), y otra parte práctica formada por una propuesta de medidas para la mitigación del riesgo. La MI, a su vez, debe constar de dos partes fundamentales e igualmente importantes, el "numerador" (obtenido mediante vigilancia sanitaria; p. ej. número de infectados en una población) y el "denominador" (obtenido mediante la monitorización poblacional; p. ej. densidad de la población estudiada). El problema de la mayoría de los sistemas de monitorización en fauna silvestre radica en que la información disponible aborda sólo uno de estos componentes (Stallknecht, 2007).

La información sobre el "numerador" se obtiene mediante vigilancia sanitaria, que se define como la recogida, constatación y análisis sistemático de información relacionada con la sanidad animal y su uso para la toma de decisiones e implementación de medidas de control efectivas (OIE, 2019).

Por otra parte, la información sobre el "denominador" profundiza en el conocimiento de las poblaciones, reuniendo información sobre tamaños poblacionales, su estructura, dinámica, distribución espacial y gestión, para llevar a cabo un análisis del riesgo de introducción o propagación de patógenos (Stallknecht, 2007). La monitorización poblacional no se considera habitualmente a la hora de llevar a cabo sistemas de monitorización en fauna silvestre a nivel mundial, hecho que Stallknecht et al. (2007) designaron como "el denominador perdido". Esto se debe principalmente a las dificultades que existen a la hora de realizar estimas poblacionales de estas especies, que finalmente suelen representarse como un índice en lugar de una estimación real (Witmer, 2005). Estos índices sirven para determinar tendencias poblacionales, pero no proporcionan información fiable sobre valores de densidad poblacional que permitan calcular tasas de incidencia, mortalidad o morbilidad, así como interpretar estos datos o compararlos entre sí.

La ausencia de datos de MI de fauna silvestre fiables y comparables entre sí es un gran impedimento a la hora de interpretar la información obtenida (Vicente et al., 2019b). Por tanto, es importante diseñar un sistema de MI armonizado para todos los países, o al menos continentes, de forma que estos datos sean comparables entre sí (Vicente et al., 2019a, 2019b). En este sentido, la recopilación de esta información a "largo plazo" juega un papel muy importante en el control de las infecciones compartidas, ya que proporciona la información necesaria para alcanzar una mayor comprensión sobre la epidemiología de los patógenos (McDonald et al., 2017). De hecho, se ha demostrado que aquellos países que, de forma habitual, llevan a cabo una MI en la interfaz doméstico-silvestre tienen una mayor capacidad para detectar y reaccionar ante la circulación de patógenos compartidos (Mörner et al., 2002). Aunque para enfermedades del ganado y del ser humano exista una amplia disponibilidad de información recogida lo largo del tiempo, para la fauna silvestre ésta es muy escasa, existiendo cinco series temporales de información sobre MI que destacan en la literatura, cuatro de ellas sobre el CMT (Delahay et al., 2013; McDonald et al., 2017; Nugent et al., 2012; Vicente et al., 2013), y una sobre fiebre hemorrágica (Christensen et al., 2020).

Tras identificar la presencia de un patógeno y caracterizar su distribución en las comunidades de hospedadores, el siguiente paso sería instaurar una serie de medidas de control para reducir su impacto en las poblaciones (Thirgood, 2009). Estas medidas se agrupan en cuatro categorías según Gortázar et al. (2015), las cuales deberían combinarse para conformar una estrategia de control integrado adecuada (Figura 2).

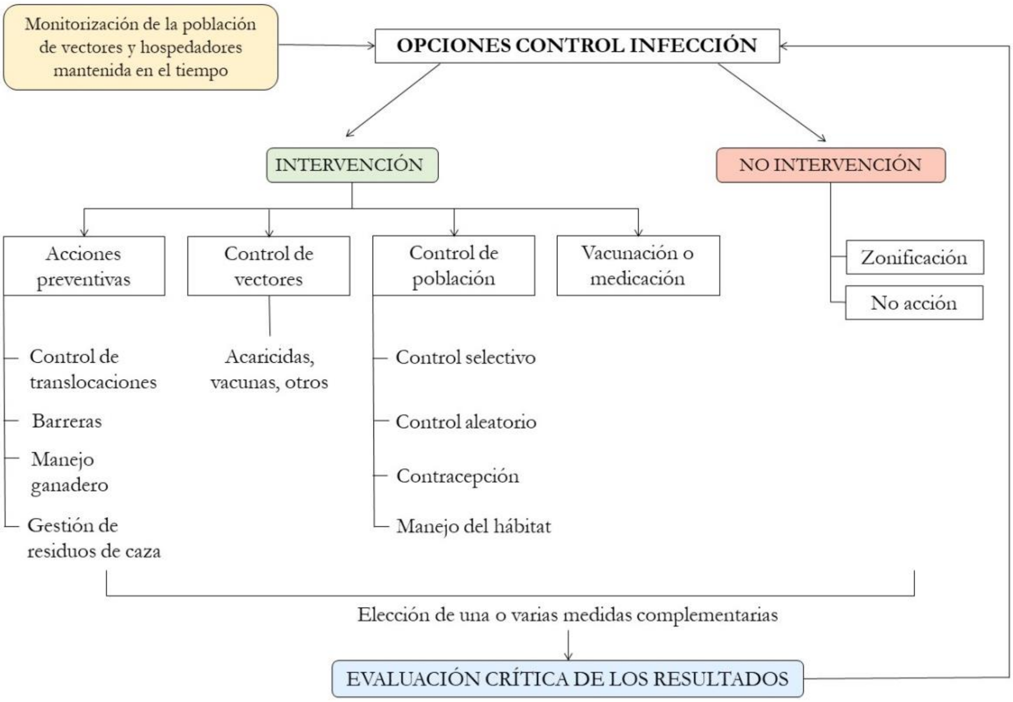


Figura 2. Opciones disponibles para el control de las infecciones compartidas (adaptación de Gortázar et al., 2015).

En los casos en los que no exista una justificación para instaurar una medida, o que ésta resulte inviable o no sea rentable económicamente (Wobeser, 2007), existe la posibilidad de no llevar a cabo ninguna intervención, aplicando la zonificación o la compartimentación, que consisten en definir subpoblaciones a las cuales se les atribuye diferentes estatus sanitarios frente a una infección con el fin de controlarla (Bienen y Tabor, 2006; O'Brien et al., 2011).

La viabilidad y aplicabilidad de todas estas medidas están condicionadas por el ser humano y por aquellos sectores que pueden verse afectados por la presencia de infecciones compartidas. Para determinar qué enfoques son exitosos en el manejo de la vida silvestre desde el punto de vista ecológico, económico y social, es necesario un buen sistema de MI (Vicente et al., 2019a). Las medidas de control instauradas dependerán de las circunstancias y la legislación local, pero siempre deben estar fundamentadas en una base científica, tratando de ser implementadas a escala continental cuando sea posible (Vicente et al., 2019a).

2. Tipos de estudios epidemiológicos: estudios a largo plazo sobre infecciones compartidas

2.1 Estudios epidemiológicos y su clasificación

La epidemiología es una disciplina científica que analiza la distribución y frecuencia de los patógenos en las poblaciones de hospedadores y establece cuáles son sus principales factores determinantes (Pfeiffer, 2010). Los estudios epidemiológicos son empleados para determinar cuáles son las causas de los patrones de una infección en la población mediante una evaluación de la relación entre la presencia de un determinado patógeno y sus posibles factores de riesgo. También se utilizan para precisar cuáles son los factores protectores que permiten prevenir o erradicar una infección determinada (Thrusfield et al., 2018). Los estudios epidemiológicos se clasifican según varios criterios que se citan a continuación (Pradas, 2019; Figura 3).

i) Según la finalidad del estudio

- Estudios descriptivos: son aquellos que se utilizan como un paso previo a la formulación de hipótesis etiológicas. Dentro de este grupo se encuentran:

  - Series de casos: estos estudios utilizan información a nivel individual, describiendo la historia clínica de individuos que presentan un efecto similar. P. ej., la descripción de un brote epidémico o una intoxicación masiva.
  - Estudios transversales: descritos en apartado de “clasificación según la direccionalidad del estudio”.
  - Estudios ecológicos: estos no utilizan información a nivel de individuo, sino datos agregados de toda la población. P. ej., el análisis de la correlación existente entre el consumo de alcohol y la tasa de mortalidad por enfermedades cardiovasculares en varios países.
- Estudios analíticos: son aquellos que evalúan las relaciones causa-efecto entre los factores de riesgo y la presencia de patógenos, es decir, investigan hipótesis etiológicas. En este caso, se dispone de dos grupos de individuos y un factor de riesgo objeto de estudio. Según el grado de control del que disponga el epidemiólogo sobre la exposición de cada uno de estos grupos a dicho factor, los estudios analíticos se clasifican en:
  - Estudios observacionales: en ellos el epidemiólogo no controla la asignación de la exposición al factor de riesgo, por lo que únicamente se lleva a cabo una observación de la relación existente entre la presencia de un patógeno y la exposición a dicho factor. Por tanto, son muy habituales en poblaciones silvestres debido a la dificultad que conlleva su observación, seguimiento y muestreo (Morrinson et al., 2008). Dentro de este grupo se encuentran los estudios de cohortes y los estudios de caso-control. Ambos se detallarán en el apartado dedicado a los estudios longitudinales.
  - Estudios experimentales: en estos estudios el epidemiólogo pauta y controla la exposición al factor de riesgo. Dentro de este grupo se encuentran:
    - Ensayos clínicos: se llevan a cabo sobre individuos enfermos con fines terapéuticos, es decir, para evaluar la eficacia de uno o más tratamientos. P. ej., la comparación de la eficacia de dos tratamientos antiparasitarios en ganado bovino.
    - Pruebas de campo y estudios de intervención comunitaria: se desarrollan sobre individuos sanos con fines preventivos. En las pruebas de campo la intervención se aplica a nivel individual, mientras que en el estudio de intervención comunitaria ésta se hace a nivel poblacional o comunitario. La evaluación de un suplemento

vitamínico en una población sana para prevenir ciertas enfermedades sería un buen ejemplo de pruebas de campo, mientras que el estudio de la efectividad de un programa de control o prevención de una enfermedad en una comunidad sería un ejemplo de estudio de intervención comunitaria.

ii) Según la direccionalidad del estudio (Kramer y Boivin, 1988)

- Estudios longitudinales: en ellos se realiza un seguimiento de los individuos durante un periodo de tiempo determinado. La exposición al factor de riesgo y su efecto se evalúan en momentos diferentes del periodo de estudio, permitiendo establecer el orden en el que se producen los acontecimientos, lo que es fundamental para determinar una relación causa-efecto. Según la magnitud temporal a la cual los efectos estudiados operan y el periodo de seguimiento de los individuos, estos estudios pueden considerarse “a corto plazo” o “a largo plazo”. A su vez, estos estudios pueden ser:
  - Estudios de cohortes: una cohorte es un conjunto de individuos con una o varias características en común. En este tipo de estudios, la agrupación de individuos generalmente se realiza según la exposición al factor de riesgo estudiado. Posteriormente, se evalúa la aparición de un determinado efecto en ambos grupos. Por tanto, los estudios de cohortes parten de la exposición para evaluar el efecto. P. ej., para el estudio de la asociación entre la aparición de una patología y el consumo de una sustancia determinada, se realiza una comparación de la incidencia de dicha patología en la cohorte expuesta a la sustancia con la incidencia en la cohorte no expuesta.
  - Estudios de caso-control: en este tipo de estudios, la agrupación de individuos se realiza según el desarrollo de un determinado efecto tras la exposición a un factor de riesgo. En este caso, tanto los individuos que no desarrollan el efecto (“controles”) como los que sí lo desarrollan (“casos”) son una muestra aleatoria de la población. En ambos grupos se evalúa la presencia de exposición al factor de riesgo. Por tanto, los estudios de caso-control parten del efecto para evaluar la exposición. Para el ejemplo anterior, en este caso los “controles” serían los individuos que no desarrollaron la patología, mientras que los individuos que la sufrieron son considerados “casos”. A partir de estos datos, se estudiaría si los individuos pertenecientes a ambos grupos estuvieron expuestos a la sustancia y el grado de exposición.

- Estudios transversales: en ellos no existe un periodo de seguimiento, de forma que la exposición al factor de riesgo y el efecto se miden simultáneamente en un momento concreto del estudio. Mediante este tipo de estudios no se pretende establecer una relación de causa-efecto. P. ej., determinar la incidencia de tuberculosis en ganado bovino en un año concreto.

iii) Según la relación temporal

- Estudios prospectivos: son aquellos en los que el efecto aparece una vez iniciado el estudio. P. ej., un estudio sobre la asociación de la calidad de la dieta durante los tres primeros años de vida con el grado de desarrollo cognitivo alcanzado a los diez años.
- Estudios retrospectivos: son aquellos en los que tanto la exposición al factor de riesgo como el efecto ya han sucedido cuando se inicia el estudio. Los estudios retrospectivos suelen llevarse a cabo a partir de registros existentes. P. ej., evaluar si los trabajadores expuestos a radiaciones ionizantes a lo largo de su vida presentan tasas superiores de mortalidad por cáncer que la población de referencia.

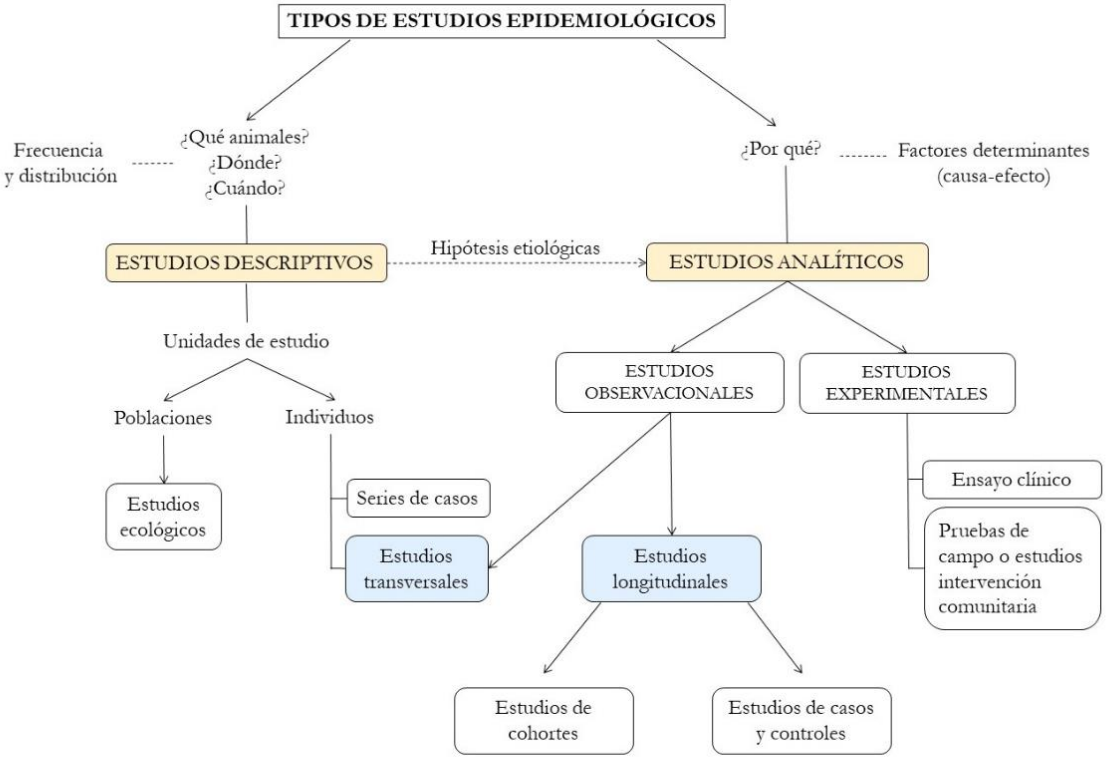


Figura 3. Clasificación de los estudios según la finalidad.

Un aspecto importante es no confundir la clasificación según la direccionalidad del estudio y según la relación temporal. De este modo, un estudio de cohortes puede ser prospectivo, cuando se realiza un seguimiento del individuo, o retrospectivo si se realiza a partir de unos datos ya existentes.

Todos los trabajos englobados en esta Tesis doctoral son estudios epidemiológicos analíticos, observacionales, longitudinales, retrospectivos, a largo plazo y de tipo caso-control, ya que disponemos de información sobre la presencia de lesiones o anticuerpos frente a un determinado patógeno en un conjunto de individuos abatidos durante un periodo de trece años, evaluándose como factores de riesgo potenciales aquellos de tipo individual, ambiental, poblacional y estocástico.

2.2 Los estudios a largo plazo y su aplicación al campo de las infecciones compartidas

Los estudios a largo plazo son estudios epidemiológicos longitudinales basados en la replicación del mismo experimento o, al menos, de experimentos con protocolos comparables, durante varios periodos consecutivos (Gordo y Avilés, 2017). De este modo, proporcionan un número suficiente de réplicas, englobando todas las etapas que forman parte de los diferentes procesos ecológicos y epidemiológicos, con el fin de alcanzar conclusiones más fiables. Para establecer el periodo de estudio y número de réplicas debe considerarse la duración de los ciclos de vida de los hospedadores y patógenos, además de la situación epidemiológica del patógeno en la población o comunidad estudiada. Por tanto, su definición es caso-dependiente. P. ej., un estudio de varios meses podría considerarse “a largo plazo” si se trabaja con *Culicoides* y transmisión del virus de la lengua azul (VLA) al ganado ovino, ya que en ese periodo podrían obtenerse numerosas generaciones y eventos de transmisión. En cambio, para estudiar el efecto del cambio climático sobre la distribución de un patógeno se necesitarían décadas, concretamente un mínimo de veinte años según Sparks y Menzel (2002). Por tanto, la perspectiva temporal empleada no es el único elemento que debemos tener en cuenta para considerar un estudio “a largo plazo”, ya que este término es relativo y dependiente del contexto, variando según el patógeno y las poblaciones estudiadas.

El enfoque “a largo plazo” del estudio de los procesos epidemiológicos y ecológicos proporciona una información más completa que la obtenida mediante valores de prevalencia u otras medidas puntuales de la transmisión de la infección (Christensen et al., 2020; McDonald et al., 2017; Vicente et al., 2013). Mediante una perspectiva temporal amplia, además, podemos conocer cuáles son y cómo operan los principales determinantes de la dinámica temporal de las infecciones y los factores implicados en la susceptibilidad o exposición a los diferentes patógenos (Delahay et al., 2013; Vicente et al., 2013). También, permite dilucidar cómo estos factores dan lugar al establecimiento de los patógenos como endémicos en las comunidades de hospedadores. Por último, este enfoque ayuda a caracterizar los principales escenarios en los que los patógenos podrían emerger y a establecer estrategias de control de riesgo más efectivas (Jones et al., 2008; Rhyan y Spraker, 2010).

2.3 Dificultades y limitaciones de los estudios a largo plazo sobre infecciones compartidas en la interfaz humano-doméstico-silvestre

Generalmente, este tipo de estudios son más costosos económicamente y presentan mayores dificultades que las investigaciones a corto plazo. Estas dificultades están causadas principalmente por aquellos factores que condicionan la continuidad de la recogida de información, como la financiación (Likens, 1989). Por tanto, es cada vez más frecuente la realización de estudios a largo plazo a partir de información retrospectiva, es decir, a partir de bases de datos ya existentes procedentes de programas de seguimiento de áreas o especies protegidas, de la actividad cinegética o pesquera, o de la ciencia ciudadana, actualmente en auge (Den Broeder et al., 2016; Keuling et al., 2013).

La información disponible sobre infecciones compartidas en fauna silvestre es escasa debido a la dificultad para su obtención. Ésta radica en el acceso limitado que tienen los investigadores a las poblaciones silvestres, lo que hace que muchas infecciones pasen inadvertidas o sean detectadas de forma tardía. Esto se debe principalmente al comportamiento elusivo y al carácter migratorio de muchas especies silvestres, lo que dificulta su captura y seguimiento a lo largo del tiempo (Fischer y Gerhold, 2003). Un problema adicional es la dificultad encontrada a la hora de seleccionar las especies de estudio, ya que existe una gran variedad taxonómica de hospedadores silvestres y no siempre se conocen las especies implicadas en la epidemiología de los patógenos (Moutou, 2004). De hecho, el sistema de registro de brotes de la Organización Internacional de Epizootias (OIE) no especifica la especie silvestre implicada, utilizando el término "fauna silvestre" para englobarlas. Por último, a pesar de los esfuerzos de los científicos por obtener muestras de un gran número de animales, la cobertura del muestreo suele ser limitada por razones logísticas o económicas (Müller et al., 1998; Tackmann et al., 1998).

En las últimas décadas se ha avanzado considerablemente en el conocimiento de los factores de riesgo implicados en la transmisión de las infecciones compartidas. También, se ha evidenciado la importancia de los reservorios silvestres en su epidemiología (Gortázar et al., 2012; Naranjo et al., 2008; Simpson, 2002). Como consecuencia, la información disponible en forma de series temporales largas es cada vez más frecuente y abundante, ya que, además, los investigadores apuestan más por esta aproximación. Sin embargo, los estudios a largo plazo sobre infecciones compartidas aún son aún muy escasos (Christensen et al., 2020; McDonald et al., 2017; Vicente et al., 2013), especialmente en la interfaz humano-ganado-fauna y en modelos multipatógeno. Por tanto, generar y analizar series temporales en este sentido es algo prioritario para poder alcanzar una mejor comprensión, control y erradicación de estas infecciones, así como para poder generar estrategias de actuación ante la entrada de otras enfermedades infecciosas (More et al., 2018).

3. Infecciones modelo de estudio

3.1 Complejo *Mycobacterium tuberculosis* como modelo de infección compartida bacteriana zoonótica

La tuberculosis animal (TB) es una enfermedad crónica, causada por bacterias pertenecientes al CMT, principalmente *Mycobacterium bovis* y *Mycobacterium caprae*, que afecta a numerosas especies de mamíferos domésticos y silvestres. Es una enfermedad de declaración obligatoria en España, al tratarse de una zoonosis, y posee gran relevancia para la salud pública, la economía y la conservación de la biodiversidad en todo el mundo (Briones et al., 2000; Delibes-Mateos, 2015; Pérez-Morote et al., 2020). La TB ocasiona graves pérdidas económicas en el sector ganadero, principalmente en ganado bovino, debido a las restricciones al movimiento de animales, a los sacrificios obligatorios y decomisos en matadero, así como a los costes derivados de la implementación de programas de control (Caminiti et al., 2016; Zinsstag et al., 2006). Estas consecuencias económicas también son perceptibles en el sector cinegético, ya que ocasiona una merma en la calidad de los trofeos, tasas de mortalidad en jabalíes adultos del 30% (Barasona et al., 2016) y ha dado lugar a la imposición de unos requisitos muy estrictos en el tratamiento de los subproductos derivados de las actividades cinegéticas (Cano-Terriza et al., 2018a). Por tanto, la TB debe ser abordada de forma multidisciplinar y conjunta por parte de todos los sectores implicados, considerando a todas las especies animales que participan en su epidemiología (MAPA, 2017).

Entre las especies domésticas, el ganado bovino es el principal reservorio del CMT, aunque el caprino, ovino y porcino también son susceptibles y poseen implicación epidemiológica en ciertas situaciones y lugares geográficos (Bezos et al., 2015; Cano-Terriza et al., 2018b; Munoz-Mendoza et al., 2016). La fauna silvestre ha sido reconocida como reservorio de la infección en todo el mundo, variando su relevancia según la región (Corner, 2006; Gormley y Corner, 2013; Gortázar et al., 2012).

En España, el jabalí actúa como principal reservorio del CMT en ambientes mediterráneos, pudiendo incluso mantener la circulación de la infección en ausencia de otros hospedadores (Naranjo et al., 2008). Igualmente, el ciervo (*Cervus elaphus*) y el gamo (*Dama dama*) participan en la epidemiología del CMT como reservorios, con una mayor implicación en el cuadrante suroeste (Garcia-Jimenez et al., 2013; Parra et al., 2006; Vicente et al., 2006). Por último, el tejón también ha sido identificado como hospedador relevante del CMT en el norte de España (Acevedo et al., 2019; Balseiro et al., 2011).

Los animales infectados son los responsables de la transmisión de la enfermedad mediante la excreción de las micobacterias. La transmisión de CMT puede producirse de forma directa o indirecta. La transmisión indirecta ocurre principalmente mediante la ingestión de vegetación, agua o barro contaminados (Caron et al., 2003; Morris et al., 1994) y suele producirse en torno a puntos de agua y lugares de alimentación, especialmente durante la estación seca, en la que se da una mayor agregación en estas zonas (Barasona et al., 2014a; Vicente et al., 2013). Por otra parte, la transmisión directa mediante el consumo de carroña o despojos de la caza o vía oro-nasal, puede ser frecuente en jabalí debido a su comportamiento (Ruiz-Fons et al., 2008b; Wilson y Wolkovich, 2011).

España se puede dividir en varias regiones biogeográficas de acuerdo con su situación epidemiológica respecto a la TB (MAPA, 2017; Figura 4). En el desarrollo de esta Tesis nos centraremos en el cuadrante suroeste de España, donde predominan los ecosistemas de bosque mediterráneo y dehesa, y donde se han reportado tasas de prevalencia de TB que se sitúan entre las más elevadas de las registradas a nivel mundial (Gortázar et al., 2008; Vicente et al., 2013), concretamente, como veremos más adelante, en el Parque Nacional de Doñana (PND). En esta zona de España existe una interfaz doméstico-silvestre de ungulados debido a la confluencia de la cría de ganado en extensivo, sobre todo bovino y porcino, con la presencia de elevadas densidades de ungulados silvestres. Según Barasona et al. (2019), las comunidades de hospedadores más complejas poseen una mayor competencia, definida como la capacidad de la comunidad de mantener y transmitir la infección por TB. Además, la gran diversidad de animales que habitan en esta biorregión comparten pastos y puntos de agua, de escasa disponibilidad en ciertas épocas del año (verano y otoño), convirtiéndose en puntos de transmisión de numerosas infecciones compartidas, entre las que destaca la TB (Vicente et al., 2007b, 2013).

Figura 4. Mapa de España en el que pueden diferenciarse las cuatro regiones en las que se divide según su situación sanitaria respecto a la TB. De menor a mayor intensidad de color: (a) Regiones insulares; (b) Costa norte (con mayor abundancia de tejón); (c) Provincias de clima mediterráneo o continental que detectan poca TB en fauna silvestre; y (d) Provincias de clima mediterráneo con mayor riesgo de TB en fauna silvestre y altas densidades de ciervo y jabalí (obtenida de MAPA, 2017).

Los estudios a largo plazo sobre la epidemiología de la TB en fauna silvestre y su interfaz con el ganado en España son bastante escasos. La serie temporal más larga analizada hasta la fecha tiene una duración de doce años y procede del suroeste del país, donde se determinaron la tendencia temporal y los principales factores de riesgo en una comunidad de hospedadores silvestres (ciervo y jabalí) que coexistían con el ganado bovino. Se inició a partir de información sobre la presencia de lesiones compatibles con TB (Vicente et al., 2013). Otros estudios más recientes desarrollados en el norte incluyeron, además de prevalencias en fauna silvestre, información sanitaria sobre ganado bovino, abarcando periodos de estudio de hasta nueve años, como los estudios de Varela-

Castro et al. (2021) y (2020) en el País Vasco, y de seis años en el caso de Acevedo et al. (2019) en Asturias. Finalmente, una serie temporal comenzada en 2005 sobre TB (lesiones compatibles; LCT) en la comunidad mixta de hospedadores del Parque Nacional de Doñana, ha dado lugar a estudios sobre aspectos sanitarios y de ecología espacial, cuyas líneas de investigación continúan en la actualidad. La realización de un mayor número de estudios a largo plazo sobre la epidemiología de la TB permitiría revelar cómo operan y cuáles son las consecuencias de los factores que actúan a medio y largo plazo sobre la infección, y cómo éstos se interrelacionan entre sí. Así proporcionarían información fiable sobre los principales determinantes de la persistencia y difusión de la TB. Actualmente, existen varios aspectos sobre esta infección en fauna silvestre que requieren una mayor atención con el fin de alcanzar un control efectivo e, incluso, su erradicación. Éstos son, principalmente, la mejora de las técnicas diagnósticas disponibles para fauna silvestre, haciéndolas más rápidas y rentables (Thomas et al., 2021); y el diseño de unas pautas de vacunación apropiadas a las características de las comunidades de hospedadores (Schreiner et al., 2020).

3.2 *Toxoplasma gondii* como modelo de infección compartida parasitaria zoonótica

Toxoplasma gondii es un protozoo parásito intracelular obligado de distribución mundial que infecta a una amplia variedad de animales de sangre caliente, incluyendo al ser humano (Dubey, 2010; Dubey y Beattie, 1988; Frenkel et al., 1970). De hecho, un tercio de la población mundial se encuentra infectada por este parásito, convirtiéndose en uno de los más exitosos de aquellos que infectan al ser humano, al ser el patógeno parasitario zoonótico con mayor incidencia a nivel mundial (Montoya y Liesenfeld, 2004; Tenter et al., 2000). Por otra parte, la infección por *T. gondii* implica graves consecuencias para la industria ganadera, especialmente en pequeños rumiantes, ocasionadas por pérdidas en la productividad derivadas de patologías reproductivas como abortos, malformaciones fetales, partos prematuros o mortinatos (Lindsay et al., 1999).

T. gondii presenta un ciclo epidemiológico indirecto en el que los félidos, tanto domésticos como silvestres (gato asilvestrado, gato montés (*Felis silvestris*) y lince ibérico en nuestro país), actúan como hospedadores definitivos (Figura 5). Por otro lado, el resto de animales de sangre caliente, principalmente animales domésticos, aves, roedores y humanos, lo hacen como hospedadores intermediarios (Dubey, 2010; Hill y Dubey, 2014). Los hospedadores definitivos juegan un papel esencial en la transmisión y persistencia de

T. gondii. Estos individuos eliminan ooquistes mediante las heces que, tras un proceso de esporulación que acontece en el medio ambiente a las 24-28 horas de su eliminación, permanecen infectivos durante largos periodos de tiempo en el medio, especialmente en ambientes húmedos y sombríos (Dubey y Beattie, 1988). En cambio, son muy sensibles a la desecación y a las altas temperaturas (Dubey et al., 2009).

La transmisión entre hospedadores puede ocurrir por varias vías (Figura 5), entre las que se encuentran la horizontal, que incluye la oral y la fecal-oral, y la vertical o transplacentaria (Dubey, 2010; Hill y Dubey, 2014). Una de las vías de transmisión más frecuentes entre especies es el consumo de tejidos procedentes de animales infectados, los cuales presentan quistes tisulares formados por bradizoitos de *T. gondii*. En este sentido, aves y roedores son considerados la principal fuente de infección para los félidos mediante esta vía (Black y Boothroyd, 2000). En cuanto al jabalí, sus hábitos carroñeros y la depredación que ejercen sobre pequeñas aves y roedores, sobre todo en épocas de escasez de recursos naturales, pueden dar lugar a una mayor exposición a *T. gondii* (Ballari y Barrios-García, 2014; Carrasco-Garcia et al., 2018). La vía fecal-oral es también una forma de transmisión frecuente, que consiste en la ingestión de agua o alimento contaminado con los ooquistes eliminados a través de las heces por un felino infectado (Dubey, 2010; Hill y Dubey, 2014). Sorprendentemente, mientras que aproximadamente el 1% de los gatos domésticos elimina ooquistes alguna vez en su vida, los gatos asilvestrados podrían eliminar una mayor cantidad de éstos al medio y de forma más continua, por lo que su papel epidemiológico es primordial (Dabritz et al., 2007). Respecto al ser humano, su principal vía de contagio es la ingestión de carne cruda o poco cocinada infectada con este parásito, lo que conlleva importantes implicaciones para la salud pública (Belluco et al., 2018; Guo et al., 2016; Schumacher et al., 2020).

Según la Directiva Europea 2003/99/CE, la vigilancia sanitaria de *T. gondii* se realiza según la situación epidemiológica de cada país, permitiendo decidir a cada estado miembro el tipo de monitorización empleada. La capacidad de decisión nacional, se ha traducido en que actualmente no se dispone de información lo suficientemente representativa sobre la presencia y distribución de este patógeno en Europa, tanto en ganado y sus productos derivados como en el ser humano (EFSA, 2007).

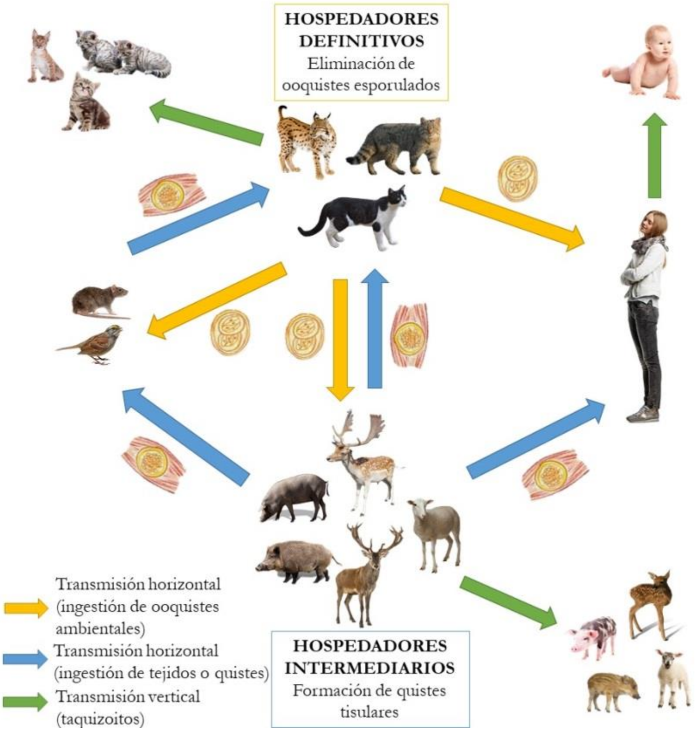


Figura 5. Ciclo epidemiológico y principales vías de transmisión de *Toxoplasma gondii*.

En España existen diferencias geográficas considerables en cuanto a las seroprevalencias de *T. gondii*, tanto en fauna silvestre como en ganado (Calero-Bernal et al., 2016; Castillo-Cuenca et al., 2020; García-Bocanegra et al., 2013; Gauss et al., 2006, 2005; Panadero et al., 2010; San Miguel et al., 2016). Centrándonos en la zona en la que se encuadra el área de estudio de esta Tesis (suroeste de la Península Ibérica), *T. gondii* ha sido detectado en una gran variedad de ungulados y carnívoros silvestres, destacando las elevadas seroprevalencias halladas en ciervo, gamo, jabalí y lince ibérico, situadas entre el 10,5 y el 81,5% (Almería et al., 2018; Gauss et al., 2005; Sobrino et al., 2007). Respecto al ganado, las seroprevalencias se encuentran entre el 16,2% y el 83,3% (Almería et al., 2018; Castillo-Cuenca et al., 2020; García-Bocanegra et al., 2013, 2010c, 2010b). Sin embargo, apenas existen estudios a largo plazo sobre la epidemiología de este parásito en los que se haya evaluado el efecto de sus principales factores de riesgo potenciales y cómo operan a lo largo del tiempo, especialmente en la interfaz doméstico-silvestre. Únicamente existen tres estudios a largo plazo sobre *T. gondii* en España. Los trabajos de Gauss et al. (2005) y (2006) analizaron un total de 507 jabalíes y 441 ciervos durante 12 años (1993-2004). El carácter temporal de estos trabajos permitió a los autores concluir sobre la relevancia de factores tan importantes como la densidad y el manejo poblacional del jabalí para la seroprevalencia de *T. gondii*. Por último, en la literatura encontramos otra serie temporal de 13 años (2007-2019), a partir de la cual se evalúa la seroprevalencia del parásito en una muestra de 393 animales silvestres en cautividad procedentes de zoológicos de toda España (Cano-Terriza et al., 2020). En los estudios anteriores la técnica empleada para el cálculo de la seroprevalencia fue la prueba de aglutinación modificada (MAT, del inglés modified agglutination test).

Aún existen ciertos aspectos de la epidemiología de *T. gondii* que requieren de una investigación más exhaustiva. Es primordial conocer el papel exacto que juegan los hospedadores silvestres en la ecología y epidemiología del parásito, sobre todo en comunidades de hospedadores domésticos y silvestres. Por otra parte, el estudio de las formas infestantes del parásito (oocistos) es esencial, principalmente su nivel de excreción por parte de gatos callejeros y silvestres, los mecanismos que permiten su transporte por los ecosistemas acuáticos y el efecto del cambio climático sobre su supervivencia. Todo ello permitiría realizar estimaciones de las cargas parasitarias ambientales y los riesgos de transmisión a otros hospedadores (Shapiro et al., 2019).

3.3 Virus de la lengua azul como modelo de infección compartida vírica transmitida por vectores

El VLA es un orbivirus de la familia *Reoviridae* que produce la enfermedad de la lengua azul, una enfermedad infecciosa emergente y re-emergente de declaración obligatoria (Rao et al., 2017). Este virus afecta sobre todo a rumiantes domésticos y silvestres, incluyendo camélidos, actuando los silvestres como posibles reservorios (Mertens et al., 2008). De entre todos sus hospedadores, la especie ovina es la que se infecta con mayor frecuencia y se ve más afectada clínicamente, mientras que en el ganado bovino y caprino la infección cursa prácticamente de forma asintomática (MacLachlan, 2004, 1994). Los principales síntomas de la enfermedad son fiebre, descarga nasal, ptialismo, presencia de lesiones orales, edema facial, depresión, anorexia y debilidad muscular (Rao et al., 2017).

La infección se transmite principalmente a través de la picadura de mosquitos del género *Culicoides* (Diptera: Ceratopogonidae), aunque existen otras posibles vías de transmisión entre vertebrados mucho menos frecuentes, como el contacto directo, la transmisión vertical (transplacentaria y por calostro), mediante semen o a través de heridas en la piel (Du Toit, 1944; Rao et al., 2017).

La lengua azul da lugar a importantes pérdidas económicas en las regiones afectadas, principalmente derivadas de restricciones en el movimiento de ganado, pérdidas en la producción, y costes derivados de la implementación de medidas de control y programas de vacunación (Pinior et al., 2018; Rushton y Lyons, 2015).

Actualmente, existen 28 serotipos del virus y otros cuatro serotipos putativos identificados por todo el mundo, entre los que no existe una inmunidad cruzada, complicando la lucha contra la enfermedad (Saminathan et al., 2020; Yang et al., 2020). Este virus se describió por primera vez en Sudáfrica en el año 1902 y se ha ido expandiendo por Europa durante las últimas décadas, probablemente favorecido por factores como el cambio climático (Rodríguez-Sánchez et al., 2008). Aunque se logró su erradicación en muchos de los países afectados, actualmente es endémico en varios países europeos entre los que se encuentra España (Saegerman et al., 2008). Las primeras incursiones del virus en España fueron causadas por los serotipos 10 y 2, en el año 1956 y 1998, respectivamente, siendo erradicados gracias a las medidas implementadas (Mellor et al., 2008). En octubre del 2004, el serotipo 4 fue detectado en una explotación centinela de Cádiz, probablemente introducido desde Marruecos, y fue nuevamente detectado en octubre de 2010, tras haber sido erradicado. En julio de 2007, el serotipo 1 fue introducido desde el norte de África y se expandió rápidamente hacia el norte y el este de España, presentando una mayor virulencia que el serotipo 4. Por último, el serotipo 8 fue detectado por primera vez en el norte de España en enero de 2008 y mostró una expansión geográfica mucho menor que los serotipos anteriores. Aunque este serotipo fue erradicado en enero de 2013, fue detectado de nuevo recientemente en octubre de 2020 (RASVE, 2021).

Las medidas de control llevadas a cabo ante la sucesiva aparición de estos serotipos dieron lugar a la puesta en marcha de un programa nacional de vigilancia, control y erradicación de la lengua azul (MAPA, 2020a). Este programa engloba la vigilancia serológica, virológica, entomológica y pasiva clínica, el control del movimiento de animales susceptibles desde las zonas de restricción, e incluye un programa de vacunación. Además, se ha realizado una zonificación del territorio español, creándose zonas de restricción para cada serotipo (Figura 6). La vacunación del ganado ovino y bovino mayor de 6 meses contra los serotipos 1, 4 y 8 es obligatoria en sus respectivas zonas de restricción. Como medida adicional, se recomienda una vacunación voluntaria frente a los serotipos 4 y 8 en aquellas zonas libres de reciente declaración para los serotipos 1 y 4, así como en la zona fronteriza con Francia, debido a la situación epidemiológica experimentada durante 2018 con 34 focos del serotipo 4 y al movimiento de terneros entre zonas fronterizas.

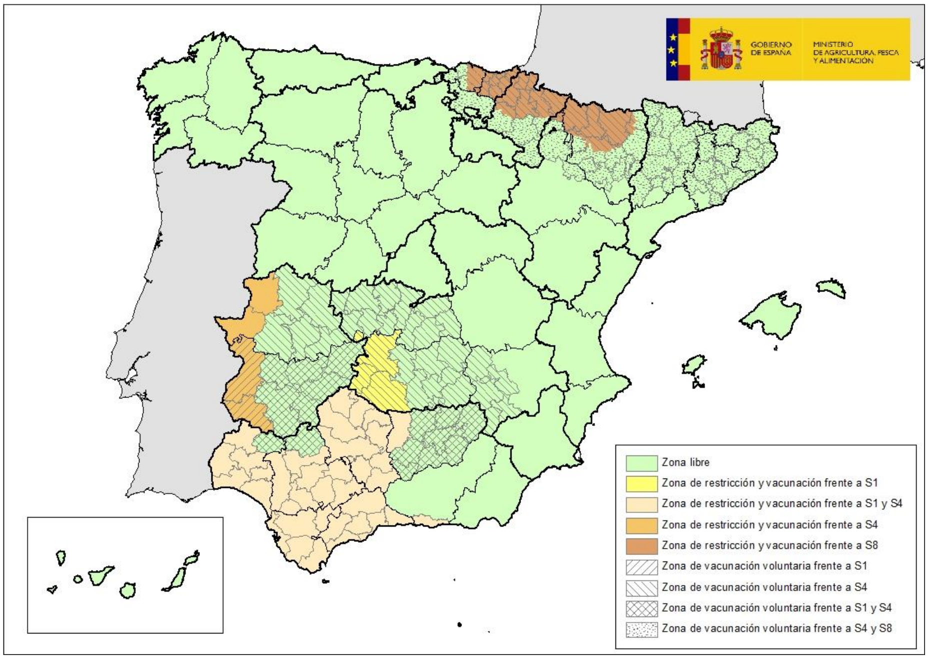


Figura 6. Mapa de la Península Ibérica con las principales zonas de restricción y vacunación contra los serotipos 1, 4 y 8 del VLA en 2021. Fuente: Ministerio de Agricultura, Pesca y Alimentación (MAPAMA, 2021b).

El papel de la fauna silvestre en la epidemiologia del VLA es aún dudoso (Rossi et al., 2019; Ruiz-Fons et al., 2014). Son varios los estudios llevados a cabo en España que han hallado la presencia de anticuerpos contra estos tres serotipos en varias especies de ungulados silvestres (Falconi et al., 2011; García-Bocanegra et al., 2011; Gómez-Guillamón et al., 2020; Rodríguez-Sánchez et al., 2010a; Ruiz-Fons et al., 2008a), algunos de los cuales han mostrado sintomatología clínica y ciertas tasas de mortalidad, como el muflón (*Ovis aries musimon*) o la cabra montés (*Capra pyrenaica hispanica*) (Fernandez-Pacheco et al., 2008; Rodríguez-Sánchez et al., 2010b). De entre todos ellos, el ciervo (66,3%) y el gamo (50,0%) mostraron las seroprevalencias más elevadas.

Aunque es un patógeno del que se dispone de bastante información en la fauna silvestre, son pocos los artículos que abordan su estudio desde una perspectiva temporal amplia, especialmente en zonas en las que se da una interfaz doméstico-silvestre (Gómez-Guillamón et al., 2020; Lorca-Oró et al., 2014; Rossi et al., 2019). Lorca-Oró et al. (2014) crearon y analizaron una serie temporal de seis años (2006-2011) con información serológica y molecular de rumiantes silvestres de toda España, concluyendo que existía una importante circulación y mantenimiento del virus en las poblaciones de ungulados silvestres. Aunque procedente de Francia, merece la pena destacar el estudio a largo plazo desarrollado por Rossi et al. (2019) durante ocho años en quince poblaciones de ciervos que incluyó también el estudio de las comunidades de *Culicoides* de las zonas muestreadas. Este trabajo determinó que el ciervo no participaba de una forma relevante en la transmisión y el mantenimiento del VLA en Francia. Por último, en un estudio de seis años (2010-2015) publicado recientemente, Gómez-Guillamón et al (2020) hallaron una seroprevalencia del VLA en cabra montés de 3,3% en Andalucía, reportando agregaciones espaciotemporales de la exposición al patógeno e identificando regiones y periodos con un riesgo más elevado.

Existen varias cuestiones sin resolver sobre el VLA y su epidemiología, extensibles a otras arbovirosis, que requieren una investigación más profunda. Entre ellas se encuentran los mecanismos de transmisión del virus entre sus hospedadores y las circunstancias en las que éstos son efectivos, la capacidad del virus de persistir en las comunidades de hospedadores mediante el ciclo silvestre en Europa (Ruder et al., 2015; Stallknecht y Howerth, 2004) o el papel que juegan las especies silvestres como reservorios verdaderos del patógeno. Respecto a los vectores, también existen numerosas cuestiones a abordar, especialmente las referidas a las consecuencias del cambio climático sobre sus poblaciones y sobre las dinámicas de infección en las comunidades de hospedadores (Baylis, 2013). En este sentido, los estudios a largo plazo podrían contribuir a ampliar la información disponible sobre estas cuestiones, proporcionando información valiosa para mejorar los programas de control y erradicación de esta enfermedad.

3.4 Virus de la hepatitis E como modelo de infección compartida vírica zoonótica

La hepatitis E es una enfermedad zoonótica emergente no notificable de distribución mundial causada por un virus ARN de cadena simple perteneciente a la familia *Hepeviridae* y al género *Orthohepevirus,* cuya especie tipo es el *Orthohepevirus* A o virus de la hepatitis E (VHE) (Adlhoch et al., 2016; Emerson y Purcell, 2003). Actualmente, el

VHE es un patógeno multihospedador clasificado en ocho genotipos (Kenney y Meng, 2019a). Los genotipos 1 y 2 han sido identificados únicamente en humanos y se han asociado a brotes epidémicos en países en vías en desarrollo de África, Asia y América del Sur (Aggarwal y Goel, 2019). Los genotipos 3 y 4 también son zoonóticos y, aunque afectan principalmente a cerdos domésticos y jabalíes, también pueden infectar a una gran variedad de animales domésticos y silvestres (Kenney y Meng, 2019a). Éstos se han descrito tanto en países en vías de desarrollo como en regiones industrializadas, incluyendo países europeos (Burri et al., 2014; Caballero-Gómez et al., 2019; Doceul et al., 2016; Kamar et al., 2017; Mansuy et al., 2011). Los genotipos 5 y 6, por su parte, afectan principalmente a jabalíes, mientras que los genotipos 7 y 8 se han hallado en camélidos (Takahashi et al., 2011; Woo et al., 2016). Estos últimos genotipos (5-8) se han descrito únicamente en Asia (Tabla 1).

Tabla 1. Principales genotipos de VHE, hospedadores, distribución, vías de transmisión y tipo de presentación. Esta tabla está basada en la información de Kenney y Meng (Kenney y Meng, 2019a).

Genotipo	Hospedador	Distribución	Transmisión	Presentación
1	Humano	África y Asia	Aguas contaminadas	Epidémico y endémico
2		África y México		
3	Humano, cerdo, jabalí, otras especies (ciervos, ratas, ovejas, conejos, caballos, mangostas y macacos)	Europa, EE. UU., Argentina, Asia y Oceanía	Ingesta de alimentos crudos o poco cocinados Infección profesional (Genotipo 3)	Autóctono y esporádico
4		Japón, China, Corea, Indonesia		
5, 6	Jabalíes	Asia	Desconocido	Desconocido
7, 8	Camello, dromedario y humanos			

El VHE es transmitido principalmente por la vía fecal-oral, mediante la ingestión de agua o alimentos contaminados con el virus para genotipos epidémicos (Emerson y Purcell, 2003; Figura 7). Los genotipos zoonóticos, por su parte, también son transmitidos mediante el consumo de carne cruda o poco cocinada, productos derivados del hígado de animales infectados o vegetales y mariscos contaminados con el virus, dando lugar a una hepatitis aguda que afecta a más de 20 millones de personas al año (Adlhoch et al., 2016; Faber et al., 2018; WHO, 2017). Debido al riesgo de transmisión zoonótica, este patógeno es considerado un problema para la salud pública en toda Europa, donde es considerado endémico. Durante los últimos años se ha observado una clara tendencia al alza en los casos de hepatitis E reportados en los países del oeste del continente (Ricci et al., 2017).

El cerdo doméstico y el jabalí son los principales reservorios implicados en la transmisión zoonótica del VHE (Boadella, 2015; De Deus et al., 2008). Sin embargo, la exposición a este patógeno ha sido evidenciada en otras especies como la vaca, el gato, el conejo, algunas especies de camélidos, aves, peces y bivalvos, aunque el papel epidemiológico que ejercen es dudoso (Thiry et al., 2017a). El carácter multihospedador de este virus, unido a su elevada supervivencia ambiental, facilitan su circulación y persistencia en numerosos ambientes, especialmente en aquellos en los que conviven animales domésticos y silvestres susceptibles a la infección (Kukielka et al., 2016; Parashar et al., 2011). Esto implica un riesgo para la salud humana, especialmente en zonas en las que el consumo de carne de caza es frecuente (Rivero-Juarez et al., 2017).

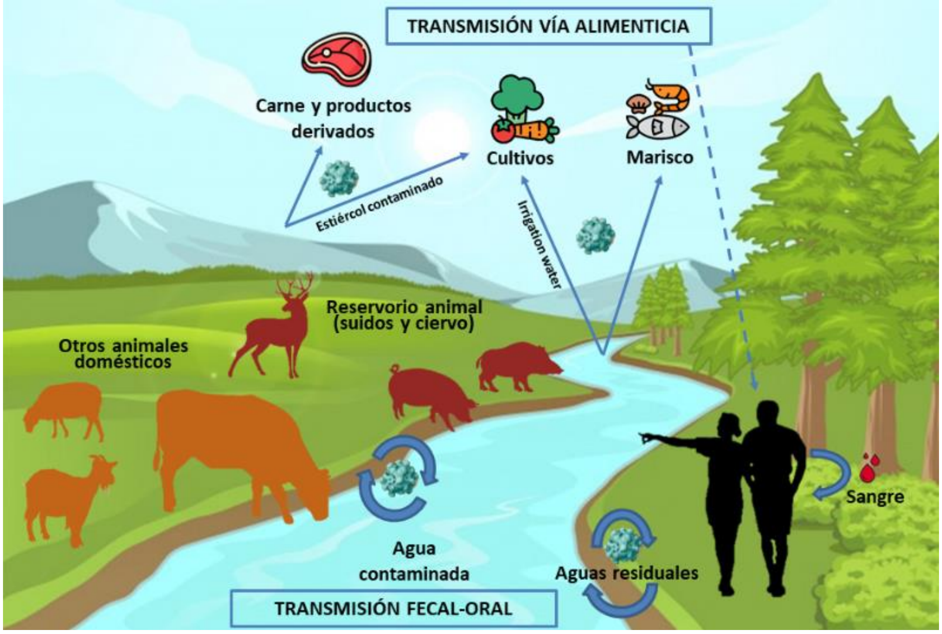


Figura 7. Ciclo epidemiológico del VHE.

Concretamente en el suroeste de España, área en la que se encuadra el área de estudio objeto de esta Tesis doctoral, la circulación del VHE-3 es endémica. De hecho, se ha evidenciado, mediante técnicas serológicas y moleculares, una elevada exposición y tasas de infección en especies domésticas, como cerdo o caballo, y silvestres, como jabalí y ciervo (Boadella et al., 2012a, 2010; De Deus et al., 2008; García-Bocanegra et al., 2019;

Kukielka et al., 2016; López-López et al., 2018). En estas especies se alcanzaron seroprevalencias del 71,0%, 57,6% y 12,9% en cerdos, jabalíes y ciervos, respectivamente, mientras que el resto de las especies estudiadas mostraron tasas inferiores al 10%.

De todos estos estudios, únicamente tres presentaron un enfoque “a largo plazo”, empleando serología y PCR como técnicas diagnósticas. De Deus et al. (2008) y Boadella et al. (2012a) obtuvieron seroprevalencias del 42,7% (2000-2005) y del 26,5% (2000-2011) en jabalíes procedentes del suroeste peninsular y de todo el país, respectivamente. Por último, Kukielka et al. (2016) estudiaron la infección por el VHE en la interfaz doméstico-silvestre en la provincia de Ciudad Real durante ocho años (2003-2010), centrándose en el cerdo (43,8%), el jabalí (57,4%) y el ciervo (12,9%). La duración de estos estudios les permitió concluir sobre aspectos tan importantes como la relación entre factores poblacionales y/o ambientales con las seroprevalencias halladas.

Aún se desconocen muchos aspectos epidemiológicos del VHE, concretamente, muchos de los reservorios animales implicados en la transmisión del virus, sobre todo en ecosistemas de interfaz doméstico-silvestre (Capai et al., 2018; Van der Poel et al., 2018). Todos estos reservorios deberían ser dilucidados con el objetivo de mejorar las actuales medidas de prevención y control de la infección. Otro tema muy importante que requiere de una mayor investigación es la transmisión zoonótica mediada por el consumo de carne procedente de animales infectados y sus productos derivados. Esto engloba la dosis infectante, el tiempo y las condiciones exactas de persistencia del virus en estos productos, la importancia de otros animales diferentes al cerdo doméstico en este tipo de transmisión y la patogénesis de la infección. Por último, es necesario un mayor número de estudios a nivel mundial que permitan evaluar la situación sanitaria global respecto a este patógeno. Los estudios a largo plazo podrían abordar muchos de los aspectos anteriormente citados y proporcionar un conocimiento más profundo sobre los factores epidemiológicos y ecológicos que intervienen en el ciclo del VHE.

3.5 Relevancia de los estudios a largo plazo sobre las infecciones modelo en la literatura internacional

Las cuatro infecciones seleccionadas como modelo de estudio para la presente Tesis doctoral se encuentran incluidas en varios rankings sobre los patógenos compartidos más relevantes para la salud animal, la salud pública y la conservación (Ciliberti et al., 2015; Gortázar et al., 2007, 2016; Wiethoelter et al., 2015). Ciliberti et al. (2015) y Gortázar et al. (2016), asignan a la infección por el CMT los valores de relevancia más elevados, seguida por el VLA, *T. gondii* y, por último, el VHE. Concretamente, Wiethoelter et al. (2015) elaboraron una lista de las diez infecciones más estudiadas en la interfaz doméstico-silvestre, en la que incluyeron la infección por el CMT y por *T.* gondii, como la cuarta y la décima más importantes, respectivamente. Además, el CMT (n=67) y *T. gondii* (n=36) se hallan entre los patógenos más abordados en estudios a largo plazo en la literatura internacional (n=538 artículos), especialmente en Europa. El VLA (n=14), por su parte, es el cuarto virus no zoonótico más estudiado mediante un enfoque "a largo plazo", mientras que el VHE (n=6) apenas se ha estudiado mediante largas series temporales (Figura 8).

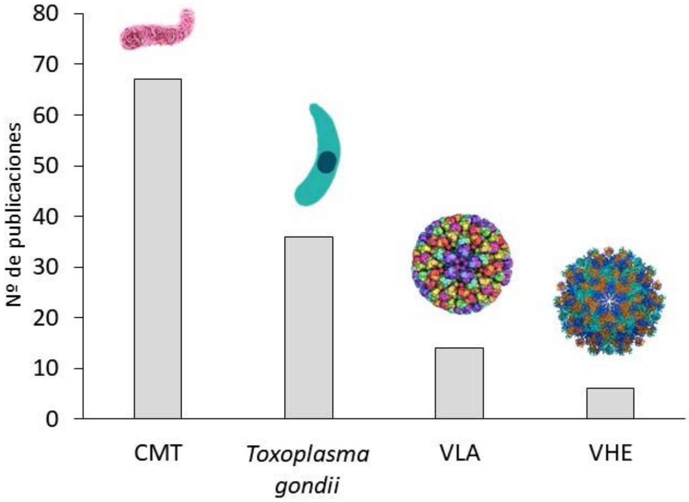


Figura 8. Número de publicaciones a largo plazo halladas en la literatura científica internacional sobre las infecciones modelo de esta Tesis doctoral.

4. El Parque Nacional de Doñana como laboratorio de campo para estudios epidemiológicos a largo plazo

El PND (37°9' N, 6°30' W) se encuentra situado en la costa atlántica, al suroeste de la Península Ibérica, concretamente en las provincias de Huelva, Sevilla y Cádiz. Es una de las reservas ecológicas europeas más importantes y presenta el nivel más elevado de protección ambiental debido a la gran diversidad de biotopos y especies que alberga.

4.1 El origen del Parque Nacional de Doñana, aprovechamientos tradicionales llevados a cabo en su territorio y su actual gestión

La forma de vida de los antiguos habitantes del PND estaba muy ligada al aprovechamiento de los recursos que el territorio ofrecía, como la pesca, la caza, la ganadería y la recolección de materias primas. Los pobladores del parque se alimentaban de los productos recolectados en huertos comunales, miel obtenida mediante apicultura, animales cazados, principalmente liebres, conejos, aves y ungulados, y de huevos durante la primavera (López-Sáez et al., 2018). Los principales aprovechamientos del PND han ido evolucionando a lo largo del tiempo, marcando distintas épocas en su historia (Figura 9).

Figura 9. Principales hitos y épocas de la historia del Parque Nacional de Doñana.

Actualmente, la gestión del parque es llevada a cabo por la Junta de Andalucía y el gobierno de España. El plan rector de uso y gestión considera como aprovechamientos tradicionales permitidos dentro de su territorio el marisqueo de la coquina, la apicultura, la recogida de piña de pino piñonero (*Pinus pinea*) y la ganadería extensiva de ganado bovino y equino, principalmente orientado a la conservación de razas tradicionales como la “marismeña” (Junta de Andalucía, 2004). Aunque el acceso del ser humano está restringido, el uso público también está incluido en este plan rector, con el fin de llevar a cabo un aprovechamiento turístico sostenible de la zona, contando con la carta europea de turismo sostenible. Sin embargo, la caza recreativa, la alimentación suplementaria y la agricultura están prohibidas (Ley 30/2014).

El PND está dividido en cinco áreas de manejo ganadero o fincas de titularidad pública y privada. De norte a sur, estas áreas son: “Coto del Rey”, “Los Sotos”, “Reserva

Biológica de Doñana", "El Puntal" y "Las Marismillas" (Figura 10). El ganado se encuentra distribuido por todo el parque a excepción de Coto del Rey, y es incapaz de atravesar los vallados que dividen las fincas entre sí, mientras que la fauna silvestre se mueve libremente por todo el territorio (Gortázar et al., 2008). En Coto del Rey, aunque actualmente se permite la presencia de un número limitado de caballos, está prohibida la cría de ganado desde el año 2002 para favorecer la conservación del lince ibérico (*Lynx pardinus*), incrementando así la disponibilidad de pastos disponibles para el conejo silvestre (*Oryctolagus cuniculus*), su principal presa. Aunque los linces se alimentan principalmente de conejos (88-100% de su dieta), en ciertas circunstancias pueden predar sobre ungulados silvestres, especialmente crías de gamo (Beltran et al., 1985; Delibes, 1980). También se cría ganado ovino, aunque en menor medida y en una zona muy restringida situada al noroeste del parque.

En cuanto a la gestión de las poblaciones silvestres, anualmente se lleva a cabo un control poblacional de los jabalíes, eliminándose aproximadamente un 10-20% de la población al año, y un plan de monitorización sanitaria de estos animales, que se extiende a los cérvidos. Ambos se incluyen en el programa de vigilancia epidemiológica de fauna silvestre de Andalucía (Junta de Andalucia, 2021) y es llevado a cabo por los agentes medioambientales y celadores forestales. El control poblacional tiene la finalidad de supervisar los posibles efectos negativos que puedan provocar estas especies en ausencia de depredadores y para obtener información sobre su situación sanitaria. Por otra parte, a partir del brote de leucemia felina que ocurrió en el parque en el año 2007 (López et al., 2009), se lleva a cabo un control poblacional de perros y gatos asilvestrados con el objetivo de reducir el riesgo de transmisión de enfermedades así como la interacción con la fauna autóctona, la cual podría resultar dañada (Junta de Andalucía, 2013).

El PND cuenta con una extensión total de 54.252 ha y está compuesto principalmente por tres biotopos: la marisma, las dunas y el monte (Figura 10). La marisma ocupa la parte oriental del parque, mientras que en la parte occidental predominan las dunas al sur y el monte al norte, entre los que pueden observarse numerosos pinares. Enlazando y separando estos biotopos se encuentra una franja longitudinal de ecotono, localmente llamado "vera".

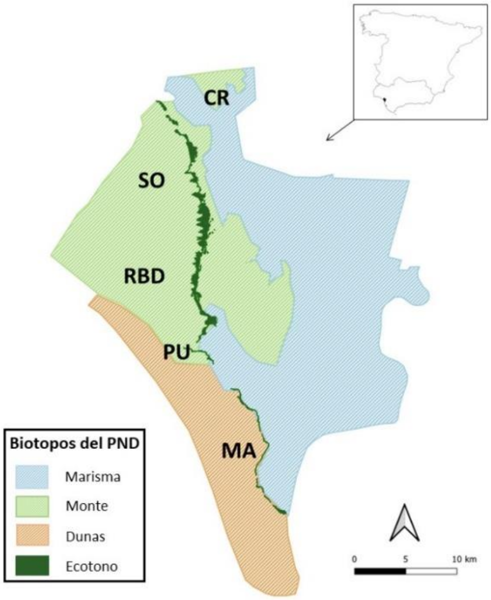


Figura 10. Mapa esquemático del Parque Nacional de Doñana con sus principales biotopos y áreas de manejo ganadero (de norte a sur: Coto del Rey -CR-, Los Sotos -SO-, Reserva Biológica de Doñana -RBD-, El Puntal -PU- y Marismillas -MA-).

4.2 Biotopos presentes

i) La marisma

La marisma es el biotopo predominante y ocupa un total de 27.000 ha, lo que supone aproximadamente la mitad de la extensión del PND. Debido a la localización estratégica del parque entre los continentes de Europa y África, la marisma es un lugar de paso, cría e invernada de numerosas especies de aves europeas y africanas. Está formada por suelos arcillosos que se inundan fácilmente con el aporte de agua de algunos arroyos y de la propia lluvia, por lo que presenta una estacionalidad muy marcada que condiciona la vida de los ungulados en el territorio. Con las primeras lluvias de otoño la marisma comienza a encharcarse, hasta inundarse por completo en invierno y primavera, mientras que en verano se seca completamente. Esta presenta pequeños desniveles que producen cambios en la salinidad del suelo, lo que determina la presencia la fauna y vegetación. La marisma dulce es la parte más profunda, lo que da lugar a una disminución de su salinidad y, como consecuencia, al crecimiento de vegetación palustre, como la castañuela (*Scirpus maritimus*) y el bayunco (*Scirpus littoralis*). En ella habitan aves acuáticas como el ánade azulón (*Anas platyrhynchos*), la focha común (*Fulica atra*), el pato cuchara europeo (*Anas clypeata*), la malvasía cabeciblanca (*Oxyura leucocephala*), algunos larolimícolas como la avefría europea (*Vanellus vanellus*), el chorlitejo chico (*Charadrius dubius*), el chorlitejo patinegro (*Charadrius alexandrinus*), la avoceta común (*Recurvirostra avosetta*) o la cigüeñuela común (*Himantopus himantopus*), y por último, flamencos (*Phoenicopterus ruber*) y ánsares comunes (*Anser anser)*. Por el contrario, la marisma salada presenta una profundidad menor y se encharca únicamente de forma superficial durante el invierno. Por tanto, la salinidad del suelo es elevada, y su vegetación está formada por almajos (*Arthrocnemum macrostachyum* y *Suaeda vera*).

ii) Las dunas y monte

Las dunas son formaciones arenosas que se encuentran al lado de la playa y contienen pequeñas manchas de vegetación que se denominan corrales. El monte o matorral se encuentra formado por una combinación de plantas leñosas con alcornoques (*Quercus suber*), sabinas (*Juniperus phoenicea subsp. turbinata*), madroños (*Arbutus unedo*), acebuches (*Olea europaea* var. *sylvestris*) y pinos piñoneros (*Pinus pinea*). Este alberga un gran número de vertebrados, entre los que destacan aves como el águila imperial ibérica (*Aquila adalberti*), la culebrera europea (*Circaetus gallicus*), el milano negro (*Milvus migrans*), carnívoros como el lince ibérico, el meloncillo (*Herpestes ichneumon*), el zorro (*Vulpes vulpes*) o el tejón, ungulados como el jabalí, el ciervo o el gamo, y otras especies como la liebre (*Lepus granatensis*) o el lirón careto (*Elyomis quercinus*), entre otros. Por último, también se hallan varias especies de anfibios y reptiles, como el sapo de espuelas (*Pelobates cultripes*), la culebra bastarda (*Malpolon monspessulanum*), la lagartija colirroja (*Acanthodactylus erytrurus*) o la tortuga mora (*Testudo gracea*).

iii) El ecotono monte-marisma o vera

La vera es una franja longitudinal estrecha de entre 200 y 1.500 metros que se encuentra separando los tres biotopos anteriormente citados. Esta zona presenta una elevada riqueza ecológica, ya que la humedad filtrada por las arenas crea un ambiente húmedo que favorece el crecimiento de junqueras y pastizales. Está formada, principalmente, por la asociación vegetal *Galiopalustris-Juncetum*, compuesto por plantas acuáticas tales como como *Scirpus maritimus*, *Galio palustris* o *Juncus maritimus*. Respecto a la fauna, predomina el abejaruco común (*Merops apiaster*), los conejos, los gamos y las avefrías europeas, además de grandes colonias de aves, especialmente buitres (*Gyps fulvus* y *Aegypius monachus*), que se establecen en los restos de antiguos bosques de alcornoques situados en el matorral adyacente.

4.3 Clima y estacionalidad

El PND presenta un clima mediterráneo subhúmedo con influencia atlántica, caracterizado por una fuerte estacionalidad y grandes fluctuaciones intra e interanuales, especialmente en lo que a la precipitación se refiere. La precipitación media oscila entre los 170 y los 1000 milímetros, mientras que la temperatura media se mantiene en torno a los 17ºC (Díaz-Delgado et al., 2016). Los cambios en la disponibilidad de agua a lo largo de las diferentes estaciones determinan la dinámica de la marisma y, como consecuencia, la distribución de los animales por el territorio, como se detallará en el apartado 4.5.

4.4 La comunidad mixta de ungulados del Parque Nacional de Doñana

La comunidad de ungulados del PND está formada por especies domésticas y silvestres. Las poblaciones de ungulados silvestres presentan densidades de moderadas a elevadas, y están compuestas por jabalíes, ciervos y gamos (EBD-CSIC, 2019). En cuanto a la comunidad de ungulados domésticos, incluye principalmente las especies bovina y equina. Estos animales se someten a todas las pruebas diagnósticas y vacunaciones de carácter obligatorio recogidas en los programas sanitarios pertinentes. Por tanto, en ganado bovino se realizan pruebas de tuberculinización dos veces año como parte del programa de erradicación de la TB (MAPA, 2021a), durante las cuales se toman muestras de sangre para el control de la brucelosis. Además, al estar situado en una zona de restricción para el serotipo 1 y 4 de lengua azul, el ganado bovino y ovino de edad superior a tres meses son vacunados anualmente (MAPA, 2020a).

Durante los últimos años, son varias las circunstancias que han dado lugar a un incremento en la abundancia de la comunidad de ungulados del parque, a excepción del gamo. Estas son la ausencia de depredadores naturales o un insuficiente control poblacional de los ungulados silvestres y sus efectos derivados, las cuales pueden confluir con un exceso de carga ganadera (Soriguer, 1988; Soriguer et al., 2001) (Figura 11). Todo ello ha dado lugar a numerosos problemas sanitarios y de conservación.

Respecto a las repercusiones sobre la conservación de la biodiversidad, el consumo y pisoteo de la flora producido por los grandes herbívoros ha conducido a una alteración significativa en la composición específica de las comunidades de plantas y a una ralentización en la regeneración natural de las especies arbóreas leñosas (Soriguer, 1988; Soriguer et al., 2001). Además, la depredación ejercida por jabalíes sobre los nidos de aves acuáticas y madrigueras de conejos, ha contribuido a la reducción de los recursos tróficos disponibles para especies amenazadas tan importantes como el lince ibérico y el águila imperial.

Por otra parte, las elevadas abundancias de ungulados facilitan las interacciones directas e indirectas entre las diferentes especies, y las elevadas tasas de infección de algunos patógenos, entre los que destaca el CMT (Barasona et al., 2016; Gortázar et al., 2011; Gortázar et al., 2008). Particularmente, el PND es uno de los puntos de la geografía española y mundial en los que se han reportado algunas de las prevalencias de TB más altas de las registradas en la literatura, especialmente en jabalí (Gortázar et al., 2008).

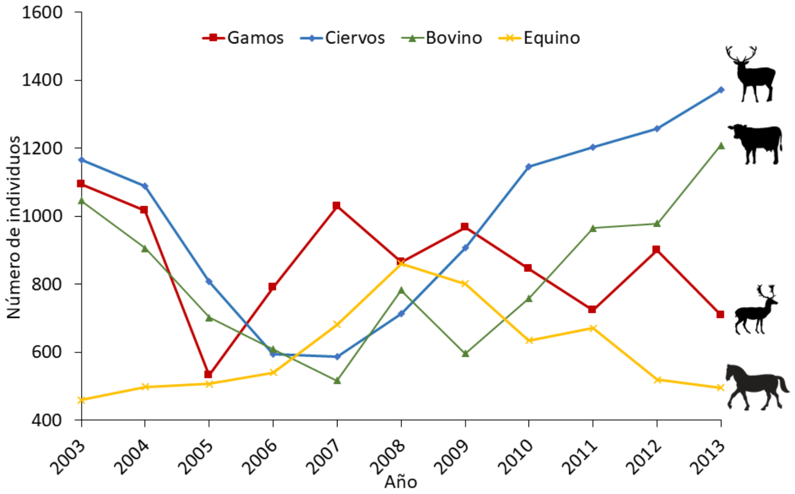


Figura 11. Evolución (2003-2014) de los últimos datos disponibles sobre abundancias correspondientes al ganado (bovino y equino) y ungulados silvestres (ciervo y gamo) en el Parque Nacional de Doñana (incluye las fincas de Coto del Rey, Sotos, Reserva Biológica de Doñana, Puntal y Marismillas) (Vicente et al., 2014).

4.5 La interfaz doméstico-silvestre-humano en el Parque Nacional de Doñana

La presencia histórica del ser humano en el parque ha contribuido a modelar su paisaje, desde los primeros habitantes cazadores-recolectores hasta la actual actividad científica que se desarrolla en gran parte de su territorio, lo que ha contribuido a la toma de conciencia conservacionista sobre este espacio (López-Sáez et al., 2018). Además, la actual presencia de viviendas dentro del parque, así como su cercanía a núcleos urbanos y pequeños asentamientos humanos, ejercen un papel esencial en la epidemiología de algunos patógenos, lo que conlleva importantes implicaciones sanitarias. A modo de ejemplo, un estudio de Cabal et al. (2017) analizó la influencia de la presión antrópica y la abundancia de ungulados sobre la distribución y diversidad de los principales genes de virulencia de *Escherichia coli*, mostrando que la diversidad genética era mayor al norte del parque, donde el agua se encuentra más contaminada por los efluentes de viviendas y granjas. Por otra parte, los ungulados del PND han mostrado una elevada exposición a algunas zoonosis letales transmitidas por vectores (p. ej., 33,5% de seroprevalencia de Flavivirus y 74,3% de fiebre hemorrágica de Crimea-Congo), siendo estas más elevadas al norte del parque, con importantes consecuencias para la salud pública (Casades-Marti et al., 2021; Gutiérrez-Guzmán et al., 2012; Peralbo-Moreno et al., 2021). Algunos autores sugieren que las características climáticas y ecológicas, las cuales favorecen la presencia y abundancia de vectores, unido a la gran diversidad de hospedadores disponibles podrían estar detrás de las cifras obtenidas.

La marcada estacionalidad que caracteriza al PND y sus consecuencias sobre la disponibilidad de agua y otros recursos naturales para los ungulados condicionan la distribución de estos a lo largo del territorio, así como la presencia de interacciones intra e interespecíficas (Barasona et al., 2014a, 2014b; Laguna et al., 2018; Figura 12a). La estación húmeda transcurre durante el invierno y la primavera. En esta época, la marisma permanece inundada y los ungulados se alimentan en aquellos parches de vegetación que permanecen sin cubrir, por encontrarse más elevados respecto al nivel medio del suelo. Por otra parte, la estación seca incluye el verano y los primeros meses del otoño. Esta es la temporada más dura para los ungulados debido a la fuerte escasez de recursos naturales, por lo que buscan zonas húmedas, ricas en pasto y agua (Bugalho y Milne, 2003; Rodriguez-Hidalgo et al., 2010), lo que da lugar a una agregación de individuos en torno al ecotono y puntos de agua (Barasona et al., 2014a). Concretamente, la zona de ecotono o “vera” proporciona alimento de alta calidad, sombra y refugio a los ungulados durante esta complicada etapa (Braza y Alvarez, 1987) (Figura 12b).

Las peculiaridades en el comportamiento intrínseco de cada especie (sociabilidad, gregarismo, hábitos carroñeros, etc.), y sus implicaciones ecológicas, resultan claves para la comprensión de la epidemiología de las infecciones compartidas en sistemas multihospedador (Gortázar et al., 2011; Laguna et al., 2018). Los puntos potenciales de contagio varían según el tipo de patógeno implicado, sus mecanismos de transmisión y las características biológicas y comportamentales de sus hospedadores, entre otras. En el caso del PND, las especies pueden mostrar preferencia por ciertos tipos de hábitat o lugares de uso compartido, patrones que pueden verse afectados por la estacionalidad y variaciones interanuales. P. ej., el ciervo y el jabalí seleccionan con más frecuencia que el gamo las zonas de bosque y matorral (Braza y Alvarez, 1989, 1987). Un estudio llevado a cabo por Barasona et al. (2014a) en el PND, reveló que el jabalí presentaba una mayor preferencia que las vacas por áreas cercanas a puntos de agua (matorral claro, pastizal y vegetación asociada a cursos de agua) en invierno y primavera, mientras que en verano y otoño no se hallaron diferencias en la selección de estos hábitats por parte de ambas especies. Asimismo, la mayor parte de las interacciones interespecíficas ocurrieron en la estación seca en torno a la vera y puntos de agua (Figura 13).

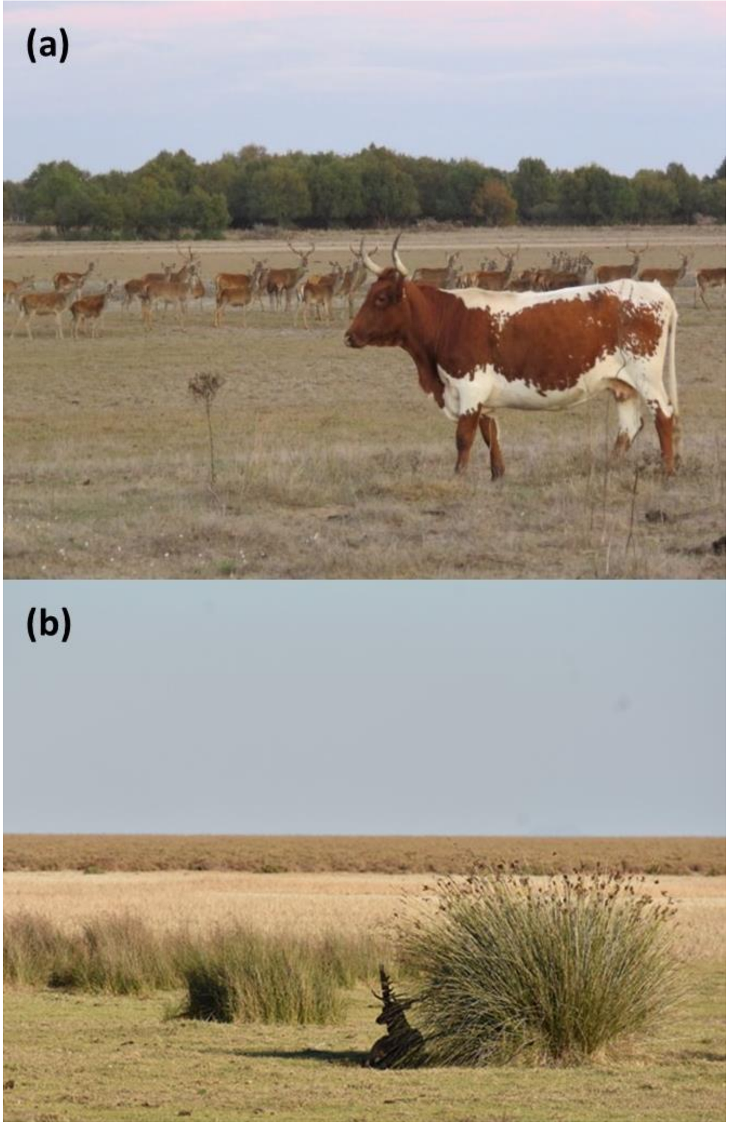


Figura 12. (**a**) Interacción (proximidad) entre una vaca y un grupo de jabalíes en un punto de agua (Autor: Pablo Palencia Mayordomo) (**b**) Gamo buscando la sombra en el ecotono durante la estación seca.

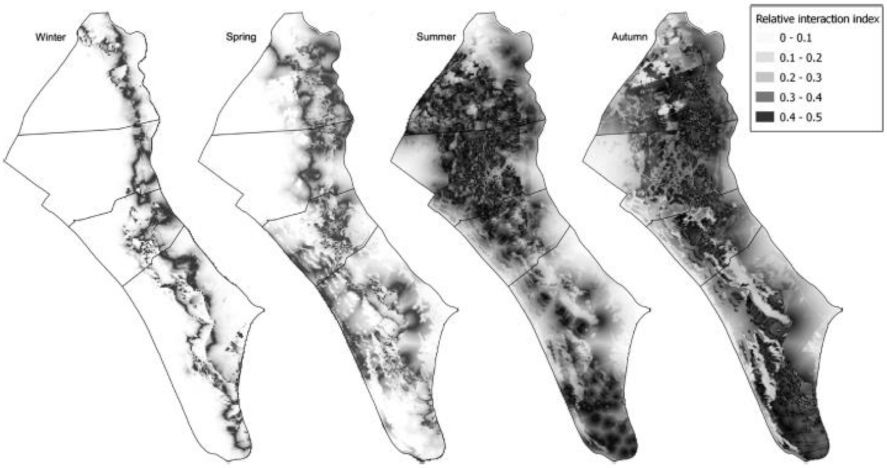


Figura 13. Patrones de interacción interespecífica entre jabalí y vaca en el Parque Nacional de Doñana (Fuente: Barasona et al. 2014a).

Otro estudio llevado a cabo por Triguero-Ocaña et al. (2019), orientado a determinar dónde y cuándo se producían estas interacciones, determinó que ocurrían principalmente en horas crepusculares y en los puntos de agregación anteriormente mencionados o en zonas abiertas. En un estudio posterior en el que se evaluaron todas las interacciones interespecíficas posibles entre ungulados, se observó que las más representativas entre domésticos y silvestres acontecieron entre gamos y vacas, los cuales mostraron tendencia a formar grupos mixtos (Triguero-Ocaña et al., 2020). En este sentido, Laguna et al. (2018) desarrollaron y evaluaron un índice de agregación basado en la abundancia y distribución de los ungulados en los diferentes grupos y de estos por el territorio del PND, permitiendo, por tanto, predecir las variaciones espaciotemporales en la transmisión de infecciones.

4.6 El Parque Nacional de Doñana como laboratorio de campo

Son varias las circunstancias que hacen del PND un escenario ideal para el estudio a largo plazo de la epidemiología de infecciones compartidas de distinta naturaleza (Figura 14). Las características climáticas y ecológicas del parque, unidas a su extensión y a la gran biodiversidad que alberga, hacen de éste un escenario ideal para el estudio de la epidemiología de numerosas infecciones compartidas de distinta naturaleza. Además, las variaciones locales en las densidades de fauna silvestre y de ganado, unido a la agregación estacional de ungulados en ciertas zonas contribuyen a que el PND sea un verdadero laboratorio de campo. Prueba de ello es la gran variedad de estudios desarrollados a lo largo del tiempo sobre enfermedades y ecología de la fauna silvestre en esta zona (Figura 15), con una tendencia claramente ascendente, teniendo en cuenta el año en curso.

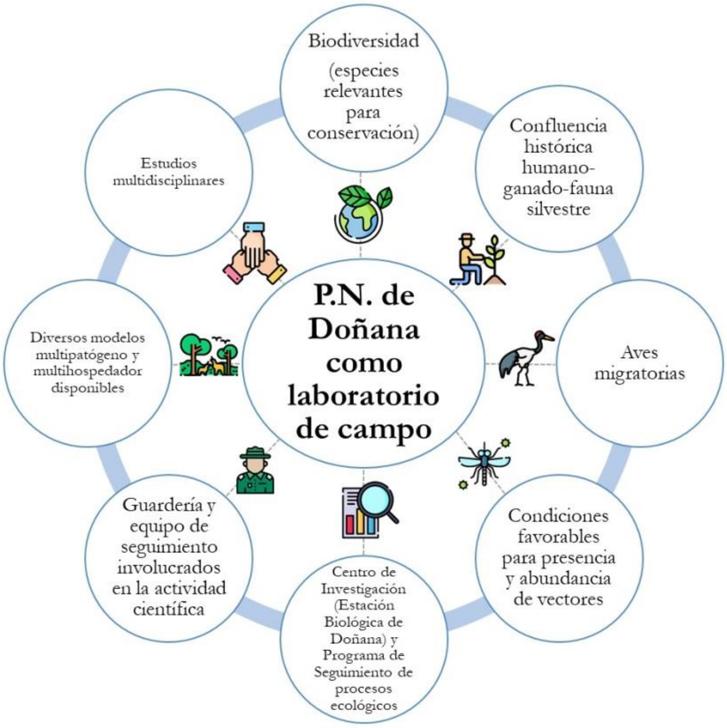


Figura 14. Características del Parque Nacional de Doñana que lo convierten en un auténtico laboratorio de campo, especialmente para estudios epidemiológicos a largo plazo.

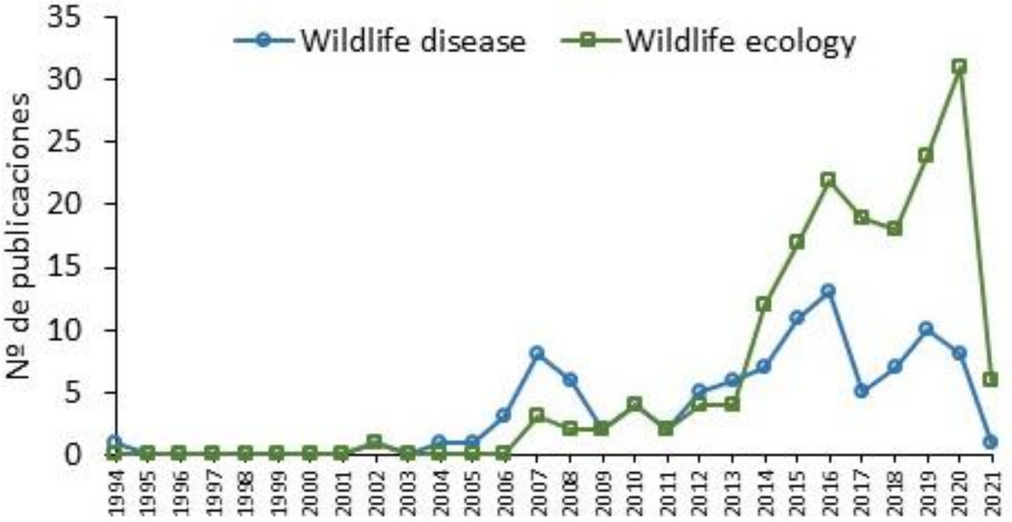


Figura 15. Tendencia temporal en el número de publicaciones halladas en la literatura al realizar una búsqueda bibliográfica en la plataforma PubMed con las combinaciones de palabras clave “wildlife” AND “disease” AND “Doñana” y “wildlife” AND “ecology” AND “Doñana”.

Por una parte, la gran variedad de especies que en él habitan, incluyendo algunas muy importantes para la conservación, y la presencia estacional de aves migratorias dan lugar a un amplio rango de hospedadores disponibles para los diferentes patógenos. De este modo, se forman comunidades multihospedador muy estables y capaces de mantener la circulación de patógenos a largo plazo y transmitirlos (Barasona et al., 2019). Además, las condiciones meteorológicas y ambientales de la zona son muy favorables para la supervivencia de vectores, los cuales presentan abundancias muy elevadas. Estos vectores también intervienen en la transmisión y la persistencia de las infecciones compartidas en las comunidades de hospedadores. Por último, la confluencia histórica del ser humano, el ganado y la fauna silvestre en el parque da lugar a una interfaz humano-doméstico-silvestre que ha coevolucionado a lo largo de los años. Por todo ello, este territorio ofrece numerosos modelos multihospedador/multipatógeno para su estudio.

Por otra parte, se dispone de una larga serie temporal de información sobre fenómenos climáticos y ecológicos, así como sobre el estado y las tendencias de la biodiversidad en el PND. En 1980 comenzó a realizarse un seguimiento de las poblaciones, principalmente centrado en aves y especies amenazadas. En 2003, el equipo de seguimiento de procesos y recursos naturales de la Estación Biológica de Doñana comenzó con el programa de seguimiento en el Espacio Natural de Doñana, en colaboración con el equipo técnico y de guardería del Espacio Natural de Doñana. Éste aborda numerosas especies y procesos ecológicos (medio ambiente, agua, hábitats, poblaciones y especies, producción primaria, y servicios ecosistémicos). Los resultados obtenidos son reportados anualmente a las oficinas del parque, a las autoridades regionales y a un repositorio de datos disponible en su página web (EBD-CSIC, 2019). Toda esta información supone una gran oportunidad para los numerosos científicos que desarrollan sus investigaciones en la zona.

Son muchas las líneas de investigación desarrolladas en los últimos años en el PND en lo que a epidemiología de infecciones compartidas se refiere, obteniendo como resultado una serie temporal de información de más de diez años. Estos estudios han sido enfocados principalmente hacia la determinación de las prevalencias, las cepas circulantes y los principales factores de riesgo de estas infecciones (Gortázar et al., 2011; Gortázar et al., 2008; Romero et al., 2008). Además, la cercanía del parque a núcleos urbanos y la existencia de pequeños asentamientos humanos en el territorio permitieron incluso abordar cuestiones desde un enfoque de “Una única salud” (Cabal et al., 2017). Con relación al

CMT, desde mediados de la década de los 2000 se han llevado a cabo numerosos estudios que han aportado conocimientos fundamentales en el campo de la epidemiología y la ecología espacial, al contar con una comunidad de hospedadores diversa y un ambiente complejo que favorecen la persistencia de la infección.

Otras líneas de investigación mencionadas en el apartado anterior se orientaron hacia la ecología espacial, cuantificando las interacciones inter e intraespecíficas entre la fauna silvestre y el ganado, así como su variabilidad espaciotemporal, mediante la utilización de sensores de proximidad y otros dispositivos de seguimiento como la telemetría (Laguna et al., 2018; Palencia et al., 2019; Triguero-Ocaña et al., 2020, 2019).

Por tanto, durante los útimos años se ha realizado una monitorización integral de esta comunidad de hospedadores, obteniéndose información de vigilancia sanitaria (numerador) y de monitorización poblacional (denominador), mediante la realización de necropsias y toma de muestras biológicas, la aplicación de técnicas de fototrampeo y colocación de dispositivos de seguimiento, y la realización de censos poblacionales (EBD-CSIC, 2019).

Justificación y objetivos

Esta Tesis doctoral aborda el estudio de una serie temporal (2005-2018) que excede la duración habitual hallada en la literatura sobre el estudio de los principales factores de riesgo de las infecciones en fauna silvestre, y particularmente las compartidas con el ganado. Se centra en varios sistemas patógeno-multihospedador (comunidad de ungulados) que presentan sus propias características diferenciales, abordando factores de naturaleza variada. Esta aproximación a medio-largo plazo es fundamental para comprender mejor los patrones de transmisión y persistencia de los patógenos compartidos entre la fauna silvestre y el ganado y, por tanto, para el diseño e implementación de estrategias de control y erradicación de éstos. Todo ello es estudiado en un auténtico laboratorio de campo en el ámbito de las infecciones compartidas, como es el PND, situado en el suroeste de la Península Ibérica, donde ya en 2005 se establece una línea de investigación principalmente centrada en la TB en la comunidad de hospedadores ungulados.

Los trabajos que componen esta Tesis doctoral poseen un enfoque multidisciplinar, combinando la epidemiología y la ecología (cualitativa y cuantitativa). Su **objetivo principal** fue la identificación de los principales factores de tipo individual, poblacional, ambiental y estocástico que, operando a medio o largo plazo, intervienen en la transmisión y persistencia de las infecciones compartidas en el medio natural. Para la consecución de este objetivo general, se plantearon los siguientes **objetivos específicos**:

1. Caracterizar la información disponible en la literatura científica indexada sobre estudios a largo plazo de enfermedades infecciosas en fauna silvestre, identificando el valor añadido que supone analizar amplias series temporales a la hora de explicar los factores determinantes y comprender mejor sus consecuencias epidemiológicas y ecológicas. Además, se pretendió proporcionar una definición del concepto de "investigación a largo plazo en fauna silvestre", considerando que la escala temporal en la que intervienen los principales determinantes de la epidemiología de un patógeno es siempre dependiente del contexto.
2. Determinar la dinámica temporal de la prevalencia o seroprevalencia de patógenos de naturaleza contrastada e identificar y determinar el papel de los principales factores de riesgo (individuales, poblacionales, ambientales y estocásticos) en la comunidad de ungulados (bovino, ciervo, gamo y jabalí) del PND, con una perspectiva a medio y largo plazo, respectivamente, para:
    - Complejo *Mycobacterium tuberculosis*

- *Toxoplasma gondii*
- Virus de la lengua azul
- Virus de la hepatitis E

Estos objetivos quedan abordados en los próximos capítulos, según se detalla en el siguiente apartado.

Estructura

La parte central de esta Tesis doctoral consta de tres capítulos, dos de ellos (Capítulo 1 y 2) presentados en formato de artículo científico (un total de cinco) escritos en lengua inglesa, incluyendo un resumen en castellano al principio de cada uno de ellos. El capítulo final (Capítulo 3) aborda la síntesis y conclusiones, y está escrito en castellano.

Capítulo 1. La importancia de los estudios a largo plazo sobre enfermedades de la fauna silvestre y en sus interfaces con el ser humano y los animales domésticos, una revisión.

En este capítulo se ha realizado una revisión bibliográfica de aquellos estudios potencialmente considerados "a largo plazo", analizando los principales objetivos planteados, las especies y patógenos estudiados, las áreas geográficas en las que se desarrollaron y las conclusiones alcanzadas por los mismos. También se ha determinado la tendencia temporal de estos estudios durante el periodo 1993-2017 y el valor añadido que supone el análisis de largas series temporales en lo que al conocimiento de la epidemiología se refiere. Por último, se ha propuesto una definición de "investigación a largo plazo en fauna silvestre", teniendo en cuenta que es un término dependiente del contexto en el que se desarrollan tanto las especies como los patógenos abordados. Con la información obtenida, se respondió a las cuestiones planteadas en el objetivo 1. El artículo científico que compone el presente artículo se cita a continuación:

Barroso, P., Acevedo, P., Vicente, J. The importance of long-term studies on wildlife diseases and their interfaces with humans and domestic animals - a review. *Transboundary and Emerging Diseases* 2020, 00:1-15. https://doi.org/10.1111/tbed.13916

Capítulo 2. Factores determinantes a largo plazo de la epidemiología de las infecciones compartidas en la comunidad hospedadora de ungulados del Parque Nacional de Doñana.

Capítulo 2.1. Factores determinantes a largo plazo de la tuberculosis en la comunidad hospedadora de ungulados del Parque Nacional de Doñana.

Para este capítulo, la infección por el CMT fue elegida como modelo de infección bacteriana compartida entre el ser humano, el ganado y la fauna silvestre. Ésta ha sido frecuentemente estudiada como ejemplo de infección compartida entre especies (Palmer et al., 2012). Su amplia distribución por la Península Ibérica y las elevadas prevalencias halladas tanto en ungulados domésticos como silvestres en el centro y sur de España, unido a su relevancia para la economía, la salud pública y la conservación de las especies, hacen de esta infección un modelo ideal de estudio. Este trabajo se centra en el estudio de principales factores individuales, poblacionales, ambientales y estocásticos que determinan y modulan el mantenimiento del CMT en el PND, así como en evaluar la tendencia temporal de sus prevalencias en la comunidad de ungulados. Como resultado de este capítulo el objetivo 2 queda cubierto, componiéndose del artículo científico que se cita a continuación:

Barroso, P., Barasona, J.A., Acevedo, P., Palencia, P., Carro, F., Negro, J.J., Torres, M.J., Gortázar, C., Soriguer, R.C., Vicente, J. Long-term determinants of tuberculosis in the ungulate host community of Doñana National Park. *Pathogens* 2020, 9 (6) 445. https://doi.org/10.3390/pathogens9060445.

Capítulo 2.2. Factores determinantes a largo plazo de la seroprevalencia de *Toxoplasma gondii* en una comunidad de ungulados silvestres.

Este estudio aborda la infección por *T. gondii* como modelo de infección parasitaria compartida entre el ser humano, el ganado y la fauna silvestre. La toxoplasmosis es la enfermedad parasitaria más extendida del mundo y presenta una gran relevancia para la salud pública, ya que aproximadamente un tercio de la población se infecta en todo el mundo (Montoya y Liesenfeld, 2004; Tenter et al., 2000). Además, es la principal causa de aborto y muerte perinatal en ganado ovino, caprino y porcino, causando graves pérdidas económicas en este sector (Dubey, 2010). Ya que una de las vías más importantes de transmisión al ser humano es el consumo de carne poco cocinada procedente de ungulados silvestres, hemos considerado importante evaluar los principales factores individuales, poblacionales, ambientales y estocásticos que determinan y modulan el mantenimiento de *T. gondii* en el PND, así como la tendencia temporal de su seroprevalencia en la comunidad de ungulados. Para este capítulo, se considera también la presencia de gatos urbanos y linces ibéricos en el área de estudio como hospedadores definitivos de *T. gondii*. Este estudio hace referencia al objetivo 2. El artículo científico que compone el presente artículo se cita a continuación:

Barroso, P., García-Bocanegra, I., Acevedo, P., Palencia, P., Carro, F., Jiménez-Ruiz, S., Almería, S., Dubey, J.P., Cano-Terriza, D., Vicente, J. Long-term determinants of the seroprevalence of *Toxoplasma gondii* in a wild ungulate community. *Animals* 2020, 10 (12) 2349; https://doi.org/10.3390/ani10122349.

Capítulo 2.3. Factores a largo plazo determinantes de la seroprevalencia del virus de la lengua azul en especies de cérvidos del sur de España.

Este capítulo se centra en la infección por el VLA como modelo de infección vírica transmitida por vectores y compartida entre el ganado y la fauna silvestre. La importancia de este patógeno es fundamentalmente económica, produciendo graves pérdidas en la producción y restricciones al movimiento (MAPA, 2020a). Los rumiantes silvestres actúan como reservorios asintomáticos y, por tanto, como centinelas de la infección por el VLA. Históricamente, el PND ha sido una zona de restricción contra el serotipo 1 y 8 del VLA, por lo que conocer su presencia y la tendencia temporal de sus seroprevalencias en la comunidad residente de rumiantes silvestres podría aportar información su circulación en el área de estudio previa a la declaración del primer brote. Ésto resulta especialmente interesante si se considera que el PND se sitúa en las provincias de Huelva, Sevilla y Cádiz, área geográfica en la que se detectaron los primeros brotes de estos serotipos en la Península Ibérica. Por otra parte, en este capítulo se evalúan los principales factores individuales, poblacionales, ambientales y estocásticos que determinan y modulan el mantenimiento del VLA en el PND. En este trabajo se aborda el objetivo 2 y está compuesto por el siguiente artículo científico.

Barroso, P., Risalde, M.A., García-Bocanegra, I., Acevedo, P., Barasona, J.A, Palencia, P., Carro, F., Jiménez-Ruiz, S., Pujols, J., Montoro, V., Vicente, J. Long-term determinants of the seroprevalence of bluetongue virus in deer species in southern Spain, *Research in Veterinary Science* 2021, 139:102-111. https://doi.org/10.1016/j.rvsc.2021.07.001

Capítulo 2.4. Factores a largo plazo determinantes de la seroprevalencia de hepatitis E en jabalíes.

En este capítulo se aborda el estudio de la infección por el VHE como modelo de infección vírica compartida entre el ser humano, el ganado y la fauna silvestre. La hepatitis E es una zoonosis emergente con gran relevancia para la salud pública, especialmente durante los últimos años. La ingestión de carne poco cocinada procedente de ungulados silvestres y domésticos representa una de las vías de contagio más frecuentes para el ser humano. Por ello, en este capítulo se evalúan los principales factores individuales, poblacionales, ambientales y estocásticos que determinan y modulan el mantenimiento del VHE en la población de jabalíes del PND. Además, se determina la tendencia temporal de su seroprevalencia. Con la información obtenida de este capítulo, compuesto por el artículo citado a continuación, se pretende abarcar la parte del objetivo 2 correspondiente a este patógeno.

Barroso, P., Risalde, M.A., García-Bocanegra, I., Acevedo, P., Barasona, J.A, Caballero-Gómez, J., Jiménez-Ruiz, S., Montoro, V., Vicente, J. Long-term determinants of the seroprevalence of Hepatitis E virus in wild boar (*Sus scrofa*). *Animals* 2021, 11, 1805. https://doi.org/10.3390/ani11061805

Capítulo 3. Discusión general y conclusiones

En este capítulo se realiza una síntesis y discusión conjunta de todos los resultados obtenidos, mencionando cada una de las conclusiones alcanzadas.

CAPÍTULO 1. La importancia de los estudios a largo plazo sobre enfermedades de la fauna silvestre y en sus interfaces con el ser humano y los animales domésticos, una revisión

CHAPTER 1. The importance of long-term studies on wildlife diseases and their interfaces with humans and domestic animals - a review.

Barroso, P., Acevedo, P., Vicente, J., 2020. The importance of long-term studies on wildlife diseases and their interfaces with humans and domestic animals - a review. *Transboundary and emerging diseases* 0, 1-15. https://doi.org/10.1111/tbed.13916

Resumen

Los estudios epidemiológicos a largo plazo en fauna silvestre (EPLFS) y sus interfaces con los seres humanos y los animales domésticos proporcionan la perspectiva temporal necesaria para alcanzar la comprensión de los principales determinantes de las enfermedades y de cómo éstos actúan. Mediante una revisión sistemática, se ha analizado la tendencia temporal de los EPLFS, sus objetivos, así como de sus principales hospedadores, patógenos y áreas geográficas estudiadas. Se ha evaluado el valor añadido que estos estudios proporcionan. Para este estudio, se seleccionaron un total de 538 artículos desde 1993 hasta 2017 con un periodo de estudio mayor que o igual a cuatro años consecutivos. Se observó un marcado incremento en el número de estudios publicados durante los últimos 20 años, lo que reflejó un creciente interés por el papel de la fauna silvestre como reservorio de estas enfermedades. Los patógenos más estudiados fueron los virus (39,2%), bacterias (38,5%) y protozoos (15,8%). Respecto a los hospedadores, los mamíferos (84,9%), concretamente ungulados (40,0%) y los carnívoros (30,9%), y las aves (12,5%) fueron los más representados en estos estudios a largo plazo. La mayoría de los artículos llegaron a conclusiones respecto a los efectos de la enfermedad en la dinámica infección-hospedador (98,7%) y alrededor del 40,0% consideró las consecuencias económicas del patógeno, proponiendo medidas de manejo y control. La actividad investigadora se focalizó principalmente en el hemisferio norte. Aunque la definición de EPLFS no está únicamente determinada por la duración del estudio, éste debe tener la suficiente duración como para abordar cuestiones ecológicas y epidemiológicas que no pueden ser resueltas mediante observaciones o experimentos a corto plazo, y aclarar los efectos de los diferentes factores determinantes del riesgo. Esta revisión bibliográfica demostró que los EPLFS proporcionan información sobre las causas y consecuencias de los patógenos en las comunidades de hospedadores. Ésto podría usarse para informar decisiones relativas a la emergencia de enfermedades y podría ayudar a crear sistemas de detección temprana del riesgo basados en investigaciones retrospectivas.

Abstract

Long-term wildlife disease research (LTWDR) and its interfaces with humans and domestic animals provides perspective to understand the diseases' main drivers and how they operate. In a systematic review, we analysed the temporal trend of the studies on LTWDR, their aims, as well as the hosts, pathogens and geographic areas studied. We also evaluated the added value that such studies provide. For analysis, we selected a total of 538 articles from 1993 to 2017 with a study period greater than or equal to 4 consecutive years. A marked increase in the number of studies published during the last 20 years was observed that reflects a growing awareness of the outstanding role of wildlife as a reservoir of diseases. The most studied pathogen agents were viruses (39.2%), bacteria (38.5%) and protozoans (15.8%). Concerning the hosts, mammals (84.9%), particularly ungulates (40.0%) and carnivores (30.9%), and birds (12.5%) were the most represented in these long-term studies. Most articles reached conclusions concerning the effect of the disease on the infection-host dynamics (98.7%) and over 40.0% considered the economic consequences or proposed management and control measures. The research was mainly located in the northern hemisphere. While the definition of LTWDR is not only determined by the duration of the monitoring, the study must be long enough to: i) address ecological and epidemiological questions that cannot be resolved with short-term observations or experiments, and ii) clarify the effects of different drivers. This review demonstrates that LTWDR has provided information about the causes and consequences of disease change that otherwise could not have been obtained. It may be used to inform decisions related to the emergence of disease and might help to design early warning systems of disease based on retrospective investigations.

Introduction

Shared infections at the human and animal interfaces are caused by transmissible pathogens where at least one host species from either can be relevant for infection maintenance (e.g. Haydon et al. 2002). Many of the wildlife infections shared with livestock are also zoonotic (Chan et al., 2013; Cleaveland et al., 2001), and therefore all interfaces are potential sources of disease emergence for humans (Jones et al., 2008; Wolfe et al., 2007). In the last few decades, several factors such as the changes in wildlife management, in land uses, farming practices and/or in human demography have led to an expansion and increased abundance of wildlife populations (Massei et al., 2015). Consequently, shared pathogens such as the Avian Influenza Virus (AIV) or African Swine Fever Virus (ASFV) (Gavier-Widen et al., 2015; Pfeiffer, 2007; Sánchez-Vizcaíno et al., 2015) have emerged, and even pandemics, such as COVID-19 (Zhou et al., 2020). These pathogens are therefore relevant to public health, the global economy, animal husbandry, game production, wildlife management and the conservation of biodiversity (e.g. Buttke et al., 2015; Caron et al., 2013; Gortázar et al., 2010).

Long-term approaches are aimed at providing the necessary perspective to understand processes that operate over long temporal scales (Christensen et al., 2020; Nugent et al., 2012; Vicente et al., 2013). We can discern what the drivers of infection dynamics are, how pathogens become endemic or fade out in host communities and populations (Gordo and Avilés, 2017; Nugent et al., 2012; Strayer et al., 1986), and generate (or evaluate) well-informed strategies for effective risk management (Delahay et al., 2013; Portier et al., 2019). This is especially relevant when analysing the epidemiological and demographic consequences of infections in long-lived species and endemic situations (Barroso et al., 2020b; McDonald et al., 2017), and also to characterise the arena of “hot scenarios” where pathogens may emerge (Jones et al., 2008). However, long-term studies are generally more costly, time-consuming and difficult to carry out than shorter investigations (Gordo and Avilés, 2017; Likens, 1989). This type of study is based on the replication of the same/comparable protocol/s or experiment/s for many years and compiling enough accurate replicas (either in different areas and hosts or not) and covering the different phases of ecological and epidemiological processes with the purpose of providing reliable conclusions. Nevertheless, considering a study “long-term” only in terms of its duration is relative since the host life cycle, infection dynamics and endemicity varies according to the system under study.

The relevance of long-term studies in the field of ecology and evolutionary biology has been widely recognized, and an increasing body of knowledge is available, both individual and population-based (Gordo and Avilés, 2017; Likens, 1989; Strayer et al., 1986). In contrast, long-term wildlife diseases research (LTWDR) in free-living hosts is scarce despite its relevance to human, livestock and wildlife health, and conservation of biodiversity (Barroso et al., 2020b; Christensen et al., 2020; Delahay et al., 2013; McDonald et al., 2017; Vicente et al., 2013). Some reviews and meta-analyses on wildlife diseases have assessed the quantity and scientific quality of the articles published and their temporal trends; paying special attention to a particular geography and the time-trend of the number of studies on specific pathogens or hosts, including the livestock-wildlife interface (Wiethoelter et al., 2015). Some wildlife pathogens have specifically received reviews (Ciliberti et al., 2015; Gortázar et al., 2016, 2007; Ruiz-Fons, 2017; Wiethoelter et al., 2015), mainly focussing on emergent shared pathogens and the drivers that give rise to their emergence (Becker et al., 2006; Daszak et al., 2000; Salkeld et al., 2013). However, a review focused on analysing LTWDR and its added value is lacking. This is of special interest to identifying research priorities and promoting the implementation or continuity of long-term studies to identify and manage the risk of disease emergence at the human-animal interfaces. By performing a systematic scoping review, our objectives were: i) to determine the temporal trend of the number of publications focused on long-term studies; ii) to describe the aims, hosts, pathogens and geographic area studied; iii) to assess the added value of these studies to understand the ecological and epidemiological processes that operate over a long temporal scale, and iv) to identify the research priorities and gaps in our knowledge which can be addressed by long-term approaches. Finally, based on the added value that it must provide, we propose a definition of LTWDR, considering that the time scale the main drivers of infection operates are always context-dependent.

Materials and methods

There are many nuances to determine what exactly constitutes LTWDR. For the purposes of this paper, LTWDR is considered to be those studies that systematically collected field data from a particular population for more than four consecutive years. However, as mentioned above, we propose a definition of LTWDR in the discussion. This somewhat arbitrary time component at least provided a repeated sampling of most pathogen cycles in a population, including the drivers of the infection dynamics that may operate over long periods. However, as the duration of the study increases, the prospects of understanding the system for long-lived host species, chronic or latent pathogens or factors operating over large periods potentially improve. Likewise, it is essential to state what is not considered long-term research in disease epidemiology. According to long-term ecology research, simulation modelling and non-continuous data collection are excluded from long-term studies (Lindenmayer et al., 2012). We note that research involving agent-based models overlooks multiyear datasets that may have been used to calibrate parameters in those models. This data is normally collected from empirical studies which are represented among our comprehensive inventory of papers, therefore avoiding duplication.

In October 2018 we reviewed research retrieved through a search on the Scopus (n=6541), PubMed (n=4742) and Web of Science (n=4206) platforms using a combination of the keywords "wildlife", "disease" and ("long-term OR (“long term")” by the Boolean operator “AND” to obtain only the intersection. In order to include complete annual periods (and compare them), we selected articles from January 1993 to December 2017 which satisfied the following eligibility criteria: i) original articles written in English, ii) a study period greater than or equal to four consecutive years, iii) a sampling of a minimum of 10 individuals/species per year, and iv) surveillance of the same population in the same study area over time. A preliminary exploration indicated that the study period reflects the initial “boom” in the number of long-term studies published. Also, for comparisons that disregard time, we considered that publications during the last two decades (papers published in 1993 included data from 1989 at least) are more comparable in terms of main interests, host species, pathogens and technical means available. Studies based on passive surveillance, clinical trials or the study of laboratory animals or captive wildlife populations were excluded.

A total of 15,489 research studies were harmonized and merged into a database for further analysis. After removing duplicates (14,595 articles), we verified the remaining 894 articles manually, excluding those which did not meet the criteria established. Finally, we reviewed 538 articles. The information compiled for each article reviewed is shown in Table 1.

Table 1. Information compiled for each article reviewed.

Information compiled	**Specific contents**	
Year of publication		
Journal´s name		
Country and continent		
Species and pathogens		
Length of the study period		
Type of conclusions reached	On infection and host dynamics	Spatial or spatiotemporal spread and persistence of the pathogen
		Reservoir status of the wildlife
		Consequences of the pathogen for the individual (energy balance, reproduction, survival, mortality)
		Regulatory or destabilizing role of the pathogens on the population dynamics
		Effects of the pathogen on the community and ecosystem
		Pathogen interactions with other regulatory factors of wildlife populations (stochastic, density-dependence, food availability, predation, intrinsic behaviour, etc.)
	Economic impact	Effect of the pathogen on the livestock industry
	Relevance to conservation	Proposal to control/eradicate the disease
	Management and control of the disease	
Other information	The evolutionary importance of the pathogens	
	The epidemiological role played by intermediate/vector hosts and/or the environment	

Furthermore, the range of the conclusions reached by the long-term studies reviewed was related to the length of the study period (in years) by using generalized linear models (GLM). In those GLM, the capability to conclude about each type of conclusion (see Table 1) was the response variable (binomial link logit) and the length of the study period (in years) was included as the explanatory variable. The assumptions of binomial GLM were met in all the models (Zuur et al., 2009).

Results and discussion

Journals

The research journals which published the majority of the publications were: Journal of Wildlife Diseases (34.9%), PLoS ONE (8.4%), Veterinary Parasitology (3.9%), and Preventive Veterinary Medicine (3.3%). Altogether, these four journals published half of the long-term studies on wildlife diseases worldwide in the period chosen.

Time-trend in the number of publications

Although we are aware that our literature search procedure might miss some articles addressing LTWDR, the results obtained are considered sufficiently representative to derive results and conclusions concerning general trends, which are presented next. The number of publications dated from 1993 to 2017 showed a ten-fold increase in less than 15 years (from 12 articles in 1993/1995 to 120 in 2016/2017). This trend reflects the increased awareness about wildlife relevant to the epidemiology of shared pathogens that may affect public health or other aspects. The initial productivity remained relatively stable until 2002-2003, when the increase became more obvious (Figure 1a). This is contemporaneous with reviews on wildlife disease studies that are not specifically focused on long-term approaches (Becker et al., 2006; Takahashi-Omoe and Omoe, 2012; Wiethoelter et al., 2015). The growth in the number of publications observed was mainly caused by the contribution of research on mammals (Figure 1b). Since then, zoonoses acquired importance, given that they were the topic of more than half of the total publications per year (Figure 1a). Significant zoonotic events took place around these years (Anderson et al., 2004; Ellis et al., 2004; Guan et al., 2004). Besides these events, there is a greater economic outlay on veterinary measures for the control of animal disease outbreaks during these years, reaching 400,000,000 €/year (Alleweldt, 2010). Therefore, important scientific and socio-economic issues arose in the early 2000s as a result of the impact of these events on human health and the global economy, leading to an increased number of long-term studies published.

Regarding temporal trends of particular taxa species (Figure 1b), we observed that some mammals (chiropterans and primates), birds or amphibians became the focus of the long-term approach later. For example, in 2006 and 2009 the first long-term studies about chiropteran and primate diseases, respectively, were published, increasing from one article in 2006 to eight articles in 2017 for chiropterans and from two articles in 2009 to six articles in 2017 for primates. Until 2006-2007, studies on Chiroptera focused on their conservation and their important contributions to ecosystems (Fujita and Turtle, 1993; O'Donnell, 2000; Sampaio et al., 2003). However, after having been involved in several outbreaks of emerging virulent infectious diseases since the early 2000s (SARS-CoV-1 and potentially in the Middle East Respiratory Syndrome-Related Coronavirus - MERS-CoV) the interest in bats increased, and long-term studies progressively appeared (e.g. Afelt et al., 2018; August et al., 2012). The emergence in 2006 of the White Nose Syndrome (WNS), a fungal infection of hibernating bats which causes high mortality rates (Blehert et al., 2009) was also a cause of the growing interest in bats. As for primates, the first articles published using the long-term approach were from 2008-2009, after previous outbreaks of the Ebola virus in humans together with outbreaks of yellow fever causing the decline of primate populations (Mora-Chavarría et al., 2017; Moreno et al., 2013; Prugnolle et al., 2011). However, most of these articles focused on parasitic amphixenosis (Carlton et al., 2008; Davies y Pedersen, 2008; Mbora y McPeek, 2009). In relation to birds, most long-term studies appeared from 2010 onwards, mostly associated with highly pathogenic AIV outbreaks with potential zoonotic consequences (Gilbert et al., 2012; Ishtiaq et al., 2012; Krauss et al., 2015). The temporal trends in conclusions on the zoonotic character of the infection, and its conservation or economic (mainly through economic losses in livestock breeding) consequences are represented in Figure 1c.

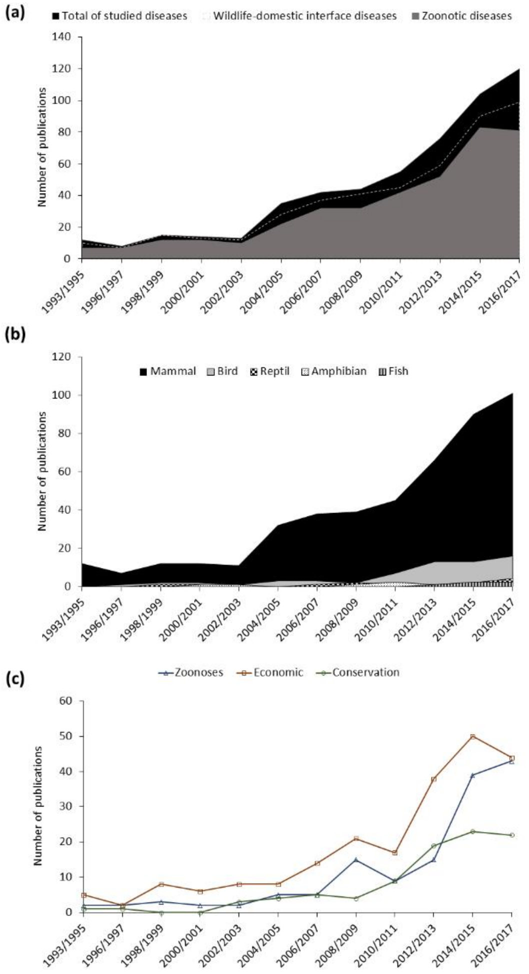


Figure 1. (**a**) Temporal trend of long-term publications (1993-2017) on wildlife diseases (n=538), indicating which diseases are zoonoses (n=391) and diseases shared at the wildlife-domestic interface (n=456) (**b**) Temporal trend of long-term publications on wildlife diseases (n=538), indicating which host species taxa were addressed (**c**) Temporal trend of conclusions on the zoonotic character of the disease, and its conservation or economic (mainly through economic losses in livestock breeding) consequences.

Pathogens and hosts

Part of the studies addressed several pathogens and/or species at once (65.0%). Hence, the frequencies here referred to are calculated as the number of articles in which a particular pathogen and/or host appears, with respect to the total number of publications (n=538). The frequency of appearance relative to each pathogen group in long-term studies on wildlife diseases is shown in Figure 2. Viruses were the most frequently addressed pathogens (39.2% of studies), followed by bacteria (38.5%) and parasites (29.7%, including protozoans and metazoans), whereas prions and fungi were less frequently studied in only 5% of the articles.

These results are in accordance with Wiethoelther et al. (2015), who has stated that over 50% of long-term studies refer to viruses and bacteria whereas prions are barely represented, in this case specifically referring to wildlife-livestock interface pathogens. The pathogenic agents within the group, separately for zoonotic and non-zoonotic are shown in Figure 3. The overall and geographical representation of these pathogens, as well as the main conclusions reached by the articles reviewed on public health, species conservation and the global economy, are discussed below in detail.

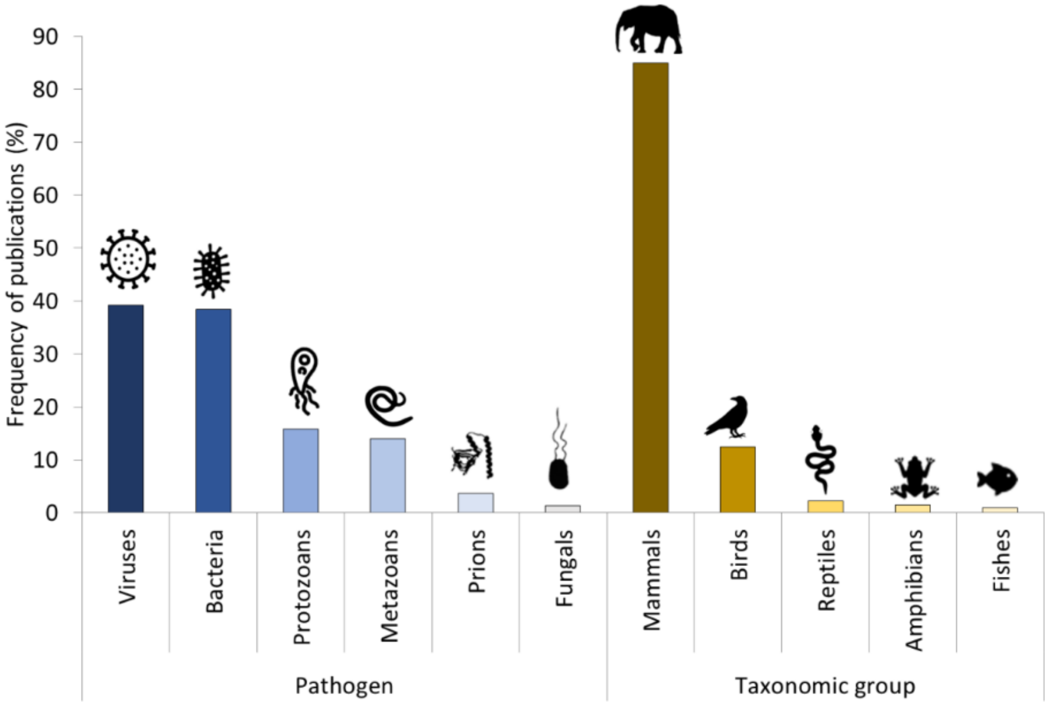


Figure 2. Frequency of occurrence of pathogen agents and host groups in long-term studies (period 1993-2017; see also Table 1).

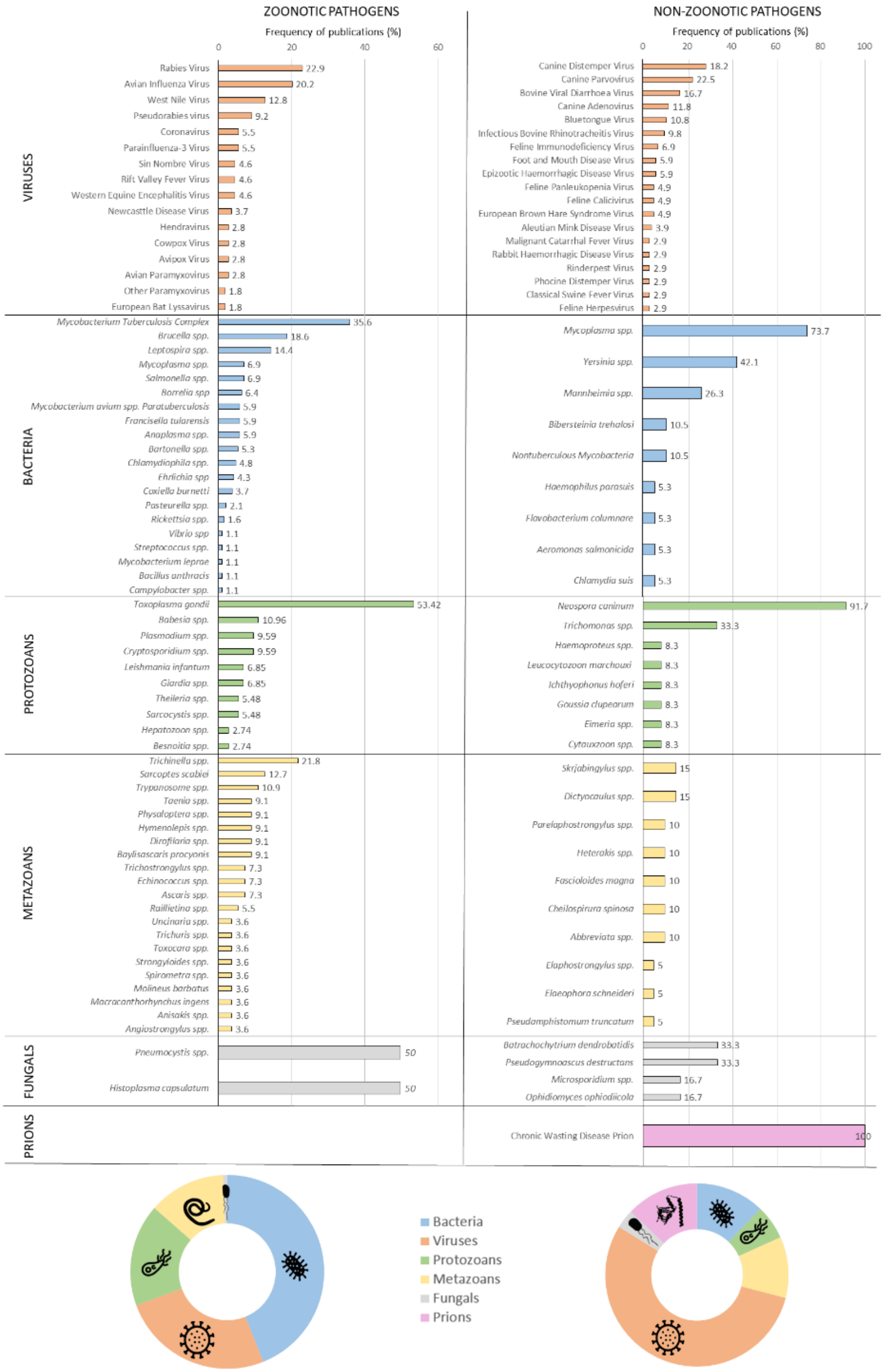


Figure 3. Frequency of publication grouped by pathogen taxa, showing the numbers for zoonotic and non-zoonotic pathogens separately, addressed by long-term studies (1993-2017). The proportion of each group of pathogens is indicated in each column (bottom). The pathogens for which controversy exists concerning their zoonotic potential have been included as non-zoonotic in the graph.

Concerning the wildlife-domestic interfaces addressed in the publications reviewed, there is a relatively low number of articles (n=67) in which the domestic side of the disease is mentioned (considering livestock and companion animals as "domestic animals"), compared to those addressing diseases potentially shared with domestic animals (n=456). Overall, these articles mentioning the domestic aspect focus on three main diseases: animal tuberculosis (TB; 34.3%), toxoplasmosis (10.4%) and rabies (9%). The most reported wildlife-domestic interfaces, as well as the most studied diseases at these interfaces, are shown in Table 2. The ungulate- and carnivore-cattle interfaces (n=32 and n=19, respectively) were the most represented in the literature reviewed, agreeing with Wiethoelter et al. (2015), who included these two interfaces in the top three of the most studied wildlife-livestock interfaces. The concern about the ungulate-cattle interface (mainly focused on red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*)) derived from the spatial and/or temporal overlap between both species, especially in free-ranging systems of livestock breeding (Carrasco-Garcia et al., 2016). This, together with the constant increase exhibited by the populations of wild ungulates which have reached previously unrecorded levels (Massei et al., 2015; Milner et al., 2006), has encouraged the spread and maintenance of diseases shared with livestock such as TB, Aujezsky's disease or porcine circovirus type 2 (e.g. Gortázar et al., 2006).

Table 2. Top 4 of wildlife-domestic interfaces studied based on the total number of long-term studies from 1993 to 2017 which addressed the domestic aspect of the disease (n=67). Pathogens involved in each interface are also shown.

Wildlife-domestic interface	**Publications**	**Pathogens**
Ungulates-cattle	32	*Mycobacterium bovis*, Bovine Viral Diarrhea Virus and *Salmonella enterica*
Carnivores-cattle	19	*Mycobacterium bovis*, Rabies Virus and *Coxiella burnetti*
Carnivores-dog	12	Rabies Virus, Canine Distemper Virus and Canine Parvovirus
Carnivores-cat	10	*Toxoplasma gondii* and Rabies Virus

Mammals (appearing in the 84.9% of the publications), followed by birds (12.5%) and reptiles (2.2%) were the most studied hosts. However, amphibians (1.5%) and fish (0.9%) were hardly represented in the literature at all (Figure 2). The fact that ungulates were the most investigated group (40.0%) is probably due to their increasing abundances and wide distribution, together with the extensive knowledge of their behaviour, ecology, physiology and population dynamics. All these factors make them highly accessible for infectious disease ecology research and are used as wild model systems in a broad range of environments (Jolles and Ezenwa, 2015). The close phylogenetic relationship between livestock species and wild ungulates may also explain the high representativeness of ungulates in the literature. Carnivores were the next most studied group (31%), and mainly for conservation purposes, as detailed below.

The representation of host species in LTWDR is shown in Figure 4. Wild boar was the most represented host species (13.2% publications) followed by red deer (9.1%), which is in accordance with other similar reviews on wildlife diseases (Gortázar et al., 2016). Currently, wild boar have become relevant to the One-health context due to their role as a true reservoir host for pathogens shared among wildlife, livestock and humans (Fredriksson-Ahomaa, 2019; Ruiz-Fons et al., 2008b).

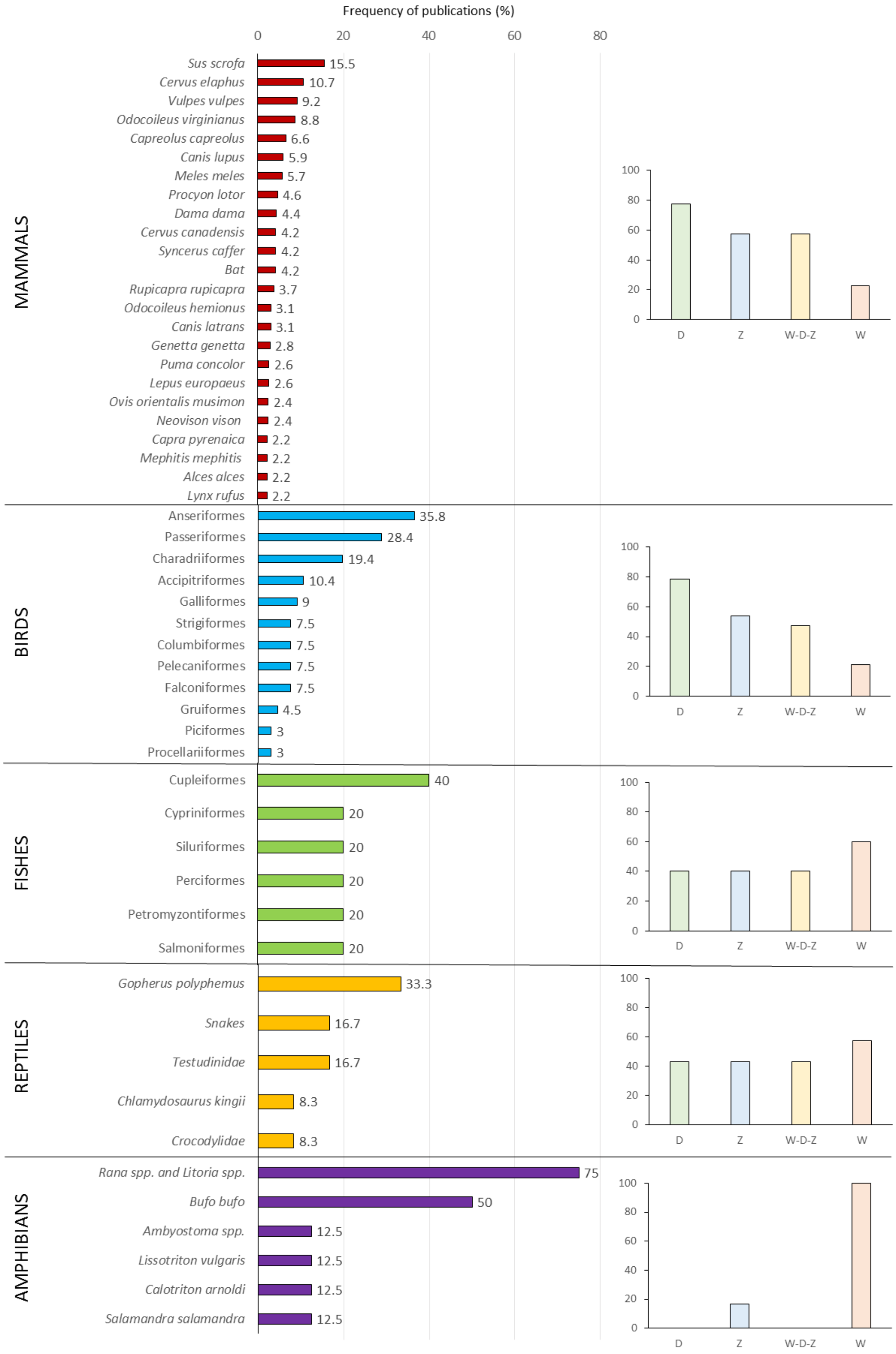


Figure 4. Frequency of publication grouped by host taxa (species or group of species) addressed by long-term studies (1993–2017). The percentage of zoonotic (Z), wildlife (W), shared among wildlife and domestic animals (D), and shared between wildlife, domestic animals and human (W-D-Z) pathogens studied within each taxa group are indicated in bar graphs.

Together, cervids constitute half of the most studied species, including red deer, white-tailed deer (*Odocoileus virginianus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and elk (*Cervus canadensis*), all of them typical of the northern hemisphere, like wild boar. Articles about these species are focused mainly on two diseases: chronic wasting disease (CWD) and TB. CWD is an emerging disease that causes severe declines in deer and elk populations in North America and may imply uncertain zoonotic risks associated with the consumption of game meat (Saunders et al., 2012). Because of the lack of knowledge about its long-term effects on cervid populations and its potential economic consequences, several long-term studies about CWD have emerged since 1995 (Spraker et al., 1997), with a special relevance from 2013 onwards (Evans et al., 2014; Magle et al., 2013; Russell et al., 2015).

The red fox (*Vulpes Vulpes*), wolf (*Canis lupus*) and badger (*Meles meles*) are the carnivore species most represented in this group, in agreement with Gortázar et al. (2016). Similarly, they are native to the northern hemisphere. Most long-term studies including red fox diseases focus on zoonotic parasite infections (49%), mainly by *Toxoplasma gondii* (20%). Previous studies have shown that the red fox harbours a wide range of pathogens relevant to domestic animals and humans (Lledó et al., 2020), it is recognized as an ideal sentinel species that is very useful to assess the evolution of an ecosystem's health (Aguirre, 2009; Lledó et al., 2020). Canine distemper virus (CDV) and canine parvovirus (CPV) are the most frequent pathogens analysed in long-term studies on wolves (44%), mainly motivated by an interest in conservation. Both CDV and CPV represent a severe threat to the conservation of carnivores, not only wolves, as they have been responsible for significant declines in the populations of other free-ranging carnivores (Loots et al., 2017; Packer et al., 1999; Young, 1994). The long-term impact of CPV and CDV on wildlife populations is poorly understood (Justice-Allen and Clement, 2019), which explains why articles on this species focused on adding knowledge about the long-term consequences of these infections in free-ranging wolf populations. Finally, badger is relevant as a maintenance host for TB, especially in the United Kingdom (Zuckerman, 1980). In fact, 62% of the articles on badgers were about TB, and some represent pioneer works of multidisciplinary long-term approaches (Delahay et al., 2013, 2000).

By the years 2000-2001 (Figure 1b), amphibian diseases had also started to receive attention. The concern about this taxonomic group may derive from the first

Batrachochytrium dendrobatidis outbreaks, first described in 1998-1999 (Berger et al., 1998; Nichols et al., 1998). This fungal pathogen is implicated in the global decline of amphibian populations and even in the extinction of some species (Stuart et al., 2004). Since 2010-2011, the range of species studied has increased, so that, for the first time, all taxonomic groups are represented in the LTWDR. Fish, and other mammal species such as some marsupials, for example koalas and the brushtail possum (*Trichosurus vulpecula*), or armadillos are also represented during these years.

Geography

LTWDR is, so far, mainly located in developed countries of the northern hemisphere; North America (42.2%; n=227) and Europe (34.2%; n=184). Within these continents, the USA (n=191), Spain (n=58), Canada (n=19) and Italy (n=18) are the countries with the most LTWDR. These kinds of studies were far less frequent in Africa, Oceania, South America and Asia which, all together, represent less than 25% (n=127) of the total publications reviewed (Figure 5). These findings are in agreement with previous reviews on wildlife diseases but not focused on long-term studies (Bliziotis et al., 2005; Vergidis et al., 2005; Wiethoelter et al., 2015). In developed areas, there may be a significant awareness about shared infections and their impact on public health and, consequently, on the economy. Additionally, these countries possess high-quality infrastructures and better financial resources to support research activities, making it possible to integrate investigation with wildlife management (Morner et al., 2002). However, underdeveloped and developing countries only finance such research to preserve the economic viability of a livestock production system, as part of their economy (Morner et al., 2002). In developing countries, the first publications on this issue date from 1998-2000 (Figure 6), and even from 2006-2007 in South America. Exceptionally, in Africa, there have been studies since 1993, probably due to the approach of collaborative research concerning the role of wildlife in zoonotic diseases in African wildlife with scientists coming from developed countries (Western, 2003).

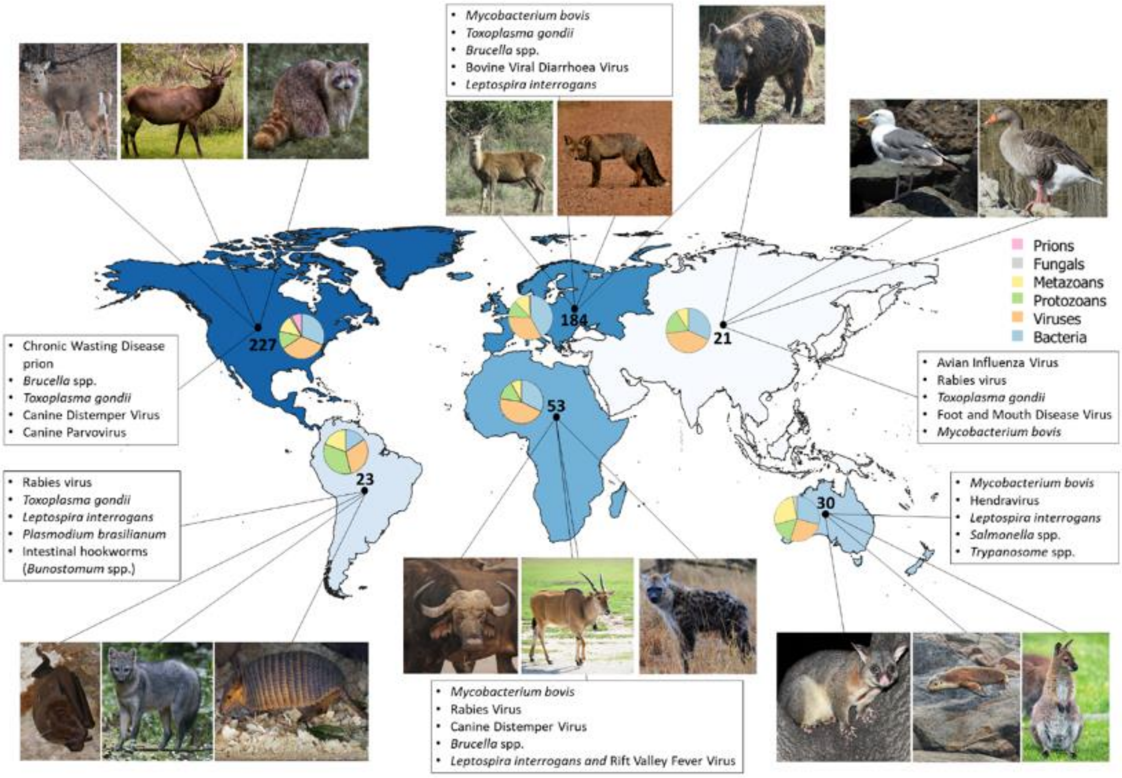


Figure 5. Most frequent pathogen agents and host species addressed by long-term studies (period 1993-2017) per continent. The research productivity per continent (number of publications) and the frequency of each pathogen group (bacteria, viruses, protozoans, metazoans, fungal and prions) per continent are also shown.

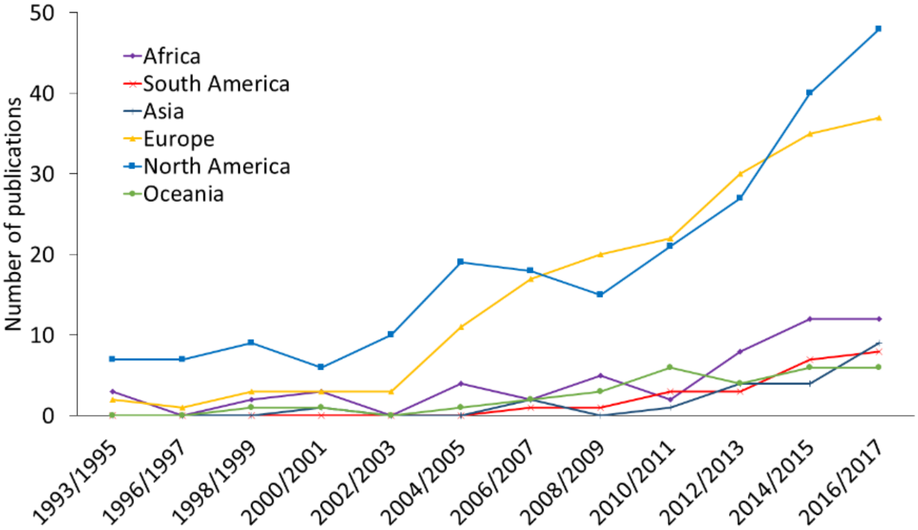


Figure 6. Temporal trend in the number of publications on long-term studies (period 1993-2017) per continent.

The largest proportion of the articles concerning North America, Europe, Africa and Asia addressed viruses, whereas in Oceania and South America bacteria, together with viruses and protozoan parasites, are, respectively, the most frequently studied pathogens (Figure 5). This concurs with the findings of previous similar studies on infectious diseases at the wildlife-livestock interface in which the representativeness of each pathogenic group per continent was analysed (Wiethoelter et al., 2015). However, in South America and Europe, parasites (metazoans and protozoans) and bacteria were the most represented pathogens, instead of viruses. The relevance of prions in North America (8%) compared to the remaining continents is remarkable (<1%), owing to CWD, a prion disease which is a major concern for the management of North American cervid populations (Miller and Williams, 2004; Williams et al., 2002). The rankings of the most frequently studied pathogens and host species by continents are shown in Figure 6. Overall, ungulates and carnivores were the most studied taxonomic groups on the majority of the continents, in agreement with Wiethoelther et al. (2015). More specifically, white-tailed deer (n=39), elk (n=19) and the raccoon (*Procyon lotor*; n=17) were the species on which North America focused long-term research. The CWD pathogen agent (n=19), *Brucella* spp. (n=19), *T. gondii* (n=16), CDV (n=15), and CPV (n=14) were the most studied pathogens.

Among the few long-term South American studies, most were centred on bat (n=4), crab-eating fox (*Cerdocyon thous*; n=3) and large hairy armadillo (*Chaetophractus villosus*; n=2) diseases, and the most reported pathogens in LTWDR were rabies virus (RV; n=3), *T. gondii* (n=3), *Leptospira interrogans* (n=2), *Plasmodium brasilianum* (n=2) and intestinal hookworms (*Bunostomum* spp.; n=2).

With respect to Europe, wild boar (n=57), red deer (n=45) and red fox (n=30), were the most studied as well as the pathogens *Mycobacterium bovis* (n=40), *T. gondii* (n=11), *Brucella* spp. (n=11), bovine viral diarrhoea virus (BVDV) (n=11) and *L. interrogans* (n=9).

In Africa, African buffalo (*Syncerus caffer*; n=16), eland (*Taurotragus oryx*; n=8) and spotted hyena (*Crocuta crocuta*; n=7) predominate as hosts in the long-term studies. In the same way, *M. bovis* (n=6), RV (n=6), CDV (n=6), *Brucella* spp. (n=5) and *L. interrogans* (n=5), as well as Rift Valley Fever Virus (RVFV, n=5) were the pathogens most frequently cited.

In relation to Asia, wild boar (n=5), gulls (*Larus* spp.; n=3) and goose (*Anser* spp.; n=3) were the species on which most long-term articles focused. Likewise, AIV (n=2), RV (n=2), *T. gondii* (n=2), foot and mouth disease virus (FMDV; n=2) and *M. bovis* (n=2) were the pathogens most reported in Asia. The limited number of articles involving long-term studies addressing the epidemiological role of wildlife species, such as bats, as reservoirs of Coronaviruses (Afelt et al., 2018; August et al., 2012; Tao et al., 2017) is noteworthy. Bats, but also birds (relevant for potential pandemics such as avian influenza) are highly biodiverse and widespread, which makes them an important source of new viruses for interspecies jumping (Chan et al., 2013). They both reach human and livestock environments (and vice versa), and they have a significant potential for pathogen spread because of their migratory habits. As different viruses may mix in these reservoirs, subsequent asymptomatic shedding and dissemination of novel mutant, recombinant, or reassortant RNA viruses may infect other host species, including humans, directly or via amplifying hosts (Hon et al., 2008).

The most reported pathogens in Oceania were *M. bovis* (n=4), Hendravirus (HeV) (n=2), *L. interrogans* (n=2), *Salmonella* spp. (n=2) and *Trypanosome* spp. (n=2). The exotic brushtail possum (n=4), Australian sea lion (*Neophoca cinerea*) (n=3) and rock wallaby (*Petrogale* spp.; n=2) were the species most represented in the literature. The most investigated pathogen is *M. bovis* because of its wide distribution and the high prevalence of TB in New Zealand (De Lisle et al., 2008; Mulholland et al., 2017; Nugent et al., 2012).

Study period and type of conclusions reached

The number of publications selected decreased as the duration of the study increased (146 for a study period of four years but only 35 over 20 years; see Figure 7a). The duration of the projects on wildlife diseases and therefore, the continuity of this type of study is conditioned by factors such as infrastructures, staff, time, etc. and is usually further limited by budgetary constraints (Gordo and Avilés, 2017), and the absence of a long-term perspective on the part of the policy-makers for science.

In almost all the long-term studies reviewed (98.7%; Figure 7b), scientists reached conclusions on the consequences of the pathogens on the individuals as well as on the population dynamics, their regulatory factors and were also able to determine whether the disease was spreading. This was possible because long-term studies provided essential information about the basic parameters of the epidemiology of the infection, including behavioural and ecosystem related factors (Rhyan and Spraker, 2010). 44.2% of the articles proposed management measures for the control of wildlife infections (Figure 7b). These measures may be more accurate as they are implemented in systems that were previously characterized by long-term studies. However, scientists and managers are usually forced to establish measures based on conclusions extrapolated from other systems. In these cases, adaptive management is a realistic approach that requires wildlife disease monitoring and may contribute to very valuable long-term data.

In 41.1% of the articles, the researchers included conclusions on the economic consequences derived from the impact of the disease on livestock and prevention and control costs (Figure 7b). Our results suggest that the economic effects of wildlife diseases can be better estimated through long-term studies (Cunningham et al., 2017). It is worth stating that the economic impact of wildlife diseases, which was often neglected, has now become relevant to the global economy (Daszak et al., 2000), and supposes a threat to wildlife resources, including game and endangered species (Cleaveland et al., 2001; Daszak et al., 2000).

A smaller proportion of the articles reviewed (16.9%) included the consequences of the diseases on the conservation of the target species or other threatened species with which they could cohabit in the conclusions (Figure 7b). Infectious diseases have been recognized as an increasing threat to wildlife conservation (the so-called “biodiversity diseases”, (Grogan et al., 2014), leading to decreasing trends in wildlife populations. Long-term studies allow us to reach conclusions on the conservation status of host species and to propose conservation measures. A more profound understanding of the impact of diseases on conservation and economics is a key aspect to developing efficient management programmes.

Finally, only 26.2% of the articles included the effects of the wildlife disease on human health, even though 65.4% of the articles investigated at least one zoonotic disease, which indicates a deficit of One Health approaches to address the causes, consequences and management of wildlife diseases.

The ability to reach conclusions concerning the pathogen consequences concerning the individual (in terms of its effect on energy balance, reproduction, survival, mortality, etc.) and in the population dynamics (regulatory or destabilizing role of pathogens) was significant and positively associated with the length of the study period (*F*=4.6, O.R.±S.E.=7.3±2.6, df=323, *p*=0.03, and *F*=4.2, O.R.±S.E.=6±2.4, df=323, *p*=0.04, respectively). This was similar concerning the interactions between the pathogen and regulatory factors of wildlife populations (specifically predation) and was marginally positively associated with the length of the study period (O.R.±S.E.=37.6±7.3).

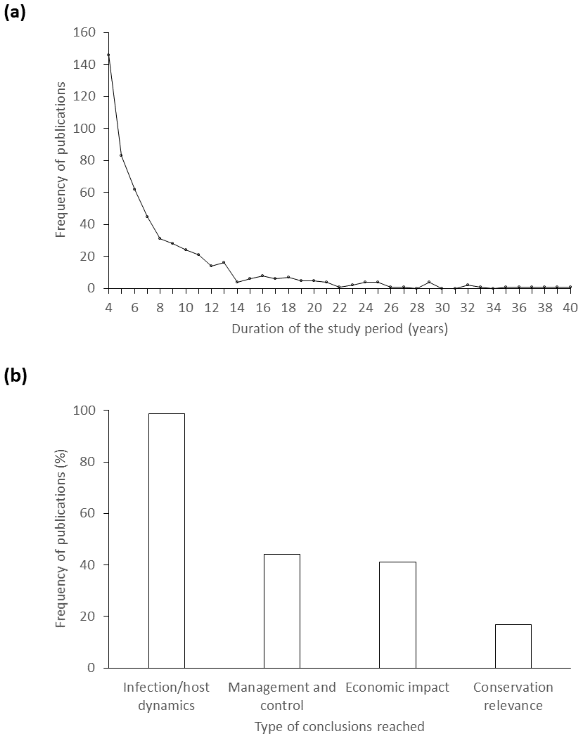


Figure 7. (**a**) Number of publications in relation to the duration of the study period (**b**) Frequency of publications in which the different type of conclusions registered was reached.

Epidemiological systems are dynamic and change on a multitude of spatial and temporal scales. Obviously, each additional year of research data helps us to better understand how epidemiological and ecological systems respond to change, such as environment-related changes. We are aware that four years are insufficient to characterize the trends and/or effects of factors operating over larger periods, such as in chronic infections or when time-delayed effects arise. However, the fact that we took into consideration the length of the study period for descriptive and analytical purposes contributes to demonstrating what added value the length of study provides.

Conclusions and future perspectives

Definitively, the concept of LTWR is context-dependent and needs to consider the time scale during which the main drivers of infection operate. A proposal of a definition of

LTWDR is made in the present review as a result of our evaluation of available research. While it is context dependent, LTWDR is long enough to:

i. Address ecological and epidemiological questions that cannot be resolved with short-term observations or experiments. LTWDR provides an understanding of how epidemiological systems respond to individual, population and environmental changes. It can also be integrated with research on short-term dynamics. In this review, we have noted that most of the articles did not address or were unable to address some specific questions.
ii. Begin to separate the effects of each driver. LTWDR describes and evaluates the temporal patterns of main drivers, indicating the temporal scale at which we must approach the study to visualize changes. Not only seasonal but also at least annual effects after several temporal replicas are recorded. LTWDR is long enough to be integrated with other long-term environmental research and monitoring.
iii. Provide information about the causes and consequences of factors such as human-driven, climate and environmental changes that could not otherwise be elucidated. Specifically, LTWDR makes it possible to investigate the complex processes of epidemiological systems in a context of global change. As a result, LTWDR not only contributes to a better understanding of processes and the advance of science but to new solutions in a context of unprecedented environmental change and emergence of pathogens. Furthermore, its results can, potentially, help inform local- to national-scale decisions related to disease emergence and other related challenges. For instance, LTWDR can help to design early warning systems for diseases on the base of retrospective investigations.

Optimizing long-term research and monitoring are essential to give continuity and expand LTWDR as far as possible. For that reason, a relevant challenge is developing reliable pathogen and host assessments with limited resources. We need to: i) meticulously design surveys capable of determining the efforts required to detect pathogens and their changes reliably; and ii) implement more efficient tools (e.g. environmental detection of pathogens) permitting the extension of the spatio-temporal range of surveillance.

Future studies should compare the added value obtained from long- and short-term studies according to the different typology of the taxa and pathogen cycles in order to assess their respective merits.

CAPÍTULO 2. Factores determinantes a largo plazo de la epidemiología de infecciones compartidas en la comunidad hospedadora de ungulados del Parque Nacional de Doñana

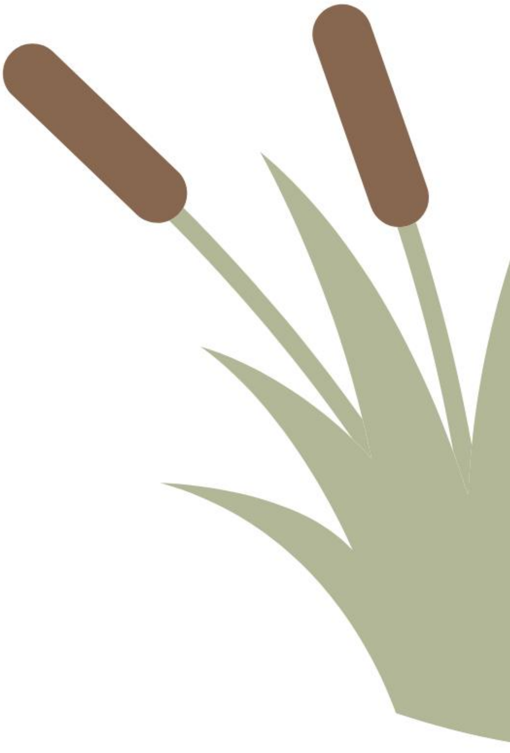
CHAPTER 2. Long-term determinants of shared infections in the ungulate host community of Doñana National Park

2.1. Factores determinantes a largo plazo de la tuberculosis en la comunidad hospedadora de ungulados del Parque Nacional de Doñana

2.2. Factores determinantes a largo plazo de la seroprevalencia de *Toxoplasma gondii* en una comunidad de ungulados silvestres

2.3. Factores determinantes a largo plazo de la seroprevalencia del virus de la lengua azul en especies de cérvidos del sur de España

2.4. Factores determinantes a largo plazo de la seroprevalencia de hepatitis E en el jabalí (*Sus scrofa*)



CAPÍTULO 2.1. Factores determinantes a largo plazo de la tuberculosis en la comunidad hospedadora de ungulados del Parque Nacional de Doñana

CHAPTER 2.1. Long-term determinants of tuberculosis in the ungulate host community of Doñana National Park

Barroso, P., Barasona, J.A., Acevedo, P., Palencia, P., Carro, F., Negro, J.J., Torres, M.J., Gortázar, C., Soriguer, R.C., Vicente, J., 2020. Long-term determinants of tuberculosis in the ungulate host community of Doñana National Park. *Pathogens* 9, 445. https://doi.org/10.3390/pathogens9060445

Resumen

La tuberculosis animal (TB) es endémica en el jabalí (*Sus scrofa*), el ciervo (*Cervus elaphus*), el gamo (*Dama dama*) y el ganado bovino en el sur y el centro de España. El objetivo de este estudio fue determinar qué procesos de los que operan a largo y medio plazo participaron en su epidemiología. Para ello, se estudió la TB en la interfaz doméstico-silvestre en el Parque Nacional de Doñana durante un periodo de 13 años (2005-2018), considerando factores poblacionales, estocásticos y ambientales (p. ej. puntos de agregación como los puntos de agua). El jabalí mostró las tasas de prevalencia más elevadas (76,7%), seguido del ciervo (42,5%), del gamo (14,4%) y del ganado bovino (10,7%). En este estudio, se evidenció la participación de procesos epidemiológicos que actuaron a medio y largo plazo y que interaccionaron con la ecología de los hospedadores y de la comunidad de hospedadores. El efecto de la sobreabundancia del jabalí sobre sus elevadas prevalencias de TB estuvo mediado por las hembras, las cuales pudieron determinar a las altas incidencias halladas en individuos juveniles en grupos maternales. La precipitación se asoció significativamente con un mayor riesgo de TB en machos de ciervo, probablemente mediado por diferencias intersexuales en los rasgos de la historia de la vida, los cuales determinaron una mayor susceptibilidad y/o exposición de éstos en comparación con las hembras. La prevalencia de TB en gamos se relacionó directamente con la del ganado bovino, lo que indicó una transmisión interespecífica significativa de la infección en ambas direcciones y/o una exposición similar a los factores de riesgo producida por las interacciones entre ambas especies. La identificación de los factores determinantes a largo plazo de la TB evidenció que su control en sistemas ganaderos en extensivo únicamente podría alcanzarse teniendo en cuenta a todos los hospedadores relevantes para su epidemiología e integrando medidas relacionadas con todos los factores implicados, como la abundancia poblacional y la agregación de ungulados domésticos y silvestres, la exposición ambiental a micobacterias, la realización de pruebas diagnósticas y campañas de saneamiento, así como el ajuste de las densidades de ungulados a cifras adecuadas.

Abstract

Animal tuberculosis (TB) is endemic in wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and cattle in south and central Spain. In order to clarify the processes that operate in the medium and long-term, we studied TB at the wildlife-livestock interface in Doñana National Park for 13 years (2005-2018) in relation to host density, stochastic factors (rainfall) and environmental features (e.g. aggregation points such as waterholes). Wild boar showed the highest prevalence of TB (76.7%), followed by red deer (42.5%), fallow deer (14.4%) and cattle (10.7%). We found evidence of relevant epidemiological processes which operate over the long-term and interact with host and community ecology. Interestingly, the effect of high wild boar population density on increased TB rates was mediated by sows, which could determine high incidence in young individuals already in maternal groups. Rainfall significantly determined a higher risk of TB in male red deer, probably mediated by sex-related differences in life history traits that determined more susceptibility and/or exposure in comparison to females. The positive association between the prevalence of TB in fallow deer and cattle may indicate significant inter-species transmission (in either direction) and/or similar exposure to risk factors mediated by ecological overlapping of grazing species. The identification of long-term drivers of TB provided evidence that its control in extensive pastoral systems can only be achieved by targeting all relevant hosts and integrating measures related to all the factors involved, such as population abundance and the aggregation of wild and domestic ungulates, environmental exposure to mycobacteria, cattle testing and culling campaigns, and adjustments of appropriate densities.

Introduction

A central problem concerning studies on the ecology of wildlife disease is that many of the most important ecological, evolutionary and human-driven processes affecting host and pathogens may occur over multiple years or even decades. Many important questions, particularly for those pathogens maintained in complex host communities (Barasona et al., 2019) can only be answered with data that extend over many years (McDonald et al., 2017; Strayer et al., 1986). Long-term studies, therefore, provide the necessary temporal perspective to understand processes that operate over wide temporal scales (Vicente et al., 2013). Some advantages of long-term approaches in wildlife disease epidemiology are the possibility of carrying out: i) analyses of density-dependent effects; ii) analyses of stochastic factors; iii) evaluations of time-delayed effects, including effects within/among species; iv) studies for detecting disease emergence patterns; v) evaluation of epidemiological processes on multiple scales, from individual to population; vi) analyses for detailing the role that social systems and between- and within-individual heterogeneity in infection transmission; vii) studies that provide valuable information applicable to the management of sanitary and socio-economic problems; and viii) assessments of risk management strategies, particularly, adaptive strategies (Delahay et al., 2013; McDonald et al., 2017; Molina-López et al., 2011). However, despite the relevance of wildlife diseases to human and animal health, livestock productivity and conservation, long-term field studies of free-living wildlife hosts are relatively rare, and particularly, in multi-host systems taking place at the interface with livestock (Delahay et al., 2013; McDonald et al., 2017; Vicente et al., 2007a).

Animal tuberculosis (TB caused by *Mycobacterium tuberculosis* complex bacteria, MTC, including *M. bovis* and *M. caprae*) is a chronic infectious disease with a complex multi-host epidemiology, infecting many wildlife and livestock species (Gortázar et al., 2012). It also supposes a zoonotic risk to humans, with relevance for public health. Mycobacteria have a long persistence in the environment, especially in humid and shady sites (Humblet et al., 2009). The infection spreads through direct and indirect routes, the latter being key for the inter-species transmission (Cowie et al., 2016; Kukielka et al., 2013). Indirect transmission usually occurs through shared resources such as water or food (Barasona et al., 2017b; Caron et al., 2003). TB is endemic in cattle and wild ungulates in south and central Spain (SCS) (Aranaz et al., 2004; Vicente et al., 2013). Previous research concluded that ungulates, mainly Eurasian wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*), are the wild reservoir hosts of TB in this area (Martín-Hernando et al., 2007; Vicente et al., 2013, 2006). They can maintain and transmit the pathogen, even in the absence of other reservoir hosts (Naranjo et al., 2008; Vicente et al., 2013, 2006). The richness of ungulate host species, including cattle, correlates with increased community competence to maintain and transmit pathogens of the MTC in game-managed and wild areas in Mediterranean Spain (Barasona et al., 2019).

In Doñana National Park (DNP, south-west Spain), the wild ungulate community (including wild boar, red deer and fallow deer, *Dama dama*) sympatrically occurs with free-ranging cattle (Barasona et al., 2014a). The prevalence rates of TB observed in DNP are among the highest ones reported in the literature, especially in wild boar (Gortázar et al., 2011). Since the first case of TB was diagnosed (León-Vizcaíno, 1990), MTC has been isolated in Iberian Lynx (*Lynx pardinus*), red fox (*Vulpes vulpes*) (Briones et al., 2000; Perez et al., 2001), red deer, fallow deer and wild boar (Barasona et al., 2014a, 2014b; Gortázar et al., 2011; Gortázar et al., 2008; Romero et al., 2008), just as in cattle. There is also serological evidence of infection in badgers (*Meles meles*) (Martín-Atance et al., 2006). Our research team has conducted studies on the prevalence of TB and infection routes in the wild ungulate community of DNP since 2006. Between 2006 and 2012, a sample of 570 wild boar, 190 red deer and 189 fallow deer was analysed, describing high prevalences (Barasona et al., 2014a, 2014b; Gortázar et al., 2011; Gortázar et al., 2008). Further samples were collected up to 2018, and there has been an attempt to test for MTC twice a year through the National Eradication Programme that removes all positive individuals (MAPA, 2021a). Research in DNP has provided an essential understanding of the epidemiology and ecology of TB in this diverse host community and such a complex environment (Barasona et al., 2014a, 2014b; Gortázar et al., 2011; Gortázar et al., 2008; Romero et al., 2008). Some of the previous findings regarding the spatial ecology of interactions at the wildlife-livestock interface (Barasona et al., 2014a, 2014b; Gortázar et al., 2011; Gortázar et al., 2008; Romero et al., 2008), the population dynamics and the molecular epidemiology (Aranaz et al., 2004; Gortázar et al., 2008; Romero et al., 2008) are noteworthy. Research in DNP has also provided evidence of the relevance of some populational and environmental factors in the transmission and maintenance of MTC (Barasona et al., 2017a). However, these studies were carried out for relatively brief periods. Hence, a long-term perspective at the population level has been required to assess the impact on the TB epidemiology of these factors as well as other possible ones which operate on broader temporal scales, providing the basis for investigations on the role and factors determining pathogen rates, persistence and spread.

In this context, using data on ungulate surveillance in DNP for 13 years our aims were i) to evaluate the factors (individual, populational and environmental) modulating the prevalence of TB, and ii) to assess factors operating in the long-term (density-dependent and stochastic) in order to explain the temporal trend of the prevalence of TB in the host community of DNP from 2005 to 2018.

Materials and Methods

Study area

This study was performed in DNP, in southwestern Spain (37°09 N, 6°309 W), covering an area of 54,252 ha on the Atlantic coast. DNP is one of the most important natural reserves in Europe in terms of biodiversity, and it was declared a United Nations Educational, Scientific and Cultural Organization (UNESCO) Biosphere Reserve in 1980, expanding its boundaries in 2012. The eastern part of the Park is taken up by seasonal marshland, whereas in the western part scrublands predominate in the north and sand dunes in the south (see Figure 1 and Barasona et al. (2014a) for a more detailed description). There is an ecotone (a narrow strip of pastureland locally called “vera”) of high ecological richness between the marshland and scrubland. This area of DNP has a dry subhumid Mediterranean climate with marked seasonality. Mean annual precipitation is 550 mm (170 to 1000 mm) (Díaz-Delgado et al., 2016). In the wet season (winter and spring) the marshlands may flood, but the ungulates can browse in some uncovered scrublands. Late summer conditions and their prolongation during autumn are the hardest season for ungulates because of limited resources. The seasonal drought causes the aggregation of wild and domestic ungulates on the ecotone and around water sources in these seasons (Barasona et al., 2014a).

Human access to the Park is restricted and it is managed by the competent conservation authorities in, the Autonomous Government of Andalusia (Gortázar et al., 2008). Cattle and horse breeding are allowed inside the Park (Fernández-Delgado, 1997). Livestock populations include autochthonous and traditional breeds of cattle and horses such as the endangered breed “Marismeña”. Furthermore, horse breeding, aimed at promoting the recovery of “Caballos de las Retuertas”, occurs in some areas of Doñana National Park. The territory of the DNP included in this study is divided into five livestock management areas: Coto del Rey (CR), Sotos (SO), Doñana Biological Reserve (RBD), Puntal (PU) and Marismillas (MA). Livestock is distributed through all the Park, except CR. In this area, located in the north of the DNP, cattle husbandry has not been allowed since 2002 as a conservation measure for the critically endangered Iberian lynx. Culling of the wild ungulate population is performed exclusively by Park rangers as part of the Park management scheme, and it is also used to carry out a health-monitoring programme (Boadella et al., 2012b; Gortázar et al., 2008).

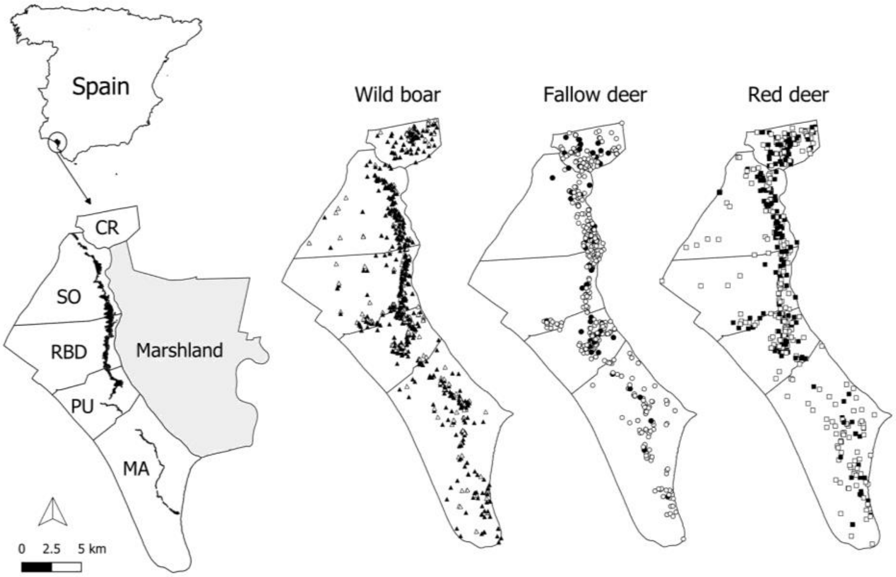


Figure 1. Map of the study area, Doñana National Park. The sampling areas (livestock management units: Coto del Rey (CR), Sotos (SO), Doñana Biological Reserva (RBD), Puntal (PU) and Marismillas (MA) are delimited, and the ecotone is displayed by a dark band. Sampled wild boar (triangles), fallow deer (circles) and red deer (squares) are shown. Black symbols mean animals positive for tuberculosis-like lesions.

Animal sampling and data collection

From 2006 to 2018, 2,594 ungulates including red deer (n=642), fallow deer (n=637) and wild boar (n=1235) were randomly (sex, age class and health status were not selected) in autumn–early winter (October–January) shot by Park rangers along with car drivers, and sampled as part of the DNP health-monitoring programme (approved by the Research Commission of DNP in accordance with the management rules established by the

Autonomous Government of Andalusia. The geographical coordinates of the animals sampled were registered by portable GPS (Garmin Ltd., Olathe, KS, USA) (Figure 1). Table S1 displays the sample size by species, livestock management area and sampling period, as well as the seroprevalences found.

The data collected included the sampling season (from 2006–2007 to 2018–2019), species, sex and age, which was determined by tooth eruption patterns (Mitchell, 2017; Saenz de Buruaga et al., 2001; Sáez-Royuela et al., 1989). Therefore, deer species were classified into three age classes: calves (<1-year-old), juveniles (1-2 years) and adults (≥3 years). Regarding wild boar, the categories were: piglets (<6 months), juveniles (6-24 months) and adults (>2 years).

The sampling was performed according to European (EC Directive 86/609/EEC) and Spanish laws (MAPA, 2005, 1988), current guidelines for the ethical use of animals in research (ASAB, 2012), the Animal Experiment Committee of Castilla-La Mancha University and the Spanish Ethics Committee (PR-2015-03-08). Necropsies and sample collection were undertaken in the field by authorized veterinarians. The presence of tuberculosis-like lesions was assessed and recorded by macroscopic inspection of the head, thoracic and mesenteric lymph nodes as well as abdominal and thoracic organs in the laboratory (Vicente et al., 2006). This analysis routinely included retropharyngeal and submandibular lymph nodes and tonsils in the head, tracheobronchial and mediastinal lymph nodes in the lungs and thorax and mesenteric lymph nodes, kidneys, liver and spleen in the abdomen. Gross lesions in other locations were also recorded. Lymph nodes were dissected, sectioned and carefully examined for gross lesions. Animals with tuberculosis-like lesions (TBL) were classified as positive. When TBL are identified in at least two of the three anatomic locations analysed (head, thorax and abdomen) we considered the TBL as generalized, indicative of a more severe and evolved infection (Vicente et al., 2007b). Concerning cattle, we compiled the information about the skin tests campaign, in which single intradermal tuberculin test was conducted by veterinary authorities (19,869 tests) during the official TB control programme in DNP, which is performed annually. Through the positive rate (%) of the annual skin test campaign, we determine the annual TB incidence in the cattle herds (newly infected animals divided by the total number of animals examined annually at each sampling site, which usually corresponds to the total cattle stock of the area, limited by DNP regulations). Positive animals are culled each year and consequently, only newly infected animals are identified in each annual campaign. The annual prevalence rates for wildlife and the annual incidence of TB in cattle were estimated for each livestock management area (CR, SO, RBD, PU and MA).

For each sampling period, the information concerning rainfall was collected from the meteorology station located at RBD (EBD-CSIC, 2019). The previous season's rainfall was selected to be included in our models because of its potential relevance to population dynamics and effects on susceptibility or exposure to many pathogens in Mediterranean environments (Rodriguez-Hidalgo et al., 2010; Vicente et al., 2013). To assess the effect of environmental factors on the risk of TBL, several variables were selected due to their influence on ungulate behaviour, distribution and epidemiology in DNP and other areas from SCS (Barasona et al., 2014a; Braza and Alvarez, 1987; Vicente et al., 2013). We used the same environmental variables that were selected in the study by Barasona et al. (2014a). For that purpose, a grid of 100 x 100 m was created, generating territorial units in which we calculated: straight-line distance (m) to nearest water hole (DWAT); straight-line distance (m) to nearest marsh-shrub ecotone (DE); and proportional cover of dense scrub, low-clear shrubland, herbaceous grassland, woodland, bare land, and watercourse vegetation. This grid was merged with the exact location of each sampled animal using a point sampling tool with QGIS version 3.4.1 (QGIS Development Team, 2019). Landcover data was obtained from Andalusia Environmental Information (REDIAM, 2019).

A distance sampling methodology was used to monitor the population density of ungulates. Specifically, we sampled seven line transects of 10-15 km distributed through the study area. For red deer and fallow deer, the surveys started two hours before sunset during September and were carried out from a vehicle (average speed was 10 $Km \cdot h^{-1}$). For wild boar, we repeated the transect one hour after the sunset in order to increase the sample size. During surveys, the distance from the observer to the animals was recorded with a telemeter. Distance Sampling 6.2 software was used to analyse the data (Thomas et al., 2010). Data were right-truncated when the probability of detection was lower than 0.15 (Buckland et al., 2001). Half-normal, uniform and hazard rate models for the detection function were fitted against the data using cosine, hermite polynomial, and simple polynomial adjustment terms fitted sequentially. The selection of the best model was based on Akaike's Information Criterion (AIC) (Akaike, 1974). Stratified analysis was done. We defined three strata, according to abundance and visibility of shrubland, marshland and the ecotone. The data of all the years (2006-2018) were considered to estimate a detection function for each stratum, and the data of each strata, year and livestock management area were considered to estimate the encounter rate and group size. Finally, a density value for each year and livestock management area was estimated.

Statistics

The presence of TBL per individual (as binomial response variable) was related to a range of explanatory variables by using generalized linear mixed models (GzLMMs). Collinearity between environmental variables was explored previously and principal component analysis (PCA) was performed to reduce the dimensions of the database in terms of environmental information. These variables were summarized in open habitats, in which open grasslands predominate, and watercourse vegetation.

Explanatory approach: in a first exploratory approach to study the statistical differences of the presence of TBL among livestock management areas (CR, SO, RBD, PU and MA), we designed GzLMMs (the presence of TBL as a response variable) for each species (red deer, fallow deer, wild boar and cattle) where the sex and age classes, DWAT, DE, open habitats and watercourse vegetation were the explanatory variables. The sampling period was fitted in the model as a random-effect factor.

While the first preliminary approach aimed to provide the statistical basis to differences in the presence of TBL among study areas, the main approach of this study is to generalize the effect of the factors studied regardless of the livestock management area. Therefore, the definitive models included livestock management area as a random factor. We then used the final models to evaluate the influence of the different determinants on the presence of TBL (as a response variable) separately for each species (red deer, fallow deer, wild boar and cattle). The explanatory variables were sex, age class, previous sampling period’s rainfall, population densities of wild ungulate and cattle, the prevalence of TBL in wild ungulates, DWAT, DE, open habitats and watercourse vegetation. The livestock management area and sampling period were fitted in the models as random effect factors. The selection of the “best models” was performed using the corrected AIC (cAIC) (Akaike, 1974). The assumptions of binomial GzLMMs were met in all the models (Zuur et al., 2009). We always used a binomial error and a logit link function. Significant *p*-value was set at 0.05. The predicted probability of TBL risk obtained from the models was used to represent results, which account for all factors in the models.

In order to explore temporal patterns, cross-correlations and autocorrelations with the real prevalence of TBL and the predicted probability of TBL in the different species studied were used. With this purpose, we also introduced the average densities of the previous two and three years in further models.

The statistical analyses were done using IBM SPSS 19.0 software (IBM Corporation, Somers, NY, USA; (George y Mallery, 2012) and R software version 3.5.2 (R Core Team, 2019). Confidence intervals for seroprevalences were estimated by the standard error (S.E. 95%), which expression was S.E. 95% = 1.96 * standard error.

Results

General

The prevalence of TBL (%±S.E. 95%) in wild boar was 76.7±2.5 (n=1235; average annual prevalence (%±S.E. 95%) of 77±8.5; n=14), followed by red deer (42.5±4.7; n=642; average annual prevalence of 42.3±7.3; n=14) and fallow deer (14.4±2.6; n=637; Figure 2a; average annual prevalence of 16.2±5.5; n=14), whereas the average annual incidence (±S.E. 95%) for cattle (based on the information about the skin tests campaign) was 10.7±1.8 (n=14). When considering TB-positive wild animals, fallow deer, red deer and wild boar presented comparable prevalence rates of generalized TBL (in at least two of the three anatomic locations studied), ranging from about 40-45% (46.7±10.5, 43.6±6 and 39.3±3.1, respectively). Considering age classes, overall, increasing age trends were indicated by the prevalence of TBL (Figure 2a, statistical results are shown below). The prevalence of generalized TBL (Figure 2b) showed an increasing age pattern in both deer species but apparently decreased with age in the wild boar. Spatially, contrasted prevalences of TBL were apparent among areas, showing a decreasing north to south gradient (Figure 2c; statistical results are presented below).

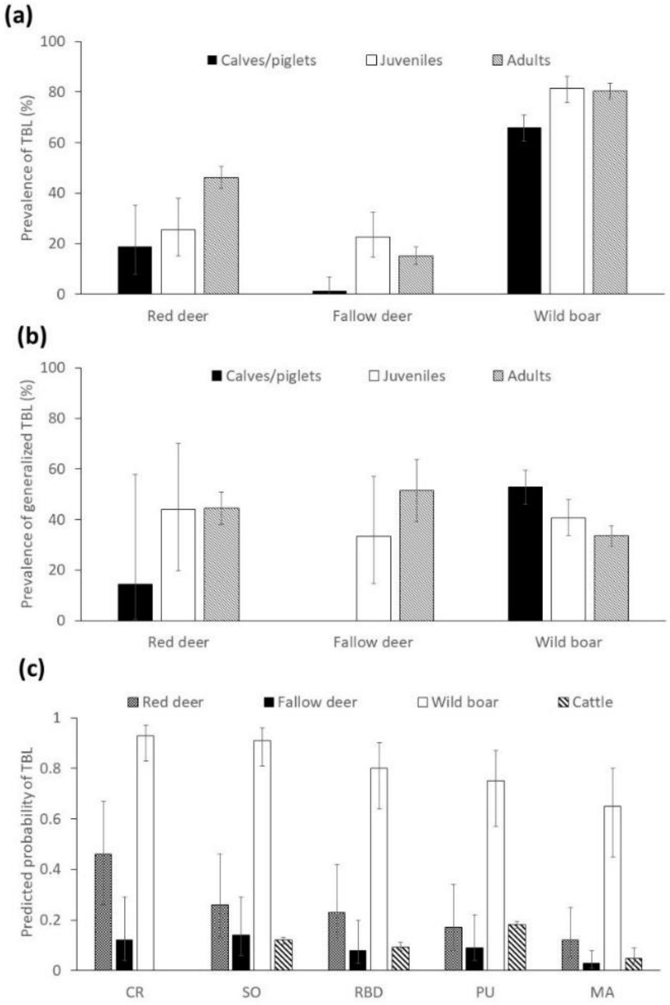


Figure 2. (a) Prevalence of tuberculosis-like lesions (TBL) (± standard error (S.E.) 95%) depending on age class in red deer, fallow deer and wild boar. (**b**) Prevalence of generalized TBL (±S.E. 95%) depending on age class in red deer, fallow deer and wild boar. (**c**) Predicted probability of TBL obtained from selected GzLMMs (± confidence interval (C.I.) 95%) for the respective species studied depending on the livestock management area, from north to south areas (see Figure 1 above for a map of the areas with full names).

As regards the temporal trends, Figure 3 and Figure 4 show the annual prevalence of TBL and prevalence of generalized TBL (%) and the population density (individuals/km^2) of wild ungulate species and cattle. The temporal trend of the rainfall (mm) is also displayed (Figure 5). Overall, the prevalence of TBL showed marked annual fluctuations, especially in wild ungulates. It was remarkable that the prevalence of TBL increased in red deer over the study period compared with the initial situation (sampling periods 2005 to 2007), and that a marked decline for two years was observed in wild boar in 2009/10-11/11 (although no data were available in the 2007-2008 sampling period) with a subsequent fast recovery to a very high prevalence. Since 2016-2017 a decline in the prevalence of TBL has been apparent in fallow deer and cattle, just as for the generalized presence of TBL in fallow deer and wild boar since 2015-2016 (Figure 5).

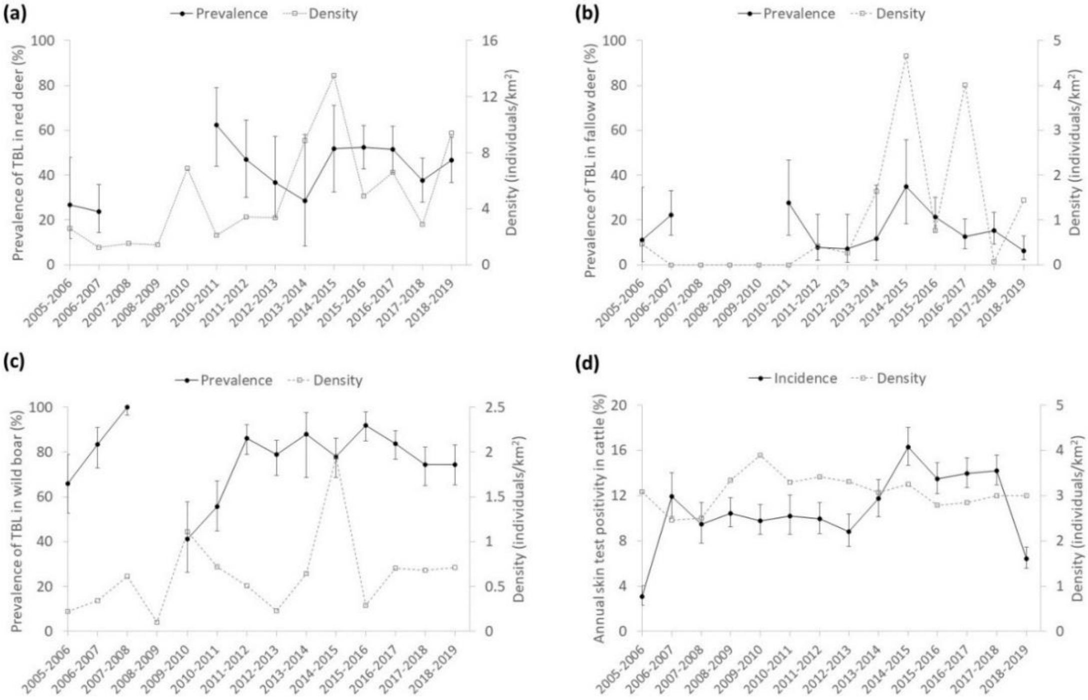


Figure 3. Temporal trend of the prevalence of tuberculosis-like lesions (TBL) (± standard error (S.E.) 95%) and population density (individuals/km^2) in (**a**) red deer, (**b**) fallow deer, (**c**) wild boar, and (**d**) annual skin test positivity (%) in cattle.

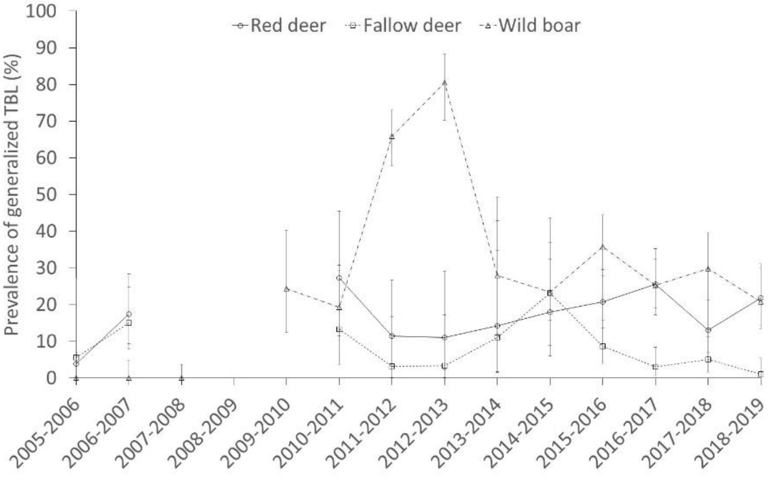


Figure 4. Temporal trend of the prevalence of generalized tuberculosis-like lesions (TBL) (± standard error (S.E.) 95%) in red deer, fallow deer and wild boar.

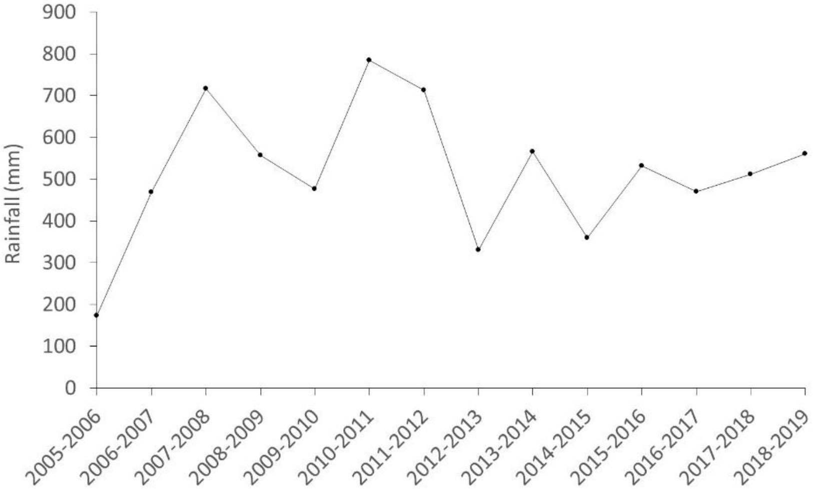


Figure 5. Temporal trend of the annual rainfall (mm).

Factors determining the presence of tuberculosis-like lesions)

We selected the full models since no differences <2 cAIC values were observed when considering other potential models during a backward stepwise model selection procedure. Concerning the exploratory GzLMM on the presence of TBL, we found statistical differences among livestock management areas for all species (wild boar, *F*=9.7, df=1211, *p*=<0.01; fallow deer, *F*=3.4, df=618, *p*=<0.01; red deer, *F*=7.1, df=624, *p*=<0.01; and cattle, *F*=23.05, df=34, *p*=<0.01). The presence of TBL decreased from north to south (Figure 2c). This pattern was more marked in red deer and wild boar compared to cattle and fallow deer.

Regarding the final GzLMMs on the presence of TBL, which incorporated other factors (previous season's rainfall and annual ungulate community densities and prevalences of TBL), and controlled for the livestock management area and sampling period as random effects, results are shown in Table 1, separately for each species.

Table 1. Test statistics from the GzLMMs for the presence of tuberculosis-like lesions (TBL) related to sex, age class, straight-line distance to nearest water hole (DWAT), straight-line distance to nearest marsh-shrub ecotone (DE), the proportion of open habitats and watercourse vegetation, previous season's rainfall (rainfall), the annual density of wild ungulates and cattle, annual prevalences of TBL in wild ungulates and some interactions among them.

Variables	**Wild boar**			**Fallow deer**			**Red deer**		
	***F* df (x,y)**	**Estimate ± S.E.**	***p***	***F* df (x,y)**	**Estimate ± S.E.**	***p***	***F* df (x,y)**	**Estimate ± S.E.**	***p***
Sex[1]	1.64 (1, 1199)	Female: 1.17±0.69	0.20	0.12 (1, 604)	Female: -0.30±1.88	0.73	1.64 (1, 612)	Female: 2.25±1.62	0.20
Age[2]	3.88 (2, 1199)	Juvenile: 2.79±1.32 Adult: 2.2±1.01	**0.02**	0.04 (2, 604)	Juvenile: 1.27±4.43 Adult: 1.47±4.25	0.96	0.15 (2, 612)	Juvenile: 2.24±2.85 Adult: 1.44±2.66	0.86
DWAT	3.66 (1, 1199)	<0.001±<0.001	**0.05**	0.51 (1, 604)	<0.001±<0.001	0.48	0.08 (1, 612)	<0.001±<0.001	0.78
DE	6.10 (1, 1199)	<0.001±<0.001	**0.01**	0.80 (1, 604)	<0.001±<0.001	0.37	5.24 (1, 612)	<0.001±<0.001	0.02
Open habitats	12.21 (1, 1199)	0.26±0.08	**<0.01**	0.61 (1, 604)	0.09±0.12	0.44	2.40 (1,612)	0.11±0.07	0.12
Watercourse vegetation	0.24 (1, 1199)	-0.04±0.08	0.63	1.68 (1, 604)	-0.15±0.11	0.20	0.09 (1, 612)	-0.03±0.1	0.76
Rainfall	0.23 (1, 1199)	<0.001±<0.001	0.63	0.09 (1, 604)	<0.001±0.01	0.77	0.09 (1, 612)	<0.001±<0.001	0.76
Density of red deer	0.04 (1, 1199)	-0.01±0.03	0.84	0.14 (1, 604)	-0.02±0.04	0.70	1.79 (1, 612)	-0.36±0.26	0.18
Density of fallow deer	0.34 (1, 1199)	0.04±0.06	0.56	0.26 (1, 604)	-0.24±0.57	0.61	0.02 (1, 612)	0.01±0.04	0.88
Density of wild boar	3.23 (1, 1199)	-1.24±0.68	0.07	0.26 (1, 604)	0.11±0.22	0.61	0.00 (1, 612)	<0.001±0.17	0.99
Prevalence of TBL in red deer	1.61 (1, 1199)	-0.01±0.01	0.20	0.05 (1, 604)	<0.001±<0.001	0.82	20.85 (1, 612)	0.05±0.03	**<0.01**
Prevalence of TBL in fallow deer	0.30 (1, 1199)	<0.001±0.01	0.59	7.03 (1, 604)	0.01±0.05	**<0.01**	0.00 (1, 612)	<0.001±0.01	0.97
Prevalence of TBL in wild boar	85.18 (1, 1199)	0.04±0.01	**<0.01**	0.00 (1, 604)	<0.001±0.01	0.98	0.04 (1, 612)	<0.001±0.01	0.84

Sex*Age	1.65 (2, 1199)	Female*Juvenile: -0.91±0.51 Female*Adult: -0.16±0.36	0.19	0.20 (2, 604)	Female*Juvenile: -0.11±1.43 Female*Adult: -0.51±1.31	0.82	0.75 (2, 612)	Female*Juvenile: -1.72±1.52 Female*Adult: -0.99±1.35	0.47
Rainfall*Sex	2.47 (1, 1199)	Rainfall*Female: <0.001±<0.001	0.12	0.29 (1,604)	Rainfall*Female: <0.001±<0.001	0.59	3.90 (1, 612)	Rainfall*Female: <0.001±<0.001	0.05
Density*Sex	0.06 (1, 1199)	Density*Female: -0.04±0.16	0.81	0.00 (1, 604)	Density*Female: <0.001±0.11	0.99	0.60 (1, 612)	Density*Female: 0.03±0.04	0.44
Rainfall*Age	1.67 (2, 1199)	Rainfall*Juvenile: <0.001±<0.001 Rainfall*Adult: <0.001±<0.001	0.19	0.04 (2, 604)	Rainfall*Juvenile: -<0.001±0.01 Rainfall*Adult: <0.001±0.01	0.96	0.67 (2, 612)	Rainfall*Juvenile: <0.001±0.01 Rainfall*Adult: <0.001±<0.001	0.51
Density*Age	0.03 (2, 1199)	Density*Juvenile: 0.03±0.26 Density*Adult: 0.05±0.18	0.97	0.00 (2, 604)	Density*Juvenile: 0.02±0.40 Density*Adult: 0.02±0.36	0.99	2.24 (2, 612)	Density*Juvenile: 0.12±0.27 Density*Adult: 0.33±0.23	0.11
Prevalence*Age	6.09 (2, 1199)	Prevalence*Juvenile: 0.04±0.01 Prevalence*Adult: 0.03±0.01	<**0.01**	1.91 (2, 604)	Prevalence*Juvenile: 0.11±0.05 Prevalence*Adult: 0.08±0.05	0.15	0.66 (2, 612)	Prevalence*Juvenile: 0.02±0.03 Prevalence*Adult: -0.01±<0.001	0.52
Rainfall*Density	2.58 (1, 1199)	<0.001±<0.001	0.11	0.23 (1, 604)	<0.001±<0.001	0.63	0.01 (1, 612)	<0.001±<0.001	0.91

The models were fitted using the sampling period and livestock management area as random effect factors. Parameter estimates for: ^{1}the sex variable were calculated using males as the reference, ^{2}the age variable were calculated using calves and piglets for red deer and wild boar, respectively, as the reference. "*" represents interactions among explanatory variables

Individual factors and inter-specific relationships

As for individual factors, the age class was statistically significant for wild boar (younger individuals presented less presence of TBL). It interacted with prevalence (see below), and sex significantly interacted with rainfall in red deer (see below).

Regarding the explanatory factor for the prevalence of TBL host species, which can be considered an intra-specific risk for TB transmission, species-specific TBL annual prevalence was always a positive significant risk for all species and, as mentioned above, it significantly interacted with age in wild boar, so that the effect was more evident in individuals younger than 1-year-old (Figure 6).

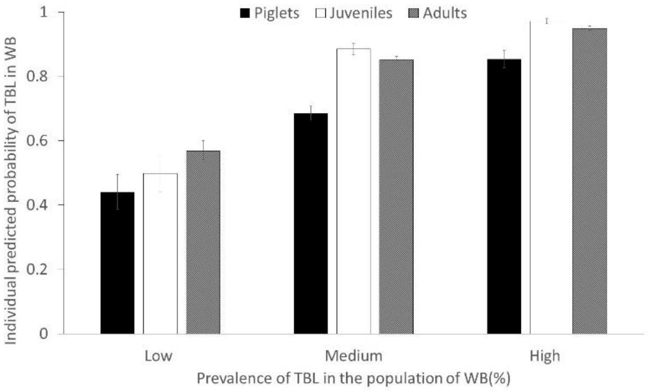


Figure 6. Predicted probability (± confidence interval (C.I.) 95%) of the presence of tuberculosis-like lesions (TBL) in wild boar depending on the interaction between prevalence rates and age class. The three categories of prevalence considered based on 33 and 66 percentiles are: low (⩽70%), medium (>70-⩽93.8%) and high (>93.8%).

Regarding inter-specific relationships, the prevalence of TBL in fallow deer was significantly (as explanatory) positively associated with the positivity of cattle to TB (as a response) ($F_{1,37}$=1.16, p=0.01; Figure 7).

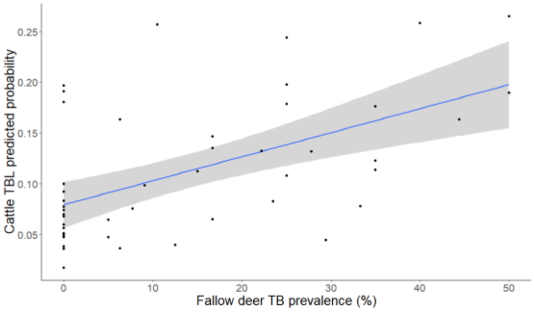


Figure 7. Predicted probability (± confidence interval (C.I.) 95%) of the positivity of cattle to TB depending on the prevalence of tuberculosis-like lesions (TBL) of fallow deer (%).

Environmental factors

In relation to habitat factors, most of the significant effects were detected in wild boar. The further the distance to water bodies, the higher presence of TBL (Figure 8a) was, whereas the closer to the ecotone (Figure 8b) and the higher availability of open habitats (Figure 8c), the higher the presence of TBL. Similarly, the proximity to ecotone corresponded to a statistically significant presence of TBL in red deer (Figure 8d).

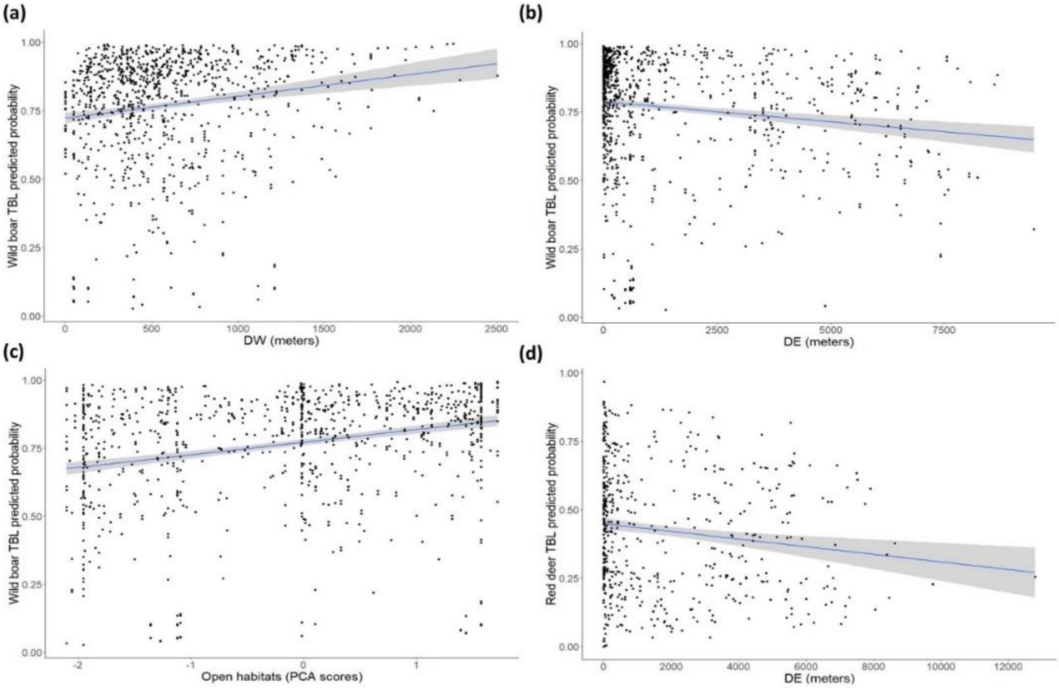


Figure 8. Predicted probability (± confidence interval (C.I.) 95%) of the presence of tuberculosis-like lesions (TBL) in (**a**) wild boar depending on the distance to water bodies (m), (**b**) wild boar depending on the distance to ecotone (m), (**c**) wild boar depending on the cover level of open habitats, measured according to the principal component analysis (PCA) scores from axis 1, and (**d**) red deer depending on the distance to ecotone (m).

Population factors

As for density-dependent factors, no direct effects were found; however, statistical effects were revealed for generalized TBL (see below).

Stochastic factors

As mentioned above, rainfall significantly interacted with sex to explain the presence of TBL in red deer, so that a high annual rainfall was specifically associated with a higher presence of TBL in males (Figure 9).

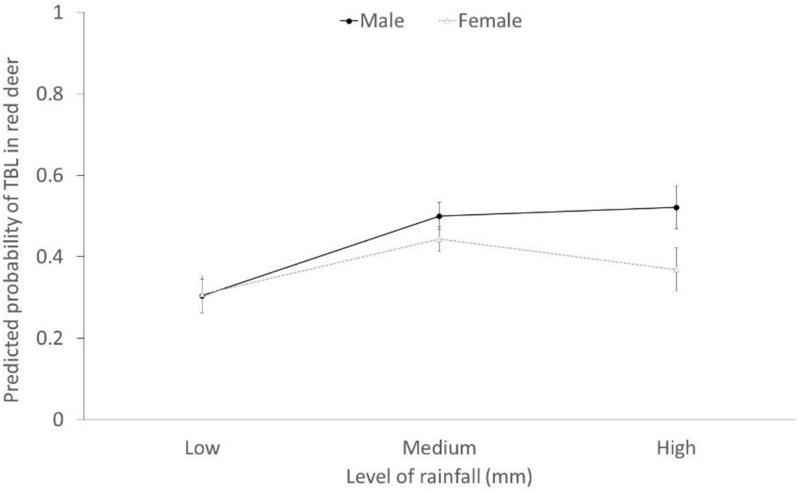


Figure 7. Predicted probability (± confidence interval (C.I.) 95%) of the presence of TBL in red deer depending on the interaction between sex and rainfall. The three categories of rainfall considered based on 33 and 66 percentiles are: low (⩽469.5 mm), medium (>469.5-⩽560.9 mm) and high (>560.9 mm).

Factors determining the presence of generalized tuberculosis-like lesions

Concerning the models on the individual presence of generalized TBL (Table 2), the annual specific prevalence of generalized TBL was positively associated in wild boar, fallow deer and red deer. The interaction between the density and sex was significant in wild boar and the positive association between high densities and the presence of generalized TBL in females was more evident compared to males (Figure 8).

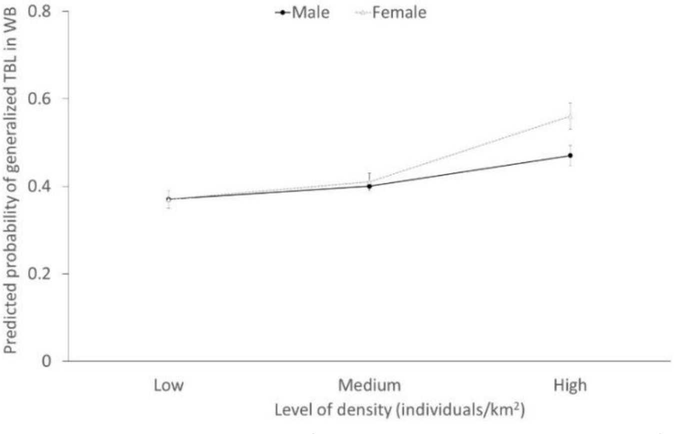


Figure 8. Predicted probability (± confidence interval (C.I.) 95%) of the presence of generalized TBL in wild boar depending on the interaction between sex and density. The three categories of density considered based on 33 and 66 percentiles are: low (⩽0.09 individuals/km^2), medium (>0.09-⩽1.06 individuals/km^2) and high (>1.06 individuals/km^2).

Table 2. Test statistics from the GzLMMs for the presence of generalized tuberculosis-like lesions (TBL) related to sex, age class, straight-line distance (m) to nearest water hole (DWAT), straight-line distance (m) to nearest marsh-shrub ecotone (DE), the proportion of open habitats and watercourse vegetation, the previous sampling period's rainfall (rainfall), annual densities of wild ungulates and cattle, annual prevalences of wild ungulates and some interactions among them.

Variables	**Wild boar**			**Fallow deer**			**Red deer**		
	F df (x,y)	**Estimate ± S.E.**	***p***	**F df (x,y)**	**Estimate ± S.E.**	***p***	**F df (x,y)**	**Estimate ± S.E.**	***p***
Sex[1]	1.11 (1, 800)	Female: -0.84±1.06	0.29	1.93 (1, 68)	Female: -8.35±5.95	0.17	0.00 (1,246)	Female: -14.70±210.89	0.95
Age[2]	0.48 (2, 800)	Juvenile: -0.59±1.46 Adult: -1.14±1.25	0.62	0.55 (1, 68)	Juvenile: 7.05±324.75 Adult: 1.90±324.80	0.46	0.09 (2, 246)	Juvenile: -175.87±613.73 Adult: -177.09±613.73	0.91
DWAT	1.76 (1, 800)	<0.001±<0.001	0.19	0.13 (1, 68)	<0.001±<0.001	0.72	1.16 (1, 246)	<0.001±<0.001	0.28
DE	1.14 (1, 800)	<0.001±<0.001	0.29	0.81 (1, 68)	<0.001±<0.001	0.37	0.01 (1, 246)	<0.001±<0.001	0.93
Open habitats	1.76 (1, 800)	-0.14±0.10	0.19	0.08 (1, 68)	0.09±0.31	0.78	0.56 (1, 246)	0.10±0.14	0.48
Watercourse vegetation	2.86 (1, 800)	-0.17±0.10	0.09	0.10 (1, 68)	-0.11±0.34	0.75	0.00 (1, 246)	0.02±0.15	0.91
Rainfall	0.38 (1, 800)	<0.001±<0.001	0.54	0.69 (1, 68)	-0.01±0.01	0.41	0.10 (1, 246)	-0.32±1.02	0.75
Density of red deer	0.60 (1, 800)	<0.001±0.04	0.44	0.04 (1, 68)	0.03±0.13	0.84	0.10 (1, 246)	-7.47±23.16	0.76
Density of fallow deer	0.26 (1, 800)	-0.04±0.08	0.61	0.60 (1, 68)	2.52±2.21	0.44	0.45 (1, 246)	-0.09±0.08	0.27
Density of wild boar	0.38 (1, 800)	0.28±0.99	0.54	0.02 (1, 68)	0.09±0.73	0.90	0.43 (1, 246)	-0.20±0.28	0.47
Density of cattle	0.31 (1, 800)	-0.07±0.13	0.58	0.26 (1, 68)	0.15±0.29	0.61	0.04 (1, 246)	0.01±0.11	0.60
Prevalence of generalized TBL in red deer	0.06 (1, 800)	-0.01±0.02	0.80	0.50 (1, 68)	-0.03±0.04	0.49	6.13 (1, 246)	0.05±0.02	**0.01**
Prevalence of generalized TBL in fallow deer	2.72 (1, 800)	-0.02±0.01	0.10	6.05 (1, 68)	0.07±0.03	**0.02**	0.15 (1, 246)	<0.001±0.01	0.70

Prevalence of generalized TBL in wild boar	59.74 (1, 800)	0.07±0.01	**<0.01**	0.05 (1,68)	-0.01±0.03	0.82	0.33 (1, 246)	-0.01±0.01	0.57
Sex*Age	0.44 (2, 800)	Female*Juvenile: -0.51±0.57 Female*Adult: -0.17±0.45	0.65	0.22 (2, 68)	Female*Adult: 0.80±1.69	0.64	0.25 (2, 246)	Female*Juvenile: -14.85±210.89 Female*Adult: 15.68±210.88	0.78
Rainfall*Sex	0.27 (1, 800)	Rainfall*Female: <0.001±<0.001	0.60	1.80 (1, 68)	Rainfall*Female: 0.02±0.01	0.19	0.04 (1, 246)	Rainfall*Female: <0.001±<0.001	0.84
Density*Sex	4.13 (1, 800)	Density*Female 0.37±0.18	**0.04**	0.50 (1, 68)	Density*Female: -0.30±0.42	0.48	1.14 (1, 246)	Density* Female -0.07±0.06	0.29
Rainfall*Age	0.27 (2, 800)	Rainfall* Juvenile: <0.001±<0.001 Rainfall* Adult: <0.001±<0.001	0.60	1.37 (2, 68)	Rainfall*Adult: 0.01±0.01	0.25	0.17 (2. 246)	Rainfall*Juvenile: 0.33±1.02 Rainfall*Adult: 0.33±1.02	0.84
Density*Age	0.55 (2, 800)	Density*Juvenile: 0.28±0.27 Density*Adult: 0.15±0.22	0.58	2.89 (2, 68)	Density*Adult: -1.97±1.16	0.09	0.06 (2, 246)	Density*Juvenile: 7.75±23.2 Density*Adult: 7.75±23.2	0.94
Rainfall*Density	0.31 (1, 800)	<0.001±<0.001	0.58	0.17 (1, 68)	<0.001±<0.001	0.68	1.47 (1, 246)	<0.001±<0.001	0.23

The models were fitted using the sampling period and livestock management area as random effect factors. Parameter estimates for: [1]the sex variable were calculated using males as the reference, [2]the age variable were calculated using calves and piglets for red deer and wild boar, respectively, as the reference. "*" represents interactions among explanatory variables

Discussion

This long-term study on TB in wildlife addresses a multi-host community including livestock, which implies added value among the scarce literature on shared wildlife-livestock infections. We found evidence of the potential effects exerted by stochastic and density-dependent factors on TBL, once controlled for other relevant drivers, which can only be assessed through a long-term perspective.

General patterns of the presence of tuberculosis-like lesions

The prevalences of TBL evidenced in this study were high compared to those obtained in similar studies on wildlife populations elsewhere (Glaser et al., 2016; Jang et al., 2017; Katale et al., 2017; Schmitt et al., 2002). The pattern of TBL presence associated with species, space and individual factors mostly confirms previous findings. Interestingly, the prevalence of TBL in fallow deer was associated with the positivity of cattle, which is probably caused by the spatial overlap between these species (see, e.g., Figure 9). The common use of pastures, mainly at the "vera" ecotone (meadows) takes place especially during late summer (August-October), and this can be a relevant season for TB spread. This ecological overlap may lead to similar environmental TB drivers in both species. The role of fallow deer in the maintenance and transmission of TB to cattle is discussed below.

Figure 9. Cattle, wild boar and fallow deer rest and graze together in close proximity on the ecotone between marshland and scrubland in Doñana National Park. Habitat sharing among ungulate species facilitates transmission of *Mycobacterium tuberculosis* complex bacteria (MTC) and explains why the eradication programme in cattle has been ineffective so far. Credit: Juan J. Negro.

Overall, a growing temporal trend was observed in the annual incidence of TBL in cattle, showing rates above 10% in the majority of years within the study period, which indicates continuous re-infection and that the current TB control in cattle is ineffective in eradicating the infection in DNP (Gortázar et al., 2008). We note that TBL figures in cattle are not comparable to those of wildlife since in the domestic species positive animals to the skin test are sacrificed each year and consequently, only “new infected” animals are identified in each campaign. The TBL figure, therefore, represents incidence (percentage of annual skin test positivity) and not prevalence, however in terms of evaluating the association of trends and evaluating risks in the long-term, they provide a useful approach.

The decline in the prevalence of TBL in wild boar that occurred as a result of the intense population control occurring in 2008-2009 that noticeably reduced wild boar densities is of particular note, and the subsequent speedy recovery of TBL levels after this population control was curtailed (Boadella et al., 2012b). A high peak of generalized TBL in wild boar was evidenced thereafter (Figure 4). In systems that harbour virulent parasites, culling can reduce the prevalence of the disease (Tanner et al., 2019), and in DNP wild boar culling temporally contributed to controlling TB, but efforts need to be maintained over time to affect long-term TB dynamics.

Spatially, a north to south gradient in the prevalence of TBL was observed. This spatial pattern had already been noted previously (Gortázar et al., 2008). However, a partial dilution of this spatial pattern is observed for all the species, indicating that the spread of TB in the DNP is also modulated by increasing prevalences in less-affected areas of the south. The cattle population has increased in the southernmost part of the park during the last five years and, therefore, an effect also modulated by domestic populations cannot be discarded. Actually, the southern part of the area, called marshland in Figure 1, is not completely isolated from Marismillas (our southernmost livestock management area) and cattle have been there for the last three years or more. This may exemplify the added sanitary risk if new cattle stocks were introduced in the areas of the park still free of cattle, as is insistently demanded by breeder associations. As recently shown by Barasona et al. (2019), the concomitant effect of adding diversity and density of hosts (including domestic breeds) to the TB host community increases the community’s ability to maintain and transmit the pathogen The similar spatial trend over time indicates that MTC epidemiology in DNP partially responds to similar drivers across species, probably mediated by similar exposure patterns, i.e., environmental factors, but also cross-specific transmission. The lower prevalence observed in fallow deer reinforces the theory of the apparent lower natural host susceptibility of this species in comparison with other wild ungulates of the Park, suggested in previous studies developed in DNP (Gortázar et al., 2008). However, we cannot ignore differences in exposure in a grazing species that mainly use open areas, in contrast to the more habitat generalist wild boar and red deer. In DNP, intra- and inter-specific contact networks, and the subsequent molecular epidemiology pattern (distribution of the *M. bovis* types), seems to act at a very local scale at the host community level (Barasona et al., 2014a; Gortázar et al., 2011; Triguero-Ocaña et al., 2020). Previous studies on molecular epidemiology of TB in DNP have suggested that a local transmission frequently occurs between species (Gortázar et al., 2011).

As for individual factors, interestingly, no differences were observed in the proportions of generalized TBL among the hosts. However, this does not necessarily mean the severity and extension of lesions do not vary among species, as well as the level of mycobacteria excretion, which is very high for wild boar in DNP (over one third does; Barasona et al., 2017a), but still unknown for deer species in that area. In Portugal, red deer have been regarded as very relevant in terms of MTC shedding (Santos et al., 2015a). Concerning the age factor, the growing TBL prevalence pattern observed in wild boar has been frequently reported in the literature (Gortázar et al., 2005; Gortázar et al., 2008; Vicente et al., 2007b). Interestingly, the high TB-induced mortality reported by Barasona et al. (2016) in wild boar could explain the absence of an increasing pattern of generalized TBL with age, as observed in this species. Beyond prevalence comparisons and general patterns, we identified some factors that can exert an effect at the long-term, and that we discuss in the following section.

Factors determining the presence of tuberculosis-like lesions

The environmental features

Our results evidenced that, for the main TB reservoir (in terms of high prevalence), the wild boar, the larger the distance to water bodies (straight-line distance to the nearest water hole, DWAT), the higher the presence of TBL (Figure 8a). Further, the closer to the ecotone (Figure 8b) and the greater availability of open habitats (Figure 8c), the higher the presence of TBL was. Similarly, the proximity to ecotone corresponded to higher presence of TBL in red deer (Figure 8d). Inter-specific transmission usually occurs by contaminated environment elements such as pasture, water or mud (Caron et al., 2003; Morris et al., 1994). Our results confirm that this indirect transmission in DNP takes place around aggregation points such as the pasture-rich ecotone and previous research indicates that this takes place especially during the dry season from June to September every year (Barasona et al., 2014a; Triguero-Ocaña et al., 2019). The ecotone between the marshland and shrublands offers high quality and palatable grasslands and shelter, especially during dry seasons (summer and autumn) (Braza and Alvarez, 1987; Lazo, 1995). Previous studies where we modelled the spatial distribution of the ungulate community throughout DNP at a fine-scale demonstrated that all ungulate species have a preference for the ecotone, where they spatially aggregate and interact (Barasona et al., 2014a; 2014b; Triguero-Ocaña et al., 2019). In this context, the spread of MTC is favoured by higher intra- and inter-specific contact rates as well as the ingestion of contaminated food or water (Barasona et al., 2017b; Morris et al., 1994).

Furthermore, wild boar sampled in areas with higher availability of open habitats, especially grasslands, had a higher presence of TBL. According to previous studies, livestock-wildlife interactions are less frequent in areas with dense vegetation, considering that dense shrublands and woodlands constitute resting sites rather than foraging habitats for wild ungulates in DNP (Barasona et al., 2014a; Triguero-Ocaña et al., 2019).

A large distance to water bodies implies that wild boar must traverse more distance to the nearest waterhole, i.e., there is a scarceness of water points around, and this may exacerbate the use of the few water points available. Therefore, higher levels of aggregation are expected at these sites, and a subsequent increase in the risk for direct and/or indirect MTC exposure and transmission (Kukielka et al., 2013; Vicente et al., 2007b). In this regard, previous studies demonstrated that the risk of TBL for wild ungulates was negatively associated with water point density in the surrounding area in Mediterranean habitats (Barasona et al., 2014b; Cowie et al., 2014; Kukielka et al., 2013). Specifically, Barasona et al (2014a) showed a higher risk of TBL for wild boar and red deer in areas of DNP with lower local waterhole density. The same association was reported in Mediterranean areas and the USA (Cooper et al., 2010) and dry sites from Africa (Munyeme et al., 2008; Renwick et al., 2007), where cattle share not only water points and irrigated fields but also infections with wildlife. Waterholes are regarded as aggregation points for wildlife and livestock in which interactions are frequent and environmental maintenance of pathogens occurs, making them potential hotspots for MTC transmission (Barasona et al., 2017a; Kukielka et al., 2013; Morris et al., 1994).

Populational factors

As for density-dependence, the models on the individual presence of generalized TBL (Table 2) indicated that the interaction between density and sex was significant in wild boar, where the positive effect of density on the generalized presence of TBL in females was more evident. Higher densities of wild boar originate increasing contact rates and depletion of resources, which favour exposure and, probably, are mediated by a reduced nutritional intake thereby increasing susceptibility (Vicente et al., 2013). The fact that these effects were more evident in females may be modulated by the physiological cost of reproduction (rearing of the piglet litter) during the dry season (Ballari and Barrios-García, 2014), which is the season when natural food availability is limited in Mediterranean habitats. This finding is very relevant since it may mediate the TB epidemiology from a very early age in wild boar. Research is needed comparing the situation in DNP with that in highly managed areas (i.e. fenced and year-round fed) for hunting purposes where the prevalence of TB in wild boar piglets is often low compared to that observed in adults (Barasona et al., 2020). Piglets already presented very high prevalences of TBL in DNP, which may be mediated by direct transmission of MTC from sows, which develops into generalized TBL, severe lesions and subsequently increased excretion of mycobacteria in a density-dependent manner (Martín-Hernando et al., 2007; Vicente et al., 2013). More research is needed to elucidate the potential mortality in juvenile wild boar due to TB, as suggested by the age decreasing pattern of generalized TBL (Figure 2b).

Stochasticity: rainfall

The effects of rainfall depended on the sex (significant interaction) to explain the presence of TBL in red deer, so that high annual rainfall was more markedly associated with the presence of TBL in males. This suggests that in rainy years, greater exposure and/or susceptibility to TB may occur in males with respect to females. We speculate this may take place during the mating season. Very intense ruts (evidenced by increased aggressive interactions between stags) have been associated with higher food availability (Carranza, 2017). In Mediterranean ecosystems, the presence of food resources is key, considering that rut occurs during a period of food scarcity (Carranza et al., 1990). In rainy years, many patches of grasslands remain available in the ecotone in DNP due to the high humidity, implying a higher aggregation of females and a maximum intrasexual competition for mating in males that is reflected in increased reproductive efforts. Furthermore, the effects of rainfall are not only mediated by water and food availability. In wet years marshlands flood and ungulates remain aggregated on the ecotone, increasing the competition to mate in stags. The conflict between immune response and the reproductive effort in red deer stags has been reported previously (Lochmiller and Deerenberg, 2000; Malo et al., 2009; Westneat and Birkhead, 1998). In this sense, the greatest investment during the rut (reproductive effort, testosterone metabolite levels and sexual signals) endanger the immunological defences, health status and fitness of red deer, making them more susceptible to infections (De la Peña et al., 2020; Galván et al., 2019; Vicente et al., 2007a). Therefore, during mating seasons of rainy years, the greater reproductive effort of stags may lead to a higher susceptibility to TB, and to higher interaction and more contact with other males. Both greater exposure and/or susceptibility to MTC infection by red deer stags in rainy years may, therefore, cause increased presence of TBL; however, our arguments remain speculative, and more research is needed on this aspect.

No sex-dependent effects of rainfall were observed in the presence of TBL for fallow deer. For this species, the same immunosuppressant effect of reproductive efforts occurring during the rut season for fallow deer stags may take place (Rehbein et al., 2014). However, the rut of fallow deer occurs later in Autumn and perhaps rainfall is more abundant and less determinant then. The fact that the prevalence of TBL is lower in fallow deer maybe also determine this absence of a relationship with rainfall (Gortázar et al., 2008).

Inter-specific relationships

The inter-specific interactions, as well as the intra-specific infection risks, are influenced by the ecological, behavioural and epidemiological factors typical of each species (Gortázar et al., 2005). These factors such as scavenging or gregariousness can lead to greater intra-specific transmission (Aranaz et al., 2004). Furthermore, individuals belonging to a particular species are exposed to the same risk factors in certain areas. Specifically, the annual intra-specific prevalence of TBL had a marked effect on the presence of TBL for wild boar, especially in piglets, which are a susceptible population in an "infectious environment" (family groups, environmental exposure). Our results also indicated a positive statistical relationship between the generalized intra-specific prevalence rates of TBL and the species-specific TBL generalized presence of TBL for all wild species. Animals with generalized TBL become important super-shedders of MTC (Santos et al., 2015b, 2015a). Hence, an increasing number of individuals with generalized TBL implies a higher excretion of mycobacteria by different routes (Martín-Hernando et al., 2007) and subsequent increased direct and/or indirect transmission.

Interestingly, we found a significant association between the positivity of cattle and the prevalence of TBL in fallow deer (see Figure 7). Previous studies on spatiotemporal interactions patterns among wild ungulates and cattle carried out in DNP showed that the dynamics of TB transmission in this area is conditioned by environmental and habitat-related peculiarities which facilitate the spatiotemporal direct and indirect overlap between wildlife and livestock species (Barasona et al., 2014a; Triguero-Ocaña et al., 2020, 2019) (Figure 9). Interestingly, high contact rates between fallow deer and cattle have been reported in DNP (Triguero-Ocaña et al., 2020). Both species share a preference for open habitats. In this sense, most studies demonstrated that cattle tend to avoid shrublands in favour of open grassland and marshland just like fallow deer, which usually graze on the meadows of the marsh periphery and on the marsh itself (Braza and Alvarez, 1987; Mulero-Pázmány et al., 2015). Furthermore, the gregarious habits of both species, more marked in open habitats, favour these contacts, often establishing mixed groups (Focardi and Pecchioli, 2005; Triguero-Ocaña et al., 2020; Tucker, 2009). This finding is in agreement with Triguero-Ocaña et al. (2020) who recently suggested the potential substantial role of fallow deer in the maintenance and transmission of TB to livestock in DNP. In summary, the association of the positivity to TB between cattle and fallow deer is indicative of i) sharing (in either direction) TB and/or ii) being exposed to similar risk factors since both species are the closest ecologically speaking among the ruminants of the Park (they are both grazers that prefer open lands, while red deer typically are mixed grazers/browsers).

Conclusions

1. The comprehensive monitoring carried out during the study period at the livestock-wildlife interface in DNP has provided evidence of relevant epidemiological processes which operate in the long term within the TB host community. The effects of the TB determinants are not straightforward but operate in complex interactions, often related to the ecology of the hosts.

  - The effect of wild boar density on TB epidemiology mediated by sows may determine a high incidence in maternal groups from a very early age.
  - Stochastic factors may determine the presence of TBL by interacting with the host's natural life history, as evidenced in male red deer.
  - The association of the presence of TB in fallow deer with that of cattle may indicate inter-species transmission (in either direction) and similar exposure to risk factors mediated by ecological overlap (they both use the same pasture areas).
2. In DNP, as well as in other protected areas, hunting is not allowed and there are no natural predators. Therefore, the health surveillance and population control of wild ungulates and comprehensive management of cattle stocks, including the application of the national TB programme, must be a priority to control the disease at the wildlife-livestock interface. Since increases in the diversity and density of the host community of TB increases the ability of the system to maintain and transmit the pathogen in Mediterranean assemblages, expanding the distribution of cattle over other areas of the Park, as demanded by breeders, could have a negative effect on the distribution and rates of TB.
3. This research illustrates that the understanding of the main determinants of the maintenance and spread of a pathogen in wildlife, livestock and human populations requires integrated wildlife monitoring. This includes the so-called "denominator" (or population) information (Vicente et al., 2019a, 2019b).
4. This long-term study provides a deeper understanding of the main drivers of a shared pathogen, MTC, which may help government agencies to develop improved risk-management strategies. We encourage administrations to conduct long-term studies and integrate monitoring and also to consider the impact of this issue on public health.

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Supplementary material 2.1

Table S1. Sample size (N) and prevalence of tuberculosis-like lesions (Prev; %) by species, sampling period and livestock management area in wild ungulates and livestock.

Species	Sampling periods	CR	SO	RBD	PU	MA	Total
		Prev (N)	Prev (N)	Prev (N)	Prev (N)	Prev (N)	Prev (N)
Red deer	2005-2006	33.3 (6)	25.0 (20)	-	-	-	26.9 (26)
	2006-2007	41.4 (29)	20.0 (15)	16.7 (12)	0 (3)	0 (10)	23.9 (69)
	2007-2008	-	-	-	-	-	-
	2009-2010	-	-	-	-	-	-
	2010-2011	62.5 (16)	71.4 (7)	33.3 (3)	100 (2)	20 (5)	62.5 (33)
	2011-2012	44.4 (9)	35.7 (14)	50.0 (4)	66.7 (3)	60.0 (5)	46.9 (35)
	2012-2013	62.5 (8)	20.0 (5)	66.7 (3)	0.0 (1)	0.0 (10)	36.8 (27)
	2013-2014	25.0 (4)	25.0 (4)	0.0 (1)	40.0 (5)	-	28.6 (14)
	2014-2015	43.8 (16)	50.0 (8)	75.0 (4)	-	-	51.9 (28)
	2015-2016	62.5 (24)	60.0 (20)	57.1 (21)	57.7 (28)	15.0 (20)	52.4 (111)
	2016-2017	71.4 (14)	70 (20)	54.5 (22)	42.9 (21)	25.0 (20)	51.6 (98)
	2017-2018	30.0 (20)	35.0 (20)	45.0 (20)	40.0 (20)	35.0 (20)	37.5 (100)
	2018-2019	55.0 (20)	81.0 (21)	30.0 (20)	20.0 (20)	25.0 (20)	46.7 (101)
	Total	50.6 (166)	48.1 (154)	44.1 (111)	41.6 (101)	21.8 (110)	42.5 (642)
Fallow deer	2005-2006	20.0 (5)	7.7 (13)	-	-	-	11.1 (18)
	2006-2007	32.3 (31)	36.4 (22)	0.0 (9)	20.0 (5)	0.0 (13)	22.2 (80)
	2007-2008	-	-	-	-	-	-
	2009-2010	-	-	-	-	-	-
	2010-2011	75.0 (4)	44.4 (9)	0.0 (3)	14.3 (7)	0.0 (7)	27.6 (30)
	2011-2012	20.0 (5)	8.3 (12)	0.0 (3)	0.0 (5)	0.0 (6)	7.7 (31)
	2012-2013	20.0 (10)	0.0 (6)	0.0 (2)	0.0 (2)	0.0 (10)	7.1 (30)
	2013-2014	0.0 (2)	20 (5)	0.0 (2)	16.7 (6)	0.0 (3)	11.8 (18)
	2014-2015	0.0 (1)	42.9 (7)	16.7 (22	40.0 (5)	28.6 (7)	34.8 (26)
	2015-2016	5.0 (20)	23.8 (21)	36.8 (19)	22.7 (22)	4.8 (21)	21.3 (103)
	2016-2017	25.0 (20)	20.0 (20)	4.8 (21)	0.0 (19)	4.8 (21)	12.6 (101)
	2017-2018	6.3 (16)	25.0 (20)	12.5 (24)	10.0 (20)	10.0 (20)	15.3 (100)
	2018-2019	5.0 (20)	0.0 (20)	0.0 (20)	20.0 (20)	5.0 (20)	6.2 (100)
	Total	18.7 (134)	20.6 (155)	11.0 (109)	14.4 (111)	5.5 (128)	14.4 (637)
Wild boar	2005-2006	-	66.7 (6)	75.0 (12)	87.5 (8)	52.0 (25)	65.9 (51)
	2006-2007	100.0 (14)	71.4 (14)	70.0 (20)	95.0 (20)	80.0 (5)	83.3 (73)
	2007-2008	-	89.7 (29)	100.0 (7)	100.0 (14)	100.0 (48)	100 (98)
	2009-2010	50.0 (2)	100.0 (4)	58.8 (17)	-	5.6 (18)	41.2 (41)
	2010-2011	80.0 (20)	100.0 (10)	12.9 (31)	23.1 (13)	44.4 (9)	55.8 (83)
	2011-2012	81.0 (21)	95.8 (24)	66.7 (63)	75.0 (12)	94.3 (35)	86.1 (155)
	2012-2013	100.0 (9)	100.0 (6)	73.7 (38)	85.7 (8)	100.0 (22)	78.8 (82)
	2013-2014	-	-	100.0 (7)	83.3 (18)		88 (25)
	2014-2015	100.0 (2)	77.3 (66)	100.0 (8)	61.5 (13)	50.0 (18)	78.1 (107)
	2015-2016	85.7 (21)	100.0	78.9 (71)	75.0 (8)	90.5 (21)	98 (137)
	2016-2017	75.0 (20)	80.0 (20)	78.2 (55)	83.6 (61)	70.0 (20)	83.6 (176)
	2017-2018	85.0 (20)	90.5 (21)	86.4 (22)	52.4 (21)	65.0 (20)	74.4 (104)
	2018-2019	50 (20)	95 (20)	85.7 (21)	65 (20)	60 (20)	74.3 (101)
	Total	79.9 (149)	88.2 (237)	71.4 (378)	75.3 (215)	73.2 (254)	76.7 (1233)
Cattle	2005-2006	-	7.6 (172)	2.7 (112)	1.4 (222)	0.6 (633)	3.1 (1139)
	2006-2007	-	12.9 (171)	6.7 (90)	20.3 (192)	8.0 (577)	12.0 (1030)
	2007-2008	-	11.4 (114)	1.6 (63)	18.6 (140)	6.3 (649)	9.5 (966)
	2009-2010	-	16.8 (119)	11.1 (90)	9.7 (185)	1.5 (742)	9.8 (1136)
	2010-2011	-	17.3 (172)	5.1 (78)	13.5 (207)	4.9 (618)	10.2 (1075)
	2011-2012	-	10.6 (142)	5.7 (88)	20.1 (254)	3.4 (938)	9.9 (1422)

	2012-2013	-	6.3 (143)	5.7 (53)	19.6 (204)	3.7 (828)	8.8 (1228)
	2013-2014	-	12.0 (175)	9.2 (65)	14.3 (245)	11.4 (1051)	11.7 (1536)
	2014-2015	-	26.5 (230)	5.4 (92)	25.4 (244)	7.8 (1037)	16.3 (1603)
	2015-2016	-	2.7 (223)	19.8 (116)	24.9 (217)	6.6 (1216)	13.5 (1772)
	2016-2017	-	14.3 (196)	18.4 (98)	18.5 (216)	4.7 (1259)	14.0 (1769)
	2017-2018	-	13.6 (235)	12.5 (80)	26.9 (249)	3.9 (1272)	14.2 (1836)
	2018-2019	-	4.7 (258)	10.4 (96)	7.1 (241)	3.6 (1424)	6.4 (2019)
Total			12.0 (2282)	8.5 (1121)	17.5 (2816)	4.9 (12244)	10.7 (18463)

CAPÍTULO 2.2. Factores determinantes a largo plazo de la seroprevalencia de *Toxoplasma gondii* en una comunidad de ungulados silvestres

CHAPTER 2.2. Long-term determinants of the seroprevalence of Toxoplasma gondii in a wild ungulate community

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Resumen

Toxoplasma gondii es un protozoo intracelular obligado que infecta a numerosos vertebrados de sangre caliente en todo el mundo, incluyendo al ser humano. En este trabajo, se estudió la epidemiología de *T. gondii* en la comunidad hospedadora de ungulados silvestres (jabalí, ciervo y gamo) del Parque Nacional de Doñana (PND, suroeste de España) durante 13 años (2005-2018). Se analizaron diferentes variables que operan, potencialmente, a medio y largo plazo (factores ambientales, poblacionales y estocásticos). En general, la comunidad hospedadora de ungulados del PND mostró seroprevalencias elevadas de *T. gondii* (STG; % ± error estándar 95%); jabalí (*Sus scrofa*) 39,0±3,3, n=698; ciervo (*Cervus elaphus*) 30,7±4,4, n=423; y gamo (*Dama dama*) 29,7±4,2, n=452). La convergencia de la compleja interacción de los hospedadores y sus nichos ecológicos y epidemiológicos con unas condiciones climáticas óptimas para la supervivencia de los oocistos en esta zona podría favorecer la transmisión del parásito en su comunidad hospedadora. La evolución temporal de la STG osciló considerablemente, sobre todo en los cérvidos. Las relaciones mostradas por los modelos estadísticos indicaron que fueron varios los factores que determinaron los patrones observados para cada especie. La coincidencia de los efectos observados en varias especies sugirió que los factores más relevantes a la hora de determinar el riesgo operaron a nivel comunitario. Este estudio abordó factores que actúan a una escala temporal amplia, permitiendo mostrar su impacto en la epidemiología de *T. gondii* y sus tendencias temporales. Este enfoque es clave para comprender la epidemiología y ecología de la infección por *T. gondi* en comunidades hospedadoras silvestres, especialmente en un contexto en el que el declive de la seroprevalencia en humanos está dando lugar a una pérdida de inmunidad.

Abstract

Toxoplasma gondii is an obligate intracellular protozoan which infects warm-blooded vertebrates, including humans, worldwide. In the present study, the epidemiology of *T. gondii* was studied in the wild ungulate host community (wild boar, red deer, and fallow deer) of Doñana National Park (DNP, south-western Spain) for 13 years (2005–2018). We assessed several variables which potentially operate in the medium and long-term (environmental features, population, and stochastic factors). Overall, the wild ungulate host community of DNP had high seroprevalence values of *T. gondii* (STG; % ± standard error (S.E.) 95%; wild boar (*Sus scrofa*) 39.0±3.3, n=698; red deer (*Cervus elaphus*) 30.7±4.4, n=423; fallow deer (*Dama dama*) 29.7±4.2, n=452). The complex interplay of hosts and ecological/epidemiological niches, together with the optimal climatic conditions for the survival of oocysts that converge in this area may favor the spread of the parasite in its host community. The temporal evolution of STG oscillated considerably, mostly in deer species. The relationships shown by statistical models indicated that several factors determined species patterns. Concomitance of effects among species, indicated that relevant drivers of risk operated at the community level. Our focus, addressing factors operating at broad temporal scale, allows showing their impacts on the epidemiology of *T. gondii* and its trends. This approach is key to understanding the epidemiology and ecology to *T. gondii* infection in wild host communities in a context where the decline in seroprevalence leads to loss of immunity in humans.

Introduction

Toxoplasma gondii is a zoonotic obligate intracellular protozoan which infects warm-blooded vertebrates (Dubey, 2010). It has an indirect life cycle where wild and domestic felids are the definitive hosts, excreting oocyst in faeces. Humans, as well as many mammal and bird species, serve as intermediate hosts of *T. gondii* and can become infected by vertical transmission, the faecal-oral route, through the ingestion of water or food contaminated with sporulated *T. gondii* oocysts, or through the consumption of tissues from animals infected with encysted bradyzoites (Dubey, 2010; Hill and Dubey, 2014).

T. gondii has been detected in wildlife and livestock worldwide (Dubey, 2010). Previous Spanish studies revealed a widespread distribution of this parasite in both wild and domestic ungulates, showing significant differences in the presence of *T. gondii* among geographic areas (Calero-Bernal et al., 2016; Castillo-Cuenca et al., 2020; García-Bocanegra et al., 2013; Gauss et al., 2005; Panadero et al., 2010). In Mediterranean ecosystems in southern Spain, antibodies against *T. gondii* have been detected in wild ungulates including wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), Barbary sheep (*Ammotragus lervia*), mouflon (*Ovis aries musimon*) and Iberian ibex (*Capra pyrenaica*). In these studies, seroprevalences of 40.2%, 15.6% and 10.5% were reached in wild boar, fallow deer and red deer, respectively (Almería et al., 2018; Gauss et al., 2005), being lower or calculated from a few samples in the other species. Regarding livestock, serosurveys in this region revealed rate levels ranging between 18.6-83.3% and 16.2-24.3% in domestic ungulates (Almería et al., 2018; García-Bocanegra et al., 2013; Jiménez-Martín et al., 2020) and pigs, respectively (Castillo-Cuenca et al., 2020; García-Bocanegra et al., 2010c, 2010b). Higher seroprevalences from this area have been reported in wild carnivores, especially in the Iberian lynx (*Lynx pardinus*), reaching rates of 81.5% (Sobrino et al., 2007).

Host-pathogen dynamics are subjected to several processes which operate over broad temporal scales; however, little attention has been paid to *T. gondii*, and particularly, in intermediate host communities at the wildlife-livestock interface (Almería et al., 2018; Panadero et al., 2010). Wide temporal data series are essential to address epidemiology and ecology in complex host communities with the necessary perspective required to understand processes taking place over many years (Barroso et al., 2020b, 2020a;

McDonald et al., 2017; Vicente et al., 2013). In Doñana National Park (DNP, south-west Spain), the wild ungulate community (including wild boar, red deer and fallow deer) occurs sympatrically with free-ranging cattle and horses, and one of the most important meta-population of the endangered Iberian lynx (Life+IBERLINCE, 2018). Studies on *T. gondii* in DNP has been exclusively conducted in felid populations with conservational purposes, showing a widespread infection in the area and reporting seroprevalence rates up to 60% (García-Bocanegra et al., 2010a; Millán et al., 2009; Sobrino et al., 2007).

The multiple transmission routes and capacity of *T. gondii* to find niches into the hosts studied provided an excellent scenario to improve our understanding of the transmission dynamics of this pathogen. While *T. gondii* has normally been considered an excellent model to study host-pathogen interactions, we also showed that it may also be used to address the study of population, community, and environmental factors. The present long-term study illustrates the interplay of factors, particularly factors operating at broad temporal scale that may contribute to the spread and maintenance of a pathogen over host communities. In this context, we present data on serosurveillance of *T. gondii* in wild ungulates (wild boar, red deer, and fallow deer) from DNP for 13 years, with the specific aims of: i) evaluating the factors (individual, populational and environmental) modulating the seroprevalence of *T. gondii* (STG), and ii) assessing the factors operating in the long-term (population and stochastic) in order to explain the temporal trend of STG in the intermediate host ungulate community from 2005 to 2018.

Material and methods

Study area

This study was conducted in DNP (54,000 ha), one of the most relevant biodiversity reserves in Europe, located on the Atlantic coast of southwestern Spain (37°09 N, 6°30 W). Human access to the Park is restricted and agriculture and hunting are prohibited inside the Park; cattle and horse breeding are allowed and are, mainly focussed on autochthonous and traditional breeds (Fernández-Delgado, 1997).

The habitat consists of a greater proportion of sand dune habitat and marshland, combined with pine forest and Mediterranean scrubland (see Figure 1 and Barasona et al. 2014a for a more detailed description). Between the scrublands and the edge of the marshland, there is a narrow north-south longitudinal strip of humid ecotone of high ecological richness.

DNP has a dry sub-humid Mediterranean climate with strong seasonality, especially in terms of water availability to animals and vegetation. The average annual temperature is 17˚C, and the mean annual precipitation is 550 mm, with high intra- and inter-annual fluctuation (170 to 1000 mm), which determine the dynamics of the marshlands (Díaz-Delgado et al., 2016). During the wet seasons (winter and spring) the marshlands may flood, so ungulates concentrate and browse in the remaining uncovered scrublands. In late summer and autumn, the hardest season for ungulates due to the shortage of resources and the seasonal drought, an aggregation of wild and domestic ungulates on the ecotone and around water points occurs (Barasona et al., 2014a).

The territory of DNP included in this study is divided into five cattle management areas from north to south: Coto del Rey (CR), Sotos (SO), Doñana Biological Reserve (RBD), Puntal (PU) and Marismillas (MA). Free-ranging livestock is distributed through the entire Park, except in the northernmost area (CR). In this area, despite the existence of a low number of horses since the last year, cattle husbandry is prohibited since 2002 as a conservation measure for the endangered Iberian lynx. A meta-population of 94 individuals of Iberian lynx currently inhabit DNP and the surrounding areas (Life+IBERLINCE, 2018). The remaining community of carnivores is comprised by red fox (*Vulpes vulpes*), Eurasian badger (*Meles meles*), Eurasian otter (*Lutra lutra*), polecat (*Mustela putorius*), European genet (*Genetta genetta*), Egyptian mongoose (*Herpestes ichneumon*), and occasionally, wild cat (*Felis silvestris silvestris*), whose presence is very scarce, probably due to the presence of a larger predator such as the Iberian lynx (Valverde, 1967). Furthermore, domestic carnivores including stray cats (*Felis silvestris catus*) and dogs (*Canis lupus familiaris*) are also occasionally present throughout DNP, although a population control plan of stray dogs and cats has been carried out in DNP since 2007 (Junta de Andalucia, 2013).

Finally, population control (by culling) of the wild ungulate population is practised exclusively by Park rangers as part of the Park management scheme, and it is also used to carry out a health monitoring programme (Barroso et al., 2020b).

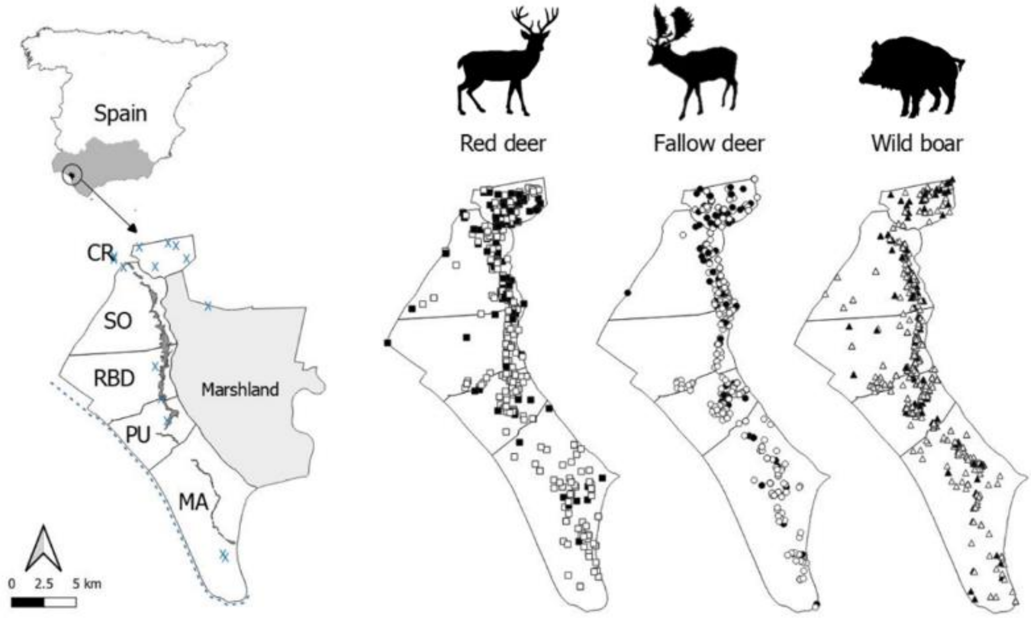

Figure 1. Map of the study area, Doñana National Park. The livestock management areas (Coto del Rey (CR), Sotos (SO), Doñana Biological Reserve (RBD), Puntal (PU) and Marismillas (MA)) are delimited and the ecotone and small human settlements are displayed by a dark band and blue "X", respectively. Red deer (squares), fallow deer (circles), and wild boar (triangles) sampled are shown. Black and white symbols mean animals positive and negative for antibodies against *Toxoplasma gondii*, respectively.

Animal sampling

From October-January of 2005 to 2018 (sampling periods 2005-2006 to 2017-2018), 423 red deer, 452 fallow deer and 698 wild boar were randomly (in terms of sex, age and health status) sampled in the population control context performed by Park rangers and necropsied as part of the DNP health-monitoring programme (approved by the Research Commission of DNP in accordance with management rules established by the Autonomous Government of Andalusia). Table S1 displays the sample size by species, livestock management area and sampling period, as well as the seroprevalences found. For each individual, the geographical location of the sighting was recorded through a portable GPS (Garmin Ltd., Olathe, KS, USA).

The sampling was performed according to European (EC Directive 86/609/EEC; EEC, 1986) and Spanish laws (RD 223/1988; MAPA, 1988), current guidelines for the ethical use of animals in research (ASAB, 2012), the Animal Experiment Committee of Castilla-La Mancha University and the Spanish Ethics Committee (PR-2015-03-08). Necropsies and sample collection were undertaken in the field by qualified veterinarians. During the examination, blood samples were collected into sterile plastic tubes (Vacutainer®, Becton-Dickinson, NJ, USA) from the heart, thoracic cavity, or preferably by endocranial venous sinuses puncture (Arenas-Montes et al., 2013).

Serological testing

Sera were obtained after centrifugation at 400 *g* for 5 minutes and stored at −20 ° C until assayed for antibodies. Antibodies to *T. gondii* were tested using the modified agglutination test (MAT) as previously described (Dubey and Desmonts, 1987). This technique has been employed broadly for the diagnosis of antibodies against *T. gondii* in both domestic and wildlife species (Dubey, 2010). Two recent large studies in wild pigs and white-tailed deer (*Odocoileus virginianus*) in the USA added evidence for the validity of serological analysis by MAT in those species since viable *T. gondii* was isolated from a large number of seropositive animals and the rate of isolating viable parasites was positively associated with MAT titers in those studies (Dubey et al., 2021; Dubey et al., 2020b, 2020c, 2020a). Each serum sample was tested at 1:25 and 1:50 dilutions, including positive and negative controls in each test. Sera with a titer of 1:25 or higher were considered positive and those with doubtful or positive results were re-tested (Gamarra et al., 2008; Gauss et al., 2006; Sobrino et al., 2007).

Data collection

Individual factors

The sex and age of the animals were determined, classifying them into three age classes on the basis of dentition eruption patterns (Saenz de Buruaga et al., 2001): calves (<1-year-old), juveniles (1-2 years) and adults (≥3 years) for deer species, and piglets (<6 months), juveniles (0.5-2 years) and adults (>2 years) for wild boar.

Considering the well-known debilitating effect of tuberculosis (TB) progression on immune response (Byrne et al., 2019), we assessed the potential effect of TB severity on the seroprevalence against *T. gondii*. For this purpose, the presence of concomitant tuberculosis-like lesions (TBL) was used as a proxy to infection by the *Mycobacterium tuberculosis* Complex (MTC), since it provides a relatively accurate diagnosis without the need for expensive laboratory confirmation (Barroso et al., 2020b; Vicente et al., 2013). The presence of TBL was recorded by macroscopic inspection of the head, thoracic and mesenteric lymph nodes as well as abdominal and thoracic organs in the laboratory (see

Vicente et al., (2006) for a detailed methodology). When TBL are identified in at least two of the three anatomical compartments examined (head, thorax and abdomen) we considered the TBL as generalized, indicative of a more severe and evolved infection (Vicente et al., 2006). According to the generalized TBL status, wild ungulates were grouped into two classes: those without TBL or showing localized TBL in a single anatomical compartment, and a second class including animals with presence of generalized TBL.

Environmental factors

As for environmental information, several variables were included in our analysis to assess their effect on STG because of their importance to ungulate behaviour, distribution, and transmission of pathogens in DNP and south Spain (Barasona et al., 2014a; Braza and Alvarez, 1987; Vicente et al., 2013). A grid of one hectare of surface was built, generating territorial units in which the proportional cover of dense scrub, low-clear shrubland, herbaceous grassland, woodland, bare land and watercourse vegetation were calculated for each territorial unit (see Barasona et al., 2014b). This grid was merged with the geographical location of the animals through a point sampling tool with QGIS version 3.4.1 (QGIS Development Team, 2019). Landcover data was obtained from Andalusia Environmental Information (REDIAM, 2019).

Given the effects reportedly associated to urban areas (Miller et al., 2002), the coast (Dubey, 2004) and surface water on the infection risk of *T. gondii* (Villari et al., 2009), the effect of the nearest location of animals to these areas was assessed. For that purpose, we calculated the straight-line distance (m) from the exact location of each animal sampled to the nearest: urban area (DURB), small human settlements (DHS), coast line (DCOAST), water point (DWAT) and marsh-shrub ecotone (DE) (see Barasona et al., 2014a; Barroso et al., 2020b; Cabal et al., 2017).

Populational factors

To estimate the population density of wild ungulates we applied distance sampling methodology (Buckland et al., 2001). Every year during September, and two hours before sunset, we sampled twice seven line transects of 10-15 Km each one, distributed throughout the study area. Additionally, for wild boar we repeated the transect one hour after sunset in order to increase the sample size. We carried out the surveys during September because it is the month of maximum detectability for these species (Acevedo et al., 2008), and to obtain density results just before the health-monitoring programme. Moreover, during September the marshland was dry, and it allowed us to sample all the habitats in DNP. The surveys were carried out from a vehicle (average speed was 10 $Km \cdot h^{-1}$), and the perpendicular distance between animals and transect was recorded with a telemeter (Garmin Ltd., Olathe, KS, USA). The analyses were carried out using Distance Sampling 6.2 software (Thomas et al., 2010) by considering stratification. We defined three strata according to their abundance and visibility: shrubland, marshland and ecotone. The data of all the years (2005-2018) were considered to estimate a detection function for each stratum, and we considered the data of each strata, sampling period and livestock management area to estimate the encounter rate and mean group size. Data were right-truncated when the probability of detection was lower than 0.15 (Buckland et al., 2001). Half-normal, uniform and hazard rate models for the detection function were fitted against the data using cosine, hermite polynomial, and simple polynomial adjustment terms, fitted sequentially. The selection of the best model was based on the Akaike's Information Criterion (AIC) (Akaike, 1974).

The abundance of the diverse community of carnivores from DNP was monitored by means of track surveys along prefixed transects on a sandy substrate according to monitoring team programme (ESPN-EBD-CSIC). Tracks left on moist sand over a 24 h period were tracked in transects of 1.5 m width and 2 km length, from dawn to midday and were expressed as Kilometric Abundance Index (KAI) of footprints. The surveys include 12 different transects distributed across the DNP which were repeated during three consecutive days, being cleaned daily.

As for livestock, we calculated the cattle and horse stocks per square kilometre for each livestock management area and sampling period.

Stochastic factors

Meteorological information (average rainfall and temperature) was collected from the meteorology station located at RBD for each sampling period (EBD-CSIC, 2019). In Mediterranean environments, rainfall and temperature have potential relevance to the dynamics of ungulate populations, as well as effects on the susceptibility or exposure to pathogens (Vicente et al., 2013). Specifically for *T. gondii*, both factors are key for the survival of oocyst in the environment (Dubey and Beattie, 1988). Therefore, they were considered here for their potential effect in *T. gondii* epidemiology.

Risk Factor Analysis

Initially, collinearity between environmental and population variables was explored (Zuur et al., 2009). Given the high collinearity observed between environmental variables and with the purpose of simplifying the environmental information, a principal component analysis (PCA) was performed, summarizing these variables in closed habitats, in which dense scrub and woodlands predominate, and watercourse vegetation.

Generalized linear mixed models (GzLMMs; binomial family) were used to assess the effect of the range of explanatory variables on the individual serological status against *T. gondii* (negative/positive). The statistical differences in STG among livestock management areas (CR, SO, RBD, PU and MA) were evaluated in a first exploratory approach, the purpose of which was showing spatial differences in the serological status against *T. gondii*. A GzLMM for each species (red deer, fallow deer, and wild boar) was designed. In these models, serological status against *T. gondii* was the response variable; the sex, age class, and the livestock management area were the explanatory variables. The sampling period and month were fitted in the model as random-effect factors.

Concerning the final model, it included livestock management area and sampling period as random-effect factors, since the main aim of this study was to generalize the effect of the variables included on the serological status against *T. gondii* regardless of the livestock management area. Models were also performed separately for each species (red deer, fallow deer, and wild boar). The explanatory variables included individual, environmental, populational and stochastic factors. Individual factors encompassed sex, age class, and TB status. Environmental factors comprised DWAT, DE, DCOAST, DHS, DURB, closed habitats, and watercourse vegetation. Regarding populational factors, the population densities of wild (fallow deer, red deer, and wild boar) and domestic ungulates (cattle and horses), as well as the abundance (KAI of footprints) of wild carnivores (all together genet, Eurasian badger, red fox, and Egyptian mongoose, and separately, the abundance of Iberian lynx) were included. Finally, the stochastic factors were the previous sampling period’s rainfall and temperature. The two-way interactions between individual-stochastic factors separately (sex-age, and rainfall-temperature) and all together (sex-rainfall and age-rainfall), as well as between population-individual factors (density-age), and population-stochastic factors (rainfall-density) were also included in the models. For the GzLMMs, a binomial error and a logit link function were used. Stepwise selection processes for the final models were performed on the basis of the corrected AIC (Akaike, 1974) (Table S2). Furthermore, the assumptions of binomial GzLMMs were met in all the best models selected (Zuur et al., 2009). The predicted probabilities of serological response to *T. gondii* obtained from these models were used to represent the results. Finally, cross-correlations and autocorrelations between STG and its predicted response probability between the different species were carried out to explore similarities of temporal patterns (Göloglu and Pott, 2008).

All models were performed using the R library *lme4* 1.1-21 version of the R software 4.0.2 (Bates et al., 2015). Significant *p*-values were set at 0.05. Confidence intervals for seroprevalences were estimated by the standard error (S.E.) 95%, which expression was S.E. 95% = 1.96 * standard error.

Results

General

The STG (MAT ≥1:25; % ± S.E. 95%) in wild boar was 39.0±3.3 (n=698), followed by red deer 30.7±4.4 (n=423), and fallow deer 29.7±4.2 (n=452). Among the seropositive animals, titers of 1:25 were detected in 34.5% wild boar, 27.7% red deer, and 55.2% fallow deer, whereas titers ≥ 1:50 were found in 72.3% red deer, 65.5% wild boar, and 44.8% fallow deer. We observed increasing age trends in STG in all wild ungulate species, except for wild boar females (Figure 2a) since, interestingly, piglets already showed high STG. With respect to gender, males tended to present higher STG than females in deer species (32.1-28.8% and 32-26.4% for red deer and fallow deer, respectively), whereas the opposite was observed in wild boar (STG=26.2% for males, STG=29.7% for females; Figure 2a; see statistical comparisons below).

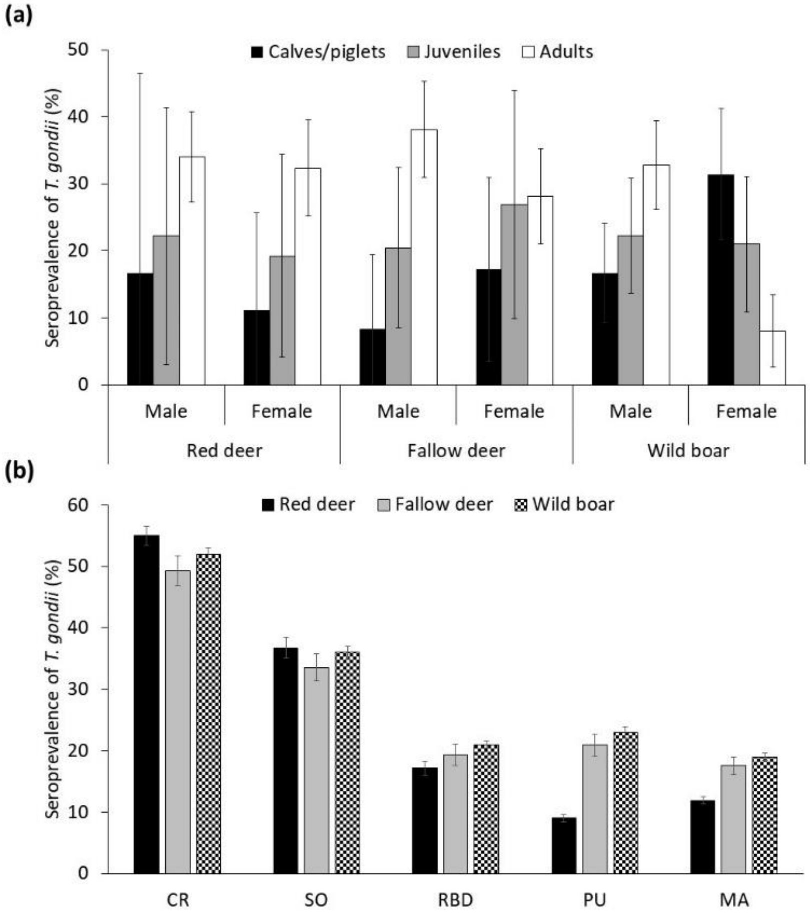

Figure 2. (**a**) Seroprevalence (± standard error (S.E.) 95%) of *Toxoplasma gondii* depending on age class and sex in red deer, fallow deer and wild boar (**b**) Seroprevalence (± S.E. 95%) of *T. gondii* obtained from selected generalized linear mixed models (GzLMMs) for the species studied depending on the livestock management area, from north to south areas (see Figure 1 for a map of the areas with their full names).

Contrasted STG were apparent among areas, which was consistent across species. In this sense, seroprevalence decreased from north to south, more markedly in red deer (Figure 2b). The temporal evolution of STG, and trends in the estimated density/abundance of each different species are summarized in Figures 3a and 3b, respectively. In this regard, the STG exhibited strong annual fluctuations, mostly in deer species (Figure 3a). Actually, it is noteworthy the significant decrease of STG in these species since the season 2013-2014. No autocorrelations or cross-correlations were observed.

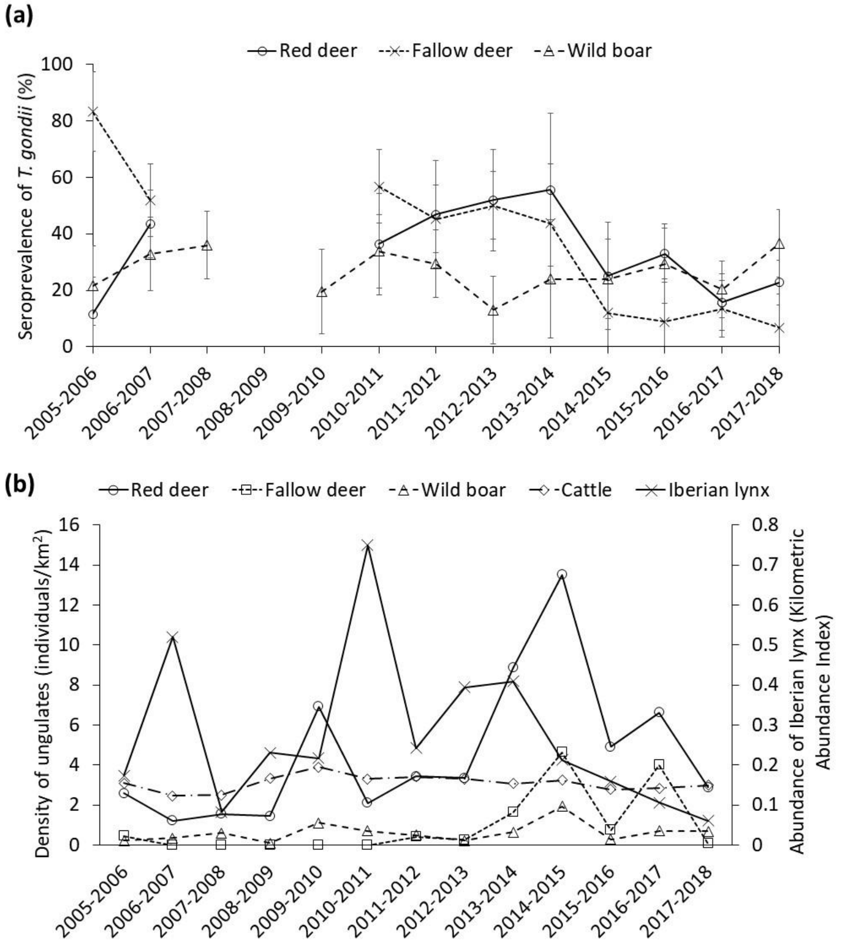

Figure 3. Temporal trend of the (**a**) seroprevalence of *Toxoplasma gondii* (± standard error (S.E.) 95%), and (**b**) population density of red deer, fallow deer, wild boar, and cattle (individuals/km^2), and Kilometric Abundance Index of Iberian lynx.

Factors determining the seroprevalence of Toxoplasma gondii

There were statistically significant differences in the STG between livestock management areas for all the wild ungulates species (red deer, $F = 13.4$, df = 410, $p \leq 0.01$; fallow deer, $F = 4.5$, df = 436, $p \leq 0.01$; and wild boar $F = 10.3$, df = 682, $p \leq 0.01$), confirming the north to south spatial decreasing gradient (Figure 2b).

The results of the GzLMMs on the status against *T. gondii*, incorporating broader environmental and populational information are shown in Table 1. The conditional R^2 obtained from these models were 0.37, 0.53 and 0.25, for red deer, fallow deer, and wild boar, respectively.

Table 1. Results of the GzLMMs of risk factors associated with seroprevalence of *Toxoplasma gondii* in each species studied related to sex, age class, straight-line distance to the nearest coastline (DCOAST), previous sampling period's rainfall (rainfall) and temperature (temperature), annual density of wild ungulates and livestock (horses and cattle), abundance of carnivores and Iberian lynx (KAI), presence of generalized tuberculosis-like lesions (TBL), cover level of closed habitat, straight-line distance to the nearest small human settlements (DHS), and two-ways interactions among them.

Variables	**Red Deer**			**Fallow Deer**			**Wild Boar**		
	F **df (x,y)**	**Estimate ± S.D.**	***p***	***F*** **df (x,y)**	**Estimate ± S.D.**	***p***	***F*** **df (x,y)**	**Estimate ± S.D.**	***p***
Sex[1]	0.44 (1, 420)	Female: -3.01±0.99	**<0.01**				0.22 (1, 695)	Female: 0.89±0.38	**0.03**
Age[2]	1.46 (2, 420)	Juveniles: 0.01±0.81 Adults: 1.03±0.70	**0.04**				2.86 (2, 695)	Juveniles: -3.76±1.24 Adults: 0.85±0.76	**<0.01**
Presence of generalizedTBL[3]	2.95 (1, 420)	Positive: 0.59±0.31	**0.05**	14.47 (1, 446)	Positive: 1.86±0.48	**<0.01**			
DCOAST	25.19 (1, 420)	<0.001±<0.001	**<0.01**	5.69 (1, 446)	<0.001±<0.001	**0.01**	26.70 (1, 695)	<0.001±<0.001	**<0.01**
DE	17.67 (1, 420)	<0.001±<0.001	0.07						
DHS							2.93 (1, 695)	<0.001±<0.001	**<0.01**
Closed habitat				5.75 (1, 446)	-0.31±0.15	**0.04**			
Rainfall	3.24 (1, 420)	0.01±<0.001	**<0.01**	0.07 (1, 446)	-0.05±0.01	**<0.01**	2.40 (1, 695)	<0.001±<0.001	0.15
Temperature	3.23 (1, 420)	-0.68±0.32	**0.04**	3.88 (1, 446)	-1.61±0.57	**<0.01**			
Density of red deer	0.43 (1, 420)	-0.11±0.04	**<0.01**				1.27 (1, 695)	-0.05±0.03	0.07
Density of fallow deer	0.43 (1, 420)	0.11±0.06	**0.04**	6.03 (1, 446)	-0.42±0.18	**0.02**	7.07 (1, 695)	-0.16±0.07	**0.02**
Density of wild boar							4.1 (1, 695)	0.34±0.16	**0.04**
Density of horses	1.14 (1, 420)	0.08±0.05	0.10						
Abundance of Iberian lynx				5.81 (1, 446)	3.56±1.60	**0.03**	0 (1, 695)	-0.86±0.45	0.06

Abundance of carnivores	13.88 (1, 420)	0.35±0.09	**<0.01**						
Sex[1]*Rainfall	10.18 (1, 420)	Rainfall*Female: -0.01±<0.001	**<0.01**						
Sex[1]*Age[2]							2.34 (2, 695)	Female*Juveniles: -0.97±0.58 Female*Adults: -1.01±0.45	0.07
Temperature* Rainfall				1.95 (1, 446)	<0.001±<0.001	**<0.01**			
Rainfall*Age[2]							7.19 (2, 695)	Rainfall*Juveniles: 0.01±<0.001 Rainfall*Adults: <0.001±<0.001	**<0.01**

The models were fitted using the sampling period and livestock management area as random effect factors. Parameter estimates for: [1]the sex variable were calculated using males as the reference, [2]the age variable were calculated using calves and piglets for red deer and wild boar, respectively, as the reference, and [3]the absence of generalized tuberculosis-like lesions (TBL) for the presence of generalized TBL variable. "*" represents interactions among explanatory variables

Individual Factors

The sex and age classes were statistically significant factors in the models on red deer and wild boar. However, no sex or age-related differences were found in fallow deer. Regarding red deer, females had lower STG than males, and irrespective of sex, the pattern increased with the age. Concerning wild boar, different sex-related age patterns were shown, increasing for males but not for females (the sex by age interaction was marginally significant).

Regarding TB status, the prevalence of TBL (% ± S.E. 95%) for wild boar, red deer and fallow deer were 77.4 ± 3.1, 42.5 ± 4.7, and 16.4 ± 3.7, respectively. Wild boar had the highest prevalence of generalized TBL (% ± S.E. 95%; 27.7 ± 3.5), followed by red deer (17.7 ± 4.2) and fallow deer (8.1 ± 2.9). The STG was higher in red deer and fallow deer presenting generalized TBL (Figure 4) compared to generalized TBL-free individuals (TBL-free plus not generalized TBL positive). As for the wild boar, a complementary model was performed with the purpose of exploring the effect of the presence of TBL (positive or negative), since no effect of the presence of generalized TBL was observed. In this model, wild boar presenting TBL showed higher STG than negative individuals (*F* = 8.96, df = 695, *p* = 0.05).

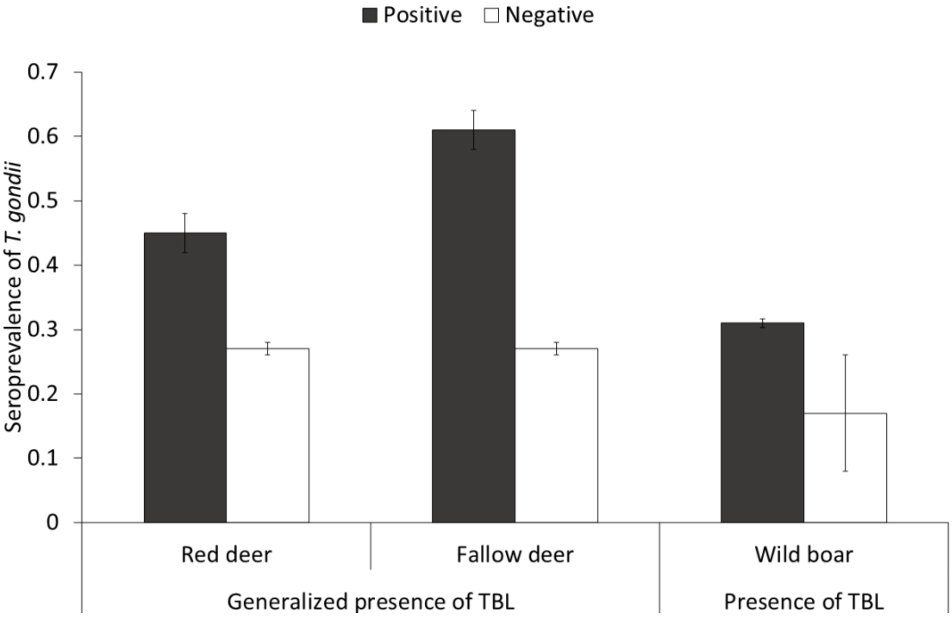

Figure 4. Predicted seroprevalence (± confidence interval (C.I.) 95%) of *Toxoplasma gondii* depending on the tuberculosis status in red deer and fallow deer (interpreted as positive animals with generalized presence of tuberculosis-like lesions (TBL)), and in wild boar (interpreted as positive animals with presence of TBL).

Environmental factors

The further to the coastline, the higher the STG was (see, e.g., Figure 5a for red deer) in all the species. Moreover, the closer to small human settlements, the higher the STG for wild boar was (Figure 5b). The increased availability of closed habitat significantly associated with lower STG in fallow deer (Figure 5c).

Populational factors

The abundance of carnivores was significantly and positively associated with the exposure to *T. gondii* in red deer (Figure 5d), and similarly, the Iberian lynx abundance positively associated with the seropositivity to this parasite in fallow deer (Figure 5e). The fallow deer density was negatively associated with the STG in wild boar (Figure 5f) and fallow deer, but positively in the case of red deer. As for red deer, a negative association was found between STG and density. In contrast, wild boar showed higher STG at higher densities.

Stochastic factors

Lower annual temperature was associated with higher STG in red deer. Furthermore, higher annual rainfall was associated with higher seropositivity to *T. gondii* in red deer. Regarding fallow deer, the interaction between rainfall and temperature was significant: overall, there was a trend to higher STG in cold years, and this pattern was more marked in dry years (see Figure 6a). Rainy years were statistically associated with higher STG in male red deer, but not in females (significant rainfall by sex interaction, Figure 6b). Concerning the wild boar, the rainfall was positively associated with the STG in juveniles, but this effect was not shown in other age classes (significant annual rainfall by age interaction, Figure 6c).

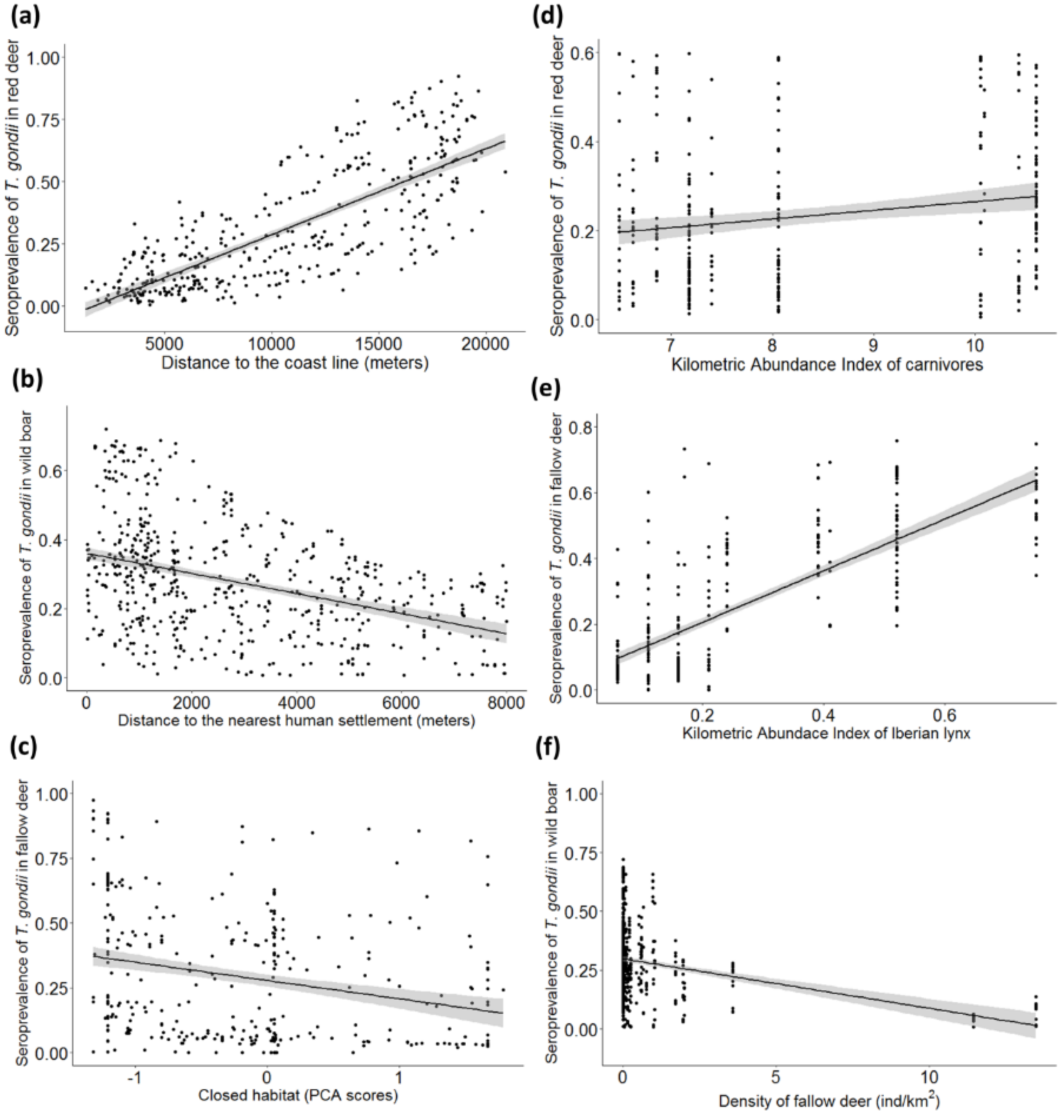

Figure 5. Predicted seroprevalence (± confidence interval (C.I.) 95%, represented by the shaded band) of *Toxoplasma gondii* obtained from selected generalized linear mixed models in (**a**) red deer depending on the distance to the coast line (m), (**b**) wild boar depending on the distance to the nearest human settlement (m), (**c**) fallow deer depending on the cover level of closed habitats, measured according to the principal component analysis (PCA) scores from axis 1, (**d**) red deer depending on the Kilometric Abundance Index (KAI) of carnivores species, (**e**) fallow deer depending on the KAI of Iberian lynx, and (**f**) wild boar depending on the density of fallow deer (individuals/km^2).

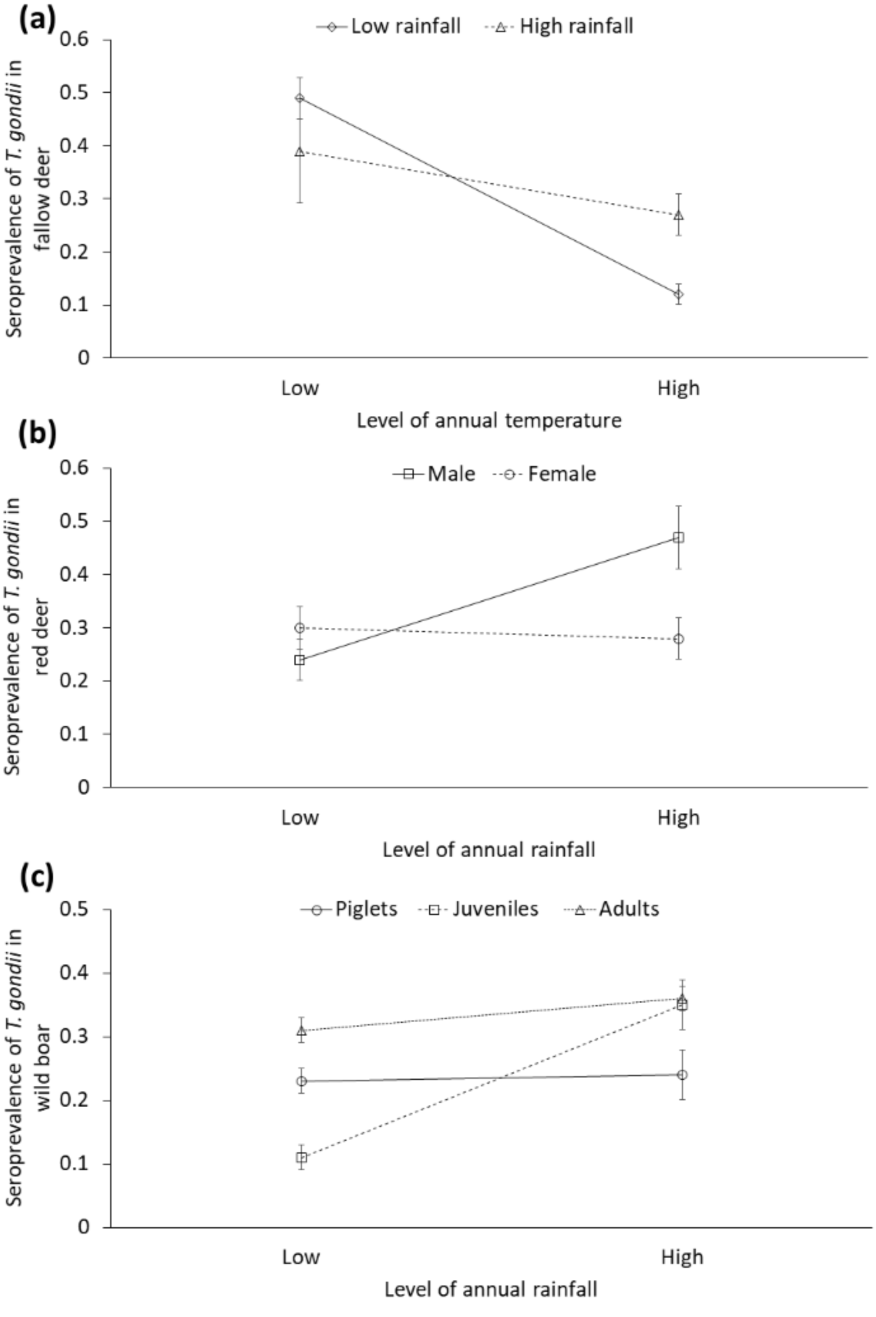

Figure 6. Predicted seroprevalence (± confidence interval (C.I.) 95%) of *Toxoplasma gondii* obtained from selected generalized linear mixed models in (**a**) fallow deer depending on the interaction between rainfall (mm) and temperature (°C), (**b**) red deer depending on the interaction between rainfall (mm) and sex, and (**c**) wild boar depending on the interaction between rainfall (mm) and age. To represent our results, and considering the mean values obtained, we established the following categories of rainfall and temperature for displaying results: low rainfall (≤ 521.10 mm), high rainfall (> 521.10 mm), low temperature (≤ 17.5 °C), and high temperature (> 17.5 °C).

Discussion

General patterns of the seroprevalence of Toxoplasma gondii

The STG reported in the present study oscillated considerably (from 29.7 to 39%) between the three species tested and sharing the same environment. This may be caused by differences in the susceptibility, the feeding behaviour, or the habitat use of those species determining the exposure (Dubey and Beattie, 1988; Panadero et al., 2010). The seroprevalence detected in wild boar (39%) concurs with studies conducted in Europe (Gauss et al., 2005; Jokelainen et al., 2012; Reiterová et al., 2016; Ruiz-Fons et al., 2006). However, in most studies from European countries, STG in wild boar ranged from 6 to 25% (Beral et al., 2012; Calero-Bernal et al., 2016; Coelho et al., 2014). In this regard, trophic relationships by predation and/or scavenging of a wide range of warm-blooded animals of the DNP may operate.

Concerning deer, the STG obtained (30.7% and 29.7%, for red deer and fallow deer, respectively) are also in accordance with those reported in the literature over Europe in general (Bartova et al., 2007; Rocchigiani et al., 2016) and Spain in particular (Almería et al., 2018; Gauss et al., 2006), ranging from 10.5 to 48.0%. Specifically for fallow deer, STG (29.7%) were among the highest reported in European studies that were mainly focussed on Spain (Almería et al., 2018; Bartova et al., 2007; Gauss et al., 2006). It was only exceeded by the rates obtained by Calero-Bernal et al. (2015) in south-central Spain (reaching the 48%). The higher rate of movement between areas reported for fallow deer in DNP may imply higher exposure to *T. gondii*, explaining the high STG observed in this species (Braza and Alvarez, 1987).

Overall, the wild ungulate host community of DNP showed higher STG compared with those reported in the literature of the European and Iberian contexts (Beral et al., 2012; Gauss et al., 2006; Rocchigiani et al., 2016). Mechanisms determining seroprevalence in different host species of the studied community are related to the life cycle of *T. gondii*, which involves both an environmental and a trophic transmission route (i.e. trophic relationships among potential hosts of the community; Wilson et al., 2020). Terrestrial herbivores should have the lowest *T. gondii* exposure, only through the ingestion of oocyst-contaminated vegetation, soil and/or drinking water. In DNP, the environmental presence of oocyst excreted by felids may be playing a major role (see below). The high biodiversity inhabiting DNP, which provides a wide range of hosts and ecological/epidemiological niches, and the optimal climatic conditions for the survival of the oocysts may favour the spread of the parasite in the DNP host community.

The specific role of the different factors in a long-term perspective is also detailed further in the discussion. Interestingly, while the STG exhibited strong annual fluctuations, mostly in deer species, it was more stable in wild boar. In populations from The Netherlands, seroprevalence in wild boar similarly established at around 35% (Opsteegh et al., 2011). Whereas authors stated that the actual mechanism behind the stabilization requires further investigation, an epidemiological SIS-model, that included a reversion to susceptible after infection (with loss of antibodies that may have been preceded by a loss of tissue cysts), fitted the data much better.

The north to south spatial gradient observed is similar to that exhibited by the prevalence of other shared pathogens tested in the wild ungulate community of DNP in previous studies (Barroso et al., 2020b; Gortázar et al., 2008). This pattern may relate to spatial variation in the contamination of the environment by *T. gondii* oocyst. The main large human settlements around DNP are concentrated in the northern part of the Park, with a subsequent higher presence of peri-domestic cat populations (Jokelainen et al., 2010), which may contaminate the environment with oocysts. Iberian lynx populations also show a north to south decreasing pattern in DNP (Garrote et al., 2011), contributing to a lesser extent to this contamination. *T. gondii* oocysts were found in faeces of 17% of cats sharing a habitat with Iberian lynx (Millán et al., 2009). In this regard, feral cats are the more likely reservoir host of parasites affecting the Iberian lynx and wildlife species in general, especially in areas where feral cats are abundant and widespread such as DNP surroundings (Millán and Casanova, 2007; Vicente et al., 2004).

Risk factors of the seroprevalence of T. gondii

Individual factors

In wild boar, overall, females had significantly higher STG than males. This result is in accordance with previous studies in this species (Gauss et al., 2005; Jokelainen et al., 2012; Reiterová et al., 2016; Ruiz-Fons et al., 2006). However, the age pattern observed was opposite to that of males. Several authors have reported that no statistically significant effect of age on STG in wild boar was observed (Antolová et al., 2007; Dubey, 2020d; Gauss et al., 2005), whereas only one study found a significantly higher prevalence in adult wild boar (Ruiz-Fons et al., 2006). Nevertheless, we must consider sex by age pattern to understand the differences. The increased exposure to *T. gondii* through life, together with the high persistence of antibodies against *T. gondii*, could explain the age pattern found in the STG in males. Even so, in females, the decline in the STG rather than indicating a decrease of exposure to the parasite, may be indicative of a subtle equilibrium of chronic infection and reduced specific humoral response that is not detected. Ecological and evolutive aspects determining differences in exposure may be behind this pattern. However, further research is required. Finally, piglets exhibiting high seroprevalences could be explained by maternal-derived antibodies, whose titers depend on those shown by sows, according to previous studies (García-Bocanegra et al., 2010c).

As for the red deer, the STG was significantly higher in adult individuals, which has been previously reported in many studies on *T. gondii* (Almería et al., 2018; Antolová et al., 2007; Jokelainen et al., 2012; Reiterová et al., 2016). An apparent similar trend, but not significant, was observed in fallow deer (Figure 2a). The increased exposure to *T. gondii* along life together with the high persistence of antibodies against *T. gondii* could explain the age pattern found in deer species.

Concerning concomitant TB infection, overall, the positive TB status of the animals significantly associated with STG in all the species studied. For deer species, the generalized presence of TBL was relevant, as well as the presence of TBL for wild boar. There are several studies on TB-*T. gondii* co-infection in humans (Hwang et al., 2012; Mashaly et al., 2017) but not in animals. The relationship of TB and STG observed may be mediated by exposure over time (age-related) and environment. In the latter case, the conditions favouring the persistence of MTC and *T. gondii* oocyst in the environment are similar (see “environmental factors” and “populational factors”).

Environmental factors

The distance to the coastline significantly positively associated with STG for all wild ungulates. Despite the usually reported contamination of seawater with *T. gondii* (Dubey, 2004), less favourable conditions to the survival of oocysts may occur in the surroundings of the coastline, according to previous studies developed in northern Spain (Panadero et al., 2010). These conditions are mainly the high temperatures reached in the sandy soils which favour the desiccation of the oocysts. The availability of closed habitat (more covered by vegetation) negatively associated with STG in fallow deer (see Figure 5c). This species typically uses and occupies meadows in the Park, and individuals sampled in more densely covered areas may have experienced lower exposure to *T. gondii*. By contrast, individuals of the other ungulate species combine the use of both types of habitats which may determine the absence of this effect.

The closer to small human settlements the higher the STG was for wild boar (see Figure 5b). This result is in accordance with previous studies where human settlements have become areas of epidemiological relevance for *T. gondii* infection, mainly mediated by the presence of peri-domestic species (Panadero et al., 2010; Villari et al., 2009). There are several human dwellings inside the Park, around which peri-domestic cats could settle and consequently contaminate the surroundings of these areas with oocysts. Moreover, wild boar could become infected through the consumption of food scraps from garbage located in these small settlements.

Populational factors

The abundances of Iberian lynx and other carnivores were statistically positively associated with STG in deer species. The presence and abundance of felids have been considered a relevant risk factor associated with *T. gondii* in livestock and wildlife worldwide (Almería et al., 2018; Castillo-Cuenca et al., 2020; Dubey, 2010; García-Bocanegra et al., 2010c; Jiménez-Martín et al., 2020). It has been reported that Iberian lynx could prey on fallow deer and less frequently on juveniles of red deer during seasons of rabbit's scarceness in DNP, especially in winter and autumn, reaching 5-10% of the biomass in the Iberian lynx diet (Delibes, 1980). This leads to an increased environmental contamination with *T. gondii* in areas with presence of wild ungulates. Furthermore, *T. gondii* infected red deer carcasses could pose a potential risk of *T. gondii* infection for carnivores species via scavenging and may therefore play a role as an amplifier of infection in the community (Bartova et al., 2007). This allows *T. gondii* to finish its life cycle, perpetuating its maintenance in the DNP host community. Little is known about the abundance of small mammals, as well as their STG in DNP. Further studies should focus on investigating their role in the maintenance and spread of this parasite in DNP.

STG significantly increases with density in wild boar. Several authors have described *T. gondii* as a density-dependent parasite for swine (Gauss et al., 2005; Villari et al., 2009). Density, together with ecological and behavioural factors typical of this species, could determine increased exposure by wild boar. Scavenging, including cannibalism, may be increased in high density situations when resources are scarce. In the dry season, both the availability of carcasses and exacerbated cannibalism behaviour (Carrasco-Garcia et al., 2018), but also ingestion of rodents and birds occur (Schley and Roper, 2003). The negative relationship found both in red deer and fallow deer between density and the risk to test positive can be explained by high recruitment of susceptible individuals (non-infected offspring) associated to high density years. Moreover, unlike for wild boar, this negative association indicates that no density-dependent effect in *T. gondii* infection occurs in deer species. These species become infected only through the ingestion of water or food contaminated with sporulated *T. gondii* oocysts, so no direct transmission route exists as in the case of wild boar, which possesses scavenging habits. However, the positive relationship evidenced between STG in red deer and fallow deer density may be mediated by increased susceptibility and/or exposure at high densities of ungulates due to the competition for scarce resources, but the exact mechanism deserves further research.

Stochastic factors

Temperature was a significant factor for red deer, displaying the lower STG during the following seasons to the warmest ones. Furthermore, rainfall significantly interacted with temperature to explain STG in fallow deer, so that the effect of the previous sampling period’s temperature on STG was more marked when the previous sampling period was dry. Previous driest and warmest sampling periods were markedly associated with a lower STG (see Figure 6a). Drought together with warm temperatures leads to higher rates of evaporation and subsequent desiccation, limiting the survival and sporulation of the oocysts in the environment. As consequence, the exposure of herbivores to infective *T. gondii* oocyst decreases (Dubey and Beattie, 1988; Gamarra et al., 2008; Gauss et al., 2006).

Additionally, the interaction between rainfall and individual factors was significantly associated with the STG in red deer and wild boar. Concerning red deer, higher previous annual rainfall was related to higher STG in stags, but not in females. This effect of rainy years on the prevalence of pathogens exhibited by males with respect to females have been evidenced in previous studies on TB in DNP, and may relate to increased exposure and/or susceptibility mediated by sexual behaviour and life history traits (Barroso et al., 2020b). An immunosuppressant effect of the intense rut that typically occurs in rainy years has been suggested. Intense rut implies greatest investments by red deer stags in terms of reproductive effort (testosterone metabolite levels and sexual signals), and the conflict between the immune response and the reproductive effort in this species is well known (Lochmiller and Deerenberg, 2000; Malo et al., 2009). *T. gondii* tissue cysts in many organs, including viscera, are believed to persist for the lifetime of the host. In addition, deer rutting typically occurs in the ecotone, which provides excellent wet conditions for oocysts to persist. Despite the same risk derived from the reproductive efforts during the rut exist for fallow deer, no sex-dependent effect of rainfall was observed in STG in this species, which is not surprising since rutting in fallow deer takes place later in Autumn, when rainfall conditions normally are less determinant.

As for wild boar, the positive effect of the rainfall on STG was more markedly in juveniles than adults and piglets. The early dispersal behaviour of young males from the natal area may leads to higher exposure to *T. gondii* (Mauget et al., 1984). This, together with the increased survival of oocysts would give rise to higher infection rates in this age group (Dubey and Beattie, 1988; Gamarra et al., 2008).

Conclusions

This study provides evidence that factors behind the risk of *T. gondii* infection in wild boar, red deer and fallow deer are related to environmental and trophic transmission routes, so as to individual, population and species characteristics. We provided evidence for most of these relationships (e.g. climate or population mediated) and trends. Concomitant pattern among species, indicated that, overall, drivers of risk also operated at the community level. However, this research raised several questions that deserve further research. Approximately one-third of the human world’s population is chronically infected while seroprevalence tends to decrease since the early 1960s in many countries (Rougier et al., 2017). As this decline in seroprevalence leads to loss of immunity, it becomes more relevant for the identification of the epidemiological role of wild hosts and the understanding of the epidemiology and ecology of *T. gondii* infection in wild host communities and at their interfaces with livestock and human. Thus, game meat, in particular venison, consumption should not be neglected as a public health risk for humans, with the subsequent impact to the public health (Dubey et al., 2021). For these purposes, addressing host population, community and environmental factors at a broad temporal scale is key.

Acknowledgments

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Supplementary material 2.2

Table S1. Sample size (N) and seroprevalence of *Toxoplasma gondii* (Seroprev; %) by species, sampling period and livestock management area in wild ungulates

Species	**Sampling period**	**CR**	**SO**	**RBD**	**PU**	**MA**	**Total**
		Seroprev (N)	Seroprev (N)	Seroprev (N)	Seroprev (N)	Seroprev (N)	Seroprev (N)
Red deer	2005-2006	0 (6)	15 (20)	-	-	-	11.5 (26)
	2006-2007	69.6 (23)	16.7 (6)	28.6 (7)	0 (2)	12.5 (8)	43.5 (46)
	2007-2008	-	-	-	-	-	
	2009-2010	-	-	-	-	-	
	2010-2011	43.8 (16)	57.1 (7)	-	0 (2)	20 (5)	36.4 (33)
	2011-2012	71.4 (7)	64.3	33.3 (3)	0 (3)	0 (5)	46.9 (32)
	2012-2013	100 (8)	40 (5)	33.3 (3)	100 (1)	20 (10)	51.9 (27)
	2013-2014	75 (4)	50 (4)	0 (1)	-	-	55.6 (9)
	2014-2015	18.8 (16)	50 (8)	0 (4)	-	-	25 (28)
	2015-2016	66.7 (15)	46.7 (15)	23.5 (17)	20 (15)	13.3 (15)	32.9 (77)
	2016-2017	40 (10)	20 (15)	6.7 (15)	6.7 (15)	13.3 (15)	15.7 (70)
	2017-2018	60 (15)	26.7 (15)	20 (15)	0 (15)	6.7 (15)	22.7 (75)
	Total	54.2 (120)	35.8 (109)	17.7 (68)	9.4 (53)	12.3 (73)	30.7 (423)
Fallow deer	2005-2006	100 (5)	76.9 (13)	-	-	-	83.3 (18)
	2006-2007	61.3 (31)	50 (20)	25 (8)	80 (5)	38.5 (13)	51.9 (77)
	2007-2008	-	-	-	-	-	
	2009-2010	-	-	-	-	-	
	2010-2011	75 (4)	55.6 (9)	100 (3)	42.9 (7)	42.9 (7)	56.7 (30)
	2011-2012	60 (5)	33.3 (12)	66.7 (3)	40 (5)	50 (6)	45.2 (31)
	2012-2013	70 (10)	83.3 (6)	0 (2)	0 (2)	30 (10)	50 (30)
	2013-2014	100 (2)	0 (5)	100 (2)	25 (4)	66.7 (3)	43.8 (16)
	2014-2015	0 (1)	0 (7)	40 (5)	20 (5)	0 (7)	12 (25)
	2015-2016	13.3 (15)	6.7 (15)	7.7 (13)	16.7 (18)	6.3 (16)	8.8 (76)
	2016-2017	40 (15)	20 (15)	0 (14)	6.7 (15)	0 (15)	13.5 (74)
	2017-2018	16.7 (12)	6.7 (15)	5.6 (18)	6.7 (15)	0 (15)	6.7 (75)
	Total	49 (100)	33.3 (117)	19.1 (68)	21.1 (76)	17.6 (91)	29.7 (452)
Wild boar	2005-2006	-	16.7 (6)	8.3 (12)	25 (8)	28 (25)	21.6 (51)
	2006-2007	42.9 (14)	21.4 (14)	29.4 (17)	38.9 (18)	25 (4)	32.8 (67)
	2007-2008	-	53.8 (26)	42.9 (7)	30.8 (13)	25.9 (27)	35.9 (73)
	2009-2010	50 (2)	50 (4)	23.5 (17)	-	5.6 (18)	19.5 (41)
	2010-2011	58.8 (17)	50 (10)	20 (20)	8.3 (12)	33.3 (9)	33.8 (68)
	2011-2012	75 (8)	31.6 (19)	26.3 (19)	18.2 (11)	9.1 (11)	29.4 (68)
	2012-2013	33.3 (9)	0 (6)	0 (13)	14.3 (7)	15.8 (19)	13 (54)
	2013-2014	-	-	0 (7)	33.3 (18)	-	24 (25)
	2014-2015	50 (2)	6.3 (16)	37.5 (8)	37.5 (17)	28.6 (7)	24 (50)
	2015-2016	53.3 (15)	16.7 (12)	30 (10)	16.7 (6)	12.5 (8)	29.4 (51)
	2016-2017	46.7 (15)	26.7 (15)	14.3 (14)	10 (20)	16.7 (15)	20.3 (79)
	2017-2018	57.1 (14)	64.3 (14)	23.1 (13)	23.1 (13)	21.4 (14)	36.6 (71)
	Total	52.1 (96)	33.1 (142)	20.8 (159)	23.8 (142)	19 (158)	39.4 (698)

Table S2. Summary of the stepwise model selection procedure, based on the corrected Akaike's information criterion (cAIC), used to explain the serological status against *Toxoplasma gondii*. Full model comprises the sex, age class, straight-line distance to: the ecotone (DE), the nearest water point (DWAT), the coast line (DCOAST), the nearest small human settlement (DHS), urban (DURB), previous sampling period's rainfall (rainfall) and temperature (temperature), coverage of closed (closed_ha) and watercourse vegetation (water_ha), annual density of fallow deer (FD_den), red deer (RD_den), wild boar (WB_den), cattle (cattle_den), and horse (horse_den), abundance of carnivores (KAI_carn) and Iberian lynx (KAI_lynx), prevalence of generalized tuberculosis-like lesions (generalized_TBL), and some interaction among them (*). In bold type is shown the variable which was removed between steps**.**

Model	cAIC
Full model for red deer: age + sex + generalized_TBL + DE + DCOAST + DHS + **DWAT** + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + age*RD_den + rainfall*RD_den + rainfall*sex + rainfall*age + rainfall*temperature [M1]	448.21
age + sex + generalized_TBL + DE + DCOAST + DHS + DURB + **closed_ha** + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + age*RD_den + rainfall*RD_den + rainfall*sex + rainfall*age + rainfall*temperature [M2]	446.22
age + sex + generalized_TBL + DE + DCOAST + DHS + DURB + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + age*RD_den + rainfall*RD_den + rainfall*sex + **rainfall*age** + rainfall*temperature [M3]	444.23
age + sex + generalized_TBL + DE + DCOAST + DHS + DURB + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + **age*RD_den** + rainfall*RD_den + rainfall*sex + rainfall*temperature [M4]	442.02
age + sex + generalized_TBL + DE + DCOAST + DHS + DURB + **water_ha** + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*RD_den + rainfall*sex + rainfall***temperature** [M5]	441.70
age + sex + generalized_TBL + DE + DCOAST + DHS + DURB + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + **KAI_lynx** + rainfall + temperature + sex*age + rainfall*RD_den + rainfall*sex + rainfall*temperature [M6]	439.72
age + sex + generalized_TBL + DE + DCOAST + DHS + DURB + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + rainfall + temperature + sex*age + rainfall*RD_den + rainfall*sex + **rainfall*temperature** [M7]	437.87
age + sex + generalized_TBL + DE + DCOAST + **DHS** + DURB + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + rainfall + temperature + sex*age + rainfall*RD_den + rainfall*sex [M8]	436.06
age + sex + generalized_TBL + DE + DCOAST + **DURB** + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + rainfall + temperature + sex*age + rainfall*RD_den + rainfall*sex [M9]	434.24
age + sex + generalized_TBL + DE + DCOAST + FD_den + RD_den + **WB_den** + horse_den + cattle_den + KAI_carn + rainfall + temperature + sex*age + rainfall*RD_den + rainfall*sex [M10]	432.48
age + sex + generalized_TBL + DE + DCOAST + FD_den + RD_den + horse_den + **cattle_den** + KAI_carn + rainfall + temperature + sex*age + rainfall*RD_den + rainfall*sex [M11]	431.19

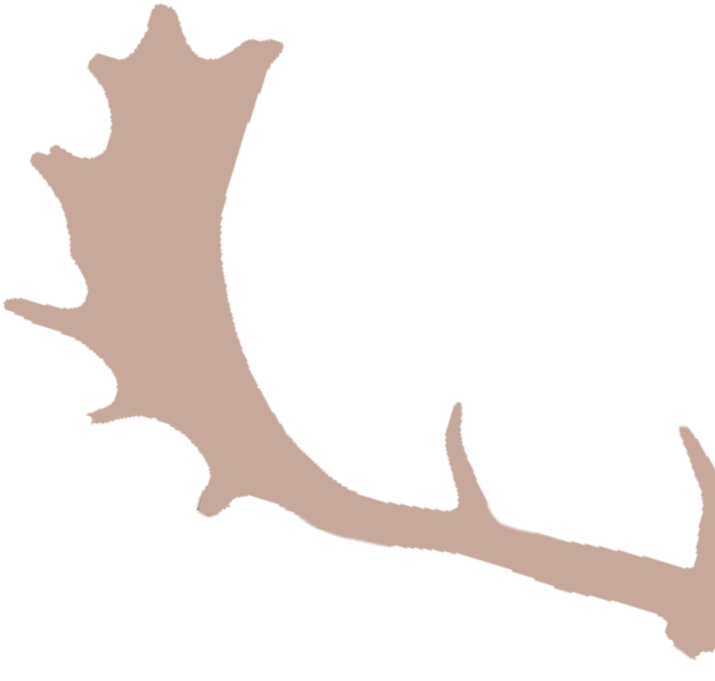
age + sex + generalized_TBL + DE + DCOAST + FD_den + RD_den + horse_den + KAI_carn + rainfall + temperature + **sex*age** + rainfall*RD_den + rainfall*sex [M12]	430.34
age + sex + generalized_TBL + DE + DCOAST + FD_den + RD_den + horse_den + KAI_carn + rainfall + temperature + **rainfall*RD_den** + rainfall*sex [M13]	429.48
age + sex + generalized_TBL + DE + DCOAST + FD_den + RD_den + horse_den + KAI_carn + rainfall + temperature + rainfall*sex [final model]	428.39
Full model for fallow deer: age + sex + generalized_TBL + DE + DCOAST + DHS + DWAT + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + **age*FD_den** + rainfall*FD_den + rainfall*sex + rainfall*age + rainfall*temperature [M1]	451.51
age + sex + generalized_TBL + DE + DCOAST + DHS + DWAT + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*FD_den + rainfall*sex + **rainfall*age** + rainfall*temperature [M2]	447.67
age + sex + generalized_TBL + **DE** + DCOAST + DHS + DWAT + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*FD_den + rainfall*sex + rainfall*temperature [M3]	445.27
age + sex + generalized_TBL + DCOAST + **DHS** + DWAT + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*FD_den + rainfall*sex + rainfall*temperature [M4]	443.27
age + sex + generalized_TBL + DCOAST + **DWAT** + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + horse_den + cattle_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*FD_den + rainfall*sex + rainfall*temperature [M5]	441.27
age + sex + generalized_TBL + DCOAST + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + horse_den + **cattle_den** + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*FD_den + rainfall*sex + rainfall*temperature [M6]	440.05
age + sex + generalized_TBL + DCOAST + DURB + closed_ha + water_ha + FD_den + RD_den + **WB_den** + horse_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*FD_den + rainfall*sex + rainfall*temperature [M7]	438.15
age + sex + generalized_TBL + DCOAST + DURB + closed_ha + water_ha + FD_den + **RD_den** + horse_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*FD_den + rainfall*sex + rainfall*temperature [M8]	436.22
age + sex + generalized_TBL + DCOAST + DURB + closed_ha + water_ha + FD_den + horse_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + **rainfall*FD_den** + rainfall*sex + rainfall*temperature [M9]	434.64
age + sex + generalized_TBL + DCOAST + DURB + closed_ha + water_ha + FD_den + horse_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + **rainfall*sex** + rainfall*temperature [M10]	433.24
age + sex + generalized_TBL + DCOAST + DURB + closed_ha + water_ha + FD_den + **horse_den** + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*temperature [M11]	432.09
age + sex + generalized_TBL + DCOAST + **DURB** + closed_ha + water_ha + FD_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*temperature [M12]	430.88
age + sex + generalized_TBL + DCOAST + closed_ha + **water_ha** + FD_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + rainfall*temperature [M13]	429.83
age + sex + generalized_TBL + DCOAST + closed_ha + FD_den + KAI_carn + KAI_lynx + rainfall + temperature + **sex*age** + rainfall*temperature [M14]	429.05
age + **sex** + generalized_TBL + DCOAST + closed_ha + FD_den + KAI_carn + KAI_lynx + rainfall + temperature + rainfall*temperature [M13]	428.50
age + generalized_TBL + DCOAST + closed_ha + FD_den + KAI_carn + KAI_lynx +	426.77

rainfall + temperature + rainfall*temperature **[M14]**	
generalized_TBL + DCOAST + closed_ha + FD_den + **KAI_carn** + KAI_lynx + rainfall + temperature + rainfall*temperature **[M15]**	425.25
generalized_TBL + DCOAST + closed_ha + FD_den + KAI_lynx + rainfall + temperature + rainfall*temperature **[final model]**	424.92
Full model for wild boar: age + sex + generalized_TBL + DE + DCOAST + DHS + DWAT + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + horse_den + **cattle_den** + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*sex + rainfall*age + rainfall*temperature [M1]	780.79
age + sex + generalized_TBL + DE + DCOAST + DHS + DWAT + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + **horse_den** + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*sex + rainfall*age + rainfall*temperature [M2]	770.04
age + sex + generalized_TBL + DE + DCOAST + DHS + DWAT + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + KAI_carn + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + **rainfall*sex** + rainfall*age + rainfall*temperature [M3]	773.84
age + sex + generalized_TBL + DE + DCOAST + DHS + DWAT + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + **KAI_carn** + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*age + rainfall*temperature [M4]	771.88
age + sex + generalized_TBL + DE + DCOAST + DHS + **DWAT** + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*age + rainfall*temperature [M5]	768.09
age + sex + generalized_TBL + **DE** + DCOAST + DHS + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*age + rainfall*temperature [M6]	766.23
age + sex + generalized_TBL + DCOAST + DHS + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*age + **rainfall*temperature** [M7]	764.56
age + sex + **generalized_TBL** + DCOAST + DHS + DURB + closed_ha + water_ha + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*age [M8]	763.29
age + sex + DCOAST + DHS + **DURB** + closed_ha + water_ha + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*age [M9]	762.15
age + sex + DCOAST + DHS + **closed_ha** + water_ha + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*age [M10]	761.39
age + sex + DCOAST + DHS + **water_ha** + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + rainfall*WB_den + rainfall*age [M11]	761.27
age + sex + DCOAST + DHS + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + age*WB_den + **rainfall*WB_den** + rainfall*age [M12]	761.15
age + sex + DCOAST + DHS + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + **age*WB_den** + rainfall*age [M13]	760.31
age + sex + DCOAST + DHS + FD_den + RD_den + WB_den + KAI_lynx + rainfall + temperature + sex*age + rainfall*age [final model]	759.83

CAPÍTULO 2.3. Factores determinantes a largo plazo de la seroprevalencia del virus de la lengua azul en especies de cérvidos del sur de España

CHAPTER 2.3. Long-term determinants of the seroprevalence of bluetongue virus in deer species in southern Spain

Barroso, P., Risalde, M.A., García-Bocanegra, I., Acevedo, P., Barasona, J.A, Palencia, P., Carro, F., Jiménez-Ruiz, S., Pujols, J., Montoro, V., Vicente, J., 2021 Long-term determinants of the seroprevalence of bluetongue virus in deer species in southern Spain. *Research in Veterinary Science* 139, 102-111.   https://doi.org/10.1016/j.rvsc.2021.07.001



Resumen

La lengua azul es una enfermedad transmitida por vectores que afecta a rumiantes domésticos y silvestres, con un gran impacto socioeconómico. En España se ha dado una circulación endémica de los serotipos 1 y 4 del virus de la lengua azul (VLA), desde 2004 y 2007, respectivamente. Sin embargo, los estudios epidemiológicos en rumiantes silvestres raramente han sido enfocados desde una perspectiva a largo plazo. Desde 2005 hasta 2018 se realizó la necropsia de un total de 881 cérvidos (ciervos (*Cervus elaphus*) y gamos (*Dama dama*)) como parte del programa de monitorización sanitaria del Parque Nacional de Doñana (PND). Las muestras de suero obtenidas fueron analizadas para evidenciar la presencia de anticuerpos contra el VLA, con los objetivos de analizar la tendencia temporal de su seroprevalencia y evaluar los factores moduladores de ésta (individuales, poblacionales, ambientales y estocásticos). El ciervo mostró seroprevalencias del VLA estadísticamente superiores (% ± error estándar 95%; 78,6±3,8) a las del gamo (53,1±4,7). La detección del VLA-1 y VLA-4 en las crías mediante seroneutralización sugirió la circulación de ambos serotipos durante el periodo de estudio. Para el ciervo, los años húmedos en los que se observaron elevadas densidades proporcionaron unas condiciones favorables para la trasmisión del virus mediada por vectores. Además, la proximidad a zonas de pastos permanentes, hábitats muy favorables para los *Culicoides*, se asoció con elevadas seroprevalencias del VLA. Las diferencias en la ecología y el comportamiento de las especies de cérvidos, que a su vez determinan su exposición a los vectores, podrían determinar las diferencias halladas en los patrones de seroprevalencias. En este estudio se evidencia el papel que los cérvidos podrían jugar en el mantenimiento del VLA. Sin embargo, se requieren futuras investigaciones con las que dilucidar el papel epidemiológico de los hospedadores en diferentes contextos, así como las consecuencias del cambio climático en la competencia de las poblaciones de vectores y su potencial efecto en las dinámicas de infección por el VLA en las comunidades de hospedadores.

Abstract

Bluetongue is a vector-borne disease affecting domestic and wild ruminants, with a major socioeconomic impact. Endemic circulation of the bluetongue virus serotype 4 (BTV-4) and BTV-1 have occurred in Spain since 2004 and 2007, respectively. However, epidemiological studies have seldom been approached from a long-term perspective in wild ruminants. A total of 881 deer (red deer (*Cervus elaphus*) and fallow deer (*Dama dama*)) were necropsied from 2005 to 2018 as part of the DNP health monitoring programme. Serum samples were tested for antibodies against BTV with the aims of assessing the temporal trend and to evaluate the modulating factors: individual, populational, environmental, and stochastic. Red deer displayed statistically significant higher seroprevalences of BTV (SBT (%) ± standard error 95%; 78.6±3.8) than fallow deer (53.1±4.7). The detection of BTV-1 and BTV-4 by the serum neutralization test in calves suggested the circulation of both serotypes over the study period. For red deer, wet years together with high densities could provide suitable conditions for vector-borne BTV transmission. Moreover, proximity to high suitability habitat for *Culicoides*, permanent pasturelands, was associated with higher SBT. The differences in the ecology and behaviour of deer species influencing the exposure to the vectors could determine the differences found in the SBT patterns. This study evidences the role that deer species may play in the maintenance of BTV, however, elucidating the epidemiological role of host in different contexts, as well as the consequences of climate change on the competent vector populations and its potential effect on the dynamics of BTV infection in hosts communities, deserve further research.

Introduction

The bluetongue virus (BTV) is an arthropod-borne virus belonging to the genus *Orbivirus* primarily affecting domestic and wild ruminants, as well as camelids (Mertens et al., 2008). Bluetongue (BT) has been detected in small domestic ruminants and different wild ruminant species, which present clinical signs of fever, nasal discharge, facial oedema, cyanotic tongue, ataxia and apathy (Mertens et al., 2008; Rao et al., 2017). This non-zoonotic pathogen is mainly transmitted by biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) (Rao et al., 2017). BT is an OIE notifiable emerging and re-emerging disease which exerts a major socioeconomic impact on the areas affected. Up to date, 28 serotypes of BTV (BTV-1 to BTV-28) and another four putative serotypes have been identified worldwide (e.g.Yang et al., 2020).

BTV has been circulating throughout Europe in the last few decades, becoming endemic in several countries, including Spain. Since the 2000s, several outbreaks of BTV-1, BTV-4 and BTV-8 have been reported in Spain in both domestic and wild ruminants (RASVE, 2021). BTV-4 was detected in southern Spain for the first time in October 2004 after being introduced from north Africa (RASVE, 2021). BTV-1 was detected in July 2007 throughout southern Spain and quickly expanded north and eastwards (RASVE, 2021). BTV-8 was detected in northern Spain in 2008, giving rise to sporadic outbreaks but has not been detected from 2013 to 2020 (RASVE, 2021). Antibodies against these three serotypes have been reported in different wild ruminant species in Spain even in the absence of outbreaks reported in livestock (Falconi et al., 2011; García-Bocanegra et al., 2011; Gómez-Guillamón et al., 2020; Rodríguez-Sánchez et al., 2010a; Ruiz-Fons et al., 2008a). In these cross-sectional studies, seropositivity to BTV was detected in red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), aoudad (*Ammotragus lervia*), Cantabrian chamois (*Rupicapra pyrenaica parva*), and Iberian wild goat (*Capra pyrenaica*). BTV-4 infection has been recently reported in an enclosed wildebeest (*Connochaetes gnou*) in southern Spain (MAPA, 2020b); and the clinical disease with mortality has been found in wild mouflon (*Ovis aries musimon*) and Iberian wild goat (Fernandez-Pacheco et al., 2008; Rodríguez-Sánchez et al., 2010b). The highest seroprevalence rates have been reported in red deer and fallow deer (66.3 and 50.0%, respectively), whereas roe deer and Iberian wild goat exhibited the lowest rates (ranging from 3.3 to 11.0%; García-Bocanegra et al., 2009; Gómez-Guillamón et al., 2020; Lorca-

Oró et al., 2011, 2014; Ruiz-Fons et al., 2008; Santiago-Moreno et al., 2011). Currently, BTV-1 and BTV-4 are circulating endemically in livestock in Spain. As a consequence, southern and south-western Spain are considered restricted zones for both serotypes, and control measures, including restricted movements, surveillance and vaccination programmes, are implemented in domestic ruminants from these regions (MAPA, 2020a).

The transmission dynamics and the role of wild and domestic hosts depend on the composition of the host-vector community, its abundance and distribution, as well as virus traits and regional environmental features, including human interventions (Ruiz-Fons et al., 2014). BTV can be maintained in a complex multi-host and multi-vector system, where the wild ruminant population can play an important role in the maintenance of the virus (García-Bocanegra et al., 2011; Ruiz-Fons et al., 2014). Climate, environment, hosts and vector population-related factors, which operate over the long-term, may substantially determine the epidemiology of BTV. The main risk factors reported for the presence of BTV in wild ruminant species are the sex, age, the habitat features, the rainfall, and the presence of certain species as well as their density (Lorca-Oró et al., 2014; Rossi et al., 2014; Ruiz-Fons et al., 2014).

Long-term approaches may provide a deeper understanding of the epidemiology and ecology of vector-borne diseases, and allow identifying the main drivers behind the emergence and persistence patterns in host populations (Barroso et al., 2020a). Although BTV has been widely studied in wild ruminants, it has seldom been approached on a long-term scale (Gómez-Guillamón et al., 2020; Lorca-Oró et al., 2014), especially in mixed-host communities. In this context, we present a long-term serosurvey study carried out in two deer species (red deer and fallow deer) in Doñana National Park (DNP) during a 13-year period, with the specific aims of assessing the temporal trend of the seroprevalence of BTV from 2005 to 2018, and to evaluate the modulating factors (individual, populational, environmental, and stochastic).

Material and methods

Study area

This study was undertaken in DNP, an important biosphere reserve located on the Atlantic coast of south-western Spain (the province of Huelva; 37°09 N, 6°30 W), covering an area of 54,252 ha. DNP wetlands occupy a strategic location between the continents of Europe and Africa, since the presence of emergent infectious diseases may associate with vectors or migrating birds that enter Europe through the Strait of Gibraltar (e.g. Höfle et al., 2013). The habitat consists of a seasonal marshland in the eastern area of the park; the western part is occupied by pine forests, the north by Mediterranean scrubland and there are sand dunes in the south. A longitudinal strip of a humid ecotone of high ecological richness separates the scrublands and the marshland (Figure 1; for a more detailed description see Barasona et al., 2014a).

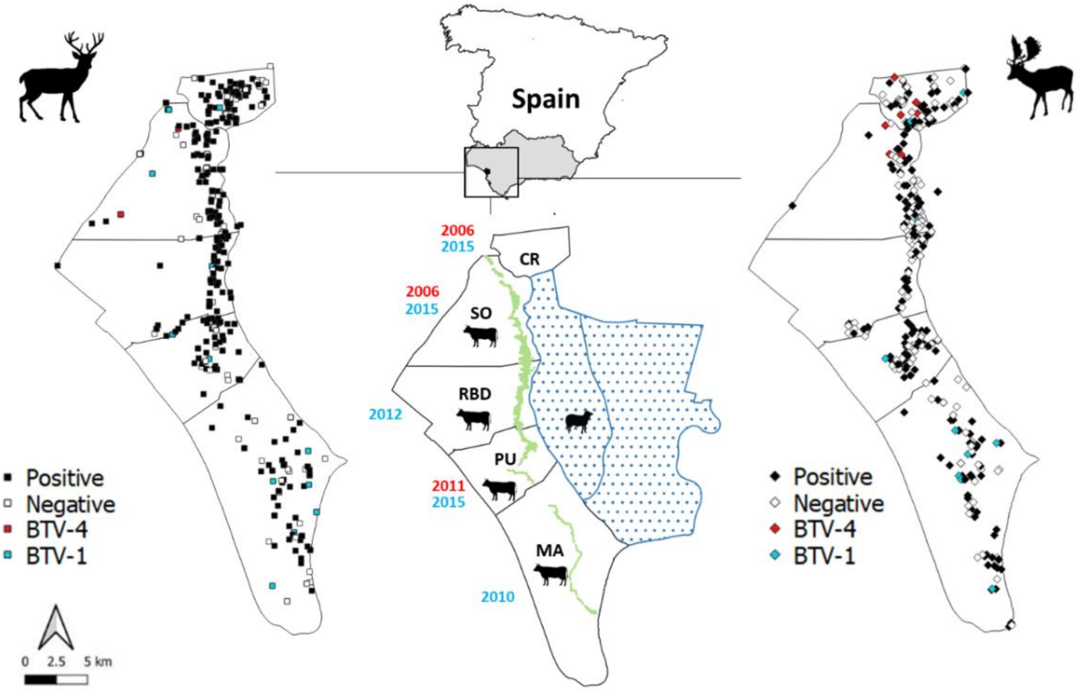


Figure 1. Map of the study area, Doñana National Park. The livestock management areas: Coto del Rey (CR), Sotos (SO), Doñana Biological Reserve (RBD), Puntal (PU) and Marismillas (MA) are delimited. The ecotone and the marshland are displayed by a green band and by a blue dotted polygon, respectively. The year in which BTV-4 and BTV-1 was detected for each livestock management area is marked in red and blue, respectively. Areas where livestock is present are marked with a silhouette of cattle and sheep.

DNP has a sub-humid Mediterranean climate with an Atlantic influence and marked seasons, which determine the variations in water and natural resources available to animals. The high inter-annual fluctuations in the rainfall (ranging from 170 to 1,000 mm) establish the dynamics of the marshland, and the average annual temperature is mild, around 17˚C. In the wet season (winter and spring) the ungulates are mostly congregated in scrubland, which remains uncovered by water when the marshlands flood. The late summer and autumn is the hardest season for the ungulates due to the significant drought and subsequent scarceness of natural resources, leading to an aggregation of wild and domestic ungulates around the ecotone and water points (Barasona et al., 2014a).

The mixed-ungulate community of DNP is composed of wild boar (*Sus scrofa*), red deer, fallow deer, as well as free-ranging livestock. This mixed host community is known to maintain some of the highest animal tuberculosis prevalence rates ever described in the literature (Barroso et al., 2020b), so a non-selective population culling of the wild ungulate population is conducted by Park rangers. Agriculture and recreational hunting are forbidden inside the Park, whereas free-ranging livestock breeding is permitted, focused on autochthonous and traditional cattle, horse, and to a lesser extent, ovine breeding with only a sporadic presence. A total of 930 heads of cattle and 854 heads of horses are kept in five livestock management areas which compose the study area, from north to south: Coto del Rey (CR), Sotos (SO), Doñana Biological Reserve (RBD), Puntal (PU) and Marismillas (MA). Cattle breeding was forbidden in 2002 in CR due to the conservation of the Iberian lynx (*Lynx pardinus*). The ovine population is located outside of these five management areas, in the Hinojos' marshland, in the north-east of DNP (see Figure 1).

The programme to control BTV undertaken in Spain since 2001 comprises several control measures implemented in restricted zones. DNP is situated in a restricted zone for BTV-1 and BTV-4 since the year 2007 (MAPA, 2021b). These measures include active and passive surveillance, restricted movements, as well as mandatory vaccination of all sheep and cattle older than three months against both serotypes with authorized inactivated vaccines and annual revaccination.

Animal sampling

From 2005 to 2018 (sampling periods 2005-2006 to 2017-2018), between September and August of the following year, 881 deer, including red deer (n=425) and fallow deer (n=456), were shot and necropsied as part of the DNP health-monitoring programme. These animals were randomly selected since culling activities did not address any specific sex by age class. The exact location where each animal was initially sighted was recorded through a portable GPS (Garmin Ltd., Olathe, KS, USA). Table S1 shows the sample size by the species, livestock management areas and sampling periods. The health monitoring programme of wild ruminants was temporally interrupted during 2006-2007 and 2010-2011.

The sampling protocol was approved by the Animal Experiment Committee of Castilla-La Mancha University and the Spanish Ethics Committee (PR-2015-03-08), and was undertaken according to the current legislation and guidelines for the ethical use of animals for research purposes (EC Directive 86/609/EEC; RD 223/1988; ASAB guidelines for the treatment of animals in behavioural research and teaching). Blood samples were collected in sterile plastic tubes (Vacutainer®, Becton-Dickinson, NJ, USA) from the endocranial venous sinuses or alternatively from the heart or thoracic cavity.

Serological analyses

Sera were obtained after centrifugation at 400 *g* for 5 minutes and stored at −20°C until assayed for antibodies against BTV. A commercial double-recognition enzymatic immunoassay was used for that purpose (ELISA; INGEZIM BTV DR 12.BTV.K0, INGENASA®, Madrid, Spain), according to the manufacturer's recommendations. This test, which has been used previously for different wild ruminant species (e.g. García-Bocanegra et al., 2009, 2011), is based on the detection of the VP7 protein of BTV and possesses a sensitivity and specificity of 99.4% and 100.0%, respectively, according to the values provided by the manufacturer. Samples of ELISA-positive sera (n=66) were then tested for the detection of specific antibodies against BTV-1 and BTV-4 by the serum neutralization test (SNT), according to the methodology described by the World Organization for Animal Health (OIE, 2019). We selected, whenever possible, sera from young animals to confirm the presence of the different serotypes during the study period. Moreover, the selection of sera was representative by species, sampling period and livestock management areas. Sera were inactivated at 56°C for 30 min and diluted (1:2 - 1:256) in microplates (Costar® Cat. Nº 3915, Cultek, Madrid, Spain) using Eagle's minimum essential medium (MEM) and mixed with 100 $TCID_{50}$ of each reference strain of BTV-1 (BTV-1/ALG/2006) and BTV-4 (BTV-4/SPA/2004). Mixtures were incubated for one hour at 37ºC, and 100 µL of a VERO E6 cell suspension in MEM supplemented with 15% foetal bovine serum (FBS; PAA Laboratories GmbH, Austria), 300 µg/l-glutamine/mL, 300 U penicillin/mL and 300 µg streptomycin/mL, were added to a final concentration of $1.5x10^4$/well. The mixture was further incubated in plates at 37°C for 6-7 days until a cytopathic effect developed in the control wells containing 10^2 $TCID_{50}$ of the virus and no serum. Samples were only considered positive if they showed neutralization (absence of cytopathic effect) at dilutions ⩾1:4 (Lorca-Oró et al., 2011). Controls for cytotoxicity in the absence of the virus were included for each sample at a 1:2 dilution. The neutralizing immune response observed was considered specific when SNT titres for a given serotype were ⩾ 4-fold higher than the titres obtained for the other serotype.

Data collection

Individual factors

Sex and age class were determined, the latter by the tooth replacement pattern (Saenz de Buruaga et al., 2001), classifying deer into three age classes: calves (<1-year-old), juveniles (1-2 years) and adults (>2 years). Since deer species are usually born between later May and the beginning of June in DNP, we assumed that a major part of the calves sampled were older than 5-6 months old.

Environmental factors

Environmental conditions are relevant to the ecology of host and vectors, and therefore to pathogen maintenance and spread (Acevedo et al., 2010; Barasona et al., 2014a). We considered a number of environmental factors that potentially determine the risk of exposure to BTV (Acevedo et al., 2010; Barroso et al., 2020b). Namely, we calculated habitat-related variables in terms of the proportion of surface covered by: dense scrub, low-clear shrubland, herbaceous grassland, woodland, bare land, and watercourse vegetation per hectare, which were obtained from Andalusia Environmental Information (REDIAM, 2019). For this purpose, a grid of one hectare surface was created and used as territorial units. The proportion of surface occupied by each cover was calculated for each territorial unit using zonal statistic tools. To characterize the habitat around each animal sampled, the locations of the animals and the grid were intersected through a point sampling tool with QGIS version 4.3.1 (QGIS Development Team, 2019). We also calculated the straight-line distance (m) from the animal location sampled to the nearest: water point (DWAT), marsh-shrub ecotone (DE), and ruminant farm (including cattle and small ruminants; DRUM), as additional variables to characterize the habitat.

Populational factors

Variations in the host-community composition and abundance may affect the exposure (mediated by competent vectors) to BTV (Ruiz-Fons et al., 2008a). Three variables were measured in this respect: population density for red deer, fallow deer, and cattle. These variables were estimated per livestock management area and sampling period. For wild species, we estimated the population density (individuals per square kilometre) from seven lineal transects of 10-15 km each one distributed along the Park using distance sampling methodology (Buckland et al., 2001). The surveys started two hours before sunset during September and were carried out from a vehicle (average speed of 10 km $\cdot h^{-1}$; for further details about sampling see Barroso et al., 2020b). The cattle census was provided by farmers and transformed into density values. An additional variable was also considered, the intra-specific seroprevalence, as another potential driver acting at populational scale. At the level of livestock management area and sampling period, the seroprevalence of BTV was calculated to assess its effect on the probability of testing positive for BTV.

Stochastic factors

Weather is relevant for host and vector population dynamics (Acevedo et al., 2010) and may subsequently determine the susceptibility and/or exposure to BTV. We selected the rainfall and temperature as meteorological parameters to be included in our models. The rainfall determines the moisture level of the soil which affects the breeding of *Culicoides*, and the temperature may influence the infection, virogenesis and transmission rates of BTV. The annual average rainfall and temperature were collected by the meteorological station located at RBD.

Statistics and risk factor analysis

We tested the relationships between the individual serological status concerning BTV (negative/positive such as the response variable, irrespective of the serotype), and the factors potentially modulating it, statistically: individual, populational, environmental and stochastic factors. The reason to include both serotypes in these models was the limited sample of animals in which SNT was undertaken (n=66). A principal component analysis (PCA) was performed for land cover variables (Barroso et al., 2020b). These variables were summarized in closed habitats (PC1) and watercourse vegetation (PC2) (Table S2). A high score of PC1 would be interpreted as a high dominance of woodland, dense and low-clear shrubland, with a low proportional cover of bare land and grassland. A high score of PC2 would mainly mean dominance of watercourse vegetation.

A first exploratory Generalized Linear Mixed Models (GzLMMs; binomial family) explored for differences among livestock management areas (CR, SO, RBD, PU and MA) and species in the serological status against BTV. In these exploratory models, we included sex, age class, species, and the livestock management area as explanatory variables, whereas the sampling period and month were fitted as nested random-effect factors.

With the purpose of generalising the effect of a number of factors on exposure to BTV, while controlling by the area of livestock management and temporal factors, the definitive models included the area of livestock management and sampling period (on a yearly basis) as random-effect factors. They were performed separately for each deer species. The explanatory variables initially included in the models encompassed the following factors: i) individual: sex and age class (both variables as categorical); ii) population (population density of deer and cattle, and intra- and inter-specific seroprevalences of BTV in deer); iii) environmental: DE, DWAT, DRUM, and the coverage of closed and watercourse habitats; and iv) stochastic: annual rainfall (mm) and average temperature (ºC) of the previous sampling period. The two-way interactions between individual, stochastic, and population factors were also included in the models. The selection of the "best model" was performed using the corrected Akaike's information criterion (cAIC).

We used binomial GzLMMs in all the models and a logit link function. The predicted probabilities of BTV obtained from the models were used to represent results, which account for all the factors in the models. Significant *p*-value was set at 0.05. Finally, cross and self-correlations between SBT and its predicted response probability between red deer and fallow deer were carried out to explore similarities of temporal patterns. The time lag (latency) at which the correlation was maximized was used to determine the latency between the two time-series explored. We used time shifts (lags) between −4 and 4 sampling periods. The absolute values of the cross and self-correlations were considered significant if they exceeded twice the estimated standard error values.

All the GzLMMs were performed using library *lme4* 1.1-21 version of R software version 3.5.2 (R Core Team, 2020). Cross and self-correlations were done using IBM SPSS 19.0 software (IBM Corporation, Somers, NY, USA).

The frequencies of seropositivity to BTV-1 and BTV-4 were calculated as the number of animals showing antibodies against each serotype divided by the total number of animals tested with SNT. Confidence intervals for seroprevalences were estimated by the standard error 95% confidence interval (S.E. 95%), which expression was S.E. 95% = 1.96*standard error.

Results

General

The overall seroprevalences of BTV (SBT; %) ± S.E. 95% in red deer and fallow deer were 78.6±3.8 (n=425) and 53.1±4.7 (n=456), respectively. Table S3 provides information about the 66 animals analysed by SNT from which four sera could not be assessed due to cytotoxicity. Seroprevalences according to sex and age are shown in Figure 2a. SBT (% ± S.E. 95%) for males and females were 84.8±4.8 (184/217) and 72.1±6.1 (150/208) in red deer, respectively, and 58.4±6.2 (143/245) and 46.9±6.7 (99/211) in fallow deer, respectively.

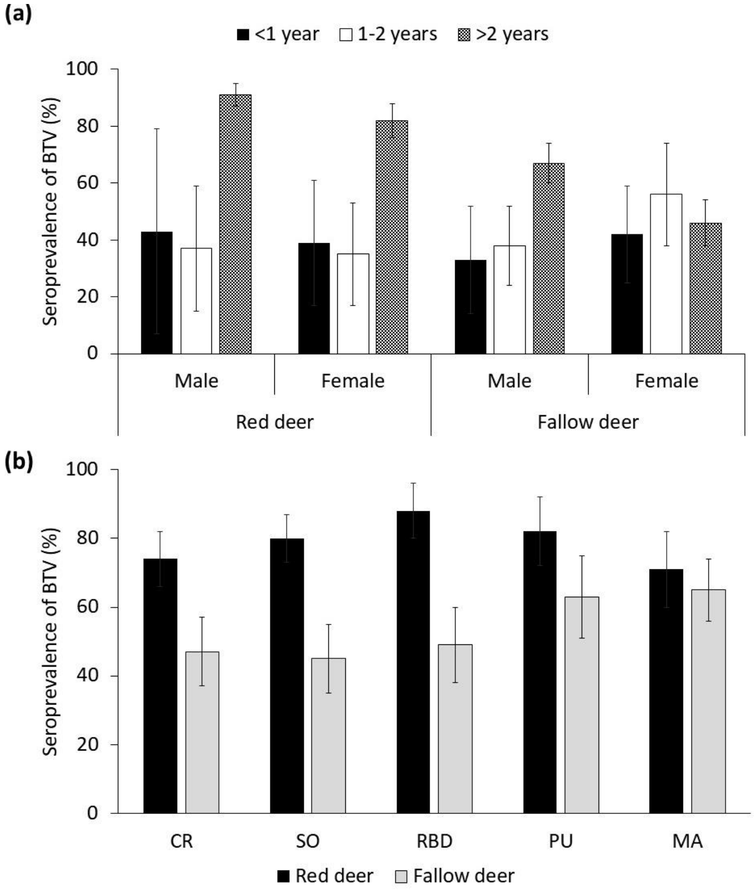


Figure 2. Seroprevalence (%± standard error (S.E.) 95%) of BTV in red deer and fallow deer depending on (**a**) age class and sex, and (**b**) the livestock management areas (see Figure 1 for a map of the areas with their full names).

The temporal trends of SBT in the deer species, the evolution in the densities of the deer and cattle, as well as in the meteorological factors are summarized in Figure 3. The SBT results followed similar temporal patterns in both deer species. While a gap in the study from 2006-2007 to 2010-2011 prevents us from interpreting the complete temporal pattern, a high prevalence for both species in years 2012 and 2013 is notable, followed by a peak in 2013 and 2014 (Figure 3a). A decreasing trend was also observed during the last three years of the study.

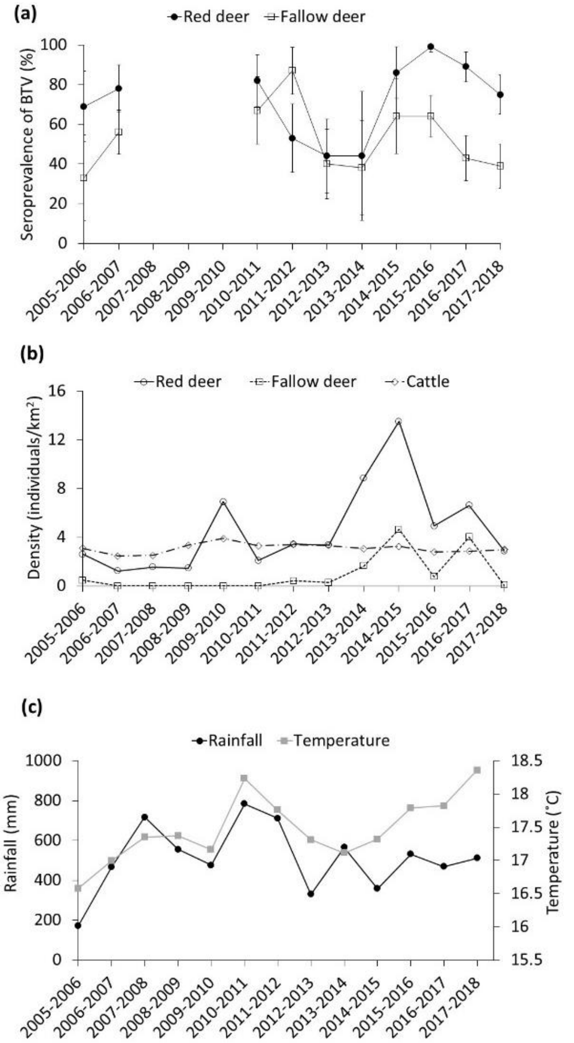


Figure 3. Temporal trend of the (**a**) seroprevalence of BTV (% ± standard error (S.E.) 95%), (**b**) population density of red deer, fallow deer and cattle (individuals/km^2), and (**c**) annual rainfall (mm) and temperature (ºC).

Table S1 and Figure 1 show SBT by species, livestock management areas and sampling periods (see Figure 4 and Table S4 for serotypes and their temporal distribution). The first complete sampling including all the livestock management areas was performed in the sampling period 2006-2007 (the second sampling period) and indicated that SBT mainly occurred in the northern and southern areas of DNP, being already widespread all over the study area (Figure 1 and 4). We evidenced statistical differences in the SBT among livestock management areas (*F*=7.89, df=873, *p*=0.05) and species (*F*=56.12, df=873, *p*<0.01), so that SBT increased in the southernmost areas of DNP (MA and PU), and red deer showed higher risk of testing positive to BTV than fallow deer (Figure 2b).

Among the animals analysed by SNT, the frequency of seropositivity to BTV-1 and BTV-4 (±S.E. 95%) were 44.8±19.5% and 48.3±19.2% in red deer, and 39.4±18.3% and 30.3±18.4% in fallow deer, respectively (see Table S3). BTV-4 was restricted to the northern areas during the first two sampling periods until the period of 2014-2015 (Figure 4). In 2011-2012, BTV-4 was also detected in one fallow deer from the south-central part of the Park (PU). This serotype was absent in our sample during the last three sampling periods of the study (from 2014-2015 onwards). As for BTV-1, it was initially detected in 2010-2011 in the southern part of DNP (MA) and spread over the study area for the remaining study period, becoming the only serotype detected during the last three sampling periods. Interestingly, BTV-1 was always detected in our typed samples in the sampling period 2014-2015, coinciding with a peak of SBT, and thereafter, it was the only serotype detected.

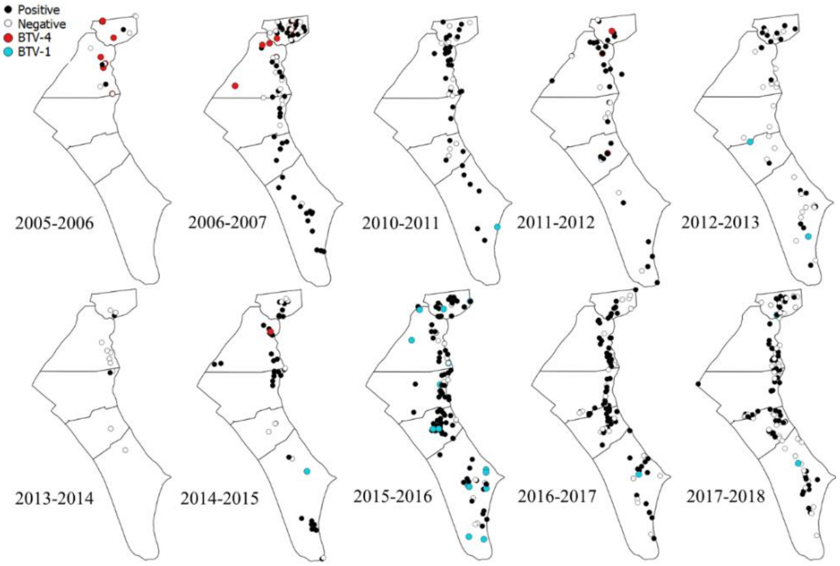


Figure 4. Maps representing the spatiotemporal detection of BTV-1 and BTV-4 in deer species (red deer and fallow deer) in Doñana National Park, obtained by serum neutralization test.

Factors determining the seroprevalence of BTV

Concerning the final GzLMMs of SBT, the results and the stepwise selection process are shown in Table 1 and Table S5, respectively, and separately for each species.

Table 1. Parameters from the best GzLMMs for the serological status against BTV in the deer species studied related to sex, age class, straight-line distance to the ecotone (DE), straight-line distance to the nearest water point (DWAT), previous sampling period's rainfall (rainfall), annual density of red deer, intra-specific seroprevalence of BTV (SBT), and some interactions among them.

Variables	**Red deer**			**Fallow deer**		
	F df (x,y)	**Estimate ±S.D.**	***p***	**F df (x,y)**	**Estimate ± S.D.**	***p***
Sex^{1}	9.59 (1, 409)	Female: 1.17±1.08	0.27	3.68 (1, 440)	Female: 0.48±0.62	0.44
Age^{2}	22.62 (2, 409)	Juvenile: -0.14±0.71 Adult: 2.72±0.64	**<0.01**	11.46 (2, 440)	Juvenile: 0.29±0.58 Adult: 1.59±0.51	**<0.01**
DE	4.16 (1, 409)	<0.001±<0.001	**0.05**			
DWAT				3.36 (1, 440)	<0.001±<0.001	0.06
Rainfall	0.12 (1, 409)	0.01±<0.001	0.08			
Density of red deer	0.87 (1, 409)	0.58±0.23	**0.01**			
Intra-specific SBT	8.02 (1, 409)	0.03±0.01	**<0.01**	57.77 (1, 440)	0.05±0.01	**<0.01**
Sex^{1}*Age^{2}				7.01 (2, 440)	Juvenile: 0.05±0.83 Adult: -1.25±0.67	**0.03**
Sex^{1}* Rainfall	4.25 (1, 409)	Rainfall*Female: <0.001±<0.001	**0.04**			
Density* Rainfall	5.44 (2, 409)	<0.001±<0.001	**0.02**			

The model was fitted using sampling period and livestock management area as random factors. Parameter estimates for the level of fixed factors were calculated using a reference value of 0 for the male level in the variable sex^{1} and calves for the variable age^{2}. '*' represents interactions among explanatory variables. The conditional R^{2} obtained from these models was 0.55 and 0.31 for red deer and fallow deer, respectively.

Individual factors

The age class was statistically significant for both species. Adult deer showed higher SBT than younger age classes (Figure 2a). However, in fallow deer, sex and age significantly interacted, so that males presented higher seroprevalence than females only during the adult age (Figure 2a).

Environmental factors

For red deer, the closeness to the marsh-shrub ecotone was associated with higher SBT (Figure 5a). Concerning fallow deer, no significant effect of environmental factors on SBT was evidenced.

Population factors

The intra-specific density was significantly and positively associated with the SBT in red deer (Figure 5b). This relationship was conditioned by stochastic factors: the interaction between red deer density and rainfall was statistically significant (see explanation below).

Stochastic factors

For red deer, the previous sampling period’s rainfall significantly interacted with populational and individual factors, respectively. In this species, rainy years were significantly related to higher SBT in males but not in females (Figure 5c). SBT was the lowest when low rainfall and density co-occurred. However, in rainy years, the relationship with density was not observed and SBT was high regardless of the density (Figure 5d).

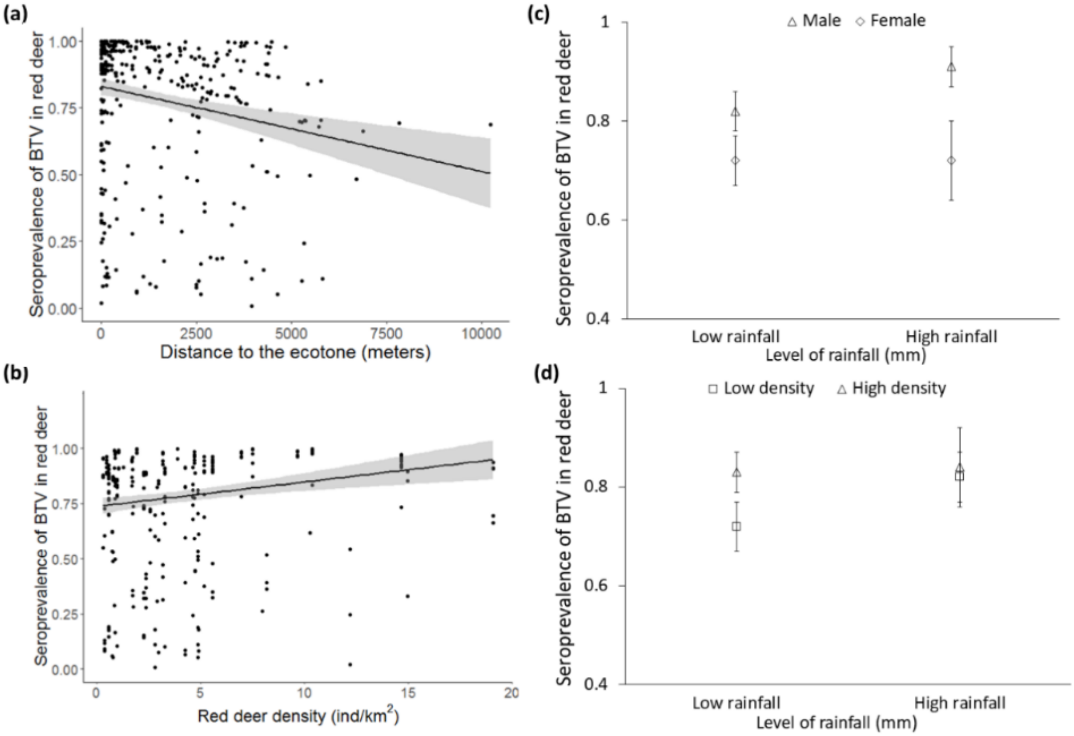


Figure 5. Individual predicted seroprevalence (± confidence interval (C.I.) 95%, represented by the shaded band) of BTV obtained from selected generalised linear mixed models for red deer depending on (**a**) the distance to the ecotone (m), (**b**) its population density (individuals/km^2); and mean predicted seroprevalence (± C.I. 95%, represented by the error bars) of BTV for red deer depending on the interaction between (**c**) annual rainfall (mm) and sex, and (**d**) annual rainfall and population density (individuals/km^2). The following categories were established to display results: low rainfall (⩽521.10 mm), high rainfall (>521.10 mm), low red deer density (⩽4.51 individuals/km^2) and high red deer density (>4.51 individuals/km^2).

Self-correlations and cross-correlations

A significant negative cross-correlation was obtained between the SBT of red deer and their predicted response probability in fallow deer, with a lag of two years (R=−0.76±0.35). Additionally, the SBT of red deer and fallow deer showed significant negative self-correlations, with a lag of three years (R: −0.60 ± 0.26; Box-lung value: 5.59; *p*=0.05) and two years (R: −0.57 ± 0.24; Box-lung value:9.74; *p*=0.02), respectively.

Discussion

To our knowledge, this is the first long-term study (2005-2018) of BTV in wildlife, from the appearance of the first outbreaks to the present day and reveals the long-term persistence of BTV in a wild ruminant host community, even in the absence of outbreaks in livestock.

General patterns of seroprevalence of BTV

Our SBT values, especially for red deer, are higher than those reported in Spain and other European countries for red deer and fallow deer (e.g. Casaubon et al., 2013; García-Bocanegra et al., 2011; Rodríguez-Sánchez et al., 2010a; Ruiz-Fons et al., 2008a) but similar to those reported by in southern Spain (García-Bocanegra et al., 2009). Unfortunately, there is no information available about the abundance, distribution, and temporal dynamics of *Culicoides* in our study area during the study period. The ecosystem of DNP comprises many wetlands which have been reported to favour the presence and abundance of *Culicoides,* which, together with the high densities of deer species in the study area, may explain the elevated SBT found in this study.

The differences in SBT observed between deer species, higher in fallow deer, may relate to ecological (habitat use and selection) and ethological (social) differences. Talavera et al. (2018) analysed the origin of blood meals in 114 females of *Culicoides* from different areas of Spain and revealed that the most abundant host was red deer (66.7%), whereas only 6.1% of the *Culicoides* fed on fallow deer. However, this study did not assess feeding preferences based on host availability (i.e. densities). In DNP, the abundance of red deer is higher and is more evenly distributed than fallow deer. Particularly, the higher local densities of individuals (aggregation) occur during the rutting season in the ecotone. However, fallow deer and cattle normally aggregate more than red deer and tend to establish mixed groups since both species prefer open habitats (Figure 6)

(Triguero-Ocaña et al., 2020), while the distribution and habitat use by red deer is more widespread. Host grouping could either exert a dilution or even a protective effect regarding biting midges (Mooring and Hart, 1992; Rossi et al., 2014; Ruiz-Fons et al., 2014), which is related to the mechanisms involved in animal grouping and protection against parasites. In addition, vaccinated cattle have been identified as a protective factor for BTV exposure of deer species due to a dilution effect or a possible vector preference for cattle (Rossi et al., 2014).

The interannual fluctuations observed in both species were similar, suggesting that deer species share the main drivers and/or common exposition to the virus. The rainiest and warmest sampling periods (2010-2011, 2011-2012, and 2015-2016; Figure 3c) concur with the highest annual SBT in red deer and fallow deer (Figure 3a, significantly in the case of red deer, see below). The significant negative self-correlation and cross-correlation of SBT observed for and between, respectively, red deer and fallow deer concur with the temporal pattern (Figure 3a) and suggest two epidemiological waves. However, the temporal gap of this study indicates that longer research is needed to elucidate the temporal pattern, and what their main drivers are (e.g. long-term acquired immunity, vector population dynamics, dissemination of new serotypes, etc.).

The spatial pattern observed may indicate higher intra-specific circulation of the virus in certain areas, but we did not evidence any clear indications of what factors determined this. Red deer are more widespread, and the central area may represent a relevant node of communication among individuals in DNP. Distribution models in DNP show that the habitat use by fallow deer in the southern part of the Park is more widespread than in other areas, and thus could determine increased exposure (Barasona et al., 2014a).

Serotype 4 was detected for the first time in Spain (in the peninsular territory) in October 2004 in the province of Cádiz, the closest to Africa in the Iberian Peninsula (Table 2). Animals first analysed in this study, from April of 2006, already tested positive for BTV-4. In 2006 no positive cases were reported in the region surrounding DNP (Table 2), however, 20 animals tested positive for BTV-4 in DNP, including calves. This indicates that BTV-4 circulated at least in wild ungulates. In Spain, the vaccination programme against BTV-4 started in 2005 for cattle and has continued until the present day in the study area and surrounding provinces, which could lead to the absence of positive cases reported in livestock in 2006 (Sánchez-Matamoros et al., 2009). During 2015-2018, only one BTV-4 outbreak in livestock was reported in 2017 in Huelva (Table 2), and we did not detect animals testing positive for this serotype. However, BTV-1 predominated during these years in our study area. BTV-1 was also first recorded in Spain mainland in July 2007 in the province of Cádiz, and the vaccination programme against this serotype was implemented in November 2007 (Sánchez-Matamoros et al., 2009). As there was a gap in our study from 2007 to 2009, the first wild ruminant testing seropositive for BTV-1 in DNP was found in 2010. Thus, the circulation of BTV-1 during these years was shared between wild and domestic ungulates, whereas circulation of BTV-4 was mainly driven by livestock, indicating that they could present independent (at least partially) BTV circulations due to vaccination campaigns in cattle.

Figure 6. Mixed group established by cattle and fallow deer in the ecotone of Doñana National Park. Credit: Christian Gortázar.

Table 2. Annual number of outbreaks caused by BTV-1 and BTV-4 in the provinces of Cádiz, Sevilla, and Huelva, as well as in the surroundings of Doñana National Park (DNP; south-western Spain) during the study period (2005-2018) (RASVE, 2021).

Year	BTV-1				BTV-4				Unknown			
	Province of Cádiz	Province of Sevilla	Province of Huelva	DNP surroundings	Province of Cádiz	Province of Sevilla	Province of Huelva	DNP surroundings	Province of Cádiz	Province of Sevilla	Province of Huelva	DNP surroundings
2005	0	0	0	0	0	0	0	0	3	17	23	0
2006	0	0	0	0	0	0	0	0	0	0	0	0
2007	0	0	0	0	0	0	0	0	448	897	1,013	26
2008	1	0	9	0	0	0	0	0	0	0	0	0
2009	0	0	0	0	0	0	0	0	0	0	0	0
2010	0	0	0	0	8	0	0	0	0	0	0	0
2011	0	0	0	0	2	0	0	0	0	0	0	0
2012	0	0	0	0	0	1	2	1	0	0	0	0
2013	0	0	0	0	15	0	31	8	0	0	0	0
2014	13	0	0	0	13	47	6	2	0	0	0	0
2015	1	1	3	1	0	0	0	0	0	0	0	0
2016	0	3	1	0	1	0	0	0	0	0	0	0
2017	3	1	0	0	2	0	1	1	0	0	0	0
2018	0	0	0	0	4	2	0	0	0	0	0	0

Factors determining the seroprevalence of BTV

Individual factors

Adults, except female fallow deer, tended to show higher SBT than calves in both deer species. This pattern is likely due to the lifelong persistence of antibodies for BTV. However, SBT was still high in calves, which may reflects a high exposure to infected vectors (Falconi et al., 2011). Among adults, males tended to display higher levels of SBT than females, more markedly in red deer. Males are more exposed to flying vectors due to their behaviour and their more extensive use of space, as they have larger home ranges, and also due to their larger body mass (Ruiz-Fons et al., 2013). For fallow deer, males showed an age-increasing pattern, whereas juveniles (1-2 years) presented the highest rate of SBT among females. This may relate to a higher exposure of adult males to biting midges in comparison to females, which takes place during the mating season, coinciding with the highest risk season of BTV circulation in southern Spain (late summer-autumn). Stags usually form small groups separated from females. However, during the rut, stags may move from their usual home ranges through the open patches of grasslands in the ecotone to meet receptive harems of females, becoming more exposed to biting midges. On the contrary, the high aggregation of females in large harems may dilute exposure to midges and BTV (Mooring and Hart, 1992).

Environmental factors

Proximity to the ecotone (the edge of the marshland) was significantly and positively associated with increased SBT in red deer. The soil in this area is highly moisture-retentive and rich in organic matter, a key factor for the suitability of vectors (Acevedo et al., 2010). Wild and domestic ungulates use this area, favouring the interaction with *Culicoides* midges and, as a consequence, increasing their exposure to BTV. Our results are also consistent with previous studies in which the edge habitats and open prairies were suggested as important risk sites for BTV infection where hosts (domestic and wild ruminants) and competent vectors meet (Pioz et al., 2012).

Population factors

SBT significantly increased with population density in red deer, which is in accordance with the elevated SBT reported at high population densities (García-Bocanegra et al., 2011). This indicates the significant role that red deer may play in the maintenance and persistence of BTV as it is the most widespread and abundant deer species in the Iberian Peninsula, often, at high densities. Red deer density may support the population dynamics of *Culicoides* midges, providing them with a highly available food resource (Talavera et al., 2018).

Stochastic factors

Increased SBT in red deer stags was associated to previous rainy years, but not in females. Wet years may relate to higher vector abundance (Gusmão et al., 2019), and more intense ruts in terms of the reproductive effort for the stags and subsequent higher exposure (Barroso et al., 2020b). The rut, which typically occurs in the ecotone during late summer, coincides with the period of major activity for *Culicoides*, providing favourable conditions for their survival and breeding. The lowest SBT in red deer occurred when low rainfall and low density co-occurred, whereas in rainy years SBT was high regardless of the density (Figure 5d). Rainy years provide favourable conditions of moisture and organic-rich soils to vectors (Nicholson and Farrar, 1994), with the subsequent increase in their abundance. When dry years and elevated densities of hosts co-occur, the widespread food resources for the vectors provided by abundant hosts may counteract the effect of rainfall scarceness, with the subsequent success in BTV transmission. On the contrary, the drought effect together with a low density of red deer leads to the lowest SBT rates, since these circumstances may hinder the survival of *Culicoides* considerably. Therefore, wet years together with high densities of this host species, created the most suitable conditions for vector borne BTV transmission.

This study evidences the widespread distribution of BTV in the deer community from DNP and highlights the epidemiological role of deer species in the establishment and persistence of BTV, even in absence of livestock. The specific ecological and ethological features, such as habitat use or gregarious behavior, of both species determine the relevance and interplay of the different risk factors. Furthermore, climatic conditions, which influences vector dynamics, together with population factors (population densities) also determine the suitability for vector-borne BTV transmission. Future work should be focused on elucidating the effect of climate change on the competent vector populations and its potential effect on the dynamics of BTV infection in hosts communities. For these purposes, continuing long-term studies is key.

Acknowledgments

The authors would like to thank the EBD ‐ CSIC monitoring team and their colleagues at the IREC (UCLM, Spain), as well as Doñana National Park and Doñana ICTS ‐ RBD, which provided logistic and technical support and the data essential for carrying out this work, and to all those who participated in the fieldwork and data collection of the wild ungulates, with special thanks to the Park ranger Jose Antonio Muriel.

Supplementary material 2.3

Table S1. Sample size (N) and seroprevalence of BTV (%) obtained by ELISA by deer species, sampling period and livestock management area.

Species	**Sampling period**	**CR**	**SO**	**RBD**	**PU**	**MA**	**Total**
		Seroprevalence (N)	Seroprevalence (N)	Seroprevalence (N)	Seroprevalence (N)	Seroprevalence (N)	Seroprevalence (N)
Red deer	2005-2006	66.7 (6)	70 (20)	-	-	-	69.2 (26)
	2006-2007	73.9 (23)	83.3 (6)	71.4 (7)	100 (2)	12.5 (7)	78.3 (46)
	2010-2011	25 (16)	100 (7)	66.7 (3)	50 (2)	100 (5)	82.8 (33)
	2011-2012	57.1 (7)	71.4 (14)	33.3 (3)	33.3 (3)	20 (5)	53.1 (32)
	2012-2013	75 (8)	20 (5)	66.7 (3)	100 (1)	30 (10)	44.4 (27)
	2013-2014	75 (4)	0 (4)	100 (1)			44.4 (9)
	2014-2015	75 (16)	100 (8)	100 (4)			85.7 (28)
	2015-2016	93.3 (15)	100 (15)	100 (17)	100 (15)	100 (15)	98.7 (79)
	2016-2017	70 (10)	100 (15)	93.3 (15)	100 (15)	73.3 (15)	88.6 (70)
	2017-2018	66.7 (15)	86.7 (15)	93.3 (15)	60 (15)	66.7 (15)	74.7 (75)
	Total	74.2 (120)	80.7 (109)	88.2 (68)	81.8 (55)	71.2 (73)	78.6 (425)
Fallow deer	2005-2006	40 (5)	30.8 (13)	-	-	-	33.3 (18)
	2006-2007	41.9 (31)	55 (29)	37.5 (8)	80 (5)	92.3 (13)	55.8 (77)
	2010-2011	50 (9)	55.6 (9)	100 (3)	71.4 (7)	71.4 (7)	66.7 (30)
	2011-2012	80 (5)	100 (12)	33.3 (3)	80 (5)	100 (6)	87.1 (31)
	2012-2013	60 (10)	16.7 (6)	0 (2)	50 (2)	40 (10)	40 (30)
	2013-2014	50 (2)	20 (5)	50 (2)	25 (4)	66.7 (3)	37.5 (16)
	2014-2015	100 (1)	85.7 (7)	60 (5)	0 (5)	85.7 (7)	64 (25)
	2015-2016	60 (15)	40 (15)	66.7 (15)	84.2 (18)	62.5 (16)	63.8 (80)
	2016-2017	33.3 (15)	33.3 (15)	35.7 (14)	46.7 (15)	66.7 (15)	43.2 (74)
	2017-2018	33.3 (12)	13.3 (15)	44.4 (18)	66.7 (15)	33.3 (15)	38.7 (75)
	Total	47 (100)	45.3 (117)	48.6 (70)	62.3 (76.0)	65.2 (92)	53.1 (456)

Table S2. Result of the Principal Component Analysis (PCA) and the two uncorrelated factors obtained.

Variable	PC1- closed habitats	PC2-watercourse vegetation
Herbaceous grassland	-0.856	-0.507
Bare land	0.259	-0.098
Watercourse vegetation	-0.096	0.991
Woodland, dense and low-clear shrubland	0.866	-0.362
Standard deviation	1.56	1.38
Explained variance	38.96	34.48
Cumulative variance	38.96	73.44

Table S3. Individual information about the animals analysed by serum neutralization test (n=66).

Species	Sampling period	Livestock management area	Age Class	Sex	Serotype
Red deer	2005-2006	CR	Adult	Male	BTV-4
	2005-2006	CR	Adult	Male	BTV-4
	2005-2006	SO	Juvenile	Female	BTV-4
	2005-2006	SO	Adult	Male	BTV-4
	2005-2006	SO	Adult	Male	BTV-4
	2005-2006	SO	Adult	Male	BTV-4
	2005-2006	SO	Adult	Male	BTV-4
	2006-2007	CR	Adult	Male	BTV-4
	2006-2007	CR	Adult	Female	BTV-4
	2006-2007	CR	Adult	Female	BTV-4
	2006-2007	SO	Juvenile	Female	BTV-4
	2006-2007	SO	Adult	Female	BTV-4
	2006-2007	SO	Adult	Male	BTV-4
	2006-2007	SO	Adult	Female	BTV-4
	2010-2011	MA	Calf	Female	BTV-1
	2012-2013	MA	Calf	Female	BTV-1
	2012-2013	RBD	Calf	Female	BTV-1
	2015-2016	CR	Calf	Female	BTV-1
	2015-2016	MA	Calf	Male	Negative
	2015-2016	MA	Calf	Female	BTV-1
	2015-2016	MA	Calf	Female	BTV-1
	2015-2016	MA	Calf	Female	BTV-1
	2015-2016	MA	Calf	Female	BTV-1
	2015-2016	PU	Calf	Female	BTV-1
	2015-2016	RBD	Calf	Female	BTV-1
	2015-2016	RBD	Calf	Female	BTV-1
	2015-2016	RBD	Calf	Male	Citotoxicty

	2015-2016	SO	Calf	Female	BTV-1
	2015-2016	SO	Calf	Female	BTV-1
	2016-2017	PU	Calf	Male	Negative
Fallow deer	2005-2006	CR	Adult	Male	BTV-4
	2005-2006	CR	Adult	Male	BTV-4
	2005-2006	SO	Calf	Female	BTV-4
	2005-2006	SO	Adult	Female	Citotoxicty
	2005-2006	SO	Adult	Female	BTV-4
	2005-2006	SO	Adult	Male	Citotoxicty
	2006-2007	CR	Calf	Female	BTV-4
	2006-2007	CR	Juvenile	Female	Negative
	2006-2007	CR	Adult	Female	Negative
	2006-2007	CR	Adult	Female	Negative
	2006-2007	MA	Calf	Male	Negative
	2006-2007	SO	Calf	Male	Negative
	2006-2007	SO	Adult	Female	BTV-4
	2011-2012	CR	Calf	Female	BTV-4
	2011-2012	PU	Calf	Male	Citotoxicty
	2011-2012	PU	Calf	Male	BTV-4
	2011-2012	SO	Calf	Female	BTV-4
	2012-2013	SO	Juvenile	Male	Negative
	2014-2015	CR	Calf	Male	Negative
	2014-2015	MA	Juvenile	Male	BTV-1
	2014-2015	SO	Juvenile	Male	BTV-4
	2015-2016	CR	Juvenile	Male	BTV-1
	2015-2016	MA	Calf	Male	BTV-1
	2015-2016	MA	Calf	Male	BTV-1
	2015-2016	MA	Juvenile	Male	BTV-1
	2015-2016	MA	Juvenile	Male	BTV-1
	2015-2016	PU	Calf	Male	BTV-1
	2015-2016	RBD	Juvenile	Male	BTV-1
	2015-2016	RBD	Juvenile	Male	BTV-1
	2015-2016	RBD	Calf	Male	Negative
	2015-2016	SO	Juvenile	Male	BTV-1
	2016-2017	MA	Juvenile	Male	BTV-1
	2017-2018	CR	Juvenile	Male	BTV-1
	2017-2018	MA	Calf	Male	Negative
	2017-2018	MA	Calf	Male	BTV-1
	2017-2018	RBD	Calf	Male	Negative

Table S4. Sample size (N) and frequency of seropositivity to BTV-1 and BTV-4 (%) obtained by serum neutralization test displayed by species and sampling period.

	Red deer				**Fallow deer**			
Sampling period		**BTV-1**	**BTV-4**	**Negative**		**BTV-1**	**BTV-4**	**Negative**
	N	Frequency (%)	Frequency (%)	Frequency (%)	N	Frequency (%)	Frequency (%)	Frequency (%)
2005-2006	7	0	100	0	4	0	100	0
2006-2007	7	0	100	0	7	0	28.6	71.4
2010-2011	1	100	0	0				
2011-2012					3	0	100	0
2012-2013	2	100	0	0	1	0	0	100
2013-2014								
2014-2015					3	33.3	33.3	33.3
2015-2016	11	90.9	0	0.1	10	90	0	10
2016-2017	1	0	0	100	1	100	0	0
2017-2018					4	50	0	50
Total	29	44.8	48.3	6.9	33	39.4	30.3	30.3

Table S5. Summary of the stepwise model selection procedure, based on the corrected Akaike's information criterion (cAIC), used to explain the serological status against BTV. Full model comprises the sex, age class, straight-line distance to the ecotone (DE), straight-line distance to the nearest water point (DWAT), straight-line distance to the nearest ruminant farm (DRUM), previous sampling period's rainfall (rainfall), coverage of closed (closed_ha) and watercourse vegetation (water_ha), annual density of fallow deer (FD_den), red deer (RD_den), wild boar (WB_den), and cattle (cattle_den), intra-specific seroprevalence of BTV (seroprev_intrasp), seroprevalence of fallow deer or red deer (seroprev_FD and seroprev_RD, respectively), temperature, and some interaction among them.

Model	**cAIC**
Full model for red deer: age + sex + DE + DWAT+ DRUM + closed_ha + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_FD + rainfall + temperature + sex*age + age*RD_den +sex*RD_den + rainfall*RD_den + rainfall*sex + **rainfall*age** + rainfall*temperature [M1]	336.99
age + sex + DE + DWAT+ DRUM + closed_ha + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_FD + rainfall + temperature + sex*age + **age*RD_den** +sex*RD_den + rainfall*RD_den + rainfall*sex + rainfall*temperature [M2]	334.11
age + sex + DE + DWAT+ DRUM + closed_ha + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_FD + rainfall + temperature + sex*age + sex*RD_den + rainfall*RD_den + rainfall*sex + **rainfall*temperature** [M3]	331.84
age + sex + DE + DWAT+ DRUM + closed_ha + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + **seroprev_FD** + rainfall + temperature + sex*age + sex*RD_den + rainfall*RD_den + rainfall*sex [M4]	330.13
age + sex + DE + DWAT+ DRUM + closed_ha + water_ha + **FD_den** + RD_den + WB_den + cattle_den + seroprev_intrasp + rainfall + temperature + sex*age + sex*RD_den + rainfall*RD_den + rainfall*sex [M5]	328.18
age + sex + DE + DWAT+ DRUM + closed_ha + water_ha + RD_den + WB_den + cattle_den + seroprev_intrasp + rainfall + **temperature** + sex*age + sex*RD_den +	326.33

rainfall*RD_den + rainfall*sex [M6]	
age + sex + DE + DWAT+ DRUM + closed_ha + water_ha + RD_den + WB_den + **cattle_den** + seroprev_intrasp + rainfall + sex*age + sex*RD_den + rainfall*RD_den + rainfall*sex [M7]	324.49
age + sex + DE + DWAT+ DRUM + closed_ha + water_ha + RD_den + WB_den + seroprev_intrasp + rainfall + sex*age + **sex*RD_den** + rainfall*RD_den + rainfall*sex [M8]	322.65
age + sex + DE + DWAT+ **DRUM** + closed_ha + water_ha + RD_den + WB_den + seroprev_intrasp + rainfall + sex*age + rainfall*RD_den + rainfall*sex [M9]	319.32
age + sex + DE + DWAT+ closed_ha + **water_ha** + RD_den + WB_den + seroprev_intrasp + rainfall + sex*age + rainfall*RD_den + rainfall*sex [M10]	318.05
age + sex + DE + **DWAT**+ closed_ha + RD_den + WB_den + seroprev_intrasp + rainfall + sex*age + rainfall*RD_den + rainfall*sex [M11]	317.38
age + sex + DE + closed_ha + RD_den + WB_den + seroprev_intrasp + rainfall + **sex*age** + rainfall*RD_den + rainfall*sex [M12]	316.01
age + sex + DE + **closed_ha** + RD_den + WB_den + seroprev_intrasp + rainfall + rainfall*RD_den + rainfall*sex [M13]	315.24
age + sex + DE + RD_den + WB_den + seroprev_intrasp + rainfall + rainfall*RD_den + rainfall*sex [final model]	314.74
Full model for fallow deer: age + sex + DE + DWAT + DRUM + closed_ha + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_RD + rainfall l+ temperature + sex*age + **age*FD_den** + sex*FD_den + rainfall*FD_den + rainfall*sex + rainfall*age + rainfall*temperature [M1]	554.03
age + sex + DE + DWAT + DRUM + closed_ha + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_RD + rainfall l+ temperature + sex*age + sex*FD_den + **rainfall*FD_den** + rainfall*sex + rainfall*age + rainfall*temperature [M2]	550.33
age + sex + DE + DWAT + DRUM + closed_ha + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_RD + rainfall l+ temperature + sex*age + sex*FD_den + rainfall*sex + rainfall*age + **rainfall*temperature** [M3]	548.33
age + sex + DE + DWAT + DRUM + **closed_ha** + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_RD + rainfall l+ temperature + sex*age + sex*FD_den + rainfall*sex + rainfall*age [M4]	546.36
age + sex + DE + DWAT + **DRUM** + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_RD + rainfall l+ temperature + sex*age + sex*FD_den + rainfall*sex + rainfall*age [M5]	544.38
age + sex + DE + DWAT + water_ha + FD_den + RD_den + WB_den + cattle_den + seroprev_intrasp + seroprev_RD + rainfall l+ temperature + sex*age + **sex*FD_den** + rainfall*sex + rainfall*age [M6]	542.47
age + sex + DE + DWAT + water_ha + FD_den + RD_den + WB_den + **cattle_den** + seroprev_intrasp + seroprev_RD + rainfall l+ temperature + sex*age + rainfall*sex + rainfall*age [M7]	540.56
age + sex + DE + DWAT + water_ha + FD_den + RD_den + WB_den + seroprev_intrasp + seroprev_RD + rainfall l+ temperature + sex*age + rainfall*sex + **rainfall*age** [M8]	538.73
age + sex + DE + DWAT + water_ha + FD_den + RD_den + WB_den + seroprev_intrasp + seroprev_RD + rainfall l+ **temperature** + sex*age + rainfall*sex [M9]	536.77
age + sex + DE + DWAT + water_ha + **FD_den** + RD_den + WB_den + seroprev_intrasp + seroprev_RD + rainfall l + sex*age + rainfall*sex [M10]	534.88
age + sex + **DE** + DWAT + water_ha + RD_den + WB_den + seroprev_intrasp + seroprev_RD + rainfall l + sex*age + rainfall*sex [M11]	533.12

age + sex + DWAT + water_ha + RD_den + WB_den + seroprev_intrasp + **seroprev_RD** + rainfall l + sex*age + rainfall*sex [M12]	532.06
age + sex + DWAT + water_ha + RD_den + WB_den + seroprev_intrasp + rainfall + sex*age + **rainfall*sex** [M13]	530.76
age + sex + DWAT + water_ha + RD_den + WB_den + seroprev_intrasp + **rainfall** + sex*age [M14]	530.38
age + sex + DWAT + water_ha + **RD_den** + WB_den + seroprev_intrasp + sex*age [M15]	529.05
age + sex + DWAT + **water_ha** + WB_den + seroprev_intrasp + sex*age [M16]	528.65
age + sex + DWAT + WB_den + seroprev_intrasp + sex*age [final model]	528.32

CAPÍTULO 2.4. Factores determinantes a largo plazo de la seroprevalencia de hepatitis E en el jabalí (*Sus scrofa*)

CHAPTER 2.4. Long-term determinants of the seroprevalence of hepatitis E virus in wild boar of Doñana National Park

Barroso, P., Risalde, M.A., García-Bocanegra, I., Acevedo, P., Barasona, J.A, Caballero-Gómez, J., Jiménez-Ruiz, S., Montoro, V., Vicente, J., 2021. Long-term determinants of the seroprevalence of Hepatitis E virus in wild boar (*Sus scrofa*). *Animals* 11, 1805. https://doi.org/10.3390/ani11061805

Resumen

El virus de la hepatitis E (VHE) es un patógeno zoonótico emergente cuyo principal reservorio son los suidos. La mayoría de los aspectos ecológicos y epidemiológicos de su ciclo silvestre aún se desconocen. Por tanto, en este trabajo se estudiaron los principales factores determinantes de la exposición al VHE en la población de jabalíes del Parque Nacional de Doñana (PND, suroeste de España), que operan a medio y largo plazo (2005-2018). Los anticuerpos contra el VHE estuvieron ampliamente distribuidos en los jabalíes del PND (% ± error estándar 95%; 46,7±3,8, 327 positivos de un total de 700 muestreados), mostrando un patrón creciente con la edad estadísticamente significativo. El patrón temporal mostró importantes fluctuaciones interanuales. Esto podría estar mediado por las marcadas variaciones observadas en los controles poblacionales del jabalí y los consecuentes cambios en las tasas de abundancia, así como su relación con las condiciones climáticas, ya que los años húmedos y las condiciones de baja abundancia de jabalí dieron lugar a las seroprevalencias más bajas observadas. El hecho de que la seroprevalencia sea elevada en situaciones de alta abundancia, y que esta relación no se vea influida por el nivel de precipitación, se debió probablemente al incremento de las interacciones entre animales y, posiblemente, a la elevada contaminación ambiental con partículas del VHE que dicha situación conlleva. La proximidad a la marisma (el principal punto de agua del área de estudio) se asoció con un elevado riesgo de presentar anticuerpos, lo que probablemente estuvo mediado por un mayor uso de esta zona durante la estación seca y por unas condiciones ambientales favorables para la supervivencia de las partículas virales. Son necesarias futuras investigaciones que aborden otras especies susceptibles para alcanzar un conocimiento más profundo de la epidemiología del VHE en las comunidades de hospedadores. El control poblacional del jabalí continúa siendo un reto muy importante a nivel internacional, teniendo en cuenta el esperado incremento de los conflictos relacionados con los patógenos compartidos asociados a esta especie, como se ha ejemplificado con el VHE. Por tanto, la vigilancia de las enfermedades del jabalí, incluyendo la monitorización poblacional y la aplicación de programas de control poblacional sostenibles, son esenciales para controlar los riesgos asociados a estos patógenos.

Abstract

The hepatitis E virus (HEV) is an emerging zoonotic pathogen whose main reservoir is suids. Most of the ecological and epidemiological aspects of its sylvatic cycle remain unknown. Thus, in this work, we study the drivers of HEV exposure in the wild boar population of Doñana National Park (DNP, southwest Spain) operating in the medium and long-term (2005-2018). Anti-HEV antibodies are widely distributed throughout the wild boar (% ± standard error 95%; 46.7 ± 3.8, 327 out of 700 sampled), showing a statistically significant age-increasing pattern. The temporal pattern displayed important interannual fluctuations. This could be mediated by marked variations in the population control of the wild boar, and subsequent changes in abundance rates, and its interplay with climatic conditions; as wet years together with a low abundance of wild boar led to the lowest seroprevalence. The fact that seroprevalence is high during conditions of high abundance, and not affected by rainfall level, is probably due to the increased interactions among the animals, and possibly, the subsequent higher environmental contamination with HEV particles. The proximity to the marshland (the main water body of the study area) is associated with a higher risk of testing positive, which is probably mediated by the preferential use of this area during the dry season and the favourable environmental conditions for the survival of HEV particles. A deeper understanding of the epidemiology of HEV in host communities deserves future research concerning other susceptible species. Most importantly, wild boar population control remains a challenge at the international level, and an increase of shared pathogen-related conflicts associated with this species is expected, as exemplified by HEV. Therefore, surveillance of wild boar diseases, including integrated population monitoring and sustainable population control programmes, will be essential to control the associated risks.

Introduction

The hepatitis E virus (HEV, RNA virus, genus Orthohepevirus, family Hepeviridae) (Emerson and Purcell, 2003) is an emerging zoonotic pathogen with a widespread distribution throughout the world (Adlhoch et al., 2016). HEV is one of the main causes of acute hepatitis worldwide, affecting more than 20 million people annually (WHO, 2017). HEV is a multi-host pathogen classified into eight genotypes (HEV-1 to 8), of which only genotypes 1 and 2 affect humans exclusively, while the rest have been isolated in a wide range of wild and domestic animals (Kenney and Meng, 2019b). Pig (*Sus scrofa domesticus*) and wild boar (*Sus scrofa*) are considered the main animal reservoir species of the virus (Boadella et al., 2012a; De Deus et al., 2008), and cases of zoonotic transmission from these species have been detected (Rivero-Juarez et al., 2017; Zheng et al., 2006).

HEV infection is primarily transmitted via the faecal-oral route through contaminated water or food in epidemic genotypes (Emerson and Purcell, 2003), whereas the transmission routes of zoonotic genotypes also include consuming raw meat or products derived from infected animals, shellfish or vegetables (Faber et al., 2018). The shedding of enterically excreted HEV particles into the environment plays a major role in the transmission of the virus, HEV sequences have been detected as clusters in humans, swine and wildlife from the same geographical region in sewage, surface and wastewater (Van der Poel, 2014). Viral particles of HEV have also been found through molecular methods in rivers from Europe and South America, originating from the excretion of faeces by infected hosts (Rusiñol et al., 2014). Thus, long-term shedding of HEV from suids could facilitate its persistence in the host communities and environment, through an increase in the exposure and risk of transmission to other sympatric susceptible wild and domestic animals (Kukielka et al., 2016).

In southern Spain, HEV-3 is endemic, and exposure and infection rates have been obtained by serological and molecular methods in domestic pigs (seroprevalence range 16.5–60.8%), wild boar (seroprevalence range 5.2–57.6%), red deer (*Cervus elaphus*; seroprevalence around 10.0%) and equines (prevalence of 0.4–3.6%) (Boadella et al., 2010; De Deus et al., 2008; García-Bocanegra et al., 2019; López-López et al., 2018; Risalde et al., 2017; Rivero-Juarez et al., 2020). Situations of a greater abundance and aggregation of wild boar are the best scenarios for HEV circulation, as they are associated with high seropositivity rates for HEV (Boadella et al., 2012a; De Deus et al., 2008; Larska et al., 2015). Additionally, higher seroprevalences of HEV were found in rural areas compared to urban areas (Forgách et al., 2010), due to ecological and/or biological variations. However, several risk factors (mainly environmental, populational, meteorological or stochastic) might have been overlooked by these studies, due to their short-term approach.

Most of the ecological and epidemiological aspects of the sylvatic cycle of HEV remain unknown, and Doñana National Park (DNP; southwestern Spain) acts as a good field laboratory for the study of pathogens transmitted throughout indirect routes in the wildlife-livestock interface (Barroso et al., 2020c; Jiménez-Ruiz et al., 2021). A previous survey conducted in 2015 reported the circulation of HEV-3 in wild boar from our study area and seroprevalence of 57.6% (Caballero-Gómez et al., 2019). The activity (e.g. defecating) of this species around water points is likely to result in environmental contamination with HEV and the potential transmission to other susceptible hosts (Ruiz-Fons et al., 2008b; Rutjes et al., 2010). The importance of wild boar in the epidemiological cycle of HEV and its key role in viral transmission provides evidence of the need to conduct long-term studies.

Long-term approaches provide a broad temporal perspective to understand processes that potentially determine the host-pathogen dynamics, allowing us to discern the main drivers of the introduction, persistence and maintenance of the pathogens in the host communities (Barroso et al., 2020a). This approach is particularly appropriate for a pathogen whose transmission depends greatly on environmental and host population factors, which may markedly change over years, due to host, density-dependent (e.g. density itself and/or aggregation) and stochastic factors (e.g. climate), as well as their complex interaction networks. However, there is a lack of long-term studies assessing the main drivers of HEV infection in wildlife. In this context, we conducted a long-term serosurvey of HEV in the wild boar from DNP during a 13-year period, with the specific aim of assessing the temporal trend of the seroprevalence of HEV and identifying the factors modulating it, namely, the individual, environmental, populational and stochastic factors.

Materials and methods

Study area

This study was carried out in DNP (37°9′ N, 6°30′ W; Figure 1), a flat sandy area of 54,252 ha located on the Atlantic coast of southwest Spain. Three main biotopes are present in DNP: scrubland, dunes and marsh. The scrubland and marshland are divided by a narrow strip of humid ecotone characterised by a high ecological richness. The climate of DNP is dry, subhumid Mediterranean, characterised by strong seasonality.

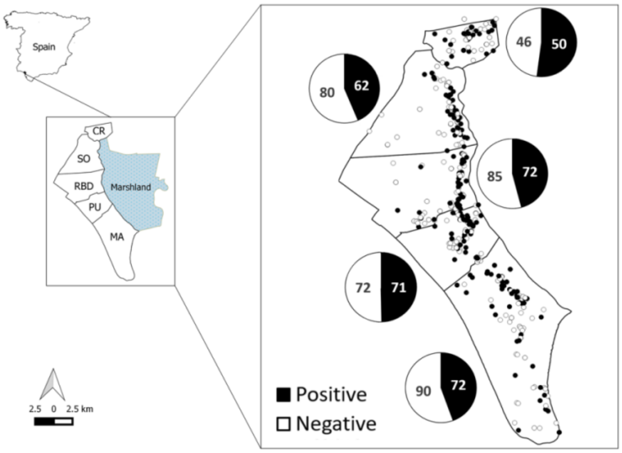


Figure 1. Map of the study area, Doñana National Park. The livestock management areas (Coto del Rey CR), Sotos (SO), Doñana Biological Reserve (RBD), Puntal (PU) and Marismillas (MA)) are delimited. Sampling distribution and the number of animals positive and negative for HEV-antibodies observed in each livestock management area are also shown. Black and white symbols mean animals positive and negative for anti-HEV antibodies, respectively. The ecotone is the north to south line between the marshland (east, in blue) and the management areas (west).

Free-ranging cattle and horse breeding are allowed, except in the northern area. Livestock is kept in the five livestock management areas of DNP, from north to south (Figure 1): Coto del Rey (CR), Sotos (SO), Doñana Biological Reserve (RBD), Puntal (PU) and Marismillas (MA). The wild ungulate community which inhabits DNP is comprised of wild boar, red deer and fallow deer (*Dama dama*). This abundant community of wild and domestic ungulates inhabits a place where the resources are limited during the dry season (late summer and autumn). This forces the aggregation of animals around water points (Laguna et al., 2018) during the dry season and acorn mast in autumn (Triguero-Ocaña et al., 2019). A more detailed description of the management, environmental conditions and habitats in DNP has been provided by the authors of Barasona et al. (2014a).

Animal sampling

We selected the wild boar population from the entire ungulate community of DNP because; i) it is the only species that has been widely recognised as a reservoir of HEV (Boadella et al., 2012a; De Deus et al., 2008; Meng, 2010), and ii) for its constant population increase and expansion (Massei et al., 2015), which increment the potential for HEV transmission in a wide range of environments, especially those where susceptible host species coexist. Taking advantage of the population control undertaken by Park rangers, 700 wild boar were necropsied between 2005-2006 and 2017-2018 sampling periods (sampling periods typically occur between October and January). This health-monitoring programme was temporally interrupted during 2008-2009. Individuals were randomly selected, since culling activities did not address any specific objective about sex or age classes. This sampling belonged to the DNP health-monitoring programme and was performed according to: European and Spanish laws (RD 223/1988; MAPA, 1988), and EC Directive 86/609/EEC; current guidelines for the ethical use of animals in research (ASAB, 2012); the Animal Experiment Committee of Castilla-La Mancha University and the Spanish Ethics Committee (PR-2015-03-08). The geographical coordinates where each animal was initially sighted were registered using a portable GPS (Garmin Ltd., Olathe, KS, USA). Blood samples were collected into sterile plastic tubes without additives (Vacutainer®, Becton-Dickinson, NJ, USA) from the endocranial venous sinus, or alternatively from the heart or thoracic cavity (Arenas-Montes et al., 2013).

Risk factors

Individual factors

During the examination, the sex and age of the wild boar were determined, the latter by dentition eruption patterns (Saenz de Buruaga et al., 2001): piglets (<6 months), juveniles (<6 months-2 years) and adults (>2 years).

Environmental factors

The habitat use by wild boar and its distribution in DNP is partly driven by environmental conditions (Laguna et al., 2018). In turn, the distribution of wild boar determines the spatiotemporal overlap of individuals around some resources, and therefore, the dynamics of pathogen transmission (Barasona et al., 2014a; Barroso et al., 2020b; Triguero-Ocaña et al., 2019). Thus, we selected a number of environmental variables as explanatory, some of them related to water sources and potential aggregation sites, to assess their potential effect on the seroprevalence of HEV (SH). Wild boar's sampling site is integrated within its home range, and most of its life will be spent within an average radius of 1.2 km (Triguero-Ocaña et al., 2020, 2019). Thus, we calculated the straight-line distances (m) from the exact location of each animal to the nearest marsh-shrub ecotone (DE), water point (DWAT), small human settlements (DHS), the marshland (DMARSH), stagnant water source (DSW) and the Guadalquivir river (DRIVER). The proportional cover of dense scrub, low-clear shrubland, herbaceous grassland, woodland, cover of bare land and watercourse vegetation for each animal were also calculated according to (Barasona et al., 2014a). All the environmental information was collected from Andalusia Environmental Information (REDIAM, 2014).

Populational factors

To assess the potential effect of host population abundance on seroprevalence, we considered, apart from the wild boar, the ungulates inhabiting DNP, which may play a role in the epidemiology of HEV (red deer, fallow deer and cattle) (Boadella et al., 2010; Huang et al., 2016; Kukielka et al., 2016; Neumann et al., 2016; Yan et al., 2016). Horses were not considered, due to the low prevalence reported for this species (0.4%; García-Bocanegra et al., 2019). The relative abundance of red deer and wild boar per area of livestock management, expressed as Kilometric Abundance Indexes (KAI) (Vincent et al., 1991), was monitored annually. KAI is used as a relative abundance index to inform in differences in wild boar abundance among areas and across years. We also calculated the population densities of fallow deer, and the cattle stocks (individuals/km^2) for each livestock management area and sampling period. For details about sampling design and effort, see Barroso et al. (2020b). The annual intra-specific seroprevalence of HEV was also calculated to assess its effect on the probability of wild boar testing positive for HEV.

Stochastic factors

The potential influence of climatic conditions concerning the availability of food resources and water to wild boar during the different seasons along the DNP is well-known (Barasona et al., 2014a), and the use of resources by animals increases their exposure to several pathogens (Vicente et al., 2013). HEV viral particles are very resistant to environmental conditions, facilitating its transmission (Barnaud et al., 2012), and dry years have been reported to be associated with increased viral contamination of water sources in different regions of Europe (Rusiñol et al., 2014). We, therefore, selected the rainfall and temperature as meteorological parameters to be included in our models to assess their potential effect on HEV epidemiology. The average rainfall and temperature per sampling period were calculated from the data obtained from the meteorology station located at RBD (EBD-CSIC, 2019). We included the rainfall and temperature of the previous sampling periods (calculated from September to August) in the models, since the animals sampled from August onwards had also been subjected to the meteorological conditions of the previous months.

Serological analyses

Sera obtained by centrifugation (400 *g* for 5 min) from blood without additives were stored at −20 °C until assayed for antibodies against HEV. Anti-HEV immunoglobulin (Ig) G antibodies were tested using a commercial indirect enzymatic immunoassay (ELISA; PrioCHECK® HEV Antibody porcine ELISA Kit. Thermo Fisher Scientific™, Waltham, Massachusetts, United States of America), following the manufacturer's instructions. This ELISA test has been used previously for wild boar (Caballero-Gómez et al., 2019; Ivanova et al., 2015; Risalde et al., 2017), and is based on recombinant antigens of the open reading frame (ORF) ORF2 and ORF3 derived from genotypes 1 and 3. According to the information provided by the manufacturer, sensitivity and specificity are 91% and 94%, respectively.

Statistical analysis

As a previous step, collinearity between individual, environmental, populational and stochastic variables was explored (Zuur et al., 2009). Given the high level of correlation obtained between land-cover variables, we performed a principal component analysis (PCA) and obtained two uncorrelated factors; closed habitats with dense scrub and woodland, and watercourse vegetation (see Table S1).

Generalised linear mixed models (GzLMMs; binomial error distribution and logit link function) were fitted to assess the relationship between the serological response against HEV (negative/positive; as a response variable) and its potential risk factors. Firstly, the spatial differences in the HEV exposure between livestock management areas (CR, SO, RBD, PU and MA) were assessed in an exploratory GzLMM. In this GzLMM, the sex, age class and livestock management area were included as fixed terms, and the sampling period and month as random terms. A second model was performed with the purpose of generalising the effect of the variables on the serological status against HEV regardless of the livestock management area and the sampling period. Thus, the final model included these variables as random-effect factors. The explanatory variables were: i) individual factors (sex and age class); ii) environmental factors (DE, DWAT, DRIVER, DHS, DSW, closed cover and the coverage of watercourse vegetation); iii) populational factors, including the relative abundances of red deer and wild boar, densities of fallow deer and cattle, and intra-specific seroprevalence of HEV, by livestock management area and sampling period; and iv) stochastic factors (previous sampling period's rainfall and temperature). In order not to overparameterise the statistical models, and to build models based on the initial hypotheses regarding the influence of density-dependent and stochastics factors, as well as the role mediated by individual factors (sex and age), we included all two-way interactions between sex, age, rainfall, temperature and population abundances and densities (wild boar, red deer, fallow deer and cattle) (Barroso et al., 2020b). A stepwise model selection process based on the corrected Akaike's Information Criterion (cAIC) was used to select the most parsimonious model (Table S2) (Akaike, 1974). Once the final model was obtained, the assumptions of the binomial GzLMMs were checked (Zuur et al., 2009). A significant *p*-value was set at 0.05, and the predicted probabilities of serological response to HEV obtained from these models were used to represent the results. GzLMMs were fitted in the R library *lme4* 1.1-21 version of the R software 4.0.2 version (Bates et al., 2015).

Finally, cross- and self-correlations between seroprevalence and the relative abundance of wild boar were carried out to explore similarities in temporal patterns. The time lag (latency) at which the correlation was maximised was used to determine the latency between the two time-series explored. We used time shifts (lags) between −4 and 4 sampling periods. The absolute values of the cross and self-correlations were considered significant if they exceeded twice the estimated standard error values. Cross-correlations and self-correlations were run using IBM SPSS 19.0 software (IBM Corporation, Somers, NY, USA; George and Mallery, 2012).

The confidence intervals for seroprevalences were estimated by the standard error 95% confidence interval (S.E. 95%).

Results

General results

A total of 327 out of 700 wild boar (SH±S.E. 95%=46.7±3.8%) presented anti-HEV antibodies in DNP during the study period (Figure 1). Table 1 displays SH observed in wild boar by livestock management area and sampling period. SH were 48.4±0.1% and 44.7±0.1% for males and females, respectively. The SH sex and age patterns are shown in Figure 2a. The temporal trend in the SH, as well as in the relative abundance of wild boar, are shown in Figure 2b. An apparent increase in SH in the 2012-2013 season after a steady decrease from 2006-2007 is noteworthy, reaching the maximum in the last sampling period (2017-2018). Spatially, the first explanatory model did not reveal statistical differences in the SH between livestock management areas (Figure 1; *F* = 0.68, df = 690, *p* = 0.62).

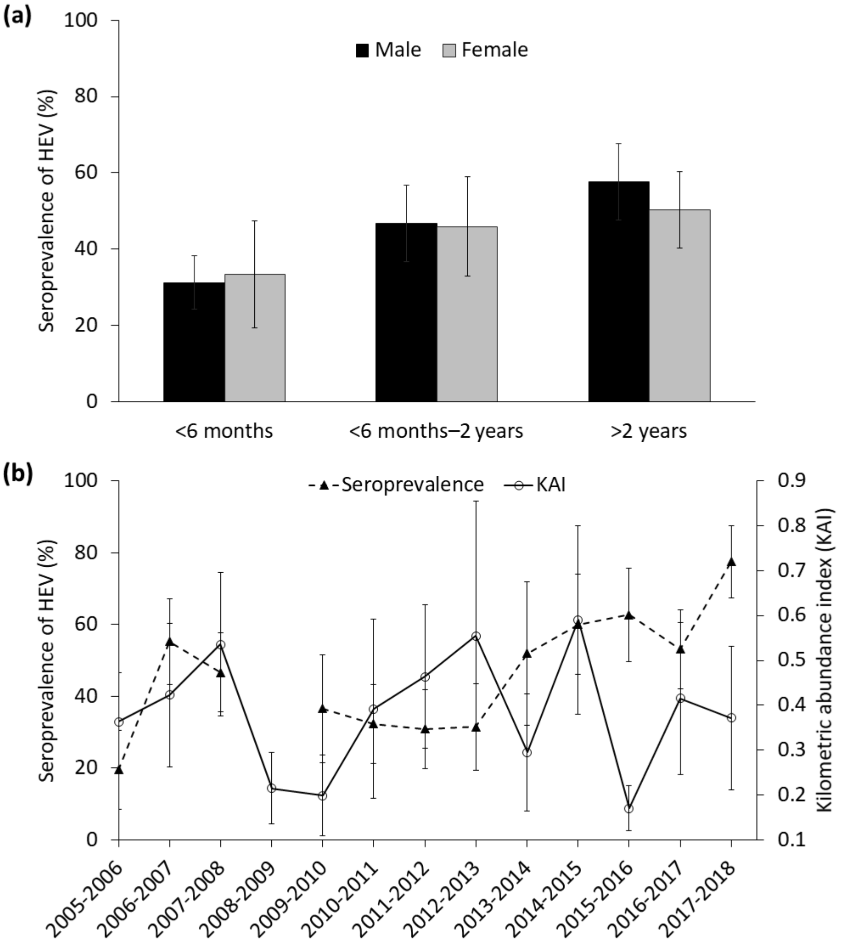


Figure 2. (**a**) Seroprevalence (% ± standard error (S.E.) 95%) of HEV depending on age class and sex. (**b**) Temporal trend of the seroprevalence of HEV (± S.E. 95%) and the average (± S.E.) relative abundance of wild boar (KAI).

Table 1. Seropositivity for HEV (Seroprev; %) obtained by ELISA and sample size (N) by sampling period and livestock management area (Coto del Rey (CR), Sotos (SO), Doñana Biological Reserve (RBD), Puntal (PU) and Marismillas (MA)).

Sampling period	**CR**	**SO**	**RBD**	**PU**	**MA**	**Total**
	Seroprev (N)	Seroprev (N)	Seroprev (N)	Seroprev (N)	Seroprev (N)	Seroprev (N)
2005-2006	-	16.7 (6)	25 (12)	12.5 (8)	20 (25)	19.6 (51)
2006-2007	71.4 (14)	57.1 (14)	35.3 (17)	66.7 (18)	25 (4)	55.2 (67)
2007-2008	-	19.2 (26)	42.9 (7)	69.2 (13)	56.3 (32)	46.7 (75)
2008-2009	-	-	-	-	-	-
2009-2010	100 (2)	50 (4)	41.2 (17)	-	2.2 (18)	36.6 (41)
2010-2011	29.4 (17)	40 (10)	35 (20)	25 (12)	33.3 (9)	32.4 (68)
2011-2012	25 (8)	31.6 (19)	26.3 (19)	54.5 (11)	18.2 (11)	30.9 (68)
2012-2013	0 (9)	0 (6)	38.5 (13)	42.9 (7)	47.4 819)	31.5 (54)
2013-2014	-	-	57.1 (7)	50 (18)	-	52.0 (25)
2014-2015	100 (2)	81.3 (16)	75 (8)	41.2 (17)	28.6 (7)	60.0 (50)
2015-2016	73.3 (15)	41.7 (12)	80 (10)	33.3 (6)	75 (8)	62.7 (51)
2016-2017	46.7 (15)	46.7 (15)	78.6 (14)	35 (20)	66.7 (15)	53.2 (79)
2017-2018	78.6 (14)	78.6 (14)	53.8 (13)	92.3 (13)	85.7 (14)	77.5 (71)
Total	52.1 (96)	43.7 (142)	54.1 (157)	49.7 (143)	44.4 (162)	46.7 (700)

Factors determining the seroprevalence of HEV

The results obtained from the best GzLMM of SH are shown in Table 2. However, we also consider three potential models obtained during the backward stepwise model selection procedure, since differences <2cAIC values were observed (Table S3). Age was statistically significant to explain SH, so that an increasing trend, regardless of sex, was evident (Figure 2a). Among the environmental factors, the distance to the marshland was the only parameter that remained in the model selected after the stepwise selection procedure (Table S2), indicating that the closer to the marshland, the higher the SH was (Figure 3a). Among populational factors, the relationship between the relative abundance of wild boar (KAI) and SH was conditioned by the rainfall (Figure 3b, see detailed explanation below). The intra-specific SH was significantly and positively associated with SH. Concerning the stochastic factors, the interaction between the previous sampling period's rainfall and the KAI of wild boar was retained in the best model and was statistically significant. SH was the lowest when high rainfall and low relative abundance co-occurred. In high relative abundance conditions, SH was high regardless of the rainfall level (Figure 3b).

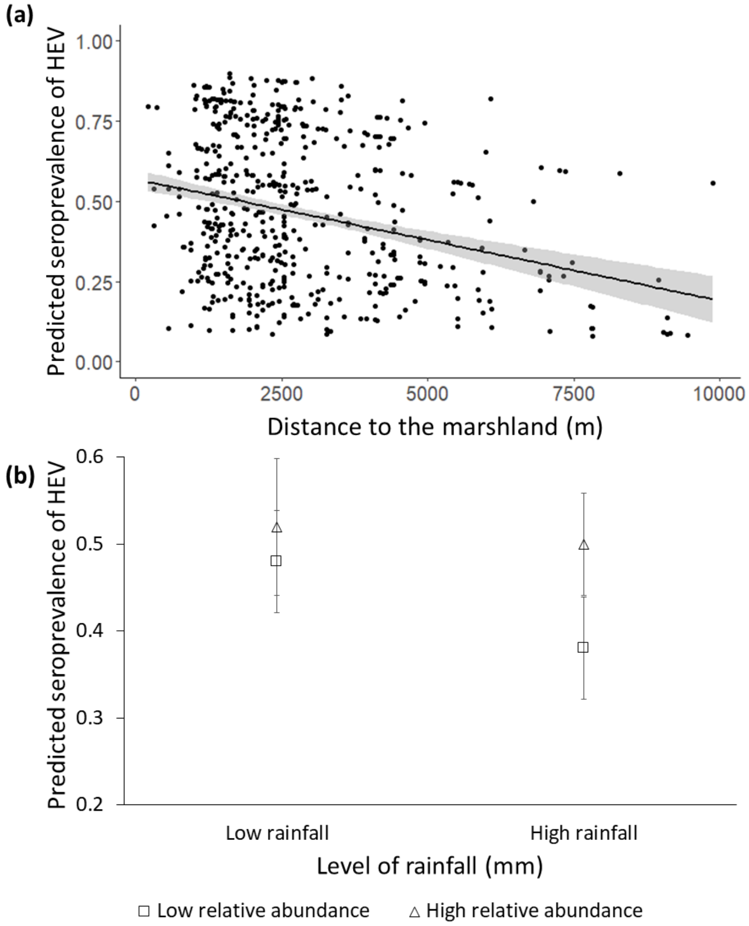


Figure 3. (**a**) Individual predicted probability to test positive for anti-HEV antibodies (± confidence interval (C.I.) 95%, represented by the shaded band) obtained from selected generalised linear mixed models (GzLMMs) depending on the distance to the marshland (m). (**b**) Mean predicted probability to test positive for anti-HEV antibodies (± C.I. 95%, represented by the error bars) obtained from selected GzLMMs depending on the interaction between annual rainfall (mm) and the relative abundance of wild boar (KAI). The following categories were established to display results: low rainfall (≤521.10 mm), high rainfall (>521.10 mm), low relative abundance of wild boar (≤0.69 KAI) and high relative abundance of wild boar (>0.69 KAI).

In the other three potential best models, the age, the distance to the marshland, and the intra-specific SH also provided statistically significant results associated with the risk of presenting antibodies to HEV, whereas no effect was observed for the relative abundance of wild boar, the sex, or the interactions between rainfall and sex with the relative abundance of wild boar (see Table S3).

Table 2. Parameters from the best GzLMM for the serological status against HEV in wild boar related to age class, relative abundance (KAI), straight-line distance to the marshland (DMARSH), the rainfall of the previous sampling period (rainfall), the intra-specific seroprevalence of HEV and the interaction between rainfall and KAI.

Variables	**F** **df (x,y)**	**Estimate ± S.D.**	***p***
Age class[1]	5.30 (2, 690)	1-2 years: 0.46±0.25 >2 years: 0.76±0.21	**<0.01**
Distance to marsh	3.09 (1, 690)	<0.001±<0.001	**0.03**
Abundance of wild boar (KAI)	4.07 (1, 690)	-0.85±0.72	0.22
Rainfall	0.01 (1, 690)	<0.001±<0.001	**0.05**
Rainfall*Abundance of wild boar	3.74 (1, 690)	<0.001±<0.001	**0.05**
Intra-specific seroprevalence	102.59 (1, 690)	0.04±0.01	**<0.01**

The model was fitted using the sampling period and livestock management area as random effect factors. [1] Parameter estimates for the age class were calculated using <6-months-old animals as the reference. [2] Standard deviation. The conditional R^2 obtained from this model was 0.267.

Self-correlations and cross-correlations

The annual values of SH showed significant positive self-correlations, with a lag of one year (R: 0.71±0.24; Box-lung value: 10.25; *p* = 0.05).

Discussion

To the best of our knowledge, this is the first longitudinal long-term survey (2005-2018) of HEV exposure in wild boar. This approach allowed us to determine a temporal pattern conditioned by marked variations in the population control of wild boar (and subsequent changes in their relative abundances), as well as in the climatic conditions throughout the study period. We have contributed to broadening the understanding of the epidemiology of HEV in such a relevant wild host and opened new perspectives for further research regarding other hosts, including the human side, and the role of management and environmental features over the long-term.

General patterns in the seroprevalence of HEV

Overall, the SH value (46.72%) is higher than those obtained in the majority of European studies, mostly ranging from 4.9% to 34.0% (Boadella et al., 2012a; Martinelli et al., 2015; Risalde et al., 2017; Thiry et al., 2017b), and concurs with some values detected in Spain in woodland (mainly hunting areas (Kukielka et al., 2016), and urban environments (Wang et al., 2019)). Occasionally, similar values have been previously reported in wild boar populations over European countries, such as Poland or Italy (Kozyra et al., 2020; Larska et al., 2015; Montagnaro et al., 2015). The high seroprevalence obtained, despite the absence of contact with domestic free-ranging pigs, suggests the capability of wild boar to maintain a constant exposure to HEV within this host community. In fact, the detection of HEV antibodies in a significant number of young wild boar (6-24 months old) for the whole study period (data not shown) suggested the recent and persistent exposure to this virus during these years, since it has been demonstrated that swine are infected at an early age after the loss of maternal antibodies (Kantala et al., 2015). The appearance of IgM antibodies in serum occurs first and is of relatively short shelf-life, followed by IgG that is more permanent in time (up to 22 weeks) (Pavio et al., 2010). Thus, IgG is more suitable for long-term seroprevalence studies (De Deus et al., 2008; Ricci et al., 2017; Salines et al., 2017), although its detection does not evidence early exposure to HEV. Further research is needed at the community level to determine the SH in sympatric cattle, horses, carnivores, lagomorphs and deer species, as well as the determinants of the persistence of HEV in such multi-host systems.

HEV is widely distributed over the study area, which contrasts with the clear spatial patterns shown by other pathogens at a relatively small scale in the wild boar in DNP (Barroso et al., 2020b, 2020c; Gortázar et al., 2008). This may indicate that factors not related to specific local conditions of the environment mostly determined heterogeneity in HEV exposure and/or SH in wild boar over the long-term. However, there were important interannual differences in the SH (ranging from 19.6 to 77.5%), characterised by steady increases or decreases, whose potential determinants are discussed below. There was a clear inflexion point of SH characterised by a continuous steady increase from 2012-2013 onwards. This change in the trend is coincident with the recovery of the wild boar population following a significant population control programme in the Park during 2004-2005 and 2005-2006 (Figure 2b; a total of 1000 individuals were removed from the population). The self-correlation of the temporal pattern (1 year-lag) suggests that patterns observed from a perspective of at least two years are independent of previous SH, which concurs with the rapid population turnover that characterises wild boar.

Factors determining seroprevalence of HEV

We evidenced a significant age-increasing pattern in SH, regardless of sex (Table 2, Figure 2a). This concurs with some studies (Caballero-Gómez et al., 2019; Montagnaro et al., 2015; Thiry et al., 2017b). However, most studies on HEV in wild boar have not reported significant age-related differences (De Deus et al., 2008; Kukielka et al., 2016; Martinelli et al., 2015; Rivero-Juarez et al., 2018). The likelihood of exposure to the virus throughout life, together with the high persistence of antibodies against HEV in wild boar, may underly this pattern (Risalde et al., 2017).

The proximity of the wild boar sampled to the marshland was positively associated with increased SH. During the last 10 years, our research group has captured and tagged more than 50 wild boar with telemetry collars in DNP (Barasona et al., 2014a; Palencia et al., 2019; Triguero-Ocaña et al., 2019). Telemetry data allow us to explain and discuss the habitat use by wild boar in the study area. During the dry season, the soil of the marshland is moisture-retentive (Braza and Alvarez, 1989), and is significantly used by wild boar, wallowing and feeding on *Scirpus maritimum* roots, earthworms, small crustaceans or mammals, predating on aquatic bird nests, or taking mud-baths (Venero, 1984). Furthermore, the level of water contamination increases during the dry season, due to an increase in the concentration of pollutants, as has been reported in human studies (Khuroo et al., 2016). The conditions of the marsh during this period may also allow for the increased survival of the virus in the environment and the subsequent high exposure of the wild boar to it.

High relative abundances of wild boar entailed increased SH risk. Previous studies have considered wild boar management practices as a proxy of density to assess its effect on SH (De Deus et al., 2008; Kukielka et al., 2016). In these studies, significantly higher SH has been found in fenced hunting estates than in open areas from south-central Spain, probably mediated by the increased aggregation (e.g. artificial feeding and watering at specific points) and the higher densities of animals (Acevedo et al., 2007; Boadella et al., 2012a; De Deus et al., 2008). Likewise, there is a negative impact associated with high densities concerning the nutrition of wild boar, as well as increased mortality rates, due to undernutrition and diseases, such as tuberculosis (Barasona et al., 2016); there may also be an increase in scavenging and risks for HEV transmission. Moreover, Larska et al. (2015) reported a positive association between the density of wild boar and the SH in a study undertaken concerning several wildlife species in Poland.

The lowest SH was observed when high rainfall and a low relative abundance of wild boar co-occurred, whereas in high relative abundance situations SH was high regardless of the rainfall level (Figure 3b). Discrepancies have been observed in the relationship between the prevalence of HEV and meteorological conditions, concluding that both rainfall and temperature did not appear to be drivers of the prevalence of HEV in wild boar (Lu et al., 2013), while another study reported a limited survival of HEV particles in the soil over 37 °C (Parashar et al., 2011). To our knowledge, there are no previous studies evaluating the effects mediated by rainfall on the risk of HEV in wild boar, which is also expected to vary depending on the local conditions and climate (Mediterranean, in our case). In this study, the temperature did not result relevant to explain the SH in the final model (see Table 2). However, the risk of direct and/or indirect HEV exposure and transmission among wild boar increases during dry years, since aggregation at the scarce watering sites available during the dry season rises as well (Barroso et al., 2020b; Ruiz-Fons et al., 2006; Vicente et al., 2013). Under these circumstances, not only do direct interactions among individuals occur more often, but there is also greater environmental contamination and persistence, since wild boar wallow in, brush against, defecate and urinate in their surroundings (Andraud et al., 2013; Barasona et al., 2014a; Kukielka et al., 2013). This may also be potentiated by cannibalism or scavenging, since wild boar carcasses become more available during periods of drought in Mediterranean regions (Barasona et al., 2016; Carrasco-Garcia et al., 2018). Thus, the concomitance of low rainfall and high densities in DNP possibly involves a greater aggregation at watering sites and the subsequent risk of direct and indirect transmission of HEV. On the contrary, rainy years together with the low relative abundance of wild boar may imply an increase in water sources, lower host aggregation and the subsequent dilution of viral particles in the environment, leading to lower SH (Rusiñol et al., 2014). However, in rainy years when a high relative abundance of wild boar occurs, this dilution effect of HEV particles caused by the rain might be not enough to counteract the high level of environmental contamination, as well as the increased direct and indirect interactions among individuals that are derived from their high abundance.

Conclusions

The effect of the relative abundance in the SH of wild boar in this study reflects the human management the population is subjected to, which is the main determinant of the wild boar population size in DNP, where predators do not exist. This factor should be considered in conjunction with others, some operating stochastically on a long-term scale (e.g. meteorological conditions), as they may interact and mask the concomitant action of different drivers.

The abundance of wild boar is still increasing, and this applies to most of the distribution range of Eurasian wild boar (which is also expanding) and feral pigs worldwide. In this study, the increase of SH observed after a major population culling programme (2004-2005 and 2005-2006) evidences the importance of sustainably controlling populations of wild boar, which are a relevant source of pathogens shared with livestock, humans and other wildlife (Boadella et al., 2012b; Ruiz-Fons et al., 2008b). Wild boar population control remains a challenge at the international level (Vicente et al., 2019a), and the current socio-economic context is not favourable. An increase in disease-related conflicts associated with this species is expected, even in peri-urban and urban habitats (Wang et al., 2019).

Wild disease surveillance, including integrated population monitoring, will be essential for disease risk evaluation and subsequent decision making. In the case of HEV, multi-host systems, including other wildlife species, such as livestock; human and environmental sources, should also be considered. Cross-species transmission of HEV from animal reservoirs is the major route of infection in humans, it has become one of the most successful zoonotic viral diseases in both developed and developing countries (Nan et al., 2017), and our results indicate that this problem may increase. Therefore, HEV and wildlife reservoirs should not be neglected as potential public health risks for humans in the future.

Acknowledgments

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Supplementary material 2.4

Table S1. Result of the Principal Component Analysis (PCA) and the two uncorrelated factors obtained.

Variable	PC1- closed habitats	PC2-watercourse habitats
Herbaceous grassland	-0.856	-0.507
Bare land	0.259	-0.098
Watercourse vegetation	-0.096	0.991
Woodland, dense and low-clear shrubland	0.866	-0.362
Standard deviation	1.56	1.38
Explained variance	38.96	34.48
Cumulative variance	38.96	73.44

Table S2. Summary of the stepwise model selection procedure, based on the corrected Akaike's information criterion (cAIC), used to explain the serological status against HEV. The full model comprises the age class, sex, straight-line distance to the nearest: Ecotone (DE), water point (DWAT), small human settlements (DHS), the marshland (DMARSH), stagnant water source (DSW) and the Guadalquivir river (DRIVER), rainfall (rainfall) and temperature (temperature) of the previous sampling period, cover of closed habitats (closed_ha), coverage of watercourse vegetation (water_ha), annual density of fallow deer (FD_den) and cattle (cattle_den), relative abundance of red deer (RD_KAI) and wild boar (WB_KAI), intra-specific seroprevalence of HEV (seroprev_intrasp) and some interaction among them (*). In bold type is shown the variable which was removed between steps.

Model	cAIC
Full model: age + sex + DE + DWAT + DHS + DMARSH + DSW + DRIVER + rainfall + temperature + closed_ha + water_ha + FD_den + cattle_den + RD_KAI + WB_KAI + seroprev_intrasp + sex*age + **age*WB_KAI** + sex*WB_KAI + rainfall*WB_KAI + rainfall*sex + rainfall*age + rainfall*temperature [M1]	856.49
age + sex + DE + DWAT + DHS + DMARSH + DSW + DRIVER + rainfall + temperature + closed_ha + water_ha + FD_den + cattle_den + RD_KAI + WB_KAI + seroprev_intrasp + sex*age + sex*WB_KAI + rainfall*WB_KAI + rainfall*sex + **rainfall*age** + rainfall*temperature [M2]	853.25
age + sex + DE + DWAT + DHS + DMARSH + DSW + DRIVER + rainfall + temperature + closed_ha + water_ha + FD_den + cattle_den + RD_KAI + WB_KAI + seroprev_intrasp + **sex*age** + sex*WB_KAI + rainfall*WB_KAI + rainfall*sex + rainfall*temperature [M3]	850.41
age + sex + DE + DWAT + DHS + DMARSH + DSW + DRIVER + rainfall + temperature + closed_ha + water_ha + FD_den + cattle_den + **RD_KAI** + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI + rainfall*sex + rainfall*temperature [M4]	847.89
age + sex + DE + DWAT + DHS + DMARSH + DSW + DRIVER + rainfall + temperature + closed_ha + water_ha + **FD_den** + cattle_den + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI + rainfall*sex + rainfall*temperature [M5]	845.89
age + sex + DE + DWAT + DHS + DMARSH + DSW + DRIVER + rainfall + temperature + closed_ha + water_ha + cattle_den + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI + **rainfall*sex** + rainfall*temperature [M6]	843.91
age + sex + DE + DWAT + DHS + DMARSH + DSW + DRIVER + rainfall + temperature + closed_ha + water_ha + **cattle_den** + WB_KAI + seroprev_intrasp + sex*WB_KAI +	841.95

rainfall*WB_KAI + rainfall*temperature [M7]	
age + sex + DE + DWAT + DHS + DMARSH + DSW + **DRIVER** + rainfall + temperature + closed_ha + water_ha + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI + rainfall*temperature [M8]	839.99
age + sex + DE + DWAT + **DHS** + DMARSH + DSW + rainfall + temperature + closed_ha + water_ha + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI + rainfall*temperature [M9]	838.05
age + sex + DE + **DWAT** + DMARSH + DSW + rainfall + temperature + closed_ha + water_ha + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI + rainfall*temperature [M10]	836.16
age + sex + DE + DMARSH + **DSW** + rainfall + temperature + closed_ha + water_ha + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI + rainfall*temperature [M11]	834.40
age + sex + DE + DMARSH + rainfall + temperature + closed_ha + water_ha + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI + **rainfall*temperature** [M12]	832.53
age + sex + DE + DMARSH + rainfall + temperature + closed_ha + **water_ha** + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI [M13]	830.74
age + sex + **DE** + DMARSH + rainfall + temperature + closed_ha + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI [M14]	828.93
age + sex + DMARSH + rainfall + **temperature** + closed_ha + WB_KAI + seroprev_intrasp + sex*WB_KAI + rainfall*WB_KAI [M15]	827.22
age + sex + DMARSH + rainfall + closed_ha + WB_KAI + seroprev_intrasp + **sex*WB_KAI** + rainfall*WB_KAI [M16]	826.07
age + **sex** + DMARSH + rainfall + closed_ha + WB_KAI + seroprev_intrasp + rainfall*WB_KAI [M17]	826.06
age + DMARSH + rainfall + **closed_ha** + WB_KAI + seroprev_intrasp + rainfall*WB_KAI [M18]	824.59
age + DMARSH + rainfall + WB_KAI + seroprev_intrasp + rainfall*WB_KAI [M19]	824.50

Table S3. Parameters from the other potential best GzLMMs for the serological status against HEV in wild boar related to age class, relative abundance (KAI), straight-line distance to the marshland (DMARSH), the rainfall of the previous sampling period (rainfall), the interaction between rainfall and KAI, the intra-specific seroprevalence of HEV, the cover of closed habitats (closed_ha) and sex.

Variables	**Model 18** **cAIC=824.59** **R^2 = 0.270**			**Model 17** **cAIC=826.06** **R^2 = 0.271**			**Model 16** **cAIC=826.07** **R^2 = 0.274**		
	F df (x,y)	Estimate ± S.D.	*p*	F df (x,y)	Estimate ± S.D.	*p*	F df (x,y)	Estimate ± S.D.	*p*
Age class[1]	5.31 (2, 689)	Juvenile:0.45±0.25 Adult: 0.77±0.21	**<0.01**	5.23 (2, 688)	Juvenile:0.44±0.25 Adult: 0.78±0.21	**<0.01**	5.23 (2, 687)	Juvenile:0.43±0.25 Adult: 0.78±0.21	**<0.01**
DMARSH	5.38 (1, 689)	-0.00±0.00	**0.01**	5.41 (1, 688)	-0.00±0.00	**0.01**	5.39 (1, 687)	-0.00±0.00	**<0.01**
Abundance of wild boar (KAI)	3.91 (1, 689)	-0.75±0.73	0.31	4.04 (1, 688)	-0.52±0.76	0.32	3.97 (1, 687)	-0.52±0.76	0.49
Rainfall	0.02 (1, 689)	-0.00±0.00	0.06	0.003 (1, 688)	-0.00±0.00	0.07	0.004 (1, 687)	-0.00±0.00	0.06
Rainfall*Abundance of wild boar (KAI)	2.99 (1, 689)	0.00±0.00	0.08	2.94 (1, 688)	0.00±0.00	0.08	2.86 (1, 687)	0.00±0.00	0.07
Intra-specific seroprevalence	102.47 (1, 689)	0.04±0.01	**<0.01**	101.91 (1, 688)	0.04±0.01	**<0.01**	101.04 (1, 687)	0.04±0.01	**<0.01**
Closed_ha	0.05 (1, 689)	0.14±0.09	0.15	0.66 (1, 688)	0.14±0.09	0.14	0.63 (1, 687)	0.14±0.09	0.14
Sex[2]				0.29 (1, 688)	Female: -0.12±0.17	0.51	0.30 (1, 687)	Female: -0.28±0.33	0.40
Sex[2]*Abundance of wild boar (KAI)							1.98 (1, 687)	Female: -0.56±0.40	0.16

The model was fitted using the sampling period and livestock management area as random effect factors. Parameter estimates for the [1]age class were calculated using <6 months-old animals as the reference and the male level in the variable sex[2].

CAPÍTULO 3.
Síntesis y conclusiones

CHAPTER 3: Synthesis and conclusions

3.1 Síntesis

En este apartado se realiza una discusión conjunta de los resultados más relevantes de cada uno de los capítulos, proporcionando una visión general de los principales hallazgos de esta Tesis doctoral. En él se resaltan todos los aspectos relativos a los factores de riesgo que operan a medio y largo plazo, así como su implicación en la epidemiología de las infecciones compartidas.

En los últimos años, el creciente interés por las enfermedades de la fauna silvestre y sus consecuencias sobre las poblaciones ha dado lugar a un importante avance en el conocimiento de los factores de riesgo que intervienen en la transmisión de las infecciones compartidas (Gonzálvez, 2021; Gortázar et al., 2016; Jiménez-Ruiz et al., 2021; Triguero-Ocaña et al., 2020). Sin embargo, existen muy pocos estudios epidemiológicos a largo plazo en la interfaz doméstico-silvestre en lugares lo suficientemente caracterizados. En definitiva, a pesar de la importancia de los reservorios silvestres como actores implicados en la transmisión de muchas infecciones compartidas (Delahay et al., 2013; Naranjo et al., 2008), apenas existen series temporales amplias de vigilancia sanitaria en fauna silvestre. Por tanto, es prioritario generarlas y analizarlas, algo que resulta imprescindible para evaluar la situación sanitaria actual y valorar la efectividad a largo plazo de cualquier intervención (**Capítulo 1**).

El **Capítulo 1**, mediante una revisión sistemática de 538 artículos, se centra en describir cuáles han sido los objetivos de la comunidad científica a la hora de realizar estudios epidemiológicos a largo plazo durante las últimas dos décadas, las principales especies y patógenos abordados, y el valor añadido de estos trabajos asociado a su duración. De entre los patógenos que resultaron más estudiados en la fauna silvestre desde un enfoque “a largo plazo”, se seleccionaron cuatro de distinta naturaleza (bacteriana, vírica y parasitaria) para el **Capítulo 2**: CMT, *T. gondii*, VLA y VHE. Éstos fueron elegidos por su relevancia para la salud pública y la economía global (Horan y Wolf, 2005; Rushton y Lyons, 2015; Shwab et al., 2018; Zheng et al., 2006). Para este capítulo, se recopilaron y analizaron largas series temporales de información obtenida a partir de monitorización sanitaria integrada en el PND durante un periodo de trece años (2005-2018). Su objetivo fue evaluar la dinámica temporal de las prevalencias y seroprevalencias de estos patógenos y evidenciar los principales factores de riesgo que, operando a medio y largo plazo, participan en su transmisión y persistencia en la comunidad residente de ungulados silvestres y domésticos. El **Capítulo 2.1** se centró en el estudio del CMT en ciervos, gamos, jabalíes y vacas. En el **Capítulo 2.2** se evaluó la exposición a *T. gondii* en la comunidad de ungulados silvestres (ciervo, gamo y jabalí). Por último, en los **Capítulos 2.3** y **2.4** se analizó la presencia de anticuerpos contra el VLA en los cérvidos y el VHE en el jabalí, respectivamente.

En el **Capítulo 1** se proporcionó una definición de estudio epidemiológico a largo plazo, enumerando sus principales características. Estos estudios aportan información sobre cómo los sistemas epidemiológicos responden a variaciones en factores individuales, ambientales, poblacionales y/o estocásticos, los cuales participan de diferente forma en el riesgo de transmisión de los patógenos compartidos (abordado en el **Capítulo 2**). Además, esta perspectiva temporal permite describir y evaluar los patrones de estos factores, discernir los efectos epidemiológicos de cada uno de ellos y determinar las causas y consecuencias de sus variaciones. También proporcionan información sobre el modo en el que estos factores se relacionan entre sí (abordado en el **Capítulo 2**). Finalmente, este enfoque “a largo plazo” posibilita el estudio de sistemas epidemiológicos complejos en un contexto de cambio global y de emergencia de enfermedades sin precedentes, ayudando, por tanto, a informar decisiones sanitarias y a mejorar las estrategias de gestión del riesgo a diferentes escalas geográficas (Epstein, 2001; Slenning, 2010). La Figura 16 recoge los cinco principios clave que debe cumplir un estudio para ser considerado “a largo plazo”.

Figura 16. Condiciones que debe reunir un estudio epidemiológico a largo plazo.

Los mamíferos silvestres (84,9%) y, concretamente, los ungulados (40,0%) fueron los hospedadores más estudiados a largo plazo en las últimas dos décadas (**Capítulo 1**). El 80% de las publicaciones revisadas analizaron el lado doméstico de la enfermedad, destacando la interfaz ungulado silvestre-ganado bovino como la más relevante (seleccionada para el **Capítulo 2**). Este interés pudo derivar de los riesgos sanitarios que implica la existencia de interacciones espaciotemporales entre ambas especies para la transmisión de infecciones compartidas. Estos contactos interespecíficos son especialmente frecuentes cuando el ganado es criado en régimen extensivo. En ambientes mediterráneos, caracterizados por una fuerte estacionalidad, la mayoría de las interacciones ocurren en la estación seca y están ligadas a factores que favorecen la agregación de animales (**Capítulo 2**). Para aquellos patógenos con elevada resistencia ambiental, las interacciones indirectas son relevantes para su persistencia en comunidades de hospedadores diversas (**Capítulo 2.1 y 2.2**). En las interfaces incluidas en esta revisión, el CMT (34,4%; abordado en el **Capítulo 2.1**) y *T. gondii* (10,4%; abordado en el **Capítulo 2.2**) fueron los patógenos más estudiados.

En el PND, área de estudio en el que se desarrolla esta Tesis doctoral, la coexistencia histórica de ganado extensivo, de una comunidad diversa de hospedadores silvestres (ungulados, carnívoros, micromamíferos, lagomorfos, aves migratorias, etc.) y del ser humano, ha contribuido a la transmisión y persistencia de numerosos agentes infecciosos que actualmente son endémicos en la zona. El **Capítulo 2** ilustra cómo cuanta mayor diversidad de especies forme una comunidad hospedadora, mayor capacidad tendrán los patógenos de persistir en ella. Ésto ocurre porque existe una gran red de interacciones inter e intraespecíficas tanto directas como indirectas entre los hospedadores, dando lugar a un sistema epidemiológico muy complejo y estable (Barasona et al., 2019). Este hecho, unido a las condiciones climáticas del parque, óptimas para la supervivencia de los patógenos (**Capítulo 2**), hace que las prevalencias y seroprevalencias obtenidas para los diferentes patógenos excedieran los valores medios reportados a nivel mundial (Figura 17; **Capítulo 2**), especialmente para el CMT o *T. gondii*, para los que se observaron cifras que se sitúan entre las más elevadas de las halladas en todo el mundo, para el CMT (**Capítulo 2.1**), o en toda Europa, en el caso de *T. gondii* en gamos (**Capítulo 2.2**).

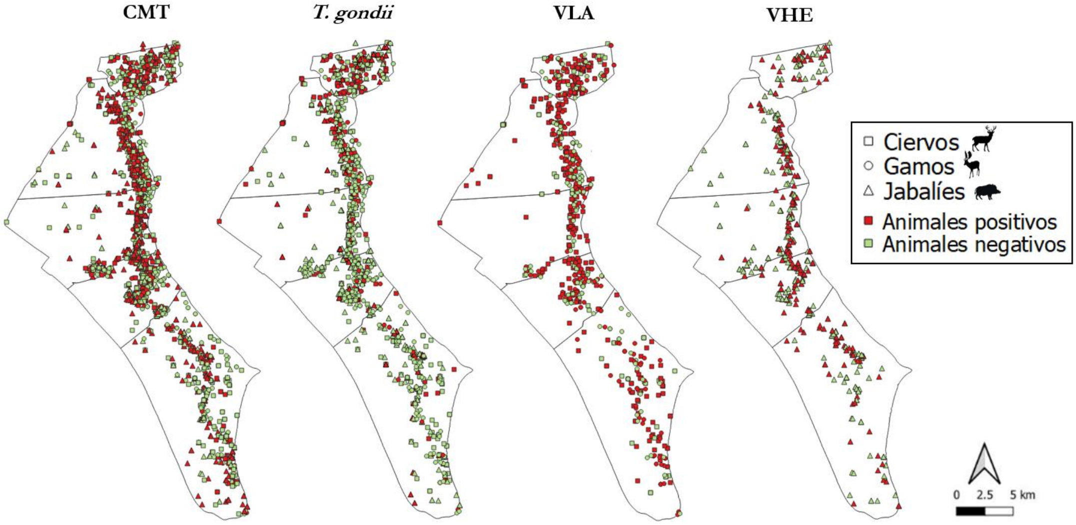

Figura 17. Mapa del Parque Nacional de Doñana con los ciervos (cuadrados), gamos (círculos) y jabalíes (triángulo) muestreados. La presencia de lesiones compatibles con tuberculosis y seropositividad a *T. gondii*, VLA y VHE se muestra en rojo, mientras que los animales negativos se muestran en verde.

En el **Capítulo 2.1** se obtuvieron valores muy altos de prevalencia de LCT en todas las especies de ungulados silvestres (76,7% en jabalí, 42,5% en ciervo y 14,4% en gamo), así como de incidencia de TB en el ganado bovino (10,7%), situándose éstas entre las más elevadas a nivel mundial. Resaltaron, sobre todo, las altas prevalencias de LCT observadas en rayones (6 meses-1 año; 66,0%), los cuales probablemente resultaron infectados directamente a través de la madre, y la ausencia de patrón creciente de generalización con la edad en el jabalí, lo que podría indicar una mortalidad de individuos adultos conforme progresa la enfermedad (Barasona et al., 2016). Estos valores de prevalencia en ungulados silvestres y la tendencia temporal ascendente observada en la incidencia del ganado bovino sugiere que las vacas se infectan constantemente, probablemente a partir de la fauna silvestre, por lo que el programa de erradicación y control de TB en vigor podría no estar resultando efectivo en el PND.

Las seroprevalencias de *T. gondii* halladas en el **Capítulo 2.2** fueron 39,0% en jabalí, 30,7% en ciervo y 29,7% en gamo. La depredación y el carroñeo entre el amplio rango de hospedadores de sangre caliente que habita en el parque podrían estar detrás de las altas seroprevalencias obtenidas. Los factores determinantes del riesgo de seropositividad a *T. gondii* actuaron a nivel de toda la comunidad de hospedadores.

En el **Capítulo 2.3** se confirmó la circulación de los serotipos 1 y 4 del VLA durante el periodo de estudio. También se evidenció el importante papel de los cérvidos, especialmente del ciervo, en el establecimiento y persistencia del virus en las comunidades de hospedadores, incluso en ausencia de ganado. Para el VLA, las seroprevalencias obtenidas (78,6% en ciervos y 53,1% en gamos) fueron similares a las reportadas previamente en el centro y sur de la Península Ibérica.

En el **Capítulo 2.4** se constató la capacidad del jabalí de mantener una circulación a largo plazo del VHE en ausencia de contacto directo o indirecto con ganado porcino en extensivo, hallándose una seroprevalencia del 46,7%. La importante proporción de individuos de entre 6 meses y 2 años que mostraron anticuerpos sugirieron una exposición constante al virus durante el periodo de estudio.

En todos los trabajos que forman parte del **Capítulo 2**, la interpretación del patrón temporal se vio dificultada por una interrupción del control poblacional de ungulados silvestres durante las temporadas 2006-2007 y 2010-2011. Las tendencias temporales de la prevalencia de LCT y de las seroprevalencias de *T. gondii*, VLA y VHE en los ungulados silvestres se mantuvieron, en su mayoría, estables (**Capítulo 2.2** y **2.3**) o fueron ascendentes (**Capítulos 2.1** y **2.4**), aunque con algunas fluctuaciones. Este hallazgo posee una gran relevancia sanitaria y socioeconómica, al obstaculizar los esfuerzos dedicados al control y erradicación de estas infecciones en el ganado. Concretamente, la tendencia temporal mostrada por la incidencia de TB en el ganado bovino fue claramente ascendente (**Capítulo 2.1**), indicando que el problema de la TB en el ciclo silvestre y su transmisión al ganado en el PND dista aún mucho de estar controlado. Las dinámicas temporales de las infecciones víricas abordadas en esta Tesis doctoral presentaron dos características comunes: mostraron oscilaciones que respondían a cambios en los factores determinantes considerados y sus seroprevalencias se correlacionaron temporalmente en todas las especies. Respecto a la seroprevalencia de VLA (**Capítulo 2.3**), sus fluctuaciones temporales se asociaron con cambios climáticos, observándose picos durante los años más cálidos y húmedos, ya que estas condiciones favorecen la supervivencia de los *Culicoides* (Acevedo et al., 2010). Estas seroprevalencias se correlacionaron negativamente con un lapso de tres años, lo que sugirió la existencia de dos ciclos epidemiológicos claramente delimitados. En cuanto al VHE (**Capítulo 2.4**), el diferente esfuerzo realizado en el control poblacional a lo largo del periodo de estudio y sus consecuencias sobre las poblaciones de jabalí determinaron la presencia de fluctuaciones temporales. En su caso, la correlación de las seroprevalencias fue negativa con un lapso de un año, evidenciando que los valores observados con dos o más años de diferencia fueron independientes. Ésto podría relacionarse con las rápidas recuperaciones que experimentan las poblaciones de jabalí tras los años en los que se ejerce un control poblacional más intenso (Gamelon et al., 2011; Servanty et al., 2011).

Todos los patógenos estudiados presentaron una amplia distribución en el PND (**Capítulo 2**). Se observaron diferencias espaciales significativas en las prevalencias de LCT y seroprevalencias de *T. gondii* y VLA, mientras que no se evidenció ningún patrón espacial para el VHE. La prevalencia de LCT y la seroprevalencia de *T. gondii* mostraron un gradiente norte-sur que ya ha sido previamente descrito para el CMT y otros patógenos en este área (Casades-Marti et al., 2021; Gortázar et al., 2008; Gutiérrez-Guzmán et al., 2012; Peralbo-Moreno et al., 2021). Esta peculiar distribución podría estar mediada por factores ambientales y poblacionales que determinan la frecuencia de las interacciones intra e interespecíficas, y por las variaciones espaciales en los niveles de contaminación ambiental por estos patógenos (**Capítulos 2.1-2.3**). Como ejemplo para ilustrar la relevancia de los factores ambientales en el aspecto espacial, la disponibilidad de agua durante la estación seca varía considerablemente entre el norte y el sur del PND. Mientras que en el sur (Marismillas) existen numerosos puntos de agua y lagunas disponibles para los animales, en el norte (Coto del Rey) es necesario aportar agua artificialmente mediante bebederos de tipo pilón (Gortázar et al., 2008). Este hecho podría dar lugar a una mayor agregación de ungulados silvestres y domésticos alrededor de los escasos puntos de agua, desencadenando un mayor riesgo de transmisión. Respecto a los factores poblacionales, las poblaciones de linces ibéricos y de especies peridomésticas en el PND también muestran un gradiente norte-sur (Garrote et al., 2011), lo que podría tener implicaciones epidemiológicas para *T. gondii*, ya que condiciona la distribución espacial de los ooquistes por el territorio. En cambio, para el VHE no existieron factores ambientales que condicionaran la exposición de los jabalíes al patógeno a nivel local (**Capítulo 2.4**).

El objetivo general de esta Tesis doctoral fue la identificación de los principales factores de riesgo implicados en la epidemiología de diferentes infecciones compartidas bacterianas, víricas y parasitarias en ambientes mediterráneos. Con los trabajos que integran el **Capítulo 2**, se ha generado información sobre cómo actúan y se interrelacionan aquellos factores que, operando a medio y largo plazo, están implicados en la transmisión y persistencia de los patógenos en el medio natural. Todos los grupos de factores considerados (individuales, poblacionales, estocásticos y ambientales) intervinieron e interactuaron entre sí para explicar el riesgo de exposición, operando la mayoría de ellos a nivel comunitario (**Capítulo 2**). Los factores determinantes para cada patógeno, así como la dirección de esta asociación se recogen en la Figura 17.

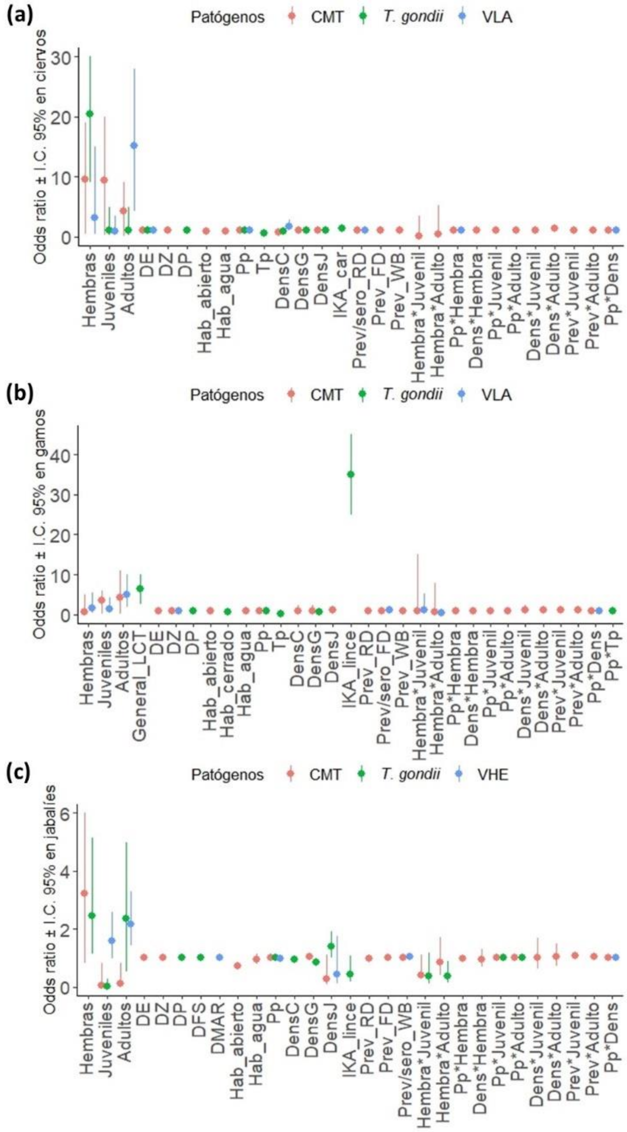

Figura 17. Factores de riesgo (individuales, poblacionales, ambientales y estocásticos, así como interacciones entre ellos) que resultaron estadísticamente significativos para la presencia de LCT (rojo) o seropositividad a *T. gondii* (verde), VLA (azul; Fig. 17 a y b) y VHE (azul; Fig. 17c) en los modelos lineales generalizados mixtos para ciervo (Fig. 17a), gamo (Fig. 17b) y jabalí (Fig. 17c) (Capítulo 2). “*” representa interacción entre factores.

Los **factores individuales** resultaron relevantes para la epidemiología de todos los patógenos abordados (**Capítulo 2**). Las diferencias observadas en los valores de prevalencia y seroprevalencia en las especies estudiadas se debieron principalmente a factores ecológicos propios de cada una de ellas, relacionados principalmente con el uso que hacen del hábitat, con su alimentación y/o con su etología (**Capítulos 2.1-2.3**). P. ej., hábitos sociales (**Capítulo 2.1 y 2.3**), preferencia por zonas de pastizal o monte (**Capítulo 2.2** y **2.3**), carroñeo y/o canibalismo (**Capítulo 2.1, 2.2** y **2.4**), etc. Todos estos factores determinaron la exposición del hospedador al patógeno y condicionaron la relevancia y el modo en el que se interrelacionaron los demás factores determinantes. El gamo mostró prevalencias y seroprevalencias inferiores a las del resto de ungulados silvestres, reforzando la teoría sugerida por Gortázar et al. (2008) sobre una posible resistencia natural de esta especie a ciertos patógenos (**Capitulo 2.1** y **2.3**). Sin embargo, para *T. gondii* las seroprevalencias obtenidas fueron muy similares a las del ciervo, convirtiéndose en la segunda más alta hallada en Europa en los últimos años. Ésto probablemente estuvo mediado por una elevada exposición al parásito debido a su amplio rango de movimiento entre áreas del PND (Braza y Alvarez, 1987).

En general, los individuos adultos mostraron prevalencias y seroprevalencias más elevadas que los jóvenes debido a la persistencia de los anticuerpos (**Capítulo 2.2** y **2.3**) y a una mayor probabilidad de infectarse a lo largo de la vida (**Capítulo 2.1**). Sin embargo, los individuos menores de 2 años presentaron altas tasas de infección debido a una transmisión directa a partir de la madre (**Capítulo 2.1**), a la acción de los anticuerpos maternales (**Capítulo 2.2**) y/o a una exposición persistente al virus (**Capítulos 2.3** y **2.4**).

El sexo interaccionó con factores poblacionales y estocásticos para explicar el riesgo de exposición a patógenos de diferente naturaleza en todas las especies (**Capítulo 2.1-2.3**). Las diferencias observadas entre sexos en la respuesta a estos factores se debieron principalmente a factores fisiológicos, que determinaron la susceptibilidad y/o exposición de los individuos a los patógenos. Los machos y hembras de especies poligínicas suelen mostrar una inversión de los recursos energéticos diferente durante su vida, condicionada principalmente por la reproducción, época en la que entran en conflicto la función inmune y la reproductiva (De la Peña et al., 2020; Malo et al., 2009; Westneat y Birkhead, 1998).

En el ciervo, la interacción del sexo con la precipitación resultó significativa para explicar el riesgo de infección por CMT, *T. gondii* y VLA (**Capítulos 2.1-2.3**). De este modo, en años lluviosos los machos presentaron seroprevalencias superiores a las de las hembras. Este hecho probablemente estuvo ocasionado por diferentes grados de exposición y/o susceptibilidad a dichos patógenos durante la reproducción. La berrea transcurre en el ecotono durante la estación seca, época de gran escasez de recursos alimenticios cuya disponibilidad determina la distribución de los animales por el territorio. Durante los años lluviosos el ecotono retiene mayor humedad, por lo que algunas manchas de vegetación permanecen disponibles para los ungulados durante el verano y el otoño. Los harenes de hembras suelen agregarse en estos pastizales, favoreciendo la competencia entre los machos, que incrementan su esfuerzo reproductivo mediante interacciones más agresivas (Millán et al., 2021). Una mayor inversión de recursos energéticos en el ámbito reproductivo conlleva una disminución de la capacidad inmune del individuo, empeorando tanto su estado sanitario como su condición corporal y aumentando su susceptibilidad a los patógenos (De la Peña et al., 2020). Por otra parte, el mayor grado de exposición de los machos está mediado por factores ambientales. El ecotono proporciona unas condiciones ideales para la supervivencia de los patógenos y vectores (Acevedo et al., 2010; Barasona et al., 2017). En los años lluviosos la marisma puede permanecer inundada hasta el final del verano y, como consecuencia, darse una mayor agregación de los animales y un mayor riesgo de transmisión (**Capítulo 2.1**). Además, durante la estación seca de años lluviosos, se cumplen las condiciones de precipitación y temperatura ideales para que exista una gran abundancia de vectores (Gusmão et al., 2019). Los machos se encuentran más expuestos a éstos que las hembras, ya que se mueven solos por el ecotono en busca de harenes, los cuales diluyen el efecto de los vectores por el agrupamiento (**Capítulo 2.3**).

En el jabalí, el efecto positivo de la densidad sobre el riesgo de sufrir una generalización de LCT fue más evidente en las hembras (**Capítulo 2.1**). Las altas densidades de individuos dan lugar a una mayor agregación y una disminución de los recursos naturales disponibles (Côté et al., 2004). Esto favorece tanto la exposición como la susceptibilidad de las hembras, especialmente durante la estación seca, cuando más sufren el coste fisiológico de la reproducción, y se relaciona, a su vez, con las elevadas prevalencias de LCT halladas en individuos menores de 6 meses.

La transmisión indirecta de CMT, *T. gondii* y VHE ocurre a través de ambientes contaminados, como pastos, puntos de agua o barro (Barasona et al., 2017b; Caron et al., 2003; Dubey, 2010; Emerson y Purcell, 2003). En el PND, las interacciones espaciotemporales intra e interespecíficas están condicionadas por **factores ambientales** relativos al hábitat (Barasona et al., 2014a; Triguero-Ocaña et al., 2020, 2019), los cuales explicaron el riesgo de infección por estos patógenos (**Capítulos 2.1, 2.2** y **2.4**). Aquellas zonas reconocidas como áreas de agregación para los animales fueron relevantes para la epidemiología de todos ellos (Barasona et al., 2014a; Triguero-Ocaña et al., 2020, 2019). Éstas fueron el ecotono, los puntos de agua y la marisma (**Capítulos 2**). Además, para *T. gondii*, la cercanía a asentamientos humanos se relacionó positivamente con el riesgo de seropositividad, ya que la contaminación ambiental ocasionada por especies peridomésticas es mucho mayor en estas zonas y sus alrededores (Jokelainen et al., 2010). También destacaron aquellos hábitats caracterizados por condiciones ambientales que determinaron la supervivencia del patógeno y/o sus vectores en el medio (**Capítulo 2**). En el caso de la línea de costa, la distancia de los individuos a ésta se asoció negativamente con el riesgo de ser seropositivos a *T. gondii*, ya que la desecación producida por el sol y la arena dificultaron considerablemente la supervivencia de los ooquistes (**Capítulo 2.2**). Sin embargo, la marisma y el ecotono son zonas con gran humedad, ricas en materia orgánica, que favorecieron la supervivencia de los patógenos (**Capítulo 2.1**, **2.2** y **2.4**), así como la viabilidad y competencia de sus vectores (**Capítulo 2.3**).

Los **factores estocásticos** también influyeron sobre la supervivencia de los patógenos en el medio y se asociaron significativamente con el riesgo de seropositividad a *T. gondii* y al VHE (**Capítulos 2.2** y **2.4**). Además, interaccionaron con los factores individuales (**Capítulo 2.2**) y poblacionales para explicar este riesgo (**Capítulo 2.3** y **2.4**). Para el jabalí, la asociación positiva existente entre la precipitación y el riesgo de *T. gondii* estuvo condicionada por la edad, siendo más marcada en individuos juveniles (1-2 años). La dispersión de éstos de su área natal, unido a la elevada supervivencia de los ooquistes en ambientes húmedos, dieron lugar a una mayor exposición de estos animales al parásito (**Capítulo 2.2**). La interacción de los factores estocásticos y poblacionales se explicó de distinta forma para VLA y VHE. Para el primero, la combinación de ambos factores fue clave para la supervivencia de los *Culicoides* (**Capítulo 2.3**), mientras que para el VHE lo fue la agregación de los individuos y sus interacciones durante la estación seca, así como la concentración de partículas virales en el medio que, a su vez, estuvo determinada por las condiciones climáticas. En este sentido, cobró una especial importancia la potencial transmisión directa del virus durante las épocas de escasez de recursos, cuando los hábitos carroñeros y el canibalismo podrían exacerbarse (**Capítulo 2.4**).

Respecto a los **factores poblacionales**, la mayoría de los patógenos mostraron un patrón densodependiente mediado por factores individuales y estocásticos (**Capítulos 2.1**, **2.3** y **2.4**). La prevalencia de LCT y las seroprevalencias de *T. gondii*, VLA y VHE se relacionaron significativamente con elevadas densidades de ungulados silvestres y con altas abundancias de carnívoros. Las situaciones de sobreabundancia de ungulados silvestres se asocian con un incremento en la exposición y susceptibilidad de los individuos a los diferentes patógenos (Vicente et al., 2013). El uso excesivo por parte de los animales de las zonas en las que se concentran los recursos, especialmente durante la estación seca, posiblemente derivó en un incremento de las tasas de contacto y en una elevada contaminación ambiental a largo plazo, ya que los patógenos sobreviven durante largos periodos en estas áreas. Además, la sobreabundancia conlleva una reducción considerable de la disponibilidad de recursos naturales y, como consecuencia, una situación de malnutrición para los individuos, con el consiguiente efecto sobre la competencia de su sistema inmune (**Capítulo 2.4**). Todo ello da lugar a un incremento de la mortalidad y, por tanto, de cadáveres disponibles para los jabalíes, que recurren a la carroña como fuente de alimento, aumentando la transmisión del CMT y VHE (**Capítulo 2.1** y **2.4**). En este sentido, las redes tróficas existentes resultaron relevantes para la interpretación de muchos de los resultados obtenidos (**Capítulos 2.1, 2.2** y **2.4**). La abundancia de carnívoros silvestres y, específicamente, la del lince ibérico se asoció positivamente con el riesgo de exposición a *T. gondii* en los cérvidos. Los felinos del PND pueden llegar a cazar gamos y crías de ciervo durante épocas de escasez de conejos, especialmente en invierno y otoño (Delibes, 1980), permitiendo la continuidad del ciclo biológico del parásito y su persistencia en esta comunidad de hospedadores (**Capítulo 2.2**).

Las **relaciones interespecíficas** e **intraespecíficas** observadas se vieron influidas por factores ecológicos (p. ej. selección de hábitats), comportamentales (p. ej. gregarismo o carroñeo) y epidemiológicos (p. ej. grado de contaminación ambiental). Se evidenciaron relaciones intraespecíficas en la transmisión del CMT, VLA y VHE (**Capítulos 2.1, 2.2** y **2.4**), es decir, elevadas prevalencias o seroprevalencias de éstos se asociaron con un mayor riesgo de exposición para los individuos de la misma especie. En el caso del CMT y VHE, este hecho se relacionó con elevados niveles de excreción del patógeno por parte de los animales infectados y el consiguiente incremento de la transmisión directa y/o indirecta (Santos et al., 2015b, 2015a). Además, los individuos con preferencia por los mismos tipos de hábitats, que generalmente pertenecen a la misma especia, suelen compartir territorios (Braza y Alvarez, 1987). Sin embargo, en el PND se han observado grupos mixtos de ungulados domésticos y silvestres (Triguero-Ocaña et al., 2020, 2019). El ciervo y el jabalí son especies muy generalistas en cuanto al uso del hábitat, por lo que, normalmente, se distribuyen uniformemente por el territorio. Sin embargo, los gamos son individuos muy gregarios y selectivos con el hábitat, que muestran preferencia por zonas abiertas de pastizal, al igual que el ganado bovino, con el que se agrupan (Triguero-Ocaña et al., 2019). La formación de estos grupos mixtos se relacionó con el riesgo de exposición al CMT y VLA, tanto positiva como negativamente (**Capítulos 2.1** y **2.3**). Para el CMT, las prevalencias de LCT se asociaron positivamente con el riesgo de positividad a TB en el ganado bovino, probablemente debido a que ambas especies estuvieron expuestas a los mismos factores de riesgo y/o a una transmisión interespecífica del patógeno (Triguero-Ocaña et al., 2020) (**Capítulo 2.1**). Sin embargo, la formación de estos grupos interespecíficos pudo explicar que los gamos mostraran seroprevalencias del VLA significativamente inferiores a las del ciervo, asociándose con un efecto de dilución ocasionado por el agrupamiento, con una preferencia de los *Culicoides* por el ganado bovino o con un efecto protector de las vacas vacunadas sobre el riesgo de exposición al virus en los gamos (Rossi et al., 2014; Ruiz-Fons et al., 2014) (**Capítulo 2.3**).

Con todos estos trabajos podemos confirmar que la duración de un estudio epidemiológico a largo plazo determinará el tipo de factores que podrán considerarse. En la medida en la que se prolongue el periodo de estudio, se podrá comprender cómo actúan los diferentes factores que operan a medio y largo plazo, así como evidenciar y valorar sus consecuencias. El orden de duración en el que se podría alcanzar la comprensión de estos factores se ilustra en la Figura 18.

Figura 18. Evolución temporal de un estudio a largo plazo y la capacidad de evidenciar los efectos de los factores determinantes sobre el riesgo de exposición a un patógeno.

El seguimiento sanitario y poblacional llevado a cabo durante los últimos trece años en la interfaz doméstico-silvestre del PND, con un total de 1.235 ungulados muestreados, ha evidenciado que la comprensión de los principales factores determinantes de la epidemiología de los patógenos en las comunidades de hospedadores requiere de un sistema de MI a largo plazo que combine la vigilancia sanitaria con la poblacional. Esto es esencial para la evaluación del riesgo, el diseño de programas de control o erradicación y la toma de decisiones efectivas (**Capítulo 1** y **2**).

Incluso en territorios protegidos como el PND, en los que no está permitida la caza, y en ausencia de depredadores naturales que puedan regular las poblaciones, la extracción sostenida y adecuada de ungulados silvestres y una gestión apropiada de la actividad ganadera deben entenderse como una prioridad dentro de la gestión sanitaria en la interfaz doméstico-silvestre (**Capítulo 2**). Por tanto, el control a largo plazo de las infecciones compartidas únicamente puede alcanzarse integrando medidas dirigidas a todos los factores implicados, como la gestión de la sobreabundancia y la agregación de ungulados, la exposición ambiental y los saneamientos del ganado bovino. De este modo, podría conseguirse una reducción considerable de las tasas de positividad a ciertas enfermedades en el ganado, lo que supondría un gran impacto económico, dados los costes que suponen los actuales programas de control y erradicación de las infecciones compartidas.

3.2 Conclusiones

1. La revisión bibliográfica realizada en esta Tesis doctoral pone de manifiesto el valor añadido que supone un enfoque “a largo plazo”, el cual permite alcanzar conclusiones robustas sobre las consecuencias de los patógenos para los individuos y las dinámicas de población, la propagación de la infección y sus consecuencias para la economía y la conservación de la biodiversidad. Curiosamente, en casi todos los estudios a largo plazo revisados, los científicos concluyeron sobre las consecuencias de los patógenos para los individuos, para la dinámica de poblaciones, así como sus principales factores reguladores. Usualmente, se requieren enfoques multidisciplinares, lo que también implica el seguimiento de los parámetros poblacionales.

Our review provided evidence of the added value of long-term approaches to reach significant conclusions on the consequences of pathogens on individuals and populations dynamic, the spread of disease, and the ramifications concerning the economy and *conservation. Interestingly, in almost all the long-term studies reviewed, scientists reached conclusions on the consequences of the pathogens on the individuals, on the population dynamics, as well as their regulatory factors. Multidisciplinary approaches were usually required, which also involve monitoring population parameters.*

2. El concepto de "estudio a largo plazo en epidemiología" es dependiente del contexto en el que éste se desarrolle. No depende únicamente de que alcance una duración concreta, sino que debe considerar los tiempos de vida y generación de los hospedadores y patógenos estudiados, así como la escala temporal en la que actúan los diferentes factores de riesgo. Por tanto, un "estudio a largo plazo en epidemiología" debe poseer una duración suficiente como para permitir, al menos, describir y evaluar patrones epidemiológicos, además de dilucidar cómo actúan y cuáles son las causas y consecuencias de los diferentes factores determinantes y predisponentes.

The concept of "long-term study in epidemiology" is dependent on the context in which it takes place. It does not only depend on reaching a specific duration, but most consider the lifetimes and generation time of the hosts and pathogens studied, and the timescale during which the main drivers of infection operate. Therefore, a "long-term study in epidemiology" must be long enough to allow, at least, to describe and evaluate epidemiological patterns, as well as to identify the different determining and predisposing factors, how they operate, and their main causes and consequences.

3. El Complejo *Mycobacterium tuberculosis*, *Toxoplasma gondii*, el virus de la lengua azul y el virus de la hepatitis E son endémicos en la comunidad de hospedadores del Parque Nacional de Doñana, en la que han circulado y persistido durante un periodo de trece años, manteniendo, en general, tendencias temporales entre ascendentes y estables. Los patrones temporales y factores determinantes de los sistemas patógeno-hospedador estudiados mostraron marcadas similitudes y diferencias entre especies hospedadoras y patógenos.

The Mycobacterium tuberculosis complex, Toxoplasma gondii, bluetongue virus, and hepatitis E virus are endemic in the host community of the Doñana National Park, where they have circulated and persisted for a 13-year period, maintaining, in general, temporal trends between increasing and stable. Temporal patterns and drivers of the host-pathogen *systems studied showed marked similarities and differences between host species and pathogens.*

4. Los patrones temporales observados para cada patógeno se asociaron a diferentes factores de tipo individual, poblacional y ambiental, que pudieron condicionar la exposición y susceptibilidad de los ungulados a los mismos a lo largo del tiempo. También fueron relevantes para su epidemiología las interacciones entre otros factores de diversa naturaleza. En este sentido, las características etológicas y ecológicas propias de la historia natural de cada especie mediaron estos efectos en función del patógeno implicado.

The temporal patterns observed for each pathogen were associated with different individual, populational and environmental factors, which could condition the exposure and susceptibility of ungulates to them over time. Interactions between other factors of diverse nature were also relevant for its epidemiology. The ethological and ecological features of the natural history of each species mediated these effects depending on the pathogen involved.

5. Algunos aspectos asociados con la ecología espacial y los hábitos sociales de los hospedadores, determinantes de cambios en su agregación a lo largo del periodo de estudio en el Parque Nacional de Doñana, se relacionaron tanto positiva como negativamente con del riesgo de infección por el Complejo *Mycobacterium tuberculosis*, *Toxoplasma gondii*, virus de la lengua azul y virus de la hepatitis E. Así, es relevante un posible efecto de dilución para el virus de la lengua azul en cérvidos, frente a un incremento del riesgo para el Complejo *Mycobacterium tuberculosis* en condiciones de elevada agregación de tales hospedadores.

Aspects related to the spatial ecology and social habits of the hosts, determinants of changes in their aggregation over the study period in environments of the Doñana National Park, were related both positively and negatively to the risk of infection by Mycobacterium tuberculosis complex, Toxoplasma gondii, bluetongue virus, and hepatitis E virus. Thus, a possible dilution effect for bluetongue virus in cervids is relevant, compared to an increased risk for the Mycobacterium tuberculosis complex under conditions of high aggregation of such hosts.

6. El seguimiento a largo plazo de la comunidad de hospedadores del Parque Nacional de Doñana también permitió evidenciar asociaciones en el riesgo y/o tasas de infección de determinados patógenos entre especies hospedadoras concretas. Esto es especialmente relevante para especies situadas a ambos lados de la interfaz doméstico-silvestre, como el caso del Complejo *Mycobacterium tuberculosis* en ganado bovino y gamo.

The long-term monitoring of the host community from Doñana National Park also revealed associations in the risk and/or infection rates of certain pathogens between specific hosts species. This is especially relevant for species situated on both sides of the livestock-wildlife interface, such as the Mycobacterium tuberculosis complex in cattle and fallow deer.

7. En esta Tesis se han abordado varios sistemas patógeno-hospedador, así como la intervención de numerosos factores de diferente naturaleza, siendo ésta una simplificación de las situaciones reales multi-patógeno multi-hospedador, donde interaccionan una gran variedad de factores complejos, lo que a menudo es difícil de caracterizar y cuantificar. Es, por tanto, un desafío para la ciencia ampliar, mediante enfoques multidisciplinares, los estudios sobre infecciones compartidas en este sentido, para así lograr una mejor comprensión de su epidemiología y gestión.

In this Thesis, several pathogen-host systems have been approached, as well as the intervention of numerous factors of different nature. This is a simplification of real multi-pathogen multi-host situations, where a great variety of complex factors interact, which is often difficult to characterize and quantify. Therefore, it is a challenge for science to broaden, through multidisciplinary approaches, studies on shared infections in this regard, in order to achieve a better understanding of their epidemiology and management.

Bibliografía

Acevedo, P., Prieto, M., Quirós, P., Merediz, I., de Juan, L., Infantes-Lorenzo, J.A., Triguero-Ocaña, R., Balseiro, A., 2019. Tuberculosis epidemiology and badger (*Meles meles*) spatial ecology in a hot-spot area in Atlantic Spain. Pathogens 8, 292. https://doi.org/10.3390/pathogens8040292

Acevedo, P., Ruiz-Fons, F., Estrada, R., Márquez, A.L., Miranda, M.A., Gortázar, C., Lucientes, J., 2010. A broad assessment of factors determining *Culicoides imicola* abundance: Modelling the present and forecasting its future in climate change scenarios. PLoS One 5, e14236. https://doi.org/10.1371/journal.pone.0014236

Acevedo, P., Ruiz-Fons, F., Vicente, J., Reyes-García, A.R., Alzaga, V., Gortázar, C., 2008. Estimating red deer abundance in a wide range of management situations in Mediterranean habitats. J. Zool. 276, 37–47. https://doi.org/10.1111/j.1469-7998.2008.00464.x

Acevedo, P., Vicente, J., Höfle, U., Cassinello, J., Ruiz-Fons, F., Gortazar, C., 2007. Estimation of European wild boar relative abundance and aggregation: A novel method in epidemiological risk assessment. Epidemiol. Infect. 135, 519–527. https://doi.org/10.1017/S0950268806007059

Adlhoch, C., Avellon, A., Baylis, S.A., Ciccaglione, A.R., Couturier, E., de Sousa, R., Epštein, J., Ethelberg, S., Faber, M., Fehér, Á., Ijaz, S., Lange, H., Manďáková, Z., Mellou, K., Mozalevskis, A., Rimhanen-Finne, R., Rizzi, V., Said, B., Sundqvist, L., Thornton, L., Tosti, M.E., van Pelt, W., Aspinall, E., Domanovic, D., Severi, E., Takkinen, J., Dalton, H.R., 2016. Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. J. Clin. Virol. 82, 9–16. https://doi.org/10.1016/j.jcv.2016.06.010

Afelt, A., Lacroix, A., Zawadzka-Pawlewska, U., Pokojski, W., Buchy, P., Frutos, R., 2018. Distribution of bat-borne viruses and environment patterns. Infect. Genet. Evol. 58, 181–191. https://doi.org/10.1016/j.meegid.2017.12.009

Aggarwal, R., Goel, A., 2019. Natural history, clinical manifestations, and pathogenesis of hepatitis E virus genotype 1 and 2 infections. Cold Spring Harb. Perspect. Med. 9, a032136. https://doi.org/10.1101/cshperspect.a032136

Aguirre, A.A., 2009. Wild canids as sentinels of ecological health: a conservation medicine perspective. Parasit. Vectors 2, S7. https://doi.org/10.1186/1756-3305-2-S1-S7

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19, 716–723. https://doi.org/10.1109/TAC.1974.1100705

Alleweldt, F., 2010. La OIE y los instrumentos de gestión de riesgos para las enfermedades epidémicas del ganado. L, in: Conferencia Internacional Sobre Gestión de Riesgos y Crisis En El Seguro Agrario. Madrid.

Almería, S., Cabezón, O., Paniagua, J., Cano-Terriza, D., Jiménez-Ruiz, S., Arenas-Montes, A., Dubey, J.P., García-Bocanegra, I., 2018. *Toxoplasma gondii* in sympatric domestic and wild ungulates in the Mediterranean ecosystem. Parasitol. Res. 117, 665–671. https://doi.org/10.1007/s00436-017-5705-6

Junta de Andalucía, 2004. Plan Rector de Uso y Gestión (PRUG) del Parque Nacional de Doñana.

Anderson, R.M., Fraser, C., Ghani, A.C., Donnelly, C.A., Riley, S., Ferguson, N.M., Leung, G.M., Lam, T.H., Hedley, A.J., 2004. Epidemiology, transmission dynamics and control of SARS: The 2002-2003 epidemic. Philos. Trans. R. Soc. B Biol. Sci. 359, 1091–1105. https://doi.org/10.1098/rstb.2004.1490

Andraud, M., Dumarest, M., Cariolet, R., Aylaj, B., Barnaud, E., Eono, F., Pavio, N., Rose, N., 2013. Direct contact and environmental contaminations are responsible for HEV transmission in pigs. Vet. Res. 44, 102. https://doi.org/10.1186/1297-9716-44-102

Antolová, D., Reiterová, K., Dubinský, P., 2007. Seroprevalence of *Toxoplasma gondii* in wild boars (*Sus scrofa*) in the Slovak Republic. Ann. Agric. Environ. Med. 14, 71–73. https://doi.org/10.1515/mamm.1994.58.2.201

Aranaz, A., de Juan, L., Montero, N., Sanchez, C., Galka, M., Delso, C., Alvarez, J., Romero, B., Bezos, J., Vela, A.I., Briones, V., Mateos, A., Dominguez, L., 2004. Bovine tuberculosis (*Mycobacterium bovis*) in wildlife in Spain. J. Clin. Microbiol. 42, 2602–2608. https://doi.org/10.1128/JCM.42.6.2602-2608.2004

Arenas-Montes, A., García-Bocanegra, I., Paniagua, J., Franco, J.J., Miró, F., Fernández-Morente, M., Carbonero, A., Arenas, A., 2013. Blood sampling by puncture in the cavernous sinus from hunted wild boar. Eur. J. Wildl. Res. 59, 299–303. https://doi.org/10.1007/s10344-013-0701-3

ASAB, 2012. Guidelines for the treatment of animals in behavioural research and teaching. Anim. Behav. 83, 301–309. https://doi.org/10.1016/j.anbehav.2011.10.031

August, T.A., Mathews, F., Nunn, M.A., 2012. Alphacoronavirus detected in bats in the United Kingdom. Vector-Borne Zoonotic Dis. 12, 530–533. https://doi.org/10.1089/vbz.2011.0829

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. Mamm. Rev. 44, 124–134. https://doi.org/10.1111/mam.12015

Balseiro, A., Rodríguez, O., González-Quirós, P., Merediz, I., Sevilla, I.A., Davé, D., Dalley, D.J., Lesellier, S., Chambers, M.A., Bezos, J., Muñoz, M., Delahay, R.J., Gortázar, C., Prieto, J.M., 2011. Infection of Eurasian badgers (*Meles meles*) with *Mycobacterium bovis* and *Mycobacterium avium* complex in Spain. Vet. J. 190, 21–25. https://doi.org/10.1016/j.tvjl.2011.04.012

Barasona, J.A., Acevedo, P., Diez-Delgado, I., Queiros, J., Carrasco-García, R., Gortazar, C., Vicente, J., 2016. Tuberculosis-associated death among adult wild boars, Spain, 2009–2014. Emerg. Infect. Dis. 22, 2178–2180. https://doi.org/10.3201/eid2212.160677

Barasona, J.A., Gortázar, C., de la Fuente, J., Vicente, J., 2019. Host richness increases tuberculosis disease risk in game-managed areas. Microorganisms 7, 182. https://doi.org/10.3390/microorganisms7060182

Barasona, J.A, Latham, M.C., Acevedo, P., Armenteros, J.A., Latham, A.D.M., Gortazar, C., Carro, F., Soriguer, R.C., Vicente, J., 2014a. Spatiotemporal interactions between wild boar and cattle: implications for cross-species disease transmission. Vet. Res. 45, 122. https://doi.org/10.1186/s13567-014-0122-7

Barasona, J.A, Mulero-Pázmány, M., Acevedo, P., Negro, J.J., Torres, M.J., Gortázar, C., Vicente, J., 2014b. Unmanned aircraft systems for studying spatial abundance of ungulates: relevance to spatial epidemiology. PLoS One 9, e115608. https://doi.org/10.1371/journal.pone.0115608

Barasona, J.A., Risalde, M.A., Ortíz, J.A., Gonzalez-Barrio, D., Che-Amat, A., Pérez-Sancho, M., Vargas-Castillo, L., Xeidakis, A., Jurado-Tarifa, E., Gortázar, C., 2020. Disease-mediated piglet mortality prevents wild boar population growth in fenced overabundant settings. Eur. J. Wildl. Res. 66, 26. https://doi.org/10.1007/s10344-020-1363-6

Barasona, J.A, Torres, M.J., Aznar, J., Gortázar, C., Vicente, J., 2017a. DNA detection reveals *Mycobacterium tuberculosis* complex shedding routes in its wildlife reservoir the Eurasian wild boar. Transbound. Emerg. Dis. 64, 906–915. https://doi.org/10.1111/tbed.12458

Barasona, J. A., Vicente, J., Díez-Delgado, I., Aznar, J., Gortázar, C., Torres, M.J., 2017b. Environmental presence of *Mycobacterium tuberculosis* complex in aggregation points at the wildlife/livestock interface. Transbound. Emerg. Dis. 64, 1148–1158. https://doi.org/10.1111/tbed.12480

Barnaud, E., Rogée, S., Garry, P., Rose, N., Pavio, N., 2012. Thermal inactivation of infectious hepatitis E virus in experimentally contaminated food. Appl. Environ. Microbiol. 78, 5153–5159. https://doi.org/10.1128/AEM.00436-12

Barroso, P., Acevedo, P., Vicente, J., 2020a. The importance of long-term studies on wildlife diseases and their interfaces with humans and domestic animals: A review. Transbound. Emerg. Dis. tbed.13916. https://doi.org/10.1111/tbed.13916

Barroso, P., Barasona, J.A., Acevedo, P., Palencia, P., Carro, F., Negro, J.J., Torres, M.J., Gortázar, C., Soriguer, R.C., Vicente, J., 2020b. Long-term determinants of tuberculosis in the ungulate host community of Doñana National Park. Pathogens 9, 445. https://doi.org/10.3390/pathogens9060445

Barroso, P., García-Bocanegra, I., Acevedo, P., Palencia, P., Carro, F., Jiménez-Ruiz, S., Almería, S., Dubey, J.P., Cano-Terriza, D., Vicente, J., 2020c. Long-Term determinants of the seroprevalence of T*oxoplasma gondii* in a wild ungulate community. Animals 10, 2349. https://doi.org/10.3390/ani10122349

Bartova, E., Sedlak, K., Pavlik, I., Literak, I., 2007. Prevalence of *Neospora caninum* and Toxoplasma gondii antibodies in wild ruminants from the countryside or captivity in the Czech Republic. J. Parasitol. 93, 1216–1218. https://doi.org/10.1645/GE-1126R.1

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Baylis, M., 2013. Research gaps in understanding how climate change will affect arboviral diseases. Anim. Heal. Res. Rev. 14, 143–146. https://doi.org/10.1017/S1466252313000145

Becker, K., Hu, Y., Biller-Andorno, N., 2006. Infectious diseases – A global challenge. Int. J. Med. Microbiol. 296, 179–185. https://doi.org/10.1016/j.ijmm.2005.12.015

Belluco, S., Simonato, G., Mancin, M., Pietrobelli, M., Ricci, A., 2018. *Toxoplasma gondii* infection and food consumption: A systematic review and meta-analysis of case-controlled studies. Crit. Rev. Food Sci. Nutr. 58, 3085–3096. https://doi.org/10.1080/10408398.2017.1352563

Beltran, J.F., Sanjose, C., Delibes, M., Braza, F., 1985. An analysis of Iberian lynx predation upon fallow deer in the coto Doñana, SW Spain. View project 17–21.

Bengis, R.G., Kock, R.A., Fischer, J., 2002. Infectious animal diseases: the wildlife/livestock interface. Rev. Sci. Tech. 21, 53–65.

Bengis, R.G., Leighton, F.A., Fischer, J.R., Artois, M., Morner, T., Tate, C.M., 2004. The role of wildlife in emerging and re-emerging zoonoses. Rev. Sci. Tech. 23, 497–511.

Beral, M., Rossi, S., Aubert, D., Gasqui, P., Terrier, M.E., Klein, F., Villena, I., Abrial, D., Gilot-Fromont, E., Richomme, C., Hars, J., Jourdain, E., 2012. Environmental factors associated with the seroprevalence of *Toxoplasma gondii* in wild boars (*Sus scrofa*), France. Ecohealth 9, 303–309. https://doi.org/10.1007/s10393-012-0786-2

Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. 95, 9031–9036. https://doi.org/10.1073/pnas.95.15.9031

Bezos, J., Casal, C., Díez-Delgado, I., Romero, B., Liandris, E., Álvarez, J., Sevilla, I.A., Juan, L. de, Domínguez, L., Gortázar, C., 2015. Goats challenged with different members of the *Mycobacterium tuberculosis* complex display different clinical pictures. Vet. Immunol. Immunopathol. 167, 185–189.

https://doi.org/10.1016/j.vetimm.2015.07.009

Bienen, L., Tabor, G., 2006. Applying an ecosystem approach to brucellosis control: can an old conflict between wildlife and agriculture be successfully managed? Front. Ecol. Environ. 4, 319–327. https://doi.org/10.1890/1540-9295(2006)4[319:AAEATB]2.0.CO

Black, M.W., Boothroyd, J.C., 2000. Lytic Cycle of *Toxoplasma gondii*. Microbiol. Mol. Biol. Rev. 64, 607–623. https://doi.org/10.1128/MMBR.64.3.607-623.2000

Blehert, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-Zier, B.M., Buckles, E.L., Coleman, J.T.H., Darling, S.R., Gargas, A., Niver, R., Okoniewski, J.C., Rudd, R.J., Stone, W.B., 2009. Bat White-Nose Syndrome: an emerging fungal pathogen? Science. 323, 227–227. https://doi.org/10.1126/science.1163874

Bliziotis, I.A., Paraschakis, K., Vergidis, P.I., Karavasiou, A.I., Falagas, M.E., 2005. Worldwide trends in quantity and quality of published articles in the field of infectious diseases. BMC Infect. Dis. 5, 16. https://doi.org/10.1186/1471-2334-5-16

Boadella, M., 2015. Hepatitis E in wild ungulates: A review. Small Rumin. Res. 128, 64–71. https://doi.org/10.1016/j.smallrumres.2015.03.007

Boadella, M., Casas, M., Martín, M., Vicente, J., Segalés, J., de la Fuente, J., Gortázar, C., 2010. Increasing contact with hepatitis E virus in red deer, Spain. Emerg. Infect. Dis. 16, 1994–1996. https://doi.org/10.3201/eid1612.100557

Boadella, M., Ruiz-Fons, J.F., Vicente, J., Martín, M., Segalés, J., Gortazar, C., 2012a. Seroprevalence evolution of selected pathogens in Iberian wild boar. Transbound. Emerg. Dis. 59, 395–404. https://doi.org/10.1111/j.1865-1682.2011.01285.x

Boadella, M., Vicente, J., Ruiz-Fons, F., de la Fuente, J., Gortazar, C., 2012b. Effects of culling Eurasian wild boar on the prevalence of *Mycobacterium bovis* and Aujeszky’s disease virus. Prev. Vet. Med. 107, 214–221. https://doi.org/10.1016/j.prevetmed.2012.06.001

Bonilla-Aldana, D.K., Dhama, K., Rodriguez-Morales, A.J., 2020. Revisiting the One Health approach in the context of COVID-19: A look into the ecology of this emerging disease. Adv. Anim. Vet. Sci. 8, 234–237. https://doi.org/10.17582/journal.aavs/2020/8.3.234.237

Brahmbhatt, D.P., Fosgate, G.T., Dyason, E., Budke, C.M., Gummow, B., Jori, F., Ward, M.P., Srinivasan, R., 2012. Contacts between domestic livestock and wildlife at the Kruger National Park Interface of the Republic of South Africa. Prev. Vet. Med. 103, 16–21. https://doi.org/10.1016/j.prevetmed.2011.08.003

Braza, F., Alvarez, F., 1989. Utilisation de l’habitat et organisation sociale du sanglier (*Sus scrofa* L.) à Doñana (Sud-Ouest de l’Espagne). Can. J. Zool. 67, 2047–2051. https://doi.org/10.1139/z89-291

Braza, F., Alvarez, F., 1987. Habitat use by red deer and fallow deer in Doñana National Park. Misc. Zoológica 11, 363–367.

Briones, V., De Juan, L., Sanchez, C., Vela, A.I., Galka, M., Montero, N., Goyache, J., Aranaz, A., Mateos, A., Domínguez, L., 2000. Bovine tuberculosis and the endangered Iberian Lynx. Emerg. Infect. Dis. 6, 189–191. https://doi.org/10.3201/eid0602.000214

Buckland, S., Anderson, D., Burnham, K., Laake, J., Borchers, D., Thomas, L., 2001. Introduction to Distance Sampling: estimating abundance of biological populations, Oxford University Press.

Bugalho, M.N., Milne, J.A., 2003. The composition of the diet of red deer (*Cervus elaphus*) in a Mediterranean environment: A case of summer nutritional constraint? For. Ecol. Manage. 181, 23–29. https://doi.org/10.1016/S0378-1127(03)00125-7

Burri, C., Vial, F., Ryser-Degiorgis, M.-P., Schwermer, H., Darling, K., Reist, M., Wu, N., Beerli, O., Schöning, J., Cavassini, M., Waldvogel, A., 2014. Seroprevalence of hepatitis E virus in domestic pigs and wild boars in Switzerland. Zoonoses Public Health. 61, 537–544. https://doi.org/10.1111/zph.12103

Buttke, D.E., Decker, D.J., Wild, M.A., 2015. The role of One Health in wildlife conservation: a challege and opportunity. J. Wildl. Dis. 51, 1–8. https://doi.org/10.7589/2014-01-004

Byrne, A.W., Graham, J., McConville, J., Milne, G., Guelbenzu-Gonzalo, M., McDowell, S., 2019. Liver fluke (*Fasciola hepatica*) co-infection with bovine tuberculosis in cattle: A prospective herd-level assessment of herd bTB risk in dairy enterprises. Transbound. Emerg. Dis. 66, tbed.13209. https://doi.org/10.1111/tbed.13209

Cabal, A., Vicente, J., Alvarez, J., Barasona, J.A., Boadella, M., Dominguez, L., Gortazar, C., 2017. Human influence and biotic homogenization drive the distribution of Escherichia coli virulence genes in natural habitats. Microbiologyopen 6, e00445. https://doi.org/10.1002/mbo3.445

Caballero-Gómez, J., Jiménez-Ruiz, S., López-López, P., Vicente, J., Risalde, M.A., Cano-Terriza, D., Frias, M., Barasona, J.A., Rivero, A., García-Bocanegra, I., Rivero-Juarez, A., 2019. Emergent subtype of hepatitis E virus genotype 3 in wild boar in Spain. Transbound. Emerg. Dis. 66, 1803–1808. https://doi.org/10.1111/tbed.13251

Cabaret, J., Morand, S., Beaudeau, F., 2012. Introduction, in: New frontiers of molecular epidemiology of infectious diseases. Springer Netherlands, Dordrecht, pp. 1–5. https://doi.org/10.1007/978-94-007-2114-2_1

Calero-Bernal, R., Pérez-Martín, J.E., Reina, D., Serrano, F.J., Frontera, E., Fuentes, I., Dubey, J.P., 2016. Detection of Zoonotic Protozoa *Toxoplasma gondii* and *Sarcocystis suihominis* in wild boars from Spain. Zoonoses Public Health 63, 346–350. https://doi.org/10.1111/zph.12243

Calero-Bernal, R., Saugar, J.M., Frontera, E., Pérez-Martín, J.E., Habela, M.A., Serrano, F.J., Reina, D., Fuentes, I., 2015. Prevalence and genotype identification of *Toxoplasma gondii* in wild animals from southwestern Spain. J. Wildl. Dis. 51, 233–238. https://doi.org/10.7589/2013-09-233

Caley, P., Hone, J., 2004. Disease transmission between and within species, and the implications for disease control. J. Appl. Ecol. 41, 94–104. https://doi.org/10.1111/j.1365-2664.2004.00867.x

Caminiti, A., Pelone, F., LaTorre, G., De Giusti, M., Saulle, R., Mannocci, A., Sala, M., Della Marta, U., Scaramozzino, P., 2016. Control and eradication of tuberculosis in cattle: a systematic review of economic evidence. Vet. Rec. 179, 70–75. https://doi.org/10.1136/vr.103616

Cano-Terriza, D., Almería, S., Caballero-Gómez, J., Jiménez-Martín, D., Castro-Scholten, S., Dubey, J.P., García-Bocanegra, I., 2020. Exposure to *Toxoplasma gondii* in zoo animals in Spain. Prev. Vet. Med. 176, 104930. https://doi.org/10.1016/j.prevetmed.2020.104930

Cano-Terriza, D., Risalde, M.A., Jiménez-Ruiz, S., Vicente, J., Isla, J., Paniagua, J., Moreno, I., Gortázar, C., Infantes-Lorenzo, J.A., García-Bocanegra, I., 2018a. Management of hunting waste as control measure for tuberculosis in wild ungulates in south-central Spain. Transbound. Emerg. Dis. 65, 1190–1196. https://doi.org/10.1111/tbed.12857

Cano-Terriza, D., Risalde, M.A., Rodríguez-Hernández, P., Napp, S., Fernández-Morente, M., Moreno, I., Bezos, J., Fernández-Molera, V., Sáez, J.L., García-Bocanegra, I., 2018b. Epidemiological surveillance of *Mycobacterium tuberculosis* complex in extensively raised pigs in the south of Spain. Prev. Vet. Med. 159, 87–91. https://doi.org/10.1016/j.prevetmed.2018.08.015

Capai, L., Charrel, R., Falchi, A., 2018. Hepatitis E in High-Income Countries: What Do We Know? And What Are the Knowledge Gaps? Viruses 10, 285. https://doi.org/10.3390/v10060285

Carlton, J.M., Adams, J.H., Silva, J.C., Bidwell, S.L., Lorenzi, H., Caler, E., Crabtree, J., Angiuoli, S. V, Merino, E.F., Amedeo, P., Cheng, Q., Coulson, R.M.R., Crabb, B.S., del Portillo, H.A., Essien, K., Feldblyum, T. V., Fernandez-Becerra, C., Gilson, P.R., Gueye, A.H., Guo, X., Kang'a, S., Kooij, T.W.A., Korsinczky, M., Meyer, E.V.-S., Nene, V., Paulsen, I., White, O., Ralph, S.A., Ren, Q., Sargeant, T.J., Salzberg, S.L., Stoeckert, C.J., Sullivan, S.A., Yamamoto, M.M., Hoffman, S.L., Wortman, J.R., Gardner, M.J., Galinski, M.R., Barnwell, J.W., Fraser-Liggett, C.M., 2008. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. Nature 455, 757–763. https://doi.org/10.1038/nature07327

Caron, A., Cross, P.C., Du Toit, J.T., 2003. Ecological implications of bovine tuberculosis in African buffalo herds. Ecol. Appl. 13, 1338–1345. https://doi.org/10.1890/02-5266

Caron, A., Miguel, E., Gomo, C., Makaya, P., Pfukenyi, D.M., Foggin, C., Hove, T., de Garine-Wichatitsky, M., 2013. Relationship between burden of infection in ungulate populations and wildlife/livestock interfaces. Epidemiol. Infect. 141, 1522–1535. https://doi.org/10.1017/S0950268813000204

Caron, A., Morand, S., De Garine-Wichatitsky, M., 2012. Epidemiological interaction at the wildlife/livestock/human interface: Can we anticipate emerging infectious diseases in their hotspots? A framework for understanding emerging diseases processes in their hot spots, in: Morand, S., Beaudeau, F. and Cabaret, J. (Ed.), New Frontiers of Molecular Epidemiology of Infectious Diseases. pp. 311–332. https://doi.org/10.1007/978-94-007-2114-2_14

Carranza, J., 2017. Ciervo – *Cervus elaphus*. En Encicl. Virtual los Vertebr. Españoles. Salvador. A., Barja, I. (Eds.). Mus. Nac. Ciencias Nat. 1–34.

Carranza, J., Alvarez, F., Redondo, T., 1990. Territoriality as a mating strategy in red deer. Anim. Behav. 40, 79–88. https://doi.org/10.1016/S0003-3472(05)80667-0

Carrasco-Garcia, R., Barasona, J.A., Gortazar, C., Montoro, V., Sanchez-Vizcaino, J.M., Vicente, J., 2016. Wildlife and livestock use of extensive farm resources in South Central Spain: implications for disease transmission. Eur. J. Wildl. Res. 62, 65–78. https://doi.org/10.1007/s10344-015-0974-9

Carrasco-Garcia, R., Barroso, P., Perez-Olivares, J., Montoro, V., Vicente, J., 2018. Consumption of big game remains by scavengers: a potential risk as regards disease transmission in central Spain. Front. Vet. Sci. 5. https://doi.org/10.3389/fvets.2018.00004

Casades-Marti, L., Cuadrado-Matías, R., Triguero-Ocaña, R., Barroso, P., Jiménez-Ruiz, S., Palencia, P., Laguna, E., Peralbo-Moreno, A., Baz-Flores, S., Fierro, Y., Ruiz-Fons, F., 2021. The spatiotemporal dynamics of exposure of wild ungulates to Flavivirus shapes the patterns of West Nile virus outbreaks in Spain, in: 69th WDA and 14th EWDA Congress. Cuenca, Spain.

Casaubon, J., Chaignat, V., Vogt, H.R., Michel, A.O., Thür, B., Ryser-Degiorgis, M.P., 2013. Survey of bluetongue virus infection in free-ranging wild ruminants in Switzerland. BMC Vet. Res. 9. https://doi.org/10.1186/1746-6148-9-166

Castillo-Cuenca, J.C., Díaz-Cao, J.M., Martínez-Moreno, Á., Cano-Terriza, D., Jiménez-Ruiz, S., Almería, S., García-Bocanegra, I., 2020. Seroepidemiology of *Toxoplasma gondii* in extensively raised Iberian pigs in Spain. Prev. Vet. Med. 175, 104854. https://doi.org/10.1016/j.prevetmed.2019.104854

Chan, J.F.-W., To, K.K.-W., Tse, H., Jin, D.-Y., Yuen, K.-Y., 2013. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol. 21, 544–555. https://doi.org/10.1016/j.tim.2013.05.005

Christensen, S.A., Ruder, M.G., Williams, D.M., Porter, W.F., Stallknecht, D.E., 2020. The role of drought as a determinant of hemorrhagic disease in the eastern United States. Glob. Chang. Biol. 26, 3799–3808. https://doi.org/10.1111/gcb.15095

Ciliberti, A., Gavier-Widén, D., Yon, L., Hutchings, M.R., Artois, M., 2015. Prioritisation of wildlife pathogens to be targeted in European surveillance programmes: Expert-based risk analysis focus on ruminants. Prev. Vet. Med. 118, 271–284. https://doi.org/10.1016/j.prevetmed.2014.11.021

Cleaveland, S., Laurenson, M.K., Taylor, L.H., 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos. Trans. R. Soc. London. 356, 991–999. https://doi.org/10.1098/rstb.2001.0889

Coelho, C., Vieira-Pinto, M., Faria, A.S., Vale-Gonçalves, H., Veloso, O., Paiva-Cardoso, M. das N., Mesquita, J.R., Lopes, A.P., 2014. Serological evidence of *Toxoplasma gondii* in hunted wild boar from Portugal. Vet. Parasitol. 202, 310–312. https://doi.org/10.1016/j.vetpar.2014.03.013

Cooper, S.M., Scott, H.M., de la Garza, G.R., Deck, A.L., Cathey, J.C., 2010. Distribution and interspecies contact of feral swine and cattle on rangeland in south Texas: implications for disease transmission. J. Wildl. Dis. 46, 152–164. https://doi.org/10.7589/0090-3558-46.1.152

Corner, L.A.L., 2006. The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: How to assess the risk. Vet. Microbiol. 112, 303–312. https://doi.org/10.1016/j.vetmic.2005.11.015

Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C., Waller, D.M., 2004. Ecological Impacts of Deer Overabundance. Annu. Rev. Ecol. Evol. Syst. 35, 113–147. https://doi.org/10.1146/annurev.ecolsys.35.021103.105725

Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Official Journal of the European Communities.

Cowie, C.E., Beck, B.B., Gortazar, C., Vicente, J., Hutchings, M.R., Moran, D., White, P.C.L., 2014. Risk factors for the detected presence of *Mycobacterium bovis* in cattle in south central Spain. Eur. J. Wildl. Res. 60, 113–123. https://doi.org/10.1007/s10344-013-0757-0

Cowie, C.E., Hutchings, M.R., Barasona, J.A., Gortázar, C., Vicente, J., White, P.C.L., 2016. Interactions between four species in a complex wildlife: livestock disease community: implications for *Mycobacterium bovis* maintenance and transmission. Eur. J. Wildl. Res. 62, 51–64. https://doi.org/10.1007/s10344-015-0973-x

Cunningham, A.A., Daszak, P., Wood, J.L.N., 2017. One Health, emerging infectious diseases and wildlife: two decades of progress? Philos. Trans. R. Soc. B Biol. Sci.

372, 20160167. https://doi.org/10.1098/rstb.2016.0167

Dabritz, H. a, Miller, M. a, Atwill, E.R., Gardner, I. a, Leutenegger, C.M., Melli, A.C., Conrad, P., 2007. Detection of *Toxoplasma gondii*-like oocysts in cat feces and estimates of the environmental oocyst burden. J. Am. Vet. Med. Assoc. 231, 1676–1684. https://doi.org/10.2460/javma.231.11.1676

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife-threats to biodiversity and human health. Science. 287, 443–449. https://doi.org/10.1126/science.287.5452.443

Davies, T.J., Pedersen, A.B., 2008. Phylogeny and geography predict pathogen community similarity in wild primates and humans. Proc. R. Soc. B Biol. Sci. 275, 1695–1701. https://doi.org/10.1098/rspb.2008.0284

Davis, A., Sharp, J., 2020. Rethinking One Health: Emergent human, animal and environmental assemblages. Soc. Sci. Med. 258, 113093. https://doi.org/10.1016/j.socscimed.2020.113093

De Deus, N., Peralta, B., Pina, S., Allepuz, A., Mateu, E., Vidal, D., Ruiz-Fons, F., Martín, M., Gortázar, C., Segalés, J., 2008. Epidemiological study of hepatitis E virus infection in European wild boars (*Sus scrofa*) in Spain. Vet. Microbiol. 129, 163–170. https://doi.org/10.1016/j.vetmic.2007.11.002

De la Peña, E., Martín, J., Barja, I., Pérez-Caballero, R., Acosta, I., Carranza, J., 2020. The immune challenge of mating effort: steroid hormone profile, dark ventral patch and parasite burden in relation to intrasexual competition in male Iberian red deer. Integr. Zool. 15, 262–275. https://doi.org/10.1111/1749-4877.12427

De Lisle, G.W., Pamela Kawakami, R., Yates, G.F., Collins, D.M., 2008. Isolation of *Mycobacterium bovis* and other mycobacterial species from ferrets and stoats. Vet. Microbiol. 132, 402–407. https://doi.org/10.1016/j.vetmic.2008.05.022

Decker, D.J., Evensen, D.T.N., Siemer, W.F., Leong, K.M., Riley, S.J., Wild, M.A., Castle, K.T., Higgins, C.L., 2010. Understanding risk perceptions to enhance communication about human-wildlife interactions and the impacts of zoonotic disease. ILAR J. 51, 255–261. https://doi.org/10.1093/ilar.51.3.255

Delahay, R.J., Langton, S., Smith, G.C., Clifton-Hadley, R.S., Cheeseman, C.L., 2000. The spatio-temporal distribution of *Mycobacterium bovis* (bovine tuberculosis) infection in a high-density badger population. J. Anim. Ecol. 69, 428–441. https://doi.org/10.1046/j.1365-2656.2000.00406.x

Delahay, R.J., Walker, N., Smith, G.S., Wilkinson, D., Clifton-Hadley, R.S., Cheeseman, C.L., Tomlinson, A.J., Chambers, M.A., 2013. Long-term temporal trends and estimated transmission rates for Mycobacterium bovis infection in an undisturbed high-density badger (*Meles meles*) population. Epidemiol. Infect. 141, 1445–1456. https://doi.org/10.1017/S0950268813000721

Delibes-Mateos, M., 2015. Conservation conflicts involving mammals in Europe. Therya 6, 123–137. https://doi.org/10.12933/therya-15-240

Delibes-Mateos, M., Ferreira, C., Carro, F., Escudero, M.A., Gortázar, C., 2014. Ecosystem effects of variant rabbit hemorrhagic disease virus, Iberian Peninsula. Emerg. Infect. Dis. 20, 2166–2168. https://doi.org/10.3201/eid2012.140517

Delibes, M., 1980. Feeding ecology of the Spanish lynx in the Coto Dońana. Acta Theriol. 25, 309–324. https://doi.org/10.4098/AT.arch.80-28

Den Broeder, L., Devilee, J., Van Oers, H., Schuit, A.J., Wagemakers, A., 2016. Citizen Science for public health. Health Promot. Int. 33. https://doi.org/10.1093/heapro/daw086

Dhama, K., Chakraborty, S.S., Kapoor, S., Tiwari, R., Kumar, A., Deb, R., Rajagunalan, S., Singh, R., Vora, K., Natesan, S., 2013. One World, One Health - veterinary perspectives. Adv. Anim. Vet. Sci. 1, 5–13.

Díaz-Delgado, R., Aragonés, D., Afán, I., Bustamante, J., 2016. Long-term monitoring of the flooding regime and hydroperiod of Doñana marshes with landsat time series (1974–2014). Remote Sens. 8, 775. https://doi.org/10.3390/rs8090775

Directiva 2003/99/CE del parlamento europeo y del consejo de 17 de noviembre de 2003 sobre la vigilancia de las zoonosis y los agentes zoonóticos y por la que se modifica la Decisión 90/424/CEE del Consejo y se deroga la Directiva 92/117/CEE del Consejo. Diario Oficial de la Unión Europea.

Doceul, V., Bagdassarian, E., Demange, A., Pavio, N., 2016. Zoonotic hepatitis E virus: classification, animal reservoirs and transmission routes. Viruses 8, 270. https://doi.org/10.3390/v8100270

Du Toit, R.M., 1944. The transmission of bluetongue and horse-sickness by *Culicoides*. Onderstepoort J. Vet. Sci. Anim. Ind. 19, 7–16.

Dubey, J. P., Murata, F., Cezar, C., Kwok, O., 2021. Epidemiologic and public health significance of *Toxoplasma gondii* infections in venison: 2009-2020. J. Parasitol. 107, 309–319. https://doi.org/10.1645/20-162.

Dubey, J.P., 2020. The history and life cycle of *Toxoplasma gondii*, in: *Toxoplasma gondii*. Elsevier, pp. 1–19. https://doi.org/10.1016/B978-0-12-815041-2.00001-3

Dubey, J.P., 2010. Toxoplasmosis of animals and humans, 2nd Edition. Ed. Boca Raton, FL.

Dubey, J.P., 2004. Toxoplasmosis – a waterborne zoonosis. Vet. Parasitol. 126, 57–72. https://doi.org/10.1016/j.vetpar.2004.09.005

Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of animals and man. Boca Raton, FL.

Dubey, J.P., Cerqueira-Cézar, C.K., Murata, F.H.A., Kwok, O.C.H., Hill, D., Yang, Y.R., Su, C., 2020a. All about *Toxoplasma gondii* infections in pigs: the past decade. Vet. Parasitol. 109185. https://doi.org/10.1016/j.vetpar.2020.109185

Dubey, J.P., Cerqueira-Cézar, C.K., Murata, F.H.A., Verma, S.K., Kwok, O.C.H., Pedersen, K., Rosenthal, B.M., Su, C., 2020b. White-tailed deer (*Odocoileus virginianus*) are a reservoir of a diversity of *Toxoplasma gondii* strains in the USA and pose a risk to consumers of undercooked venison. Parasitology 147, 775–781. https://doi.org/10.1017/S0031182020000451

Dubey, J.P., Cerqueira-Cézar, C.K., Murata, F.H.A., Verma, S.K., Kwok, O.C.H., Pedersen, K., Rosenthal, B.M., Su, C., 2020c. Genotyping of viable *Toxoplasma gondii* from the first national survey of feral swine revealed evidence for sylvatic transmission cycle, and presence of highly virulent parasite genotypes. Parasitology 147, 295–302. https://doi.org/10.1017/S0031182019001586

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339. https://doi.org/10.1111/j.2042-3306.1987.tb01426.x

Dubey, J.P., Lindsay, D.S., Lappin, M.R., 2009. Toxoplasmosis and other intestinal coccidial infections in cats and dogs. Vet. Clin. North Am. Small Anim. Pract. 39, 1009–1034. https://doi.org/10.1016/j.cvsm.2009.08.001

EBD-CSIC, 2019. Equipo de Seguimiento del Espacio Nacional de Doñana. http://icts.ebd.csic.es/datos-meteorologicos (accessed 7.5.19).

EFSA, 2007. Surveillance and monitoring of toxoplasma in humans, food and animals - scientific opinion of the panel on biological hazards. EFSA J. 583, 1–64. https://doi.org/10.2903/j.efsa.2007.583

Ellis, T.M., Barry Bousfield, R., Bissett, L.A., Dyrting, K.C., Luk, G.S.M., Tsim, S.T., Sturm-ramirez, K., Webster, R.G., Guan, Y., Peiris, J.S.M., 2004. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. Avian Pathol. 33, 492–505. https://doi.org/10.1080/03079450400003601

Emerson, S.U., Purcell, R.H., 2003. Hepatitis E virus. Rev. Med. Virol. 13, 145–154. https://doi.org/10.1002/rmv.384

Epstein, P.R., 2001. Climate change and emerging infectious diseases. Microbes Infect. 3, 747–754. https://doi.org/10.1016/S1286-4579(01)01429-0

Evans, T.S., Schuler, K.L., Walter, W.D., 2014. Surveillance and monitoring of white-tailed deer for chronic wasting disease in the Northeastern United States. J. Fish Wildl. Manag. 5, 387–393. https://doi.org/10.3996/032014-JFWM-021

Faber, M., Askar, M., Stark, K., 2018. Case-control study on risk factors for acute hepatitis E in Germany, 2012 to 2014. Euro Surveill. 23, 19. https://doi.org/10.2807/1560-

7917.ES.2018.23.19.17-00469

Falconi, C., López-Olvera, J.R., Gortázar, C., 2011. BTV infection in wild ruminants, with emphasis on red deer: A review. Vet. Microbiol. 151, 209–219. https://doi.org/10.1016/j.vetmic.2011.02.011

Fernández-Delgado, C., 1997. Conservation management of a European natural area: Doñana National Park, Spain. Princ. Conserv. Biol. 458–467.

Fernández-Gil, A., Naves, J., Ordiz, A., Quevedo, M., Revilla, E., Delibes, M., 2016. Conflict misleads large carnivore management and conservation: brown bears and wolves in Spain. PLoS One 11, e0151541. https://doi.org/10.1371/journal.pone.0151541

Fernandez-Pacheco, P., Fernandez-Pinero, J., Agüero, M., Jimenez-Clavero, M.A., 2008. Bluetongue virus serotype 1 in wild mouflons in Spain. Vet. Rec. 162, 659–660. https://doi.org/10.1136/vr.162.20.659

Fischer, J.R., Gerhold, R.W., 2003. Wildlife as a risk factor in animal health and zoonoses, in: Compendium of Technical Items Presented to the International Committee or to Regional Commissions 2001-2002. Office International des Épizooties, Paris, France, pp. 273–289.

Focardi, S., Pecchioli, E., 2005. Social cohesion and foraging decrease with group size in fallow deer (*Dama dama*). Behav. Ecol. Sociobiol. 59, 84–91. https://doi.org/10.1007/s00265-005-0012-0

Forgách, P., Nowotny, N., Erdélyi, K., Boncz, A., Zentai, J., Szűcs, G., Reuter, G., Bakonyi, T., 2010. Detection of hepatitis E virus in samples of animal origin collected in Hungary. Vet. Microbiol. 143, 106–116. https://doi.org/10.1016/j.vetmic.2009.11.004

Fredriksson-Ahomaa, M., 2019. Wild boar: a reservoir of foodborne zoonoses. Foodborne Pathog. Dis. 16, 153–165. https://doi.org/10.1089/fpd.2018.2512

Frenkel, J.K., Dubey, J.P., Miller, N.L., 1970. *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. Science. 167, 893–896. https://doi.org/10.1126/science.167.3919.893

Fujita, M.S., Turtle, M.D., 1993. Flying foxes (Chiroptera: Pteropodidae): threatened animals of key ecological and economic importance. Biol. Conserv. 5, 455–463. https://doi.org/10.1016/0006-3207(93)90766-T

Galván, I., Solano, F., Zougagh, M., de Andrés, F., Murtada, K., Ríos, Á., de la Peña, E., Carranza, J., 2019. Unprecedented high catecholamine production causing hair pigmentation after urinary excretion in red deer. Cell. Mol. Life Sci. 76, 397–404. https://doi.org/10.1007/s00018-018-2962-1

Gamarra, J.A., Cabezón, O., Pabón, M., Arnal, M.C., Luco, D.F., Dubey, J.P., Gortázar, C., Almeria, S., 2008. Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. Vet. Parasitol. 153, 152–156. https://doi.org/10.1016/j.vetpar.2008.01.028

Gamelon, M., Besnard, A., Gaillard, J.-M., Servanty, S., Baubet, E., Brandt, S., Gimenez, O., 2011. High hunting pressure selects for earlier birth date: wild boar as a case study. Evolution. 65, 3248–3447. https://doi.org/10.1111/j.1558-5646.2011.01366.x

García-Bocanegra, I., Arenas-Montes, A., Lorca-Orá, C., Pujols, J., González, M., Napp, S., Gámez-Guillamán, F., Zorrilla, I., Miguel, E., Arenas, A., 2011. Role of wild ruminants in the epidemiology of bluetongue virus serotypes 1, 4 and 8 in Spain. Vet. Res. 42, 1–7. https://doi.org/10.1186/1297-9716-42-88

García-Bocanegra, I., Cabezón, O., Hernández, E., Martínez-Cruz, M.S., Martínez-Moreno, Á., Martínez-Moreno, J., 2013. *Toxoplasma gondii* in ruminant species (cattle, sheep, and goats) from southern Spain. J. Parasitol. 99, 438–440. https://doi.org/10.1645/12-27.1

García-Bocanegra, I., Dubey, J.P., Martínez, F., Vargas, A., Cabezón, O., Zorrilla, I., Arenas, A., Almería, S., 2010a. Factors affecting seroprevalence of *Toxoplasma gondii* in the endangered Iberian lynx (*Lynx pardinus*). Vet. Parasitol. 167, 36–42. https://doi.org/10.1016/j.vetpar.2009.09.044

García-Bocanegra, I., Dubey, J.P., Simon-Grifé, M., Cabezón, O., Casal, J., Allepuz, A., Napp, S., Almería, S., 2010b. Seroprevalence and risk factors associated with Toxoplasma gondii infection in pig farms from Catalonia, north-eastern Spain. Res. Vet. Sci. 89, 85–87. https://doi.org/10.1016/j.rvsc.2010.01.017

García-Bocanegra, I., Napp, S., Casal, J., Perea, A., Allepuz, A., Alba, A., Carbonero, A., Arenas, A., 2009. Bluetongue epidemiology in wild ruminants from southern Spain. Eur. J. Wildl. Res. 55, 173–178. https://doi.org/10.1007/s10344-008-0231-6

García-Bocanegra, I., Rivero, A., Caballero-Gómez, J., López-López, P., Cano-Terriza, D., Frías, M., Jiménez-Ruiz, S., Risalde, M.A., Gómez-Villamandos, J.C., Rivero-Juarez, A., 2019. Hepatitis E virus infection in equines in Spain. Transbound. Emerg. Dis. 66, 66–71. https://doi.org/10.1111/tbed.12962

García-Bocanegra, I., Simon-Grifé, M., Dubey, J.P., Casal, J., Martín, G.E., Cabezón, O., Perea, A., Almería, S., 2010c. Seroprevalence and risk factors associated with *Toxoplasma gondii* in domestic pigs from Spain. Parasitol. Int. 59, 421–426. https://doi.org/10.1016/j.parint.2010.06.001

García-Jimenez, W.L., Fernandez-Llario, P., Benitez-Medina, J.M., Cerrato, R., Cuesta, J., Garcia-Sanchez, A., Goncalves, P., Martinez, R., Risco, D., Salguero, F.J., Serrano, E., Gomez, L., Hermoso-de-Mendoza, J., 2013. Reducing Eurasian wild boar (*Sus scrofa*) population density as a measure for bovine tuberculosis control: effects in wild boar and a sympatric fallow deer (*Dama dama*) population in Central Spain.

Prev. Vet. Med. 110, 435–446. https://doi.org/10.1016/j.prevetmed.2013.02.017

Garrote, G., Perez de Ayala, R., Pereira, P., Robles, F., Guzman, N., García, F.J., Iglesias, M.C., Hervás, J., Fajardo, I., Simón, M., Barroso, J.L., 2011. Estimation of the Iberian lynx (*Lynx pardinus*) population in the Doñana area, SW Spain, using capture–recapture analysis of camera-trapping data. Eur. J. Wildl. Res. 57, 355–362. https://doi.org/10.1007/s10344-010-0440-7

Gauss, C.B.L., Dubey, J.P., Vidal, D., Cabezón, O., Ruiz-Fons, F., Vicente, J., Marco, I., Lavin, S., Gortazar, C., Almería, S., 2006. Prevalence of *Toxoplasma gondii* antibodies in red deer (*Cervus elaphus*) and other wild ruminants from Spain. Vet. Parasitol. 136, 193–200. https://doi.org/10.1016/j.vetpar.2005.11.013

Gauss, C.B.L., Dubey, J.P., Vidal, D., Ruiz, F., Vicente, J., Marco, I., Lavin, S., Gortazar, C., Almería, S., 2005. Seroprevalence of *Toxoplasma gondii* in wild pigs (*Sus scrofa*) from Spain. Vet. Parasitol. 131, 151–156. https://doi.org/10.1016/j.vetpar.2005.04.023

Gavier-Widen, D., Stahl, K., Neimanis, A.S., Segerstad, C.H. a., Gortazar, C., Rossi, S., Kuiken, T., 2015. African swine fever in wild boar in Europe: a notable challenge. Vet. Rec. 176, 199–200. https://doi.org/10.1136/vr.h699

George, D., Mallery, P., 2012. IBM SPSS for windows statistics 19 step by step: A simple guidance and reference, 12 th. ed. Boston, USA.

Gilbert, M., Jambal, L., Karesh, W.B., Fine, A., Shiilegdamba, E., Dulam, P., Sodnomdarjaa, R., Ganzorig, K., Batchuluun, D., Tseveenmyadag, N., Bolortuya, P., Cardona, C.J., Leung, C.Y.H., Peiris, J.S.M., Spackman, E., Swayne, D.E., Joly, D.O., 2012. Highly pathogenic avian influenza virus among wild birds in Mongolia. PLoS One 7, e44097. https://doi.org/10.1371/journal.pone.0044097

Glaser, L., Carstensen, M., Shaw, S., Robbe-Austerman, S., Wunschmann, A., Grear, D., Stuber, T., Thomsen, B., 2016. Descriptive epidemiology and whole genome sequencing analysis for an outbreak of bovine tuberculosis in beef cattle and white-tailed deer in northwestern Minnesota. PLoS One 11, e0145735. https://doi.org/10.1371/journal.pone.0145735

Göloglu, F., Pott, A., 2008. Results on the Crosscorrelation and Autocorrelation of Sequences, in: Sequences and Their Applications-SETA 2008. 5th International Conference. Springer Berlin Heidelberg, Lexington, KY, USA, pp. 95–105.

Gómez-Guillamón, F., Caballero-Gómez, J., Agüero, M., Camacho-Sillero, L., Risalde, M.A., Zorrilla, I., Villalba, R., Rivero-Juárez, A., García-Bocanegra, I., 2020. Re-emergence of bluetongue virus serotype 4 in Iberian ibex (*Capra pyrenaica*) and sympatric livestock in Spain, 2018–2019. Transbound. Emerg. Dis. 68, 458–466. https://doi.org/10.1111/tbed.13696

Gómez-Guillamón, F., Díaz-Cao, J.M., Camacho-Sillero, L., Cano-Terriza, D., Alcaide, E.M., Cabezón, Ó., Arenas, A., García-Bocanegra, I., 2020. Spatiotemporal monitoring of selected pathogens in Iberian ibex (*Capra pyrenaica*). Transbound. Emerg. Dis. tbed.13576. https://doi.org/10.1111/tbed.13576

Gonzálvez-Juan, M., 2021. The role of wild carnivore carcasses in the transmission of infectious agents : epidemiological and ecological aspects. Tesis Doctoral, Universidad de Murcia.

Gordo, O., Avilés, J.M., 2017. El valor de los estudios a largo plazo en la ecología del Comportamiento. Ecosistemas 26, 21–31. https://doi.org/10.7818/ECOS.2017.26-3.04

Gormley, E., Corner, L.A.L., 2013. Control strategies for wildlife tuberculosis in Ireland. Transbound. Emerg. Dis. 60, 128–135. https://doi.org/10.1111/tbed.12095

Gortázar, C., Acevedo, P., Ruiz-Fons, F., Vicente, J., 2006. Disease risks and overabundance of game species. Eur. J. Wildl. Res. 52, 81–87. https://doi.org/10.1007/s10344-005-0022-2

Gortázar, C., Delahay, R.J., McDonald, R.A., Boadella, M., Wilson, G.J., Gavier-Widen, D., Acevedo, P., 2012. The status of tuberculosis in European wild mammals. Mamm. Rev. 42, 193–206. https://doi.org/10.1111/j.1365-2907.2011.00191.x

Gortázar, C., Diez-Delgado, I., Barasona, J.A., Vicente, J., De La Fuente, J., Boadella, M., 2015. The wild side of disease control at the wildlife-livestock-human interface: A review. Front. Vet. Sci. 1, 27. https://doi.org/10.3389/fvets.2014.00027

Gortázar, C., Ferroglio, E., Höfle, U., Frölich, K., Vicente, J., 2007. Diseases shared between wildlife and livestock: a European perspective. Eur. J. Wildl. Res. 53, 241–256. https://doi.org/10.1007/s10344-007-0098-y

Gortázar, C., Ferroglio, E., Lutton, C.E., Acevedo, P., 2010. Disease-related conflicts in mammal conservation. Wildl. Res. 37, 668. https://doi.org/10.1071/WR10031

Gortázar, C., Ruiz-Fons, J.F., Höfle, U., 2016. Infections shared with wildlife: an updated perspective. Eur. J. Wildl. Res. 62, 511–525. https://doi.org/10.1007/s10344-016-1033-x

Gortázar, C., Torres, M.J., Acevedo, P., Aznar, J., Negro, J.J., de la Fuente, J., Vicente, J., 2011. Fine-tuning the space, time, and host distribution of mycobacteria in wildlife. BMC Microbiol. 11, 27. https://doi.org/10.1186/1471-2180-11-27

Gortázar, C., Torres, M.J., Vicente, J., Acevedo, P., Reglero, M., de la Fuente, J., Negro, J.J., Aznar-Martín, J., 2008. Bovine tuberculosis in Doñana Biosphere Reserve: The role of wild ungulates as disease reservoirs in the last Iberian lynx strongholds. PLoS One 3, e2776. https://doi.org/10.1371/journal.pone.0002776

Gortázar, C., Vicente, J., Samper, S., Garrido, J.M., Fernández-De-Mera, I.G., Gavín, P., Juste, R.A., Martín, C., Acevedo, P., De La Puente, M., Höfle, U., 2005. Molecular characterization of *Mycobacterium tuberculosis* complex isolates from wild ungulates in south-central Spain. Vet. Res. 36, 43–52. https://doi.org/10.1051/vetres:2004051

Grogan, L.F., Berger, L., Rose, K., Grillo, V., Cashins, S.D., Skerratt, L.F., 2014. Surveillance for emerging biodiversity diseases of wildlife. PLoS Pathog. 10, e1004015. https://doi.org/10.1371/journal.ppat.1004015

Guan, Y., Poon, L.L.M., Cheung, C.Y., Ellis, T.M., Lim, W., Lipatov, A.S., Chan, K.H., Sturm-Ramirez, K.M., Cheung, C.L., Leung, Y.H.C., Yuen, K.Y., Webster, R.G., Peiris, J.S.M., 2004. H5N1 influenza: A protean pandemic threat. Proc. Natl. Acad. Sci. U. S. A. 101, 8156–8161. https://doi.org/10.1073/pnas.0402443101

Guo, M., Mishra, A., Buchanan, R.L., Dubey, J.P., Hill, D.E., Gamble, H.R., Jones, J.L., Pradhan, A.K., 2016. A systematic meta-analysis of *Toxoplasma gondii* prevalence in food animals in the United States. Foodborne Pathog. Dis. 13, 109–118. https://doi.org/10.1089/fpd.2015.2070

Gusmão, G.M.C., Brito, G.A., Moraes, L.S., Bandeira, M.D.C.A., Rebêlo, J.M.M., 2019. Temporal variation in species abundance and richness of *Culicoides* (Diptera: Ceratopogonidae) in a Tropical Equatorial area. J. Med. Entomol. 56, 1013–1018. https://doi.org/10.1093/jme/tjz015

Gutiérrez-Guzmán, A.V., Vicente, J., Sobrino, R., Perez-Ramírez, E., Llorente, F., Höfle, U., 2012. Antibodies to West Nile virus and related flaviviruses in wild boar, red foxes and other mesomammals from Spain. Vet. Microbiol. 159, 291–297. https://doi.org/10.1016/j.vetmic.2012.04.019

Haydon, D.T., Cleaveland, S., Taylor, L.H., Laurenson, M.K., 2002. Identifying reservoirs of infection: a conceptual and practical challenge. Emerg. Infect. Dis. 8, 1468–1473. https://doi.org/10.3201/eid0812.010317

Hernandez, S.M., Welch, C.N., Peters, V.E., Lipp, E.K., Curry, S., Yabsley, M.J., Sanchez, S., Presotto, A., Gerner-Smidt, P., Hise, K.B., Hammond, E., Kistler, W.M., Madden, M., Conway, A.L., Kwan, T., Maurer, J.J., 2016. Urbanized white ibises (*Eudocimus albus*) as carriers of *Salmonella enterica* of significance to public health and wildlife. PLoS One 11. https://doi.org/10.1371/journal.pone.0164402

Hill, D.E., Dubey, J.P., 2014. Toxoplasmosis. U.S. Geol. Surv. Circ. 84, 1389.

Höfle, U., Gamino, V., de Mera, I.G.F., Mangold, A.J., Ortíz, J.-A., de la Fuente, J., 2013. Usutu virus in migratory song thrushes, Spain. Emerg. Infect. Dis. 19, 1173–1175. https://doi.org/10.3201/eid1907.130199

Hon, C.-C., Lam, T.-Y., Shi, Z.-L., Drummond, A.J., Yip, C.-W., Zeng, F., Lam, P.-Y., Leung, F.C.-C., 2008. Evidence of the recombinant origin of a bat severe acute respiratory syndrome (SARS)-like coronavirus and its implications on the direct ancestor of SARS coronavirus. J. Virol. 82, 1819–1826. https://doi.org/10.1128/JVI.01926-07

Horan, R.D., Wolf, C.A., 2005. The Economics of managing infectious wildlife disease. Am. J. Agric. Econ. 87, 537–551. https://doi.org/10.1111/j.1467-8276.2005.00746.x

Huang, F., Li, Y., Yu, W., Jing, S., Wang, J., Long, F., He, Z., Yang, C., Bi, Y., Cao, W., Liu, C., Hua, X., Pan, Q., 2016. Excretion of infectious hepatitis E virus into milk in cows imposes high risks of zoonosis. Hepatology 64, 350–359. https://doi.org/10.1002/hep.28668

Humblet, M.-F., Boschiroli, M.L., Saegerman, C., 2009. Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Vet. Res. 40, 50. https://doi.org/10.1051/vetres/2009033

Hwang, E.H., Ahn, P.G., Lee, D.M., Kim, H.S., 2012. Cerebral toxoplasmosis combined with disseminated tuberculosis. J. Korean Neurosurg. Soc. 51, 316–319. https://doi.org/10.3340/jkns.2012.51.5.316

Ishtiaq, F., Gilbert, M., Brown, J., Joyner, P., Sodnomdarjaa, R., Luttrell, M.P., Stallknecht, D., Joly, D.O., 2012. Antibodies to influenza A virus in wild birds across Mongolia, 2006-2009. Jwd 48, 768–75. https://doi.org/10.7589/0090-3558-48.3.768

Ivanova, A., Tefanova, V., Reshetnjak, I., Kuznetsova, T., Geller, J., Lundkvist, Å., Janson, M., Neare, K., Velström, K., Jokelainen, P., Lassen, B., Hütt, P., Saar, T., Viltrop, A., Golovljova, I., 2015. Hepatitis E virus in domestic pigs, wild boars, pig farm workers, and hunters in Estonia. Food Environ. Virol. 7, 403–412. https://doi.org/10.1007/s12560-015-9210-8

Jang, Y., Ryoo, S., Lee, Hyunkyoung, Kim, N., Lee, Hang, Park, S., Song, W., Kim, J.-T., Lee, H.S., Myung Kim, J., 2017. Isolation of *Mycobacterium bovis* from Free-Ranging Wildlife in South Korea. J. Wildl. Dis. 53, 181–185. https://doi.org/10.7589/2015-11-295

Jiménez-Martín, D., García-Bocanegra, I., Almería, S., Castro-Scholten, S., Dubey, J.P., Amaro-López, M.A., Cano-Terriza, D., 2020. Epidemiological surveillance of *Toxoplasma gondii* in small ruminants in southern Spain. Prev. Vet. Med. 183, 105137. https://doi.org/10.1016/j.prevetmed.2020.105137

Jiménez-Ruiz, S., García-Bocanegra, I., Acevedo, P., Espunyes, J., Triguero-Ocaña, R., Cano-Terriza, D., Torres-Sánchez, M.J., Vicente, J., Risalde, M.A., 2021. A survey of shared pathogens at the domestic-wild ruminants' interface in Doñana National Park (Spain). Transbound. Emerg. Dis. 0: 1–9. https://doi.org/10.1111/tbed.14126

Jokelainen, P., Näreaho, A., Hälli, O., Heinonen, M., Sukura, A., 2012. Farmed wild boars exposed to *Toxoplasma gondii* and *Trichinella* spp. Vet. Parasitol. 187, 323–327. https://doi.org/10.1016/j.vetpar.2011.12.026

Jokelainen, P., Näreaho, A., Knaapi, S., Oksanen, A., Rikula, U., Sukura, A., 2010. *Toxoplasma gondii* in wild cervids and sheep in Finland: North-south gradient in seroprevalence. Vet. Parasitol. 171, 331–336.

https://doi.org/10.1016/j.vetpar.2010.04.008

Jolles, A.E., Ezenwa, V.O., 2015. Ungulates as model systems for the study of disease processes in natural populations. J. Mammal. 96, 4–15. https://doi.org/10.1093/jmammal/gyu007

Jones, B.A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M.Y., McKeever, D., Mutua, F., Young, J., McDermott, J., Pfeiffer, D.U., 2013. Zoonosis emergence linked to agricultural intensification and environmental change. Proc. Natl. Acad. Sci. 110, 8399–8404. https://doi.org/10.1073/pnas.1208059110

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, 990–993. https://doi.org/10.1038/nature06536

Junta de Andalucia, 2021. Programa de vigilancia epidemiológica de la fauna silvestre en Andalucía (PVE). https://www.juntadeandalucia.es/medioambiente/portal/landing-page-índice/-/asset_publisher/zX2ouZa4r1Rf/content/programa-de-vigilancia-epidemiol-c3-b3gica-de-la-fauna-silvestre-en-andaluc-c3-ada-pve--1/20151?categoryVal=

Junta de Andalucia, 2013. Memoria de actividades y resultados del Espacio Natural de Doñana. Consejeria de medio ambiente y ordenación del territorio.

Justice-Allen, A., Clement, M.J., 2019. Effect of Canine Parvovirus and Canine Distemper Virus on the Mexican wolf (*Canis lupus baileyi*) population in the USA. J. Wildl. Dis. 55, 682–688. https://doi.org/10.7589/2018-07-175

Kamar, N., Izopet, J., Pavio, N., Aggarwal, R., Labrique, A., Wedemeyer, H., Dalton, H.R., 2017. Hepatitis E virus infection. Nat. Rev. Dis. Prim. 3, 17086. https://doi.org/10.1038/nrdp.2017.86

Kantala, T., Heinonen, M., Oristo, S., von Bonsdorff, C.-H., Maunula, L., 2015. Hepatitis E virus in young pigs in Finland and characterization of the isolated partial genomic sequences of genotype 3 HEV. Foodborne Pathog. Dis. 12, 253–260. https://doi.org/10.1089/fpd.2014.1841

Katale, B.Z., Mbugi, E. V., Siame, K.K., Keyyu, J.D., Kendall, S., Kazwala, R.R., Dockrell, H.M., Fyumagwa, R.D., Michel, A.L., Rweyemamu, M., Streicher, E.M., Warren, R.M., van Helden, P., Matee, M.I., 2017. Isolation and potential for transmission of *Mycobacterium bovis* at human–livestock–wildlife interface of the Serengeti ecosystem, Northern Tanzania. Transbound. Emerg. Dis. 64, 815–825. https://doi.org/10.1111/tbed.12445

Kenney, S.P., Meng, X.-J., 2019a. Hepatitis E virus genome structure and replication strategy. Cold Spring Harb. Perspect. Med. 9, a031724. https://doi.org/10.1101/cshperspect.a031724

Kenney, S.P., Meng, X.-J., 2019b. Hepatitis E virus: animal models and zoonosis. Annu. Rev. Anim. Biosci. 7, 427–448. https://doi.org/10.1146/annurev-animal-020518-115117

Keuling, O., Baubet, E., Duscher, A., Ebert, C., Fischer, C., Monaco, A., Podgórski, T., Prevot, C., Ronnenberg, K., Sodeikat, G., Stier, N., Thurfjell, H., 2013. Mortality rates of wild boar Sus scrofa L. in central Europe. Eur. J. Wildl. Res. 59, 805–814. https://doi.org/10.1007/s10344-013-0733-8

Khuroo, Mohammad, Khuroo, Mehnaaz, Khuroo, N., 2016. Transmission of hepatitis E virus in developing countries. Viruses 8, 253. https://doi.org/10.3390/v8090253

Kock, R.A., 2005. What is this infamous "wildlife/livestock disease interface?" a review of current knowledge for the African continent, in: Conservation and development interventions at the wildlife/livestock interface. pp. 1–10.

Konjević, D., Bujanić, M., Erman, V., Gudan Kurilj, A., Živičnjak, T., Severin, K., Tomić, S., Martinković, F., 2017. New data on wild boar (*Sus scrofa* L.) a dead-end host for large American liver fluke (*Fascioloides magna*). Helminthologia 54, 77–80. https://doi.org/10.1515/helm-2017-0006

Kozyra, I., Jabłoński, A., Bigoraj, E., Rzeżutka, A., 2020. Wild boar as a sylvatic reservoir of hepatitis E virus in Poland : a cross-sectional population study. Viruses. 12, 1113. https://doi.org/10.3390/v12101113

Kramer, M.S., Boivin, J.-F., 1988. The importance of directionality in epidemiologic research design. J. Clin. Epidemiol. 41, 717–718. https://doi.org/10.1016/0895-4356(88)90156-4

Krauss, S., Stucker, K.M., Schobel, S.A., Danner, A., Friedman, K., Knowles, J.P., Kayali, G., Niles, L.J., Dey, A.D., Raven, G., Pryor, P., Lin, X., Das, S.R., Stockwell, T.B., Wentworth, D.E., Webster, R.G., 2015. Long-term surveillance of H7 influenza viruses in American wild aquatic birds: Are the H7N3 influenza viruses in wild birds the precursors of highly pathogenic strains in domestic poultry? Emerg. Microbes Infect. 4, 1–9. https://doi.org/10.1038/emi.2015.35

Kukielka, E., Barasona, J.A., Cowie, C.E., Drewe, J.A., Gortazar, C., Cotarelo, I., Vicente, J., 2013. Spatial and temporal interactions between livestock and wildlife in South Central Spain assessed by camera traps. Prev. Vet. Med. 112, 213–221. https://doi.org/10.1016/j.prevetmed.2013.08.008

Kukielka, E., Rodriguez-Prieto, V., Vicente, J., Sánchez-Vizcaíno, J.M., 2016. Constant hepatitis E virus (HEV) circulation in wild boar and red deer in Spain: An increasing concern source of HEV zoonotic transmission. Transbound. Emerg. Dis. 63, e360–e368. https://doi.org/10.1111/tbed.12311

Laguna, E., Barasona, J.A., Triguero-Ocaña, R., Mulero-Pázmány, M., Negro, J.J., Vicente, J., Acevedo, P., 2018. The relevance of host overcrowding in wildlife epidemiology: A new spatially explicit aggregation index. Ecol. Indic. 84, 695–700. https://doi.org/10.1016/j.ecolind.2017.09.039

Lari, A., Lorenzi, D., Nigrelli, D., Brocchi, E., Faccini, S., Poli, A., 2006. Pseudorabies virus in European wild boar from Central Italy. J. Wildl. Dis. 42, 319–324. https://doi.org/10.7589/0090-3558-42.2.319

Larska, M., Krzysiak, M.K., Jabłoński, A., Kesik, J., Bednarski, M., Rola, J., 2015. Hepatitis E virus antibody prevalence in wildlife in Poland. Zoonoses Public Health 62, 105–110. https://doi.org/10.1111/zph.12113

Lazo, A., 1995. Ranging behaviour of feral cattle (*Bos taurus*) in Doñana National Park, S.W. Spain. J. Zool. 236, 359–369. https://doi.org/10.1111/j.1469-7998.1995.tb02718.x

León-Vizcaíno, L., 1990. Outbreak of tuberculosis caused by *Mycobacterium bovis* in wild boars. 32 Int. Symp. Erkrankungen Zoo-Wildtiere, Eskilstuna 185–190.

Ley 30/2014, de 3 de diciembre, de Parques Nacionales. BOE n 293.

Life+IBERLINCE, 2018. Life+IBERLINCE, Recuperación de la distribución histórica del Lince ibérico (*Lynx pardinus*) en España y Portugal. (LIFE10NAT/ES/570). http://www.iberlince.eu/index.php/esp/lince-iberico-esp/ (accessed 6.18.20).

Likens, G.E., 1989. Long-term studies in ecology: approaches and alternatives. Springer Verlag, Berlin.

Lindenmayer, D.B., Likens, G.E., Andersen, A., Bowman, D., Bull, C.M., Burns, E., Dickman, C.R., Hoffmann, A.A., Keith, D.A., Liddell, M.J., Lowe, A.J., Metcalfe, D.J., Phinn, S.R., Russell-Smith, J., Thurgate, N., Wardle, G.M., 2012. Value of long-term ecological studies. Austral Ecol. 37, 745–757. https://doi.org/10.1111/j.1442-9993.2011.02351.x

Lindsay, D.S., Blagburn, B.L., Dubey, J.P., 1999. Coccidia and other protozoa, in: Straw, B., D´Allaire, S., Mengeling, W., Taylor, D. (Eds.), Disease of Swine. pp. 655–657.

Lledó, L., Serrano, J.L., Giménez-Pardo, C., Gegúndez, I., 2020. Wild red foxes (*Vulpes vulpes*) as sentinels of rodent-borne Hantavirus and lymphocytic choriomeningitis virus in the province of Soria, northern Spain. J. Wildl. Dis. 56, 658–661. https://doi.org/10.7589/2019-09-239

Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos 88, 87–98. https://doi.org/10.1034/j.1600-0706.2000.880110.x

Loots, A.K., Mitchell, E., Dalton, D.L., Kotzé, A., Venter, E.H., 2017. Advances in canine distemper virus pathogenesis research: A wildlife perspective. J. Gen. Virol. 98, 311–321. https://doi.org/10.1099/jgv.0.000666

López-López, P., Risalde, M. de los A., Frias, M., García-Bocanegra, I., Brieva, T., Caballero-Gomez, J., Camacho, A., Fernández-Molera, V., Machuca, I., Gomez-Villamandos, J.C., Rivero, A., Rivero-Juarez, A., 2018. Risk factors associated with hepatitis E virus in pigs from different production systems. Vet. Microbiol. 224, 88–92. https://doi.org/10.1016/j.vetmic.2018.08.020

López-Sáez, J.A., Pérez-Díaz, S., Rodríguez-Ramírez, A., Blanco-González, A., Villarías-Robles, J.J.R., Luelmo-Lautenschlaeger, R., Jiménez-Moreno, G., Celestino-Pérez, S., Cerrillo-Cuenca, E., Pérez-Asensio, J.N., León, Á., 2018. Mid-late Holocene environmental and cultural dynamics at the south-west tip of Europe (Doñana National Park, SW Iberia, Spain). J. Archaeol. Sci. Reports 22, 58–78. https://doi.org/10.1016/j.jasrep.2018.09.014

López, G., Martínez, F., Meli, M.L., Bach, E., Martínez-Granados, C., López-Parra, M., Fernández, L., Ruiz, G., Vargas, A., Molina, I., Díaz-Portero, M.A., Gil-Sánchez, J. M. Cadenas, R., Pastor, J., Lutz, H., Simón, M.A., 2009. A feline leukemia virus (FeLV) outbreak in the Doñana Iberian lynx population, in: Vargas, A., Breitenmoser, C., Breitenmoser, U. (Eds.), Conservación ex-situ del lince ibérico: un enfoque multidisciplinar. Iberian lynx ex situ conservation: an interdisciplicanary approach. Fundación biodiversidad, Madrid, pp. 234–247.

Lorca-Oró, C., López-Olvera, J.R., Ruiz-Fons, F., Acevedo, P., García-Bocanegra, I., Oleaga, Á., Gortázar, C., Pujols, J., 2014. Long-term dynamics of bluetongue virus in wild ruminants: relationship with outbreaks in livestock in Spain, 2006-2011. PLoS One 9, 2006–2011. https://doi.org/10.1371/journal.pone.0100027

Lorca-Oró, C., Pujols, J., Arenas, A., Gómez-Guillamón, F., Zorrilla, I., Domingo, M., Arenas-Montés, A., Ruano, M.J., García-Bocanegra, I., 2011. Epidemiological surveillance of bluetongue virus serotypes 1, 4 and 8 in Spanish ibex (*Capra pyrenaica hispanica*) in southern Spain. Vet. Microbiol. 149, 230–235. https://doi.org/10.1016/j.vetmic.2010.10.023

Lu, Y.H., Qian, H.Z., Hu, A.Q., Qin, X., Jiang, Q.W., Zheng, Y.J., 2013. Seasonal pattern of hepatitis E virus prevalence in swine in two different geographical areas of China. Epidemiol. Infect. 141, 2403–2409. https://doi.org/10.1017/S0950268813000113

Lubroth, J., 2013. FAO and the One Health Approach, in: Mackenzie, J.S., Jeggo, M., Daszak, P., Richt, J.A. (Eds.), One Health: the human-animal-environment interfaces in emerging infectious diseases: food safety and security, and international and national plans for implementation of One Health activities. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 65–72. https://doi.org/10.1007/82_2012_262

MacLachlan, N.J., 2004. Bluetongue: pathogenesis and duration of viraemia. Vet. Ital. 40, 462–467.

MacLachlan, N.J., 1994. The pathogenesis and immunology of bluetongue virus infection of ruminants. Comp. Immunol. Microbiol. Infect. Dis. 17, 197–206. https://doi.org/10.1016/0147-9571(94)90043-4

Magle, S.B., Samuel, M.D., Van Deelen, T.R., Robinson, S.J., Mathews, N.E., 2013. Evaluating spatial overlap and relatedness of white-tailed deer in a chronic wasting disease management zone. PLoS One 8, e56568. https://doi.org/10.1371/journal.pone.0056568

Malo, A.F., Roldan, E.R.S., Garde, J.J., Soler, A.J., Vicente, J., Gortazar, C., Gomendio, M., 2009. What does testosterone do for red deer males? Proc. R. Soc. B Biol. Sci. 276, 971–980. https://doi.org/10.1098/rspb.2008.1367

Mansuy, J.-M., Bendall, R., Legrand-Abravanel, F., Sauné, K., Miédouge, M., Ellis, V., Rech, H., Destruel, F., Kamar, N., Dalton, H.R., Izopet, J., 2011. Hepatitis E virus antibodies in blood donors, France. Emerg. Infect. Dis. 17, 2309–2312. https://doi.org/10.3201/eid1712.110371

MAPA, 2021a. Programa nacional de erradicación de la tuberculosis bovina.

MAPA, 2020a. Programa de vigilancia, control, y erradicación de la lengua azul en España 2020.

MAPA, 2020b. Detección de recirculación del Serotipo 4 del virus de la Lengua Azul en la provincia de Málaga (06/02/2020).

MAPA, 2005. Real Decreto 1201/2005, de 10 de octubre, sobre protección de los animales utilizados para experimentación y otros fines científicos.

MAPA, 1988. Real Decreto 223/1988, de 14 de marzo, sobre protección de los animales utilizados para experimentación y otros fines científicos. Ministerio de Agricultura, Pesca y Alimentación, Spain.

MAPA, 2021b. Lengua azul. https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/sanidad-animal/enfermedades/lengua-azul/lengua_azul.aspx

MAPA, 2017. Plan de Actuación sobre Tuberculosis en Especies Silvestres (PATUBES). Madrid, Spain.

Martín-Atance, P., León-Vizcaíno, L., Palomares, F., Revilla, E., González-Candela, M., Calzada, J., Cubero-Pablo, M.J., Delibes, M., 2006. Antibodies to *Mycobacterium bovis* in wild carnivores from Doñana National Park (Spain). J. Wildl. Dis. 42, 704–708. https://doi.org/10.7589/0090-3558-42.3.704

Martín-Hernando, M.P., Höfle, U., Vicente, J., Ruiz-Fons, F., Vidal, D., Barral, M., Garrido, J.M., de la Fuente, J., Gortazar, C., 2007. Lesions associated with *Mycobacterium tuberculosis* complex infection in the European wild boar. Tuberculosis 87, 360–367. https://doi.org/10.1016/j.tube.2007.02.003

Martin, C., Pastoret, P.-P., Brochier, B., Humblet, M.-F., Saegerman, C., 2011. A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. Vet. Res. 42, 70. https://doi.org/10.1186/1297-9716-42-70

Martinelli, N., Pavoni, E., Filogari, D., Ferrari, N., Chiari, M., Canelli, E., Lombardi, G., 2015. Hepatitis E virus in wild boar in the central northern part of Italy. Transbound. Emerg. Dis. 62, 217–222. https://doi.org/10.1111/tbed.12118

Mashaly, M., Nabih, N., Fawzy, I.M., El Henawy, A.A., 2017. Tuberculosis/toxoplasmosis co-infection in Egyptian patients: A reciprocal impact. Asian Pac. J. Trop. Med. 10, 315–319. https://doi.org/10.1016/j.apjtm.2017.03.012

Massei, G., Kindberg, J., Licoppe, A., Gačić, D., Šprem, N., Kamler, J., Baubet, E., Hohmann, U., Monaco, A., Ozoliņš, J., Cellina, S., Podgórski, T., Fonseca, C., Markov, N., Pokorny, B., Rosell, C., Náhlik, A., 2015. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. Pest Manag. Sci. 71, 492–500. https://doi.org/10.1002/ps.3965

Mauget, R., Campan, R., Spitz, F., Dardaillom, M., Janeau, G., Pepin, D., 1984. Synthise des connaissances actuelles sur la biologie du sanglier, perspectives de recherche, in: Sym. Int. Sanglier. Tolouse: Les colloques de l'INRA.

Mbora, D.N.M., McPeek, M.A., 2009. Host density and human activities mediate increased parasite prevalence and richness in primates threatened by habitat loss and fragmentation. J. Anim. Ecol. 78, 210–218. https://doi.org/10.1111/j.1365-2656.2008.01481.x

McDonald, J.L., Robertson, A., Silk, M.J., 2017. Wildlife disease ecology from the individual to the population: Insights from a long-term study of a naturally infected European badger population. J. Anim. Ecol. 87, 101–112. https://doi.org/10.1111/1365-2656.12743

Mellor, P.S., Carpenter, S., Harrup, L., Baylis, M., Mertens, P.P.C., 2008. Bluetongue in Europe and the Mediterranean Basin: History of occurrence prior to 2006. Prev. Vet. Med. 87, 4–20. https://doi.org/10.1016/j.prevetmed.2008.06.002

Meng, X.J., 2010. Hepatitis E virus: Animal reservoirs and zoonotic risk. Vet. Microbiol. 140, 256–265. https://doi.org/10.1016/j.vetmic.2009.03.017

Mertens, P., Baylis, M., Mellor, P., 2008. Bluetongue. Academic Press, Cambridge, MA, USA.

Millán, J., Candela, M.G., Palomares, F., Cubero, M.J., Rodríguez, A., Barral, M., de la Fuente, J., Almería, S., León-Vizcaíno, L., 2009. Disease threats to the endangered Iberian lynx (*Lynx pardinus*). Vet. J. 182, 114–124. https://doi.org/10.1016/j.tvjl.2008.04.005

Millán, M., Carranza, J., Pérez-González, J., Valencia, J., Torres-Porras, J., Seoane, J.M., de la Peña, E., Alarcos, S., Sánchez-Prieto, C.B., Castillo, L., Flores, A., Membrillo, A., 2021. Rainfall decrease and red deer rutting behaviour: Weaker and delayed rutting activity though higher opportunity for sexual selection. PLoS One 16, e0244802. https://doi.org/10.1371/journal.pone.0244802

Millán, J., Casanova, J.C., 2007. Helminth parasites of the endangered Iberian lynx (*Lynx pardinus*) and sympatric carnivores. J. Helminthol. 81, 377–380. https://doi.org/10.1017/S0022149X07869203

Miller, M.A., Olea-Popelka, F., 2013. One Health in the shrinking world: Experiences with tuberculosis at the human-livestock-wildlife interface. Comp. Immunol. Microbiol. Infect. Dis. 36, 263–268. https://doi.org/10.1016/j.cimid.2012.07.005

Miller, M.A., Gardner, I.A., Kreuder, C., Paradies, D.M., Worcester, K.R., Jessup, D.A., Dodd, E., Harris, M.D., Ames, J.A., Packham, A.E., Conrad, P.A., 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). Int. J. Parasitol. 32, 997–1006. https://doi.org/10.1016/S0020-7519(02)00069-3

Miller, M.W., Williams, E.S., 2004. Chronic wasting disease of cervids. Curr. Top. Microbiol. Immunol. 284, 193–214.

Milner, J.M., Bonenfant, C., Mysterud, A., Gaillard, J.M., Csányi, S., Stenseth, N.C., 2006. Temporal and spatial development of red deer harvesting in Europe: Biological and cultural factors. J. Appl. Ecol. 43, 721–734. https://doi.org/10.1111/j.1365-2664.2006.01183.x

Mitchell, B., 2017. Growth Layers in Dental Cement for Determining the Age of Red Deer (*Cervus elaphus* L.). J. Anim. Ecol. 36, 279–293.

Molina-López, R.A., Casal, J., Darwich, L., 2011. Causes of morbidity in wild raptor populations admitted at a wildlife rehabilitation centre in Spain from 1995-2007: A long term retrospective study. PLoS One 6, e24603. https://doi.org/10.1371/journal.pone.0024603

Montagnaro, S., De Martinis, C., Sasso, S., Ciarcia, R., Damiano, S., Auletta, L., Iovane, V., Zottola, T., Pagnini, U., 2015. Viral and antibody prevalence of hepatitis E in European wild boars (*Sus scrofa*) and hunters at zoonotic risk in the Latium Region. J. Comp. Pathol. 153, 1–8. https://doi.org/10.1016/j.jcpa.2015.04.006

Montoya, J.G., Liesenfeld, O., 2004. Toxoplasmosis. Lancet 363, 1965–1976.

Mooring, M.S., Hart, B.L., 1992. Animal grouping for protection from parasites: selfish herd and encounter-dilution effects. Behaviour 123, 173–193. https://doi.org/10.1163/156853992X00011

Mora-Chavarría, E., Umaña-Castro, R., Abou-Madi, N., Solano-González, S., Retamosa-Izaguirre, M., Jiménez-Soto, M., Blanco-Peña, K., 2017. Health assessment of captive psittacine species in prerelease programs at Costa Rican rescue centers. J.

Zoo Wildl. Med. 48, 1135–1145. https://doi.org/10.1638/2016-0259R.1

More, S., Miranda, M.A., Bicout, D., Bøtner, A., Butterworth, A., Calistri, P., Edwards, S., Garin-Bastuji, B., Good, M., Michel, V., Raj, M., Nielsen, S.S., Sihvonen, L., Spoolder, H., Stegeman, J.A., Velarde, A., Willeberg, P., Winckler, C., Depner, K., Guberti, V., Masiulis, M., Olsevskis, E., Satran, P., Spiridon, M., Thulke, H., Vilrop, A., Wozniakowski, G., Bau, A., Broglia, A., Cortiñas Abrahantes, J., Dhollander, S., Gogin, A., Muñoz Gajardo, I., Verdonck, F., Amato, L., Gortázar Schmidt, C., 2018. African swine fever in wild boar. EFSA J. 16, e05344. https://doi.org/10.2903/j.efsa.2018.5344

Moreno, E.S., Spinola, R., Tengan, C.H., Brasil, R.A., Siciliano, M.M., Coimbra, T.L.M., Silveira, V.R., Rocco, I.M., Bisordi, I., Souza, R.P. de, Petrella, S., Pereira, L.E., Maeda, A.Y., Silva, F.G. da, Suzuki, A., 2013. Yellow fever epizootics in non-human primates, São Paulo state, Brazil, 2008-2009. Rev. Inst. Med. Trop. Sao Paulo 55, 45–50. https://doi.org/10.1590/S0036-46652013000100008

Morner, T., Obendorf, D.L., Artois, M., Woodford, M.H., 2002. Surveillance and monitoring of wildlife diseases. Rev. Sci. Tech. l’OIE 21, 67–76. https://doi.org/10.20506/rst.21.1.1321

Morrinson, M.L., Block, W.M., Strickland, M.D., Collier, B.A., Peterson, M.J., 2008. Wildlife study design, 2nd ed. Springer Science & Business Media, USA.

Morris, R.S., Pfeiffer, D.U., Jackson, R., 1994. The epidemiology of *Mycobacterium bovis* infections. Vet. Microbiol. 40, 153–177. https://doi.org/10.1016/0378-1135(94)90053-1

Moutou, F., 2004. Même en épidémiologie, un peu de zoologie ne peut pas faire de mal. Épidémiologie Santé Anim. 45, 121–123.

Mulero-Pázmány, M., Barasona, J.Á., Acevedo, P., Vicente, J., Negro, J.J., 2015. Unmanned Aircraft Systems complement biologging in spatial ecology studies. Ecol. Evol. 5, 4808–4818. https://doi.org/10.1002/ece3.1744

Mulholland, C. V., Ruthe, A., Cursons, R.T., Durrant, R., Karalus, N., Coley, K., Bower, J., Permina, E., Coleman, M.J., Roberts, S.A., Arcus, V.L., Cook, G.M., Aung, H.L., 2017. Rapid molecular diagnosis of the *Mycobacterium tuberculosis* Rangipo strain responsible for the largest recurring TB cluster in New Zealand. Diagn. Microbiol. Infect. Dis. 88, 138–140. https://doi.org/10.1016/j.diagmicrobio.2017.03.012

Müller, T., Teuffert, J., Ziedler, K., Possardt, C., Kramer, M., Staubach, C., Conraths, F.J., 1998. Pseudorabies in the European wild boar from eastern Germany. J. Wildl. Dis. 34, 251–258. https://doi.org/10.7589/0090-3558-34.2.251

Munoz-Mendoza, M., Romero, B., Del Cerro, A., Gortazar, C., Garcia-Marin, J.F., Menendez, S., Mourelo, J., de Juan, L., Saez, J.L., Delahay, R.J., Balseiro, A., 2016. Sheep as a potential source of bovine tb: epidemiology, pathology and evaluation of diagnostic techniques. Transbound. Emerg. Dis. 63, 635–646. https://doi.org/10.1111/tbed.12325

Munyeme, M., Muma, J.B., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Samui, K.L., Dorny, P., Tryland, M., 2008. Risk factors associated with bovine tuberculosis in traditional cattle of the livestock/wildlife interface areas in the Kafue basin of Zambia. Prev. Vet. Med. 85, 317–328. https://doi.org/10.1016/j.prevetmed.2008.03.006

Nan, Y., Wu, C., Zhao, Q., Zhou, E.-M., 2017. Zoonotic hepatitis E virus: an ignored risk for public health. Front. Microbiol. 8, 2396. https://doi.org/10.3389/fmicb.2017.02396

Naranjo, V., Gortazar, C., Vicente, J., de la Fuente, J., 2008. Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex. Vet. Microbiol. 127, 1–9. https://doi.org/10.1016/j.vetmic.2007.10.002

Neumann, S., Hackl, S.S., Piepenschneider, M., Vina-Rodriguez, A., Dremsek, P., Ulrich, R.G., Groschup, M.H., Eiden, M., 2016. Serologic and molecular survey of hepatitis E Virus in German deer populations. Source J. Wildl. Dis. Wildl. Dis. Assoc. 52, 106–113. https://doi.org/10.7589/2014-12-282

Nichols, D.K., Pessier, A.P., Longcore, J.E., 1998. Cutaneous chytridiomycosis in amphibians: an imerging disease?, in: Joint Conderence 1998. Proceedings of American Association of Zoo Veterinarians. pp. 269–271.

Nicholson, S., Farrar, T., 1994. The influence of soil type on the relationships between NDVI, rainfall, and soil moisture in semiarid Botswana. I. NDVI response to rainfall. Remote Sens. Environ. 50, 107–120. https://doi.org/10.1016/0034-4257(94)90038-8

Nugent, G., 2011. Maintenance, spillover and spillback transmission of bovine tuberculosis in multi-host wildlife complexes: A New Zealand case study. Vet. Microbiol. 151, 34–42. https://doi.org/10.1016/j.vetmic.2011.02.023

Nugent, G., Whitford, J., Yockney, I.J., Cross, M.L., 2012. Reduced spillover transmission of Mycobacterium bovis to feral pigs (*Sus scofa*) following population control of brushtail possums (*Trichosurus vulpecula*). Epidemiol. Infect. 140, 1036–1047. https://doi.org/10.1017/S0950268811001579

O'Brien, D.J., Schmitt, S.M., Rudolph, B.A., Nugent, G., 2011. Recent advances in the management of bovine tuberculosis in free-ranging wildlife. Vet. Microbiol. 151, 23–33. https://doi.org/10.1016/j.vetmic.2011.02.022

O'Donnell, C.F.J., 2000. Conservation status and causes of decline of the threatened New Zealand Long-tailed Bat *Chalinolobus tuberculatus* (Chiroptera: Vespertilionidae). Mamm. Rev. 30, 89–106. https://doi.org/10.1046/j.1365-2907.2000.00059.x

O'Neill, X., White, A., Ruiz-Fons, F., Gortázar, C., 2020. Modelling the transmission and persistence of African swine fever in wild boar in contrasting European scenarios. Sci. Rep. 10, 5895. https://doi.org/10.1038/s41598-020-62736-y

OIE, 2019. Vigilancia sanitaria de los animales terrestres, in: Código Sanitario Para Los Animales Terrestres.

Opsteegh, M., Swart, A., Fonville, M., Dekkers, L., van der Giessen, J., 2011. Age-related *Toxoplasma gondii* seroprevalence in dutch wild boar inconsistent with lifelong persistence of antibodies. PLoS One 6, e16240. https://doi.org/10.1371/journal.pone.0016240

Packer, C., Altizer, S., Appel, M., Brown, E., Martenson, J., O'Brien, S.J., Roelke-Parker, M., Hofmann-Lehmann, R., Lutz, H., 1999. Viruses of the Serengeti: Patterns of infection and mortality in African lions. J. Anim. Ecol. 68, 1161–1178. https://doi.org/10.1046/j.1365-2656.1999.00360.x

Palencia, P., Vicente, J., Barroso, P., Barasona, J.Á., Soriguer, R.C., Acevedo, P., 2019. Estimating day range from camera-trap data: the animals' behaviour as a key parameter. J. Zool. 309, 182–190. https://doi.org/10.1111/jzo.12710

Palmer, M. V., Thacker, T.C., Waters, W.R., Gortázar, C., Corner, L.A.L., 2012. *Mycobacterium bovis*: A model pathogen at the interface of livestock, wildlife, and humans. Vet. Med. Int. 2012, 236205. https://doi.org/10.1155/2012/236205

Panadero, R., Painceira, A., López, C., Vázquez, L., Paz, A., Díaz, P., Dacal, V., Cienfuegos, S., Fernández, G., Lago, N., Díez-Baños, P., Morrondo, P., 2010. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in wild and domestic ruminants sharing pastures in Galicia (Northwest Spain). Res. Vet. Sci. 88, 111–115. https://doi.org/10.1016/j.rvsc.2009.05.010

Parashar, D., Khalkar, P., Arankalle, V.A., 2011. Survival of hepatitis A and E viruses in soil samples. Clin. Microbiol. Infect. 17, E1–E4. https://doi.org/10.1111/j.1469-0691.2011.03652.x

Parra, A., García, A., Inglis, N.F., Tato, A., Alonso, J.M., Hermoso de Mendoza, M., Hermoso de Mendoza, J., Larrasa, J., 2006. An epidemiological evaluation of *Mycobacterium bovis* infections in wild game animals of the Spanish Mediterranean ecosystem. Res. Vet. Sci. 80, 140–146. https://doi.org/10.1016/j.rvsc.2005.05.010

Pavio, N., Meng, X.-J., Renou, C., 2010. Zoonotic hepatitis E: animal reservoirs and emerging risks. Vet. Res. 41, 46. https://doi.org/10.1051/vetres/2010018

Peralbo-Moreno, A., Cuadrado-Matías, R., Baz-Flores, S., Barroso, P., Triguero-Ocaña, R., Jiménez-Ruiz, S., Acevedo, P., Ruiz-Fons, F., 2021. Spatial modelling of *Hyalomma lusitanicum* ticks shape Crimean-Congo haemorrhagic fever virus exposure in Doñana National Park, Spain, in: 69th WDA and 14th EWDA Congress. Cuenca, Spain.

Pérez-Morote, R., Pontones-Rosa, C., Gortázar-Schmidt, C., Muñoz-Cardona, Á.I., 2020. Quantifying the economic impact of bovine tuberculosis on livestock farms in south-western Spain. Animals 10, 2433. https://doi.org/10.3390/ani10122433

Perez, J., Mozos, E., Calzada, J., Leon-Vizcaino, L., Cubero, M.J., Velarde, J., 2001. Tuberculosis in an Iberian lynx (*Lynx pardina*). Vet. Rec. 148, 414–415. https://doi.org/10.1136/vr.148.13.414

Perry, B.D., Grace, D., Sones, K., 2013. Current drivers and future directions of global livestock disease dynamics. Proc. Natl. Acad. Sci. 110, 20871–20877. https://doi.org/10.1073/pnas.1012953108

Pfeiffer, D., 2010. Veterinary epidemiology: an introduction, 1st ed. Wiley-Blackwell, University of London, UK.

Pfeiffer, D.U., 2007. Assessment of H5N1 Hpai risk and the importance of wild birds. J. Wildl. Dis. 43, S47–S50.

Pinior, B., Firth, C.L., Loitsch, A., Stockreiter, S., Hutter, S., Richter, V., Lebl, K., Schwermer, H., Käsbohrer, A., 2018. Cost distribution of bluetongue surveillance and vaccination programmes in Austria and Switzerland (2007-2016). Vet. Rec. 182, 1–8. https://doi.org/10.1136/vr.104448

Pioz, M., Guis, H., Crespin, L., Gay, E., Calavas, D., Durand, B., Abrial, D., Ducrot, C., 2012. Why did bluetongue spread the way it did? environmental factors influencing the velocity of bluetongue virus serotype 8 epizootic wave in france. PLoS One 7, e43360. https://doi.org/10.1371/journal.pone.0043360

Portier, J., Ryser-Degiorgis, M.-P., Hutchings, M.R., Monchâtre-Leroy, E., Richomme, C., Larrat, S., van der Poel, W.H.M., Dominguez, M., Linden, A., Santos, P.T., Warns-Petit, E., Chollet, J.-Y., Cavalerie, L., Grandmontagne, C., Boadella, M., Bonbon, E., Artois, M., 2019. Multi-host disease management: the why and the how to include wildlife. BMC Vet. Res. 15, 295. https://doi.org/10.1186/s12917-019-2030-6

Power, A.G., Mitchell, C.E., 2004. Pathogen spillover in disease epidemics. Am. Nat. 164, S79–S89. https://doi.org/10.1086/424610

Pradas, F., 2019. Metodología en salud pública. Inst. Aragon. en Ciencias la Salud, pp. 1–9.

Prugnolle, F., Durand, P., Ollomo, B., Duval, L., Ariey, F., Arnathau, C., Gonzalez, J.-P., Leroy, E., Renaud, F., 2011. A fresh look at the origin of *Plasmodium falciparum*, the most malignant malaria agent. PLoS Pathog. 7, e1001283. https://doi.org/10.1371/journal.ppat.1001283

QGIS Development Team, 2019. QGIS Geographic Information System. Open Source Geospatial Foundation Project. https://qgis.org/es/site/ (accessed 10.1.19).

R Core Team, 2020. R: A language and environment for statistical computing.

R Core Team, 2019. R: A language and environment for statistical computing.

Rao, P.P., Hegde, N.R., Singh, K.P., Putty, K., Hemadri, D., Maan, N.S., Reddy, Y.N., Maan, S., Mertens, P.P.C., 2017. Bluetongue: aetiology, epidemiology, pathogenesis, diagnosis and control, in: Emerging and re-emerging infectious diseases of livestock. Springer International Publishing, Cham, pp. 3–54. https://doi.org/10.1007/978-3-319-47426-7_1

RASVE, 2021. Red de Alerta Sanitaria Veterinaria (RASVE). https://servicio.mapama.gob.es/rasve/ (accessed 1.1.21).

REDIAM, 2019. Consejería de Medio Ambiente y Ordenación del Territorio, Andalucía, España: Red de Información Ambiental de Andalucía, REDIAM. http://www.juntadeandalucia.es/medioambiente/site/rediam (accessed 20.03.19).

REDIAM, 2014. Consejería de Medio Ambiente y Ordenación del Territorio, Andalucía, España: Red de Información Ambiental de Andalucía, REDIAM. http://www.juntadeandalucia.es/medioambiente/site/rediam (accessed 7.8.14).

Rehbein, S., Visser, M., Jekel, I., Silaghi, C., 2014. Endoparasites of the fallow deer (*Dama dama*) of the Antheringer Au in Salzburg, Austria. Wien. Klin. Wochenschr. 126, 37–41. https://doi.org/10.1007/s00508-014-0506-8

Reiterová, K., Špilovská, S., Blaňarová, L., Derdáková, M., Čobádiová, A., Hisira, V., 2016. Wild boar (*Sus scrofa*) – reservoir host of *Toxoplasma gondii*, *Neospora caninum* and *Anaplasma phagocytophilum* in Slovakia. Acta Parasitol. 61, 255–260. https://doi.org/10.1515/ap-2016-0035

Renwick, A.R., White, P.C.L., Bengis, R.G., 2007. Bovine tuberculosis in southern African wildlife: a multi-species host–pathogen system. Epidemiol. Infect. 135, 529–540. https://doi.org/10.1017/S0950268806007205

Rhyan, J.C., Spraker, T.R., 2010. Emergence of diseases from wildlife reservoirs. Vet. Pathol. 47, 34–39. https://doi.org/10.1177/0300985809354466

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernandez Escamez, P.S., Herman, L., Koutsoumanis, K., Lindqvist, R., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Di Bartolo, I., Johne, R., Pavio, N., Rutjes, S., van der Poel, W., Vasickova, P., Hempen, M., Messens, W., Rizzi, V., Latronico, F., Girones, R., 2017. Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen. EFSA J. 15, e04886. https://doi.org/10.2903/j.efsa.2017.4886

Risalde, M.A., Rivero-Juárez, A., Romero-Palomo, F., Frías, M., López-López, P., Cano-Terriza, D., García-Bocanegra, I., Jiménez-Ruíz, S., Camacho, Á., Machuca, I., Gomez-Villamandos, J.C., Rivero, A., 2017. Persistence of hepatitis E virus in the liver of non-viremic naturally infected wild boar. PLoS One 12, e0186858. https://doi.org/10.1371/journal.pone.0186858

Rivero-Juarez, A., Dashti, A., López-López, P., Muadica, A.S., Risalde, M.A., Köster, P.C., Machuca, I., Bailo, B., Hernández de Mingo, M., Dacal, E., García-Bocanegra, I., Saugar, J.M., Calero-Bernal, R., González-Barrio, D., Rivero, A., Briz, V., Carmena, D., 2020. Protist enteroparasites in wild boar (*Sus scrofa ferus*) and black Iberian pig (*Sus scrofa domesticus*) in southern Spain: A protective effect on hepatitis e acquisition? Parasites and Vectors 13, 1–9. https://doi.org/10.1186/s13071-020-04152-9

Rivero-Juarez, A., Frias, M., Martinez-Peinado, A., Risalde, M.A., Rodriguez-Cano, D., Camacho, A., García-Bocanegra, I., Cuenca-Lopez, F., Gomez-Villamandos, J.C., Rivero, A., 2017. Familial hepatitis E outbreak linked to wild boar meat consumption. Zoonoses Public Health 64, 561–565. https://doi.org/10.1111/zph.12343

Rivero-Juarez, A., Risalde, M.A., Frias, M., García-Bocanegra, I., López-López, P., Cano-Terriza, D., Camacho, A., Jimenez-Ruiz, S., Gomez-Villamandos, J.C., Rivero, A., 2018. Prevalence of hepatitis E virus infection in wild boars from Spain: A possible seasonal pattern? BMC Vet. Res. 14, 4–9. https://doi.org/10.1186/s12917-018-1377-4

Rocchigiani, G., Nardoni, S., D'Ascenzi, C., Nicoloso, S., Picciolli, F., Papini, R., Mancianti, F., 2016. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in red deer from Central Italy. Ann. Agric. Environ. Med. 23, 699–701. https://doi.org/10.5604/12321966.1226870

Rodriguez-Hidalgo, P., Gortazar, C., Tortosa, F.S., Rodriguez-Vigal, C., Fierro, Y., Vicente, J., 2010. Effects of density, climate, and supplementary forage on body mass and pregnancy rates of female red deer in Spain. Oecologia 164, 389–398. https://doi.org/10.1007/s00442-010-1663-8

Rodriguez-Prieto, V., 2014. Nuevas aportaciones epidemiológicas y diagnósticas para el estudio de la interacción entre la fauna doméstica y silvestre. Tesis Doctoral, Universidad Complutense de Madrid.

Rodríguez-Sánchez, B., Gortázar, C., Ruiz-Fons, F., Sánchez-Vizcaíno, J.M., 2010a. Bluetongue virus serotypes 1 and 4 in red deer, Spain. Emerg. Infect. Dis. 16, 518–520. https://doi.org/10.3201/eid1603.090626

Rodríguez-Sánchez, B., Iglesias-Martín, I., Martínez-Avilés, M., Sánchez-Vizcaíno, J.M., 2008. Orbiviruses in the mediterranean basin: updated epidemiological situation of bluetongue and new methods for the detection of BTV serotype 4. Transbound. Emerg. Dis. 55, 205–214. https://doi.org/10.1111/j.1865-1682.2008.01029.x

Rodríguez-Sánchez, B., Sánchez-Cordón, P.J., Molina, V., Risalde, M.A., Diego, A.C.P. de, Gómez-Villamandos, J.C., Sánchez-Vizcaíno, J.M., 2010b. Detection of bluetongue serotype 4 in mouflons (*Ovis aries musimon*) from Spain. Vet. Microbiol. 141, 164–167. https://doi.org/10.1016/j.vetmic.2009.08.003

Romero, B., Aranaz, A., Sandoval, Á., Álvarez, J., de Juan, L., Bezos, J., Sánchez, C., Galka, M., Fernández, P., Mateos, A., Domínguez, L., 2008. Persistence and molecular evolution of *Mycobacterium bovis* population from cattle and wildlife in Doñana National Park revealed by genotype variation. Vet. Microbiol. 132, 87–95. https://doi.org/10.1016/j.vetmic.2008.04.032

Rossi, S., Balenghien, T., Viarouge, C., Faure, E., Zanella, G., Sailleau, C., Mathieu, B., Delécolle, J.C., Ninio, C., Garros, C., Gardès, L., Tholoniat, C., Ariston, A., Gauthier, D., Mondoloni, S., Barboiron, A., Pellerin, M., Gibert, P., Novella, C., Barbier, S., Guillaumat, E., Zientara, S., Vitour, D., Bréard, E., 2019. Red deer (*Cervus elaphus*) did not play the role of maintenance host for bluetongue virus in France: The burden of proof by long-term wildlife monitoring and culicoides snapshots. Viruses 11, 903. https://doi.org/10.3390/v11100903

Rossi, S., Pioz, M., Beard, E., Durand, B., Gibert, P., Gauthier, D., Klein, F., Maillard, D., Saint-Andrieux, C., Saubusse, T., Hars, J., 2014. Bluetongue dynamics in French wildlife: exploring the driving forces. Transbound. Emerg. Dis. 61, e12–e24. https://doi.org/10.1111/tbed.12061

Rougier, S., Montoya, J.G., Peyron, F., 2017. Lifelong persistence of Toxoplasma cysts: a questionable dogma? Trends Parasitol. 33, 93–101. https://doi.org/10.1016/j.pt.2016.10.007

Ruder, M.G., Lysyk, T.J., Stallknecht, D.E., Foil, L.D., Johnson, D.J., Chase, C.C., Dargatz, D.A., Gibbs, E.P.J., 2015. Transmission and epidemiology of bluetongue and epizootic hemorrhagic disease in North America: Current perspectives, research gaps, and future directions. Vector-Borne Zoonotic Dis. 15, 348–363. https://doi.org/10.1089/vbz.2014.1703

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. Transbound. Emerg. Dis. 64, 68–88. https://doi.org/10.1111/tbed.12369

Ruiz-Fons, F., Acevedo, P., Sobrino, R., Vicente, J., Fierro, Y., Fernández-de-Mera, I.G., 2013. Sex-biased differences in the effects of host individual, host population and environmental traits driving tick parasitism in red deer. Front. Cell. Infect. Microbiol. 3, 1–12. https://doi.org/10.3389/fcimb.2013.00023

Ruiz-Fons, F., Reyes-García, Á.R., Alcaide, V., Gortázar, C., 2008a. Spatial and temporal evolution of bluetongue virus in wild ruminants, Spain. Emerg. Infect. Dis. 14, 951–953. https://doi.org/10.3201/eid1406.071586

Ruiz-Fons, F., Sánchez-Matamoros, A., Gortázar, C., Sánchez-Vizcaíno, J.M., 2014. The role of wildlife in bluetongue virus maintenance in Europe: Lessons learned after the natural infection in Spain. Virus Res. 182, 50–58. https://doi.org/10.1016/j.virusres.2013.12.031

Ruiz-Fons, F., Segalés, J., Gortázar, C., 2008b. A review of viral diseases of the European wild boar: Effects of population dynamics and reservoir rôle. Vet. J. 176, 158–169. https://doi.org/10.1016/j.tvjl.2007.02.017

Ruiz-Fons, F., Vicente, J., Vidal, D., Höfle, U., Villanúa, D., Gauss, C., Segalés, J., Almería, S., Montoro, V., Gortázar, C., 2006. Seroprevalence of six reproductive pathogens in European wild boar (*Sus scrofa*) from Spain: The effect on wild boar female reproductive performance. Theriogenology 65, 731–743. https://doi.org/10.1016/j.theriogenology.2005.07.001

Rushton, J., Lyons, N., 2015. Economic impact of Bluetongue: a review of the effects on production. Vet. Ital. 51, 401–406. https://doi.org/https://doi.org/10.12834/VetIt.646.3183.1

Rusiñol, M., Fernandez-Cassi, X., Hundesa, A., Vieira, C., Kern, A., Eriksson, I., Ziros, P., Kay, D., Miagostovich, M., Vargha, M., Allard, A., Vantarakis, A., Wyn-Jones, P., Bofill-Mas, S., Girones, R., 2014. Application of human and animal viral microbial source tracking tools in fresh and marine waters from five different geographical areas. Water Res. 59, 119–129. https://doi.org/10.1016/j.watres.2014.04.013

Russell, R.E., Gude, J.A., Anderson, N.J., Ramsey, J.M., 2015. Identifying priority chronic wasting disease surveillance areas for mule deer in Montana. J. Wildl. Manage. 79, 989–997. https://doi.org/10.1002/jwmg.914

Rutjes, S.A., Lodder-Verschoor, F., Lodder, W.J., van der Giessen, J., Reesink, H., Bouwknegt, M., de Roda Husman, A.M., 2010. Seroprevalence and molecular detection of hepatitis E virus in wild boar and red deer in The Netherlands. J. Virol. Methods 168, 197–206. https://doi.org/10.1016/j.jviromet.2010.05.014

Saegerman, C., Berkvens, D., Mellor, P.S., 2008. Bluetongue Epidemiology in the European Union. Emerg. Infect. Dis. 14, 539–544. https://doi.org/10.3201/eid1404.071441

Saenz de Buruaga, M., Lucio-Calero, A., Purroy, F.J., 2001. Reconocimiento de sexo y edad en especies cinegéticas, 1st ed. Edilesa, Madrid, Spain.

Sáez-Royuela, C., Gomáriz, R.P., Tellería, J.L., 1989. Age-determination of European wild boar. Wildl. Soc. Bull. 17, 326–329.

Salines, M., Andraud, M., Rose, N., 2017. From the epidemiology of hepatitis E virus (HEV) within the swine reservoir to public health risk mitigation strategies: a comprehensive review. Vet. Res. 48, 31. https://doi.org/10.1186/s13567-017-0436-3

Salkeld, D.J., Padgett, K.A., Jones, J.H., 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecol. Lett. 16, 679–686. https://doi.org/10.1111/ele.12101

Saminathan, M., Singh, K.P., Khorajiya, J.H., Dinesh, M., Vineetha, S., Maity, M., Rahman, A.F., Misri, J., Malik, Y.S., Gupta, V.K., Singh, R.K., Dhama, K., 2020. An updated review on bluetongue virus: epidemiology, pathobiology, and advances in diagnosis and control with special reference to India. Vet. Q. 40, 258–321. https://doi.org/10.1080/01652176.2020.1831708

Sampaio, E.M., Kalko, E.K. V., Bernard, E., Rodriguez-Herrera, B., Handley, C.O., 2003. A biodiversity assessment of bats (Chiroptera) in a tropical lowland rainforest of Central Amazonia, including methodological and conservation considerations. Stud. Neotrop. Fauna Environ. 38, 17–31. https://doi.org/10.1076/snfe.38.1.17.14035

San Miguel, J.M., Gutiérrez-Expósito, D., Aguado-Martínez, A., González-Zotes, E., Pereira-Bueno, J., Gómez-Bautista, M., Rubio, P., Ortega-Mora, L.M., Collantes-Fernández, E., Álvarez-García, G., 2016. Effect of different ecosystems and management practices on *Toxoplasma gondii* and *Neospora caninum* infections in wild ruminants in Spain. J. Wildl. Dis. 52, 293–300. https://doi.org/10.7589/2015-07-176

Sánchez-Matamoros, A., Martínez, M., Sánchez-Vizcaíno, J.M., 2009. Impact of vaccination in the control of the bluetongue virus. Rev. Complut. Ciencias Vet. 3, 31–40.

Sánchez-Vizcaíno, J.M., Mur, L., Gomez-Villamandos, J.C., Carrasco, L., 2015. An update on the epidemiology and pathology of African swine fever. J. Comp. Pathol. 152, 9–21. https://doi.org/10.1016/j.jcpa.2014.09.003

Sánchez-Vizcaíno, J.M., Mur, L., Martínez-López, B., 2012. African Swine Fever: an epidemiological update. Transbound. Emerg. Dis. 59, 27–35. https://doi.org/10.1111/j.1865-1682.2011.01293.x

Santiago-Moreno, J., Carvajal, A., Astorga, R.J., Coloma, M.A., Toledano-Díaz, A., Gómez-Guillamon, F., Salas-Vega, R., López-Sebastián, A., 2011. Potential impact of diseases transmissible by sperm on the establishment of Iberian ibex (*Capra pyrenaica*) genome resource banks. Eur. J. Wildl. Res. 57, 211–216. https://doi.org/10.1007/s10344-010-0413-x

Santos, N., Almeida, V., Gortázar, C., Correia-Neves, M., 2015a. Patterns of *Mycobacterium tuberculosis*-complex excretion and characterization of super-shedders in naturally-infected wild boar and red deer. Vet. Res. 46, 129. https://doi.org/10.1186/s13567-015-0270-4

Santos, N., Santos, C., Valente, T., Gortázar, C., Almeida, V., Correia-Neves, M., 2015b. Widespread environmental contamination with *Mycobacterium tuberculosis* complex revealed by a molecular detection protocol. PLoS One 10, e0142079. https://doi.org/10.1371/journal.pone.0142079

Saunders, S.E., Bartelt-Hunt, S.L., Bartz, J.C., 2012. Occurrence, transmission, and zoonotic potential of chronic wasting disease. Emerg. Infect. Dis. 18, 369–376.

https://doi.org/10.3201/eid1803.110685

Schley, L., Roper, T.J., 2003. Diet of wild boar Sus scrofa in Western Europe, with particular reference to consumption of agricultural crops. Mamm. Rev. 33, 43–56. https://doi.org/10.1046/j.1365-2907.2003.00010.x

Schmitt, S.M., O'Brien, D.J., Bruning-Fann, C.S., Fitzgerald, S.D., 2002. Bovine tuberculosis in Michigan wildlife and livestock. Ann. N. Y. Acad. Sci. 969, 262–268. https://doi.org/10.1111/j.1749-6632.2002.tb04390.x

Schreiner, C.L., Nuismer, S.L., Basinski, A.J., 2020. When to vaccinate a fluctuating wildlife population: Is timing everything? J. Appl. Ecol. 57, 307–319. https://doi.org/10.1111/1365-2664.13539

Schumacher, A.C., Elbadawi, L.I., DeSalvo, T., Straily, A., Ajzenberg, D., Letzer, D., Moldenhauer, E., Handly, T.L., Hill, D., Dardé, M.-L., Pomares, C., Passebosc-Faure, K., Bisgard, K., Gomez, C.A., Press, C., Smiley, S., Montoya, J.G., Kazmierczak, J.J., 2020. Toxoplasmosis outbreak associated with *Toxoplasma gondii*-contaminated venison–high attack rate, unusual clinical presentation, and atypical genotype. Clin. Infect. Dis. 72, 1557–1565. https://doi.org/10.1093/cid/ciaa285

Servanty, S., Gaillard, J.-M., Ronchi, F., Focardi, S., Baubet, É., Gimenez, O., 2011. Influence of harvesting pressure on demographic tactics: implications for wildlife management. J. Appl. Ecol. 48, 835–843. https://doi.org/10.1111/j.1365-2664.2011.02017.x

Shapiro, K., Bahia-Oliveira, L., Dixon, B., Dumètre, A., de Wit, L.A., VanWormer, E., Villena, I., 2019. Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. Food Waterborne Parasitol. 15, e00049. https://doi.org/10.1016/j.fawpar.2019.e00049

Shwab, E.K., Saraf, P., Zhu, X.-Q., Zhou, D.-H., McFerrin, B.M., Ajzenberg, D., Schares, G., Hammond-Aryee, K., van Helden, P., Higgins, S.A., Gerhold, R.W., Rosenthal, B.M., Zhao, X., Dubey, J.P., Su, C., 2018. Human impact on the diversity and virulence of the ubiquitous zoonotic parasite *Toxoplasma gondii*. Proc. Natl. Acad. Sci. 115, E6956–E6963. https://doi.org/10.1073/pnas.1722202115

Simpson, V.R., 2002. Wild animals as reservoirs of infectious diseases in the UK. Vet. J. 163, 128–146. https://doi.org/10.1053/tvjl.2001.0662

Slenning, B.D., 2010. Global climate change and implications for disease emergence. Vet. Pathol. 47, 28–33. https://doi.org/10.1177/0300985809354465

Smith, K.F., Acevedo-Whitehouse, K., Pedersen, A.B., 2009. The role of infectious diseases in biological conservation. Anim. Conserv. 12, 1–12. https://doi.org/10.1111/j.1469-1795.2008.00228.x

Sobrino, R., Cabezón, O., Millán, J., Pabón, M., Arnal, M.C., Luco, D.F., Gortázar, C., Dubey, J.P., Almeria, S., 2007. Seroprevalence of *Toxoplasma gondii* antibodies in wild carnivores from Spain. Vet. Parasitol. 148, 187–192. https://doi.org/10.1016/j.vetpar.2007.06.038

Soriguer, R.C., 1988. Ramoneo y daño por los grandes herbívoros en las plantas del matorral mediterráneo, in: I Congreso Mundial Sobre El Bosque y Matorral Mediterráneo, Cáceres.

Soriguer, R.C., Delibes, J.R., Fandos, P., Zamora, R., Rodríguez, A., 2001. Evaluación de la capacidad de carga de la marisma del Parque Nacional de Doñana. En: Análisis de la incidencia de los grandes herbívoros en la marisma y en la vera del Parque Nacional de Doñana. Organismo autónomo de Parques Nacionales, Madrid.

Sorvillo, T.E., Rodriguez, S.E., Hudson, P., Carey, M., Rodriguez, L.L., Spiropoulou, C.F., Bird, B.H., Spengler, J.R., Bente, D.A., 2020. Towards a sustainable One Health approach to Crimean–Congo Hemorrhagic Fever prevention: focus areas and gaps in knowledge. Trop. Med. Infect. Dis. 5, 113. https://doi.org/10.3390/tropicalmed5030113

Sparks, T., Menzel, A., 2002. Observed changes in seasons: An overview. Int. J. Climatol. 22, 1715–1725. https://doi.org/https://doi.org/10.1002/joc.821

Spraker, T.R., Miller, M.W., Williams, E.S., Getzy, D.M., Adrian, W.J., Schoonveld, G.G., Spowart, R.A., O'Rourke, K.I., Miller, J.M., Merz, P.A., 1997. Spongiform encephalopathy in free-ranging mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) in northcentral Colorado. J. Wildl. Dis. 33, 1–6. https://doi.org/10.7589/0090-3558-33.1.1

Stallknecht, D.E., Howerth, E., 2004. Epidemiology of bluetongue and epizootic haemorrhagic disease in wildlife: surveillance methods. Vet. Ital. 40, 203–207.

Stallknecht, D.E., 2007. Impediments to wildlife disease surveillance, research, and diagnostics. Curr. Top. Microbiol. Immunol. 315, 445–461. https://doi.org/10.1007/978-3-540-70962-6_17

Strayer, D., Glitezenstein, J.S., Jones, C.G., Kolasa, J., Likens, G.E., McDonnell, M.J., Parker, G.G., Pickett, S.T.A., 1986. Long-term ecological studies: an illustrated account of their design, operation, and importance to ecology. Occas. Publ. Inst. Ecosyst. Stud. 2, 1–38.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. Science. 306, 1783–1786. https://doi.org/10.1126/science.1103538

Tackmann, K., Löschner, U., Mix, H., Staubach, C., Thulke, H.-H., Conraths, F.J., 1998. Spatial distribution patterns of *Echinococcus multilocularis* (Leuckart 1863)

(Cestoda: Cyclophyllidea: Taeniidae) among red foxes in an endemic focus in Brandenburg, Germany. Epidemiol. Infect. 120, 101–109. https://doi.org/10.1017/S0950268897008406

Takahashi-Omoe, H., Omoe, K., 2012. Worldwide trends in infectious disease research revealed by a new bibliometric method, in: Priti, R. (Ed.), Insight and Control of Infectious Disease in Global Scenario. InTech, pp. 121–138. https://doi.org/10.5772/2240

Takahashi, M., Nishizawa, T., Sato, H., Sato, Y., Jirintai, Nagashima, S., Okamoto, H., 2011. Analysis of the full-length genome of a hepatitis E virus isolate obtained from a wild boar in Japan that is classifiable into a novel genotype. J. Gen. Virol. 92, 902–908. https://doi.org/10.1099/vir.0.029470-0

Talavera, S., Muñoz-Muñoz, F., Verdún, M., Pujol, N., Pagés, N., 2018. Revealing potential bridge vectors for BTV and SBV: a study on *Culicoides* blood feeding preferences in natural ecosystems in Spain. Med. Vet. Entomol. 32, 35–40. https://doi.org/10.1111/mve.12263

Tanner, E., White, A., Lurz, P.W.W., Gortázar, C., Díez-Delgado, I., Boots, M., 2019. The critical role of infectious disease in compensatory population growth in response to culling. Am. Nat. 194, E1–E12. https://doi.org/10.1086/703437

Tao, Y., Shi, M., Chommanard, C., Queen, K., Zhang, J., Markotter, W., Kuzmin, I. V., Holmes, E.C., Tong, S., 2017. Surveillance of bat coronaviruses in Kenya identifies relatives of human coronaviruses NL63 and 229E and their recombination history. J. Virol. 91, 1–16. https://doi.org/10.1128/JVI.01953-16

Taylor, L.H., Latham, S.M., Woolhouse, M.E.J., 2001. Risk factors for human disease emergence. Philos. Trans. R. Soc. London. Ser. B Biol. Sci. 356, 983–989. https://doi.org/10.1098/rstb.2001.0888

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30, 1217–1258. https://doi.org/10.1016/S0020-7519(00)00124-7

Thirgood, S., 2009. New perspectives on managing wildlife diseases. J. Appl. Ecol. 46, 454–456. https://doi.org/10.1111/j.1365-2664.2009.01629.x

Thiry, D., Mauroy, A., Pavio, N., Purdy, M.A., Rose, N., Thiry, E., de Oliveira-Filho, E.F., 2017a. Hepatitis E virus and related viruses in animals. Transbound. Emerg. Dis. 64, 37–52. https://doi.org/10.1111/tbed.12351

Thiry, D., Mauroy, A., Saegerman, C., Licoppe, A., Fett, T., Thomas, I., Brochier, B., Thiry, E., Linden, A., 2017b. Belgian wildlife as potential zoonotic reservoir of hepatitis E virus. Transbound. Emerg. Dis. 64, 764–773. https://doi.org/10.1111/tbed.12435

Thomas, J., Balseiro, A., Gortázar, C., Risalde, M.A., 2021. Diagnosis of tuberculosis in wildlife: a systematic review. Vet. Res. 52, 31. https://doi.org/10.1186/s13567-020-00881-y

Thomas, L., Buckland, S.T., Rexstad, E.A., Laake, J.L., Strindberg, S., Hedley, S.L., Bishop, J.R.B., Marques, T.A., Burnham, K.P., 2010. Distance software: design and analysis of distance sampling surveys for estimating population size. J. Appl. Ecol. 47, 5–14. https://doi.org/10.1111/j.1365-2664.2009.01737.x

Thrusfield, M., Christley, R., Brown, H., Diggle, P.J., French, N., Howe, K., Kelly, L., O'Connor, A., Sargeant, J., Wood, H., 2018. The scope of epidemiology, in: Veterinary Epidemiology. Wiley-Blackwell, Hoboken, NJ, pp. 28–41.

Triguero-Ocaña, R., Barasona, J.A., Carro, F., Soriguer, R.C., Vicente, J., Acevedo, P., 2019. Spatio-temporal trends in the frequency of interspecific interactions between domestic and wild ungulates from Mediterranean Spain. PLoS One 14, e0211216. https://doi.org/10.1371/journal.pone.0211216

Triguero-Ocaña, R., Martínez-López, B., Vicente, J., Barasona, J.A., Martínez-Guijosa, J., Acevedo, P., 2020. Dynamic network of interactions in the wildlife-livestock interface in mediterranean spain: An epidemiological point of view. Pathogens 9, 1–16. https://doi.org/10.3390/pathogens9020120

Trilla, A., 2020. One world, one health: The novel coronavirus COVID-19 epidemic. Med. Clínica (English Ed. 154, 175–177. https://doi.org/10.1016/j.medcle.2020.02.001

Tucker, C.B., 2009. Behaviour of cattle, in: Biology, I. (Ed.), The ethology of domestic animals, 2nd Ed.; Jensen, P., Linköping University: Linköping, Sweden.

Valente, A.M., Acevedo, P., Figueiredo, A.M., Fonseca, C., Torres, R.T., 2020. Overabundant wild ungulate populations in Europe: management with consideration of socio-ecological consequences. Mamm. Rev. 50, 353–366. https://doi.org/10.1111/mam.12202

Valverde, J.A., 1967. Estructura de una comunidad de vertebrados terrestres. Monografías de la Estación Biológica de Doñana, CSIC, Madrid, España.

Van der Poel, W.H.M., 2014. Food and environmental routes of Hepatitis E virus transmission. Curr. Opin. Virol. 4, 91–96. https://doi.org/10.1016/j.coviro.2014.01.006

Van der Poel, W.H.M., Dalton, H.R., Johne, R., Pavio, N., Bouwknegt, M., Wu, T., Cook, N., Meng, X.J., 2018. Knowledge gaps and research priorities in the prevention and control of hepatitis E virus infection. Transbound. Emerg. Dis. 65, 22–29. https://doi.org/10.1111/tbed.12760

Varela-Castro, L., Alvarez, V., Sevilla, I.A., Barral, M., 2020. Risk factors associated to a high *Mycobacterium tuberculosis* complex seroprevalence in wild boar (*Sus scrofa*) from a low bovine tuberculosis prevalence area. PLoS One 15, e0231559. https://doi.org/10.1371/journal.pone.0231559

Varela-Castro, L., Gerrikagoitia, X., Alvarez, V., Geijo, M. V., Barral, M., Sevilla, I.A., 2021. A long-term survey on *Mycobacterium tuberculosis* complex in wild mammals from a bovine tuberculosis low prevalence area. Eur. J. Wildl. Res. 67, 43. https://doi.org/10.1007/s10344-021-01489-z

Venero, J.L., 1984. Dieta de los grandes fitófagos silvestres del Parque Nacional de Doñana. Acta Vertebr. 11, 45–69.

VerCauteren, K.C., Gortázar, C., Beltrán-Alcrudo, D., Vicente, J., 2021. Diseases at the Wildlife - Livestock Interface, in: Vicente, Joaquín, Vercauteren, K.C., Gortázar, Christian (Eds.), Host community interfaces: The Wildlife-Livestock, Wildlife Research Monographs. Springer International Publishing, Cham, Switzerland, pp. 3–32. https://doi.org/10.1007/978-3-030-65365-1

Vergidis, P.I., Karavasiou, A.I., Paraschakis, K., Bliziotis, I.A., Falagas, M.E., 2005. Bibliometric analysis of global trends for research productivity in microbiology. Eur. J. Clin. Microbiol. Infect. Dis. 24, 342–346. https://doi.org/10.1007/s10096-005-1306-x

Vicente-Santos, A., Moreira-Soto, A., Soto-Garita, C., Chaverri, L.G., Chaves, A., Drexler, J.F., Morales, J.A., Alfaro-Alarcón, A., Rodríguez-Herrera, B., Corrales-Aguilar, E., 2017. Neotropical bats that co-habit with humans function as dead-end hosts for dengue virus. PLoS Negl. Trop. Dis. 11, e0005537. https://doi.org/10.1371/journal.pntd.0005537

Vicente, J., Apollonio, M., Blanco-Aguiar, J.A., Borowik, T., Brivio, F., Casaer, J., Croft, S., Ericsson, G., Ferroglio, E., Gavier-Widen, D., Gortázar, C., Jansen, P.A., Keuling, O., Kowalczyk, R., Petrovic, K., Plhal, R., Podgórski, T., Sange, M., Scandura, M., Schmidt, K., Smith, G.C., Soriguer, R., Thulke, H.-H., Zanet, S., Acevedo, P., 2019a. Science-based wildlife disease response. Science. 364, 943–944. https://doi.org/10.1126/science.aax4310

Vicente, J., Barasona, J.A., Acevedo, P., Ruiz-Fons, J.F., Boadella, M., Diez-Delgado, I., Beltran-Beck, B., González-Barrio, D., Queirós, J., Montoro, V., de la Fuente, J., Gortazar, C., 2013. Temporal trend of tuberculosis in wild ungulates from Mediterranean Spain. Transbound. Emerg. Dis. 60, 92–103. https://doi.org/10.1111/tbed.12167

Vicente, J., Delahay, R.J., Walker, N.J., Cheeseman, C.L., 2007a. Social organization and movement influence the incidence of bovine tuberculosis in an undisturbed high-density badger *Meles meles* population. J. Anim. Ecol. 76, 348–360. https://doi.org/10.1111/j.1365-2656.2006.01199.x

Vicente, J., Höfle, U., Garrido, J.M., Fernández-de-Mera, I.G., Acevedo, P., Juste, R., Barral, M., Gortazar, C., 2007b. Risk factors associated with the prevalence of tuberculosis-like lesions in fenced wild boar and red deer in south central Spain. Vet. Res. 38, 451–464. https://doi.org/10.1051/vetres:2007002

Vicente, J., Höfle, U., Garrido, J.M., Fernández-De-Mera, I.G., Juste, R., Barral, M., Gortazar, C., 2006. Wild boar and red deer display high prevalences of tuberculosis-like lesions in Spain. Vet. Res. 37, 107–119. https://doi.org/10.1051/vetres:2005044

Vicente, J., Palencia, P., Plhal, R., Blanco-Aguiar, J.A., Laguna, E., Soriguer, R., López, J.F., Podgórski, T., Petrović, K., Apollonio, M., Scandura, M., Ferroglio, E., Zanet, S., Brivio, F., Keuling, O., Smith, G.C., Guibert, M., Villanúa, D., Rosell, C., Colomer, J., Armenteros, J.Á., Quirós, P.G., Palacios, O.H., Ferreres, J., Torres, J.A., Pareja, P., Martínez-Carrasco, C., Fafián, J.A., Escribano, F., Esteve, C., Acevedo, P., 2019b. Harmonization of the use of hunting statistics for wild boar density estimation in different study areas. EFSA Support. Publ. 16, 1706E. https://doi.org/10.2903/sp.efsa.2019.EN-1706

Vicente, J., Palomares, F., Ruiz de Ibañez, A., Ortiz, J., 2004. Epidemiology of Ancylostoma spp. in the endangered Iberian lynx (*Lynx pardinus*) in the Doñana National Park, south-west Spain. J. Helminthol. 78, 179–183. https://doi.org/10.1079/JOH2003216

Vicente, J., Perez-Rodriguez, L., Gortázar, C., 2007c. Sex, age, spleen size, and kidney fat of red deer relative to infection intensities of the lungworm *Elaphostrongylus cervi*. Naturwissenschaften 94, 581–587. https://doi.org/10.1007/s00114-007-0231-5

Vicente, J., Soriguer, R.C., Gortázar, C., Carro, F., Acevedo, P., Barasona, J.A., Torres, M.J., Negro, J.J., 2014. Bases técnicas para una extracción sostenible de ungulados del Parque Nacional de Doñana.

Villari, S., Vesco, G., Petersen, E., Crispo, A., Buffolano, W., 2009. Risk factors for toxoplasmosis in pigs bred in Sicily, Southern Italy. Vet. Parasitol. 161, 1–8. https://doi.org/10.1016/j.vetpar.2009.01.019

Vincent, J.P., Gaillard, J.M., Bideau, E., 1991. Kilometric index as biological indicator for monitoring forest roe deer populations. Acta Theriol. 36, 315–328.

Wang, H., Castillo-Contreras, R., Saguti, F., López-Olvera, J.R., Karlsson, M., Mentaberre, G., Lindh, M., Serra-Cobo, J., Norder, H., 2019. Genetically similar hepatitis E virus strains infect both humans and wild boars in the Barcelona area, Spain, and Sweden. Transbound. Emerg. Dis. 66, 978–985. https://doi.org/10.1111/tbed.13115

Western, D., 2003. Conservation Science in Africa and the Role of International Collaboration. Conserv. Biol. 17, 11–19. https://doi.org/10.1046/j.1523-1739.2003.02089.x

Westneat, D.F., Birkhead, T.R., 1998. Alternative hypotheses linking the immune system and mate choice for good genes. Proc. R. Soc. London. Ser. B Biol. Sci. 265, 1065–1073. https://doi.org/10.1098/rspb.1998.0400

WHO, 2017. Global hepatitis report, 2017, pp. 1–83.

Wiethoelter, Anke K, Beltran-Alcrudo, D., Kock, R., Mor, S.M., 2015. Global trends in infectious diseases at the wildlife-livestock interface. Proc. Natl. Acad. Sci. U. S. A. 112, 9662–9667. https://doi.org/10.1073/pnas.1422741112

Williams, E.S., Miller, M.W., Kreeger, T.J., Kahn, R.H., Thorne, E.T., 2002. Chronic Wasting Disease of deer and elk: a review with recommendations for management. J. Wildl. Manage. 66, 551–563. https://doi.org/10.2307/3803123

Wilson, A.G., Lapen, D.R., Mitchell, G.W., Provencher, J.F., Wilson, S., 2020. Interaction of diet and habitat predicts *Toxoplasma gondii* infection rates in wild birds at a global scale. Glob. Ecol. Biogeogr. 29, 1189–1198. https://doi.org/10.1111/geb.13096

Wilson, E.E., Wolkovich, E.M., 2011. Scavenging: how carnivores and carrion structure communities. Trends Ecol. Evol. 26, 129–135. https://doi.org/10.1016/j.tree.2010.12.011

Witmer, G.W., 2005. Wildlife population monitoring: some practical considerations. Wildl. Res. 32, 259–263. https://doi.org/10.1071/WR04003

Wobeser, G., 2007. Disease in wild animals. Investigation and management, 2nd ed. Springer-Verlag Berlin Heidelberg, New York.

Wolfe, N.D., Dunavan, C.P., Diamond, J., 2007. Origins of major human infectious diseases. Nature 447, 279–283. https://doi.org/10.1038/nature05775

Woo, P.C.Y., Lau, S.K.P., Teng, J.L.L., Cao, K.-Y., Wernery, U., Schountz, T., Chiu, T.H., Tsang, A.K.L., Wong, P.-C., Wong, E.Y.M., Yuen, K.-Y., 2016. New Hepatitis E virus genotype in Bactrian Camels, Xinjiang, China, 2013. Emerg. Infect. Dis. 22, 2219–2221. https://doi.org/10.3201/eid2212.160979

World Organization for Animal Health (OIE), 2009. Bluetongue and Epizootic Haemorrhagic Disease. Chapter 2.1.3. http://www.oie.int/fileadmin/%0AHome/eng/Health_standards/tahm/2.01.03_BLUETONGUE.pdf

Yan, B., Zhang, L., Gong, L., Lv, J., Feng, Y., Liu, J., Song, L., Xu, Q., Jiang, M., Xu, A., 2016. Hepatitis E virus in yellow cattle, shandong, Eastern China. Emerg. Infect. Dis. 22, 2211–2212. https://doi.org/10.3201/eid2212.160641

Yang, H., Gu, W., Li, Zhanhong, Zhang, L., Liao, D., Song, J., Baoxin, S., Hasimu, J., Li, Zhuoran, Yang, Z., Zhong, Q., Li, H., 2020. Novel putative bluetongue virus serotype 29 isolated from inapparently infected goat in Xinjiang of China. Transbound. Emerg. Dis. 68, 2543–2555. https://doi.org/10.1111/tbed.13927

Young, T.P., 1994. Natural die-offs of large mammals: implications for conservation. conserv. Biol. 8, 410–418. https://doi.org/10.1046/j.1523-1739.1994.08020410.x

Zheng, Y., Ge, S., Zhang, J., Guo, Q., Ng, M.H., Wang, F., Xia, N., Jiang, Q., 2006. Swine as a principal reservoir of hepatitis E virus that infects humans in eastern China. J. Infect. Dis. 193, 1643–1649. https://doi.org/10.1086/504293

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Deng, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F., Shi, Z.-L., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273. https://doi.org/10.1038/s41586-020-2012-7

Zinsstag, J., Schelling, E., Roth, M.A., Kazwala, D.V.M., 2006. Economics of bovine tuberculosis, in: *Mycobacterium bovis* infection in animals and humans, Second Edition. Blackwell, Boston, Massachusetts, pp. 68–83.

Zinsstag, J., Schelling, E., Waltner-Toews, D., Tanner, M., 2011. From “one medicine” to “one health” and systemic approaches to health and well-being. Prev. Vet. Med. 101, 148–156. https://doi.org/10.1016/j.prevetmed.2010.07.003

Zuckerman, Lord, 1980. Badgers, cattle and tuberculosis. Her Majesty´s Stationer Office, London.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. GLM and GAM for count data, in: Environmental Forum. pp. 209–243. https://doi.org/10.1007/978-0-387-87458-6_9

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